UNIVERSITY OF CAPE TOWN

MASTER'S DISSERTATION

Exploring the effects of single and dual phase culturing on the concentrations of Southern Ocean sea-ice algae and transporting living sea-ice algae from the Southern Ocean to land-based research facilities

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Declaration of Authorship

I, Mark HAMBROCK, declare that this dissertation titled, "Exploring the effects of single and dual phase culturing on the concentrations of Southern Ocean sea-ice algae and transporting living sea-ice algae from the Southern Ocean to land-based research facilities" and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University.
- Where any part of this dissertation has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this dissertation is entirely my own work.
- I have acknowledged all main sources of help.
- Where the dissertation is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Signed: Signed by candidate

Date: October 31, 2021

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Abstract

Sea ice is a complex material with a significant impact on the global climate. Understanding the development of sea-ice properties based on the change in growth conditions is vital for the development of predictive models, which are key to providing forecasts of the influence global warming will have on sea ice. Algae found in sea ice form an important part of the oceanic food network, providing secondary producers with a source of food, particularly during winter, and are suspected of seeding algae blooms during spring and summer. Researching sea ice and sea-ice algae *in situ* is an expensive and logistically difficult undertaking, especially in the Southern Ocean. Consequently, many researchers elect to perform research on artificial sea ice, where conditions are more controlled while logistical and financial constraints are reduced. Despite the extent to which sea-ice research has been performed on artificial sea ice, relatively little research on sea-ice algae in artificial sea ice has been done. Sea-ice algae are strongly affected by the temperature, salinity, nutrient availability and intensity of photosynthetically active radiation in their environment. This makes the transportation of living sea-ice algae difficult. Little documentation of transportation of living sea-ice algae exists, with most of the laboratory research of sea-ice algae being performed on single-species liquid cultures. Such research is important but fails to address the complexity of real sea-ice algae communities.

This dissertation investigates the effects of three sea-ice algae transportation methods on the concentration development of sea-ice algae, as well as the potential for experimentation with the algae transported with these methods. Two methods were adapted from literature: transportation in solid (1) and liquid (2) environments. In addition to these methods, a third method was explored: Transportation of living sea-ice algae in a hybrid solid-liquid system. The aim of transporting in the hybrid system was to minimise the changes from the natural to the artificial environment.

Solid sea-ice storage was evaluated by means of an artificial sea-ice study: Artificial sea ice was grown, extracted and stored at -20 °C for 7 different durations, between 0 minutes and 35 weeks. Samples were segmented into 20 mm thick slices, melted, analysed for salinity and brine profile development was assessed. It was found that storage significantly impacted brine profiles, causing an average bulk desalination of 19% between samples stored for 1 day and 35 weeks, as well as a change in the shape of the salinity profile from a W to a C shape. Solid sea-ice transportation was thus eliminated as a transportation method for this work due to the high change algae communities would likely undergo during due to desalination and unfavourable environmental conditions associated with low temperatures.

A solid-liquid hybrid system for the transportation of sea-ice algae was designed and constructed, consisting of a 30 litre Perspex tank, insulation, and a heating system. Two sea-ice cores were obtained from the Southern Ocean in winter of 2019 and transported in the hybrid system to land facilities. Issues with the system were identified, dedicated lights added, and an additional hybrid tank constructed. Six sea-ice cores were obtained from the Southern Ocean in spring of 2019 and parts of them transported in the hybrid tanks to land facilities, where they were melted and cultured in a liquid environment for 56 days. Sections of the cores were melted, and the algae preserved before transportation. Algae concentrations were determined via microscopy of preserved samples taken before transport, after transport and after liquid culturing. Taxonomic distributions of algae varied greatly between initial samples and concentrations ranged from 46 000 to 1 200 000 cells per ml. The hybrid transportation method caused the lowest degree of change in the community compositions and increased the overall concentrations of algae, whilst liquid incubation mostly decreased algae concentrations.

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Chapter 1

Introduction

1.1 Background

Sea ice forms on the surfaces of oceans, particularly in the polar regions. Up to 2.9% and 3.7% (Stammerjohn and Maksym, 2016) of the earth's surface is seasonally covered by Arctic and Antarctic sea ice respectively, making it one of the largest biomes on the globe (Thomas, 2002) and one of the most important food sources for marine life in the Arctic and Southern Oceans (Arrigo, 2016). With the changing climate, sea ice is disappearing at an alarming rate, with an approximate decrease in sea-ice extent of 2.7% per year since 1953 (Meier, 2016). Sea ice plays an important role in the global climate due to its reflective properties: The sea ice-albedo feedback loop describes the phenomenon of global sea ice-surface reduction through light and heat absorption, causing a global temperature increase and, in turn, more global sea ice-surface reduction (Curry, Schramm, and Ebert, 1995). Predictive models for such mechanisms are crucial for the overall prediction of the annual change in the global climate (Notz and Bitz, 2016).

In addition to the direct reflection of sunlight, sea ice plays a secondary important role in reducing the effects of climate change through its facilitation of carbon fixation. Sea ice is a favourable habitat for algae, providing them with conditions allowing concentrations in the sea ice to reach up to 80 times those found in the water column below (Weissenberger and Grossmann, 1998). Le Quéré et al. (2016) approximate that 28% of human activity-related carbon dioxide (CO_2) is absorbed into oceans. Of this 12% is attributed to the polar regions with sea-ice algae and chemical sea-ice processes accounting for 3.5% of ocean, or 0.9% of global carbon fixation (Søren et al., 2011). Sea-ice algae contribute significantly to primary production in the Arctic and Southern Oceans, particularly during winter (Gradinger, 2009). Understanding their reaction to environmental changes is thus of importance for estimates of bioproductivity in the Arctic and Southern Oceans. Estimates of the overall bioproductivity in sea ice are currently inaccurate due to a lack of data and understanding about the behaviour of microorganisms in sea ice (Vancoppenolle et al., 2013). The shrinking of the sea-ice habitat due to global warming has an impact on the survivability of algae species, particularly those in the Arctic, since a nearly complete disappearance of sea ice during summer is forecasted by Wang and Overland (2012) to occur within the next 5 to 27 years. Losing long-standing algae communities associated with persisting sea ice is likely to affect the Arctic food web.

Sea ice is generally expensive and logistically difficult to procure (Miller et al., 2015), with some regions being logistically inaccessible for parts of the year (Lange, 1988; Gradinger, 2009). Lowering the cost and increasing the availability of sea ice and sea-ice algae through growth in laboratories is thus necessary. Polar ships are limited in the space available for experimental equipment and personnel, further emphasising the importance of laboratory experiments. Current methods of transporting sea-ice algae and experiments with them are not able to provide sufficiently relevant data to earth system models (Vancoppenolle and Tedesco, 2016). In

this work we address the need for a modified method of transporting living sea-ice algae. In addition, designing and validation of the modified transportation method in comparison to current practices is also undertaken. This system would need to more closely simulate the environment that sea-ice algae experience *in situ* to improve their survivability and lower the community composition changes.

1.2 Scope and Limitations

This study focussed on the comparison of methods of transport of sea-ice algae and their potential impacts on sea-ice algae communities, contrasting two literature methods to a novel method. The literature methods are transportation in solid and in liquid phase, whereas the novel method involves transportation in vessels containing both phases. The investigation into the potential impacts of solid sea-ice transportation on sea-ice algae is limited to a study of brine profile changes in artificial sea ice stored at -20 °C for up to 35 weeks. Simulation of the natural sea-ice environment in the hybrid system was limited to providing general sea-ice temperature, irradiance, nutrient, and salinity conditions. It did not mimic the sample-specific environments and elements, such as snow, wind and seawater circulation. Experimentation with algae in this study is limited to samples obtained from the marginal sea-ice zone in the Southern Ocean during the SCALE Spring Cruise of 2019. The liquid culture experiment was performed with a single level of irradiance and one temperature, with bulk nutrient concentrations being adjusted in the same manner as during transportation. This study does not consider the effects of different nutrient compositions, storage at different temperatures for each method of transportation, or the effects of different light intensities and compositions on sea-ice algae.

Chapter 2

Literature Review

2.1 Environmental Importance of Sea Ice

Sea ice is a better reflector of sunlight than ocean water, with sea ice reflecting 10 times as much sunlight as water (Petrich and Eicken, 2016). The sea ice-albedo feedback loop describes the phenomenon of global sea ice surface-reduction causing a global temperature increase and, in turn, more global sea ice surface-reduction (Curry, Schramm, and Ebert, 1995). In the Antarctic region, the physical motion of the ocean water causes sea ice to collide, break and float into warmer waters where it melts. Prediction models for such mechanisms are crucial for the overall prediction of the annual change in the global climate. Sea-ice algae are the most abundant organisms in the sea ice-brine channels, with a seasonally higher bio-productivity than the phytoplankton in the water below (Nomura et al., 2011). These algae are an important part of the food chain in the Arctic and Southern Oceans, seasonally being the main food supply for ocean herbivores and further being estimated to account for 4% - 50% of total primary production in sea ice-covered seas (Gradinger, 2009). Sea-ice algae are also assumed to contribute to the bloom of phytoplankton through seeding after the sea ice melts (Kuosa et al., 1992; Caron, Gast, and Garneau, 2016)

Additionally, sea ice plays a significant role in facilitating carbon storage, accounting for approximately 0.9% of global human activity-related carbon fixation: Le Quéré et al. (2016) claim that approximately a quarter of the human activity-related CO_2 is absorbed into our oceans, amounting to 2.4 ± 0.5 gigatons of carbon per annum. Søren et al. (2011), through their modelling efforts, have concluded that 83 kilotons of carbon are deposited annually through sea ice-related mechanisms such as brine rejection, precipitation of $CaCO_3$ and the uptake of carbon dioxide into depleted surface waters following the sea-ice melt. This estimate of Søren et al. (2011) does not take into account the continuous melting and freezing of sea ice, but only the change in volume from its minimal to maximal extent over a season and is thus likely an underestimate. Arrigo (2016) warns that model estimates of sea-ice algae production are extrapolations from few and highly localised field observations and that such estimates therefore require further verification.

2.2 Sea-Ice Formation

Seawater is a complex mixture of water, various organic and inorganic compounds, and gases. Figure 2.1 shows the phases of the major compounds in $35\ \frac{\mathrm{g}}{\mathrm{kg}}$ saline seawater at equilibrium as it freezes at atmospheric pressure (Deville, 2013). Several compounds precipitate from the solution in the temperature band between 0 and $-54\ ^{\circ}\mathrm{C}$. Sea ice is a highly dynamic 3-phase

material consisting of gases, concentrated brine solution and various salts and ice, in concentrations that evolve as external conditions vary. Furthermore, sea-ice structures are strongly dependent on the season and location (Lange, 1988; Gradinger, 2009).

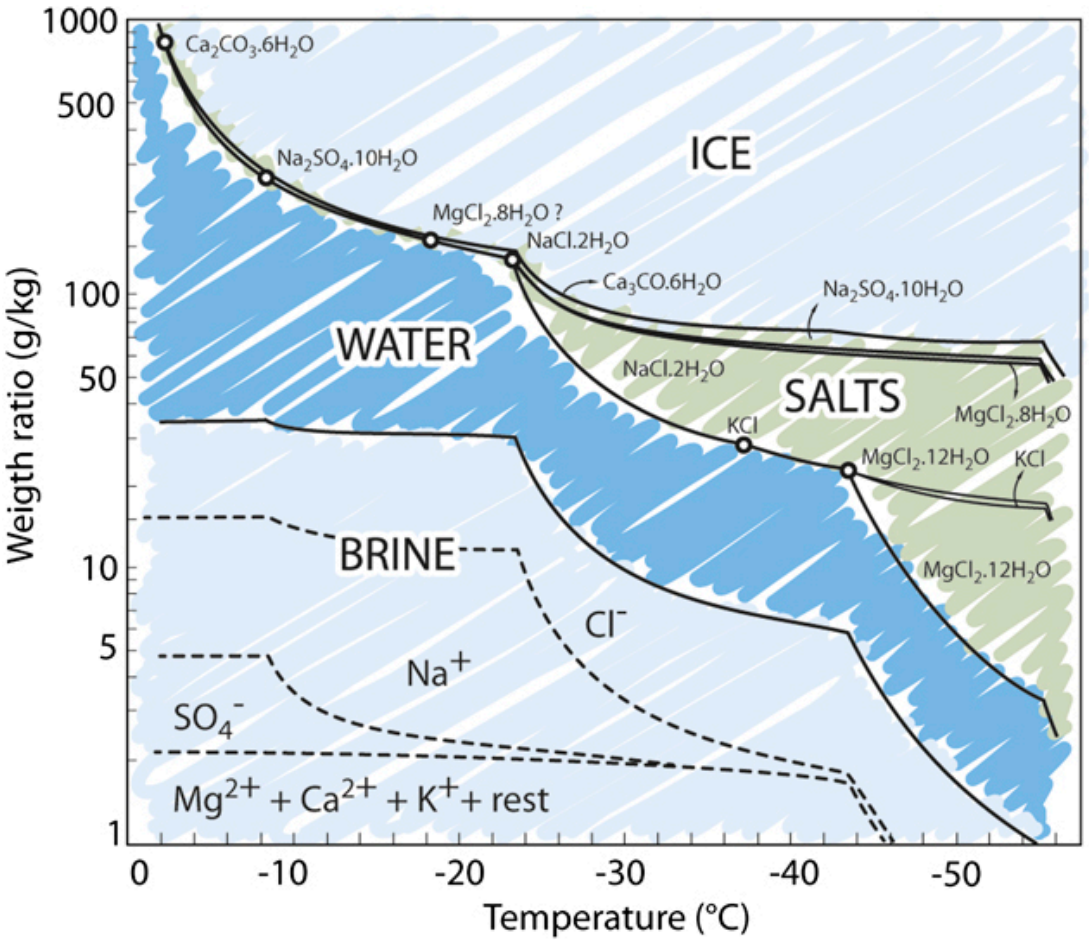

Figure 2.1: Phase diagram of sea ice (Deville, 2013)

As sea water freezes, pure H_2O crystals form in the 1h lattice structure depicted in Figure 2.2, a parallel arrangement of planes of water molecules in hexagonal-chair position. The continued growth of the lattice primarily occurs in the direction of the c-axis (Chaplin, 2018).

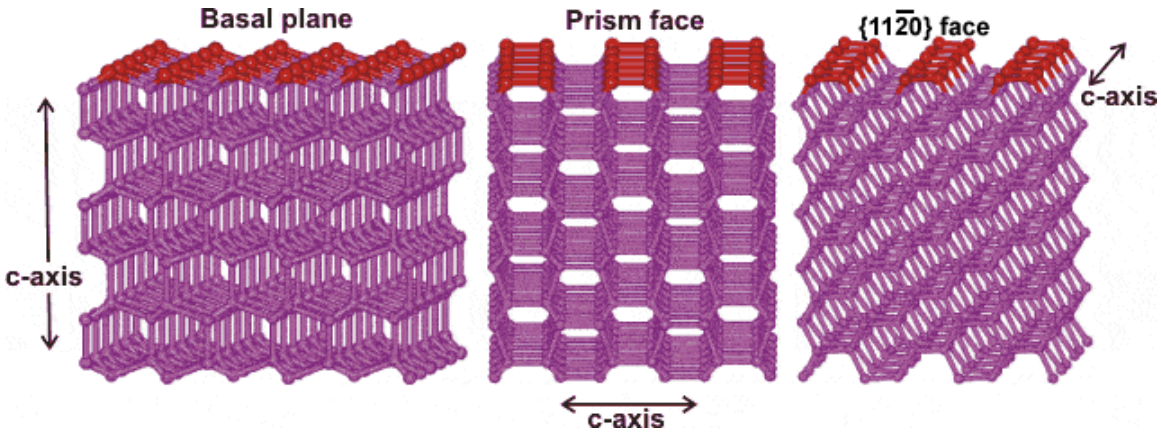

Figure 2.2: Ice Ih lattice structure (Chaplin, 2018)

When arbitrarily aligned ice crystals freeze together, the resulting polycrystalline structure is called granular ice, having the grainy texture shown in Figure 2.3.d. The thickness of this layer is dependent on ocean conditions such as turbulence (Petrich and Eicken, 2010). Figure 2.3 shows the different polycrystalline structures of sea ice under polarised light. As the sea ice continues to grow, the structure changes from the direction-less granular texture (d) to columnar vertical sea ice (a,b) as those crystals with their growth axis perpendicular to the ocean surface out-grow non-perpendicular crystals. The area between the granular and columnar sea ice is called the "transition zone" (c).

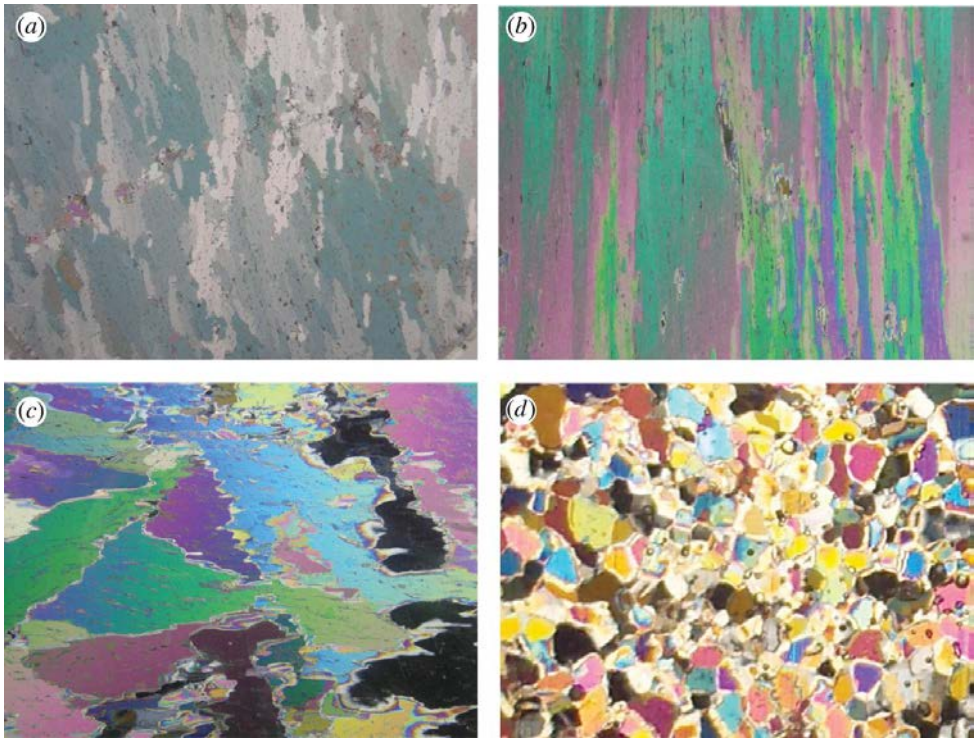

Figure 2.3: Cross-polarised images of of sea-ice cross-sections with different polycrystalline structures from Antarctica (a,b), the Ross Sea (c) and the Bellingshausen Sea (d) (Gully et al., 2015)

Salt is expelled from the freezing solution as ice crystals form. It is either retained between crystals in channels or pockets (Golden et al., 2007; Eicken et al., 2000) or ejected into the seawater below owing to thermo-volumetrically induced pressure zones or drained due to gravity (Notz and Worster, 2009). Brine channel and pocket volumes are dependent on the salt composition and concentration, temperature and pressure within the brine (Cottier, Eicken, and Wadhams, 1999). During summer, sea-ice salinity may be further reduced due to flushing melt water (Notz and Worster, 2009). Typical first-year sea-ice salinities range from 4 to 6 practical salinity units (PSU), in contrast to typical ocean salinities of 32 to 35 PSU (Timco and Weeks, 2010). Petrich and Eicken (2016) report first-year sea ice to typically have a C-shaped salinity profile. Timco and Weeks (2010)[and references therein] show close coupling between salinity and several sea-ice properties, such as density, porosity, tensile strength (through porosity), flexural strength and others.

The Arctic Ocean is mostly enclosed by land masses, limiting sea ice and water movement in the region (Stammerjohn and Maksym, 2016). Due to this, weather conditions in the Arctic Ocean are calm relative to those in the Southern Ocean, with sea ice in the Southern Ocean

often experiencing wind and wave motions bordering on the global extremes (Stammerjohn and Maksym, 2016). Sea ice in the Arctic Ocean mostly forms through congelation into sea-ice sheets, with accumulation of sea ice through ridging resulting in sea ice typically being thick in the region (NSIDC, 2018). Figure 2.4 shows the progression which sea ice undergoes in the rough ocean conditions common to the Southern Ocean: Frazil ice conglomerates through wave and wind motion (Weeks and Ackley, 1986) and forms larger masses of sea ice that bump into each other. During this process, the edges of the conglomerated sea ice are rounded, resulting in disks of sea ice called "pancakes" (Weeks and Ackley, 1986; Petrich and Eicken, 2016) which later consolidate into sea ice sheets. Areas of the ocean which are partially, but not fully covered by sea ice are referred to as the marginal ice zone (Sturm and Massom, 2016).

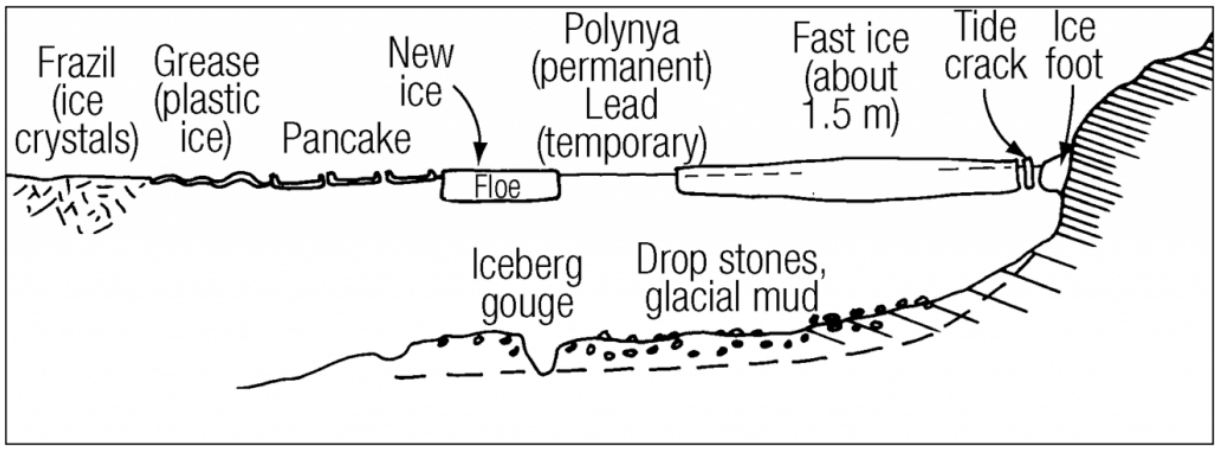

Figure 2.4: Sea-ice formation in rough conditions (AAD, 2014)

Antarctic sea-ice formation conditions differ significantly from those in the Arctic: Antarctica is a central landmass, with sea ice forming on the edges and extending outwards (Stammerjohn and Maksym, 2016). Due to the lack of land constraints, the sea ice around Antarctica moves more freely than that at its northern counterpart, resulting in fewer sea-ice ridges and thus thinner sea-ice (NSIDC, 2018), with most sea ice melting within a year of formation. Sea ice of this kind is termed first-year sea ice. The ocean conditions around Antarctica are rough (Stammerjohn and Maksym, 2016), causing most sea ice to form via the pancake pathway. One of the major differences between Arctic and Antarctic sea-ice composition is the degree of frozen flooded snow, a phenomenon far more common in the Southern Ocean than the Arctic (Petrich and Eicken, 2016).

2.3 Sea Ice, a Habitat

2.3.1 Physical processes of phytoplankton incorporation into sea ice

During winter and autumn, sea-ice algae extending into the water provide a concentrated food source to bottom feeders (Kauko et al., 2018). The growth of these sea-ice algae is primarily governed by the availability of light and nutrients (Gradinger, 2009), as well as the temperature and salinity of their environment (Campbell et al., 2014). Light, thermal and saline growth conditions become favourable for sea-ice algae in early spring and cause algae to bloom for several days or weeks. The sea ice starts melting and nutrients and algal communities from

the sea ice are released into the water, ending the sea-ice algae bloom (Campbell et al., 2014). Sea-ice algae have been found to mostly inhabit the bottom 3 (Horner and Alexander, 1972) to 10 centimetres of sea ice in the Arctic (Campbell et al., 2014), with chlorophyll α values reportedly exceeding those in the ocean below by up to 80 times (Weissenberger and Grossmann, 1998). Prior to consolidation of frazil crystals, concentrations of sea-ice algae species in frazil ice are significantly higher than in the surface water below (Ackley and Sullivan, 1994). A supporting theory for this phenomenon is that algae stick to the surface of frazil crystals as they rise through the water column and are further mixed with water through current and wave action (Ackley and Sullivan, 1994).

In a series of artificial sea-ice experiments, conducted in a 2-metre water column, Garrison, Close, and Reimnitz (1989) investigated the degree to which scavenging of algae from the freezing solution with frazil ice may contribute to the initial chlorophyll profile. They concluded that observed concentrations in nature may be attributed solely to the scavenging of particles by frazil ice if the process were to occur in the upper 50 metres of the water column. An issue with their hypothesis is that it requires frazil-ice formation to occur 50 metres below the water surface. Since cooling in the polar oceans commonly occurs at the air-water interface, it is unlikely that frazil ice rising from the depths is the dominant method of algae harvesting, particularly in the turbulent Southern Ocean. It is speculated that processes such as wave field pumping and Langmuir circulation play an important part in facilitating organic entrapment (Lannuzel et al., 2010). Weissenberger and Grossmann (1998) found that in their laboratory sea ice, tank wave action increases the uptake rate of algae into sea ice relative to the underlying solution. They further determined that a current underneath the sea ice may significantly increase the concentration of biomatter in the lower segment of the sea ice and postulated that this is due to algae adhering to the surfaces of dendrites. Algal uptake due to currents was found to be higher than that due to wave field pumping with their specific parameters.

Laboratory experiments by Ackley, Dieckmann, and Shen (1987) and Shen and Ackermann (1990) on the effects of wave action on the uptake rate of algae into sea ice showed that this process alone could account for the high algal concentrations often found in newly formed sea ice. Heavy snow loading (at least half of the sea ice thickness) may result in seawater flooding the snow layer at the ice-snow interface and freezing of the submerged snow, producing snow ice. Snow flooding is more common in the Southern Ocean than in the Arctic due to the generally thinner sea ice and more substantial snow fall in the Antarctic region (Petrich and Eicken, 2016). The flooded layer has been estimated to have a production of $2.4 \pm 1.4 \frac{\mathrm{g(Carbon)}}{\mathrm{m^2 day}}$. Chlorophyll concentrations seasonally exceed $400 \frac{\mathrm{mg}}{\mathrm{m^2}}$ (Arrigo et al., 1997) depending on light, nutrient and temperature changes and the seed population. Legendre et al. (1992) suggest this system to be the largest carbon consumer of all sea-ice communities based on computational modelling.

2.3.2 Factors affecting the growth of algae in sea ice

The growth of sea-ice algae is primarily governed by their local salinity and temperature, the availability of light, nutrient supply (Gradinger, 2009) and some trace metals, particularly iron (Lannuzel et al., 2010; Lannuzel et al., 2015; Lannuzel et al., 2016; Krembs, Eicken, and Deming, 2011; Genovese et al., 2018).

2.3.2.1 Temperature and salinity

As sea-ice temperature decreases, so do the volumes of brine channels and pockets, whilst their salinity increases (see Figure 2.1), concentrating nutrients and thus providing a preferable environment for algae in comparison to the water column in terms of nutrient availability; however, increased salinities also put algal cells under osmotic-pressure-induced stress and will result in a reduced growth rate as salinity rises (Mock and Kroon, 2002).

In her thesis on the influence of temperature and salinity on sea-ice diatoms, Andersson (2015) explored the effects of varying these two parameters on three different algae species common to sea ice and phytoplankton blooms. She found that reaction to changes to temperature is species-specific and that differences in salinities of treatment did not affect the outcomes of those treatments. Andersson (2015) further notes that algae had to be adapted to experimental conditions for 3 to 7 days before performing as expected.

The reaction of sea-ice algae is also species-dependent, since Stoecker et al. (1997) measured continued bioproductivity in brine channels with temperatures as low as $-7.1\ ^{\circ}\mathrm{C}$ and practical salinities of up to 129 PSU. Conversely, Grant and Horner (1976) investigated the responses of four sea-ice diatoms on changes to their salinities under liquid culture conditions, varying the treatment salinities from 5 to 70 PSU and measured no growth above 60 PSU.

2.3.2.2 Radiation

Photosynthetically active radiation (PAR) is a necessity for photosynthesis. Polar algae experience a wide range of irradiance levels, ranging from complete darkness in the polar winter to values as high as $1487\frac{\mu\mathrm{mol}}{\mathrm{m^2s}}$ (Lazzara et al., 2007). Depending on the geographic location, days may pass without the sun going down in summer or coming up in winter. Besides geographic and weather influences on surface light levels, sea ice introduces further complexity to algal light availability: Light levels are not equal from the top to the bottom in sea ice. Snow and ice attenuate light depending on their respective thicknesses and states (Perovich, 2016, and references therein), as illustrated in Figure 2.5. As such, the degree to which sea-ice algae are exposed to sunlight or fractions thereof is dependent on their relative location within the sea ice. Palmisano and Sullivan (1982) state the range 0 to $46\frac{\mu\mathrm{mol}}{\mathrm{m^2s}}$ as a good approximation of the range bottom sea-ice algae experience through the annual cycle.

Different algae are optimised for different parts of the sea-ice complex and the conditions therein. Mangoni et al. (2009) state that sympagic community primary production is mostly limited by light availability. Hancke et al. (2018) report algae growth of bottom communities occurring at irradiance below $0.17\frac{\mu\mathrm{mol}}{\mathrm{m^2s}}$ and that the species *Nitzschia frigidia* was able to acclimatise to a light level of $110\frac{\mu\mathrm{mol}}{\mathrm{m^2s}}$, whereas Palmisano, SooHoo, and Sullivan (1985) noted photoinhibition for some Antarctic sea-ice species at irradiance levels above $25\frac{\mu\mathrm{mol}}{\mathrm{m^2s}}$. Beyond the absolute irradiance value, the spectral distribution contributes to controlling algal growth, with UVB radiation having been determined to reduce algal productivity (McMinn, Ashworth, and Ryan, 1999). Mangoni et al. (2009) found that subjecting bottom sea-ice algae acclimatised to low light levels ($7\frac{\mu\mathrm{mol}}{\mathrm{m^2s}}$) to $19\frac{\mu\mathrm{mol}}{\mathrm{m^2s}}$ resulted in significant photodamage for some algae, with substantial differences in the adaptability of different algae to the environmental change.

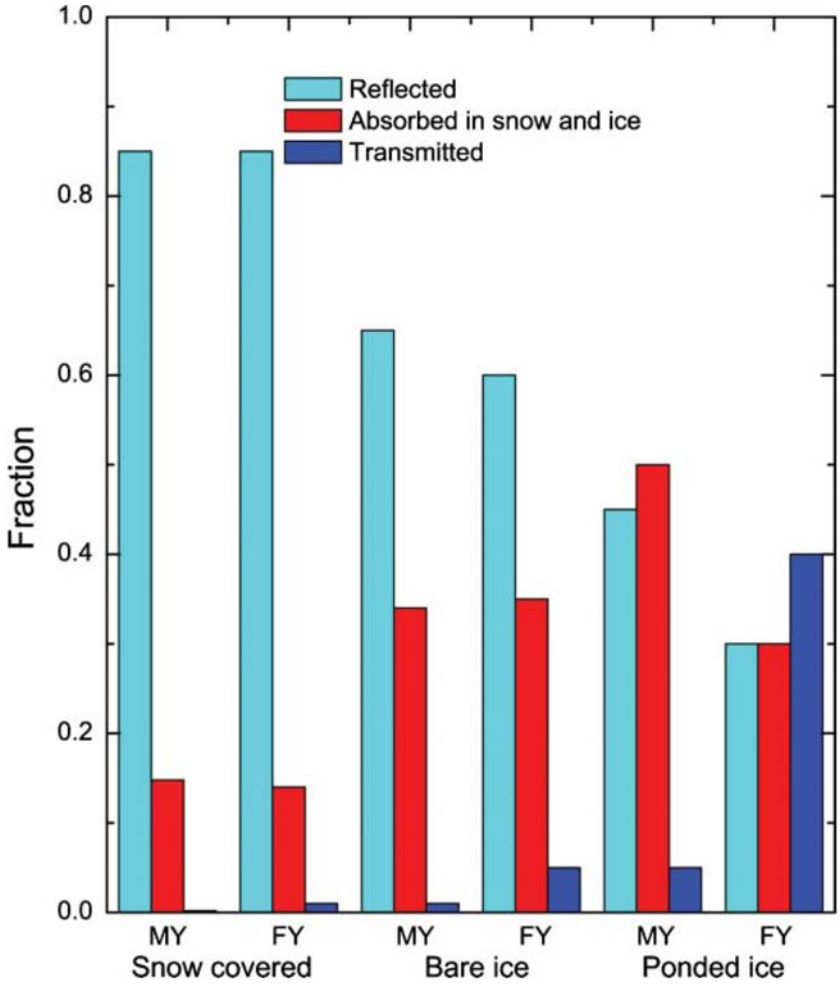

Figure 2.5: Solar partitioning under varying sea-ice conditions (Perovich, 2016)

2.3.2.3 Nutrients

During sea-ice formation nutrients are largely distributed in proportion to brine and are therefore initially determined by the local composition of seawater. Due to the generally lower bulk salinity of sea ice in comparison to seawater, the bulk concentrations of nutrients within sea ice are also usually lower than in the water below. Despite this, the localised concentrations of brine within sea ice result in an environment in which algae initially experience higher nutrient concentrations than in the water column (Meiners and Michel, 2016).

Consumption, production and modification of organic and inorganic nutrients through biological processes change the initial nutrient concentrations in brine channels and pockets. This potentially limits algal growth within the sea ice during periods of peak production (Meiners and Michel, 2016). Fluxes at the interfaces between sea ice, the ocean and the atmosphere may additionally alter the nutrient profile. Primary producers harvest nutrients from the water below, nutrient-depleted brine is ejected from brine channels and replaced by lower-salinity nutrient-rich seawater. Particulate deposition and gas exchange occur with the atmosphere and contribute to nutrient replenishment (Meiners and Michel, 2016).

2.3.2.4 Iron as key micro-nutrient

Trace metals form part of the dissolved ions in sea water and are absorbed into the sea ice as it grows, accumulating to concentrations sometimes greater than in the ocean below (Vancoppenolle et al., 2013). Iron in the sea-ice zone undergoes a seasonal regeneration cycle (Schoemann et al., 2008; Lannuzel et al., 2010) and is replenished from several deep-ocean and continental sources, providing the necessary nutrients for the seasonal algal blooms. The seasonal cycle and inter-system exchange of iron is described below and, to some extent, can be assumed to hold for other trace nutrients as well.

During autumn, the initial entrapment of iron during the coagulation of frazil ice occurs. The entrapped iron occurs in the form of detritus and is bonded to suspended organic matter. Lannuzel et al. (2010) hypothesised that two processes govern the winter iron absorption in sea ice: Firstly, the physical process of saline solution concentration during congelation in the brine channels. Secondly, the biological process of algal iron absorption at the bottom of the sea ice. This second process is limited by iron replenishment through up-welling from the ocean. Algal blooms occur in spring due to the increase in sunlight and average temperature. Increased temperatures induce melting and reduce iron-induced algal growth limitations, with most dissolved iron being released from melting sea ice within a few days (Lannuzel et al., 2010). In summer, wind and ocean currents induce large-scale sea-ice drifts which contribute to distributing biogeochemical matter (Vancoppenolle et al., 2013). Other biological matter sinks or remains in suspension, being absorbed into the sea ice during the following autumn (Lannuzel et al., 2010).

2.3.2.5 Influence of sea-ice algae on sea ice

The influence of sea-ice properties, such as porosity and salinity, on sea-ice algae has received much attention in the scientific community; however, the influence of sea-ice algae on sea-ice properties is not well researched. The recent discovery of ice-binding proteins (IBP) released by some diatoms, provided insight as to how algae may change their environment (Krembs, Eicken, and Deming, 2011). Raymond (2011) experimentally determined that IBP have a significant impact on brine channel connectivity. As such, it stands to reason that the presence of algae in sea ice has an impact on brine drainage during extraction and storage. Furthermore, the correlation between brine drainage during extraction and the resulting loss of sea-ice algae is of interest since a linear correlation should not be assumed, as IBP allow algae to better adhere to ice.

2.4 Sea-ice algae sampling and bioproductivity monitoring methods

2.4.1 Sea-ice sampling methods

A standard sea-ice extraction method is by excavating a vertical cylinder, or core, of sea ice using a rotating bladed tube. When the cylinder of sea ice is pulled up, gravity causes brine to drain from the core. The effect of brine drainage from cores is a serious issue for all studies using cores to estimate algal quantities in sea ice as some quantity of algae is inevitably lost in extraction (Miller et al., 2015). This quantity is further elevated by the following drivers: the fact that the brine forms the habitat for algae, the fact that sea-ice algae are usually most

concentrated in the lower part of the sea ice (Arrigo, 2016) and that this lower part of the sea ice is most porous (Petrich and Eicken, 2016) and thus most liable to brine losses during extraction.

The subsequent standard steps in core analysis are segmentation and melting for chemical and biological analyses (Miller et al., 2015; Gradinger, 2009; Campbell et al., 2014). Current melting methodologies introduce several uncertainties to the results of analyses for biological purposes, as some algae may experience osmotic stress, removing even traces of some species when melted without adequate preparations (Campbell et al., 2014). Commonly used methods to reduce osmotic stress include melting the samples into pre-filtered or artificial seawater (Gradinger, 2009; Campbell et al., 2014; Miller et al., 2015), or melting the sea ice over several days (Miller et al., 2015). To minimise continued algal growth post-extraction, the melting process should be performed in absolute darkness and at temperatures at, or slightly above the melting point of the final solution (Gradinger, 2009; Miller et al., 2015).

Diving assisted sub-sampling is a method used to sample the brine, seawater, and unconsolidated sea ice directly below sea-ice sheets. The sample is collected by a submersible pump operated by a scuba diver (Campbell et al., 2014). Samples gained through this method provide insight into the average chemical and biological composition of sea ice as well as the water immediately below the sea ice; however, the lack of horizontal sampling confinement limits the accuracy of this sampling method for vertical estimates of compositions below the sea ice.

2.4.2 *In situ* bioproductivity monitoring methods

Tracer incubation is a method where nutrients are doped with a radioactive isotope such as C^{14}, which are then introduced into a previously extracted and segmented core. The core is then reassembled in its original orientation and order and replaced into its coring hole. Subsequent re-extraction and radiation analysis will thus allow for an algal distribution estimate. However, the issue of brine drainage persists and is even elevated through the prior extraction and segmentation (Gradinger, 2009).

Oxygen monitoring is a method of estimating algal productivity by monitoring the oxygen flux at the ice-water interface through the diffusive boundary layer (McMinn and Hegseth, 2007). Limitations to this method include the inherent lack of vertical distribution, the need to position probes at a distance from the hole where they were deployed. Additionally, this method may not be executed in conjunction with diving-assisted sub-sampling or near a site where sub-sampling or diver entry has occurred.

Normalised Difference Index measurements are a non-invasive method of quantifying algal biomass in sea ice and can be carried out *in situ* based on chlorophyll α content, by measuring the absorbance of specific light frequencies and normalising this with respect to average $(\mathrm{chlorophyll}\alpha) : (\mathrm{algalbiomass})$ ratios at different conditions (Normalised Difference Index). Due to the dependence of these ratios on environmental conditions and community compositions, specific calibration during testing is required (Campbell et al., 2014), which currently falls back onto data obtained from coring. As such, it is also insufficient in quantifying overall biomass as the coring calibration suffers from brine drainage.

2.5 Transport of Living Sea-Ice Algae and Subsequent *ex situ* Studies

In situ monitoring of sea-ice algae is a well-established practice; however, existing *in situ* monitoring methods face several challenges which suggest the need for laboratory experimentation: Tracer incubation and normalised difference index measurements are affected by brine and thus algae loss due to coring procedures (Miller et al., 2015), whilst diving-assisted subsampling (McMinn and Hegseth, 2007) and oxygen monitoring (Miller et al., 2015) are constrained by their lack of horizontal and vertical measurement accuracy respectively. Additionally, these methods can only be executed in calm and stable conditions due to safety concerns and equipment limitations, requiring deployment of scientists and equipment onto or underneath the ice. As such, they are unsuitable for studies on pancake or brash ice and cannot be practically implemented to study sea-ice algae blooms where sea ice has melted substantially.

Sea-ice algae have been transported to land facilities in two forms: bulk solid and fully liquid medium, meaning transporting them either within sea ice or melted sea ice. Both forms of transportation have distinct advantages and disadvantages when it comes to ease of transport, overall survivability of algae as well as community preservation. Community preservation will be focused on in this work, as it has been noted by Vancoppenolle and Tedesco (2016) that experimentation with algae communities in the sea-ice medium is still lacking and of great importance to improving the uncertainty of current biogeochemistry sea-ice models, and consequently earth system models.

2.5.1 Transport in solid medium

Yan et al. (2020) transported algae in a core obtained from the Sea of Okhotsk at -5 °C, blocking light to the core during transport to prevent growth for the 3 weeks between extraction and preparation for experimentation. The choice of -5 °C as the temperature of transportation was guided by the rule of fives explored by Golden, Ackley, and Lytle (1998), which states that sea ice with a bulk salinity of 5 PSU, at -5 °C will have a brine volume fraction of 5% and thus not be permeable. As such, any temperature above -5 °C would likely be insufficient in preventing loss of algae through gravity drainage of the brine; however, the rule of fives is not guaranteed to hold.

Sea-ice salinity is highly variable vertically, horizontally and temporally throughout a single floe (Thomas, 2016). The top and bottom of a sea-ice core typically have higher bulk salinities than the centre of the core, resulting in salinity profiles often being C-shaped (Petrich and Eicken, 2016). The bottom (Campbell et al., 2014; Horner and Alexander, 1972; Lund-Hansen et al., 2017) and, seasonally and regionally, the top of sea ice also contain the largest fraction of algae (Legendre et al., 1992; Ackley and Sullivan, 1994; Thomas, 2016). Since the rule of fives does not hold at bulk salinities above 5 PSU, the top and bottom algae communities cannot be expected to be preserved at a temperature of -5 °C. The rule of fives also does not consider the effects which primary producers may have on sea ice. Exopolysacharides (EPS) and other substances released by bacteria and microalgae in sea ice change the degree of interconnectivity of brine channels, as well as the local freezing point (Krembs, Eicken, and Deming, 2011; Raymond, 2011). They may thus affect the porosity of sea ice, potentially violating the rule of fives.

Sea ice typically has a non-uniform temperature profile, as the sea ice directly in contact with the seawater will approximate its temperature, whilst sea ice closer to the surface will be at a

temperature closer to that of the surrounding air or snow (Thomas, 2016). In order to limit the draining of brine out of the sea ice, it is imperative that the temperature of the core is lowered quickly. Quick changes to the core temperature result in quick changes to brine salinity, which induces osmotic stress to algae. Osmotic stress has been linked with the destruction of sea-ice algae (Thomas, 2016; Campbell et al., 2019; Yan et al., 2020), affecting some types of algae more than others, with diatoms being least affected (Steele, Franklin, and Underwood, 2014). As such, the quick cooling of the samples results in a degradation of the community species distribution through selective destruction of osmotic stress-affected species. Species sensitive to osmotic stress frequently inhabit the lower segments of the sea ice (Kauko et al., 2018), where the sea-ice temperature is warmer, and are thus even more affected by this phenomenon in the context of cooling for transportation.

Changes to the temperature of sea ice are typically linked to changes in the brine profile due to changes in local densities, resulting in brine pressure increases and expulsion of brine (Petrich and Eicken, 2016). As such, partial or total cooling or heating of a core to -5 °C cannot prevent changes from the *in situ* to the *ex situ* brine and algae profiles, even if all other assumptions of the rule of fives hold. Although preservation of the original brine and algae profile was not the intent of Yan et al. (2020), the fact that changes to both inherently occur with this method of transportation should be noted. Additional changes to the brine profile may occur due to the relatively high storage temperature, as Cox and Weeks (1986) suggest storing sea ice at -23 °C if changes to the brine profile are to be avoided, whilst Miller et al. (2015) suggest storage below -20 °C for biogeochemical core storage. Petrich and Eicken (2016) report this as the temperature cores are commonly stored at to prevent brine drainage.

Grant and Horner (1976), Vargo et al. (1986), and Andersson (2015) found that diatoms in their laboratory experiments required several days to adapt to changes in environmental conditions before resuming natural production rates. This suggests that natural sea-ice samples undergoing transportation, where they are deprived of light or not adapted to other conditions of intended experiments, will need to undergo adaptation before natural production rates can be assumed. But, continued production during the adaption period might change the community composition, since continued production would produce changes to the nutrient composition (Andersson, 2015).

There are some advantages to transporting sea-ice algae within their solid medium: A large portion of the algae initially in the core should be preserved through this method. The initial required processing time is negligible, consisting solely of covering the core with a light-impermeable shield and transport to the on-ship cold storage facilities. Transport between on-ship and on-land storage facilities is only complex in its requirement to keep the samples at the exact storage temperature, as fluctuations above -5 °C may cause brine and algae loss, whilst cooling samples well below -5 °C may cause harm to the algae in the sea ice.

2.5.2 Transport in liquid medium

Grant and Horner (1976), Xu et al. (2014), and Dawson et al. (2020) chose to melt their sea-ice samples immediately after extraction. Direct quick melting of sea-ice provides potential for osmotic stress, which has been linked with the destruction of some algae. Methods to limit the degree of osmotic stress algae experience, such as melting into filtered seawater, have been explored and are effective (Campbell et al., 2019); however, they require more preparation and operational time.

Post-melting production of algae may significantly alter the community composition, since some algae adapt more easily than others to the changes of temperature and other conditions (Grant and Horner, 1976; Vargo et al., 1986; Andersson, 2015). This may pose an issue when algae are to be adapted to experimental conditions, as further production during a period where light and nutrients are available, is unavoidable. Xu et al. (2014) and Dawson et al. (2020) chose to forego the preservation of the initial community composition by isolating a specific strain of algae for further experimentation.

Transport of algae in liquid medium is relatively easy, though a higher initial processing time than for the transport of algae in solid medium is required. If samples are treated appropriately, osmotic stress can be minimised and the algal community well-preserved. Supplying samples with nutrients, air and current motion is also simplified for those cases where immediate experimentation is desired.

2.5.3 Laboratory studies on sea-ice algae

Sea-ice algae are understudied when compared to other marine ecosystems (Vancoppenolle and Tedesco, 2016). The understanding of their behaviour under varying conditions is of importance for earth system models, which currently assume sea ice to be unchanging in its chemistry and biology (Vancoppenolle et al., 2013). Since algae community concentrations and compositions are highly localised, the relatively small number of studies exacerbate the degree of uncertainty associated with current estimates of primary production, biomass and the responses of algae to changes in their environment (Lund-Hansen et al., 2017).

Ryan et al. (2011) state that melting of the sea-ice matrix and subsequent artificial culturing of the released algae is the dominant practice in studies on the stress responses of sea-ice algae, with most of these studies concentrating on bottom-ice algae. They warn that damage or modification of cellular physiology may occur due to this practice and suggest that algae should be studied *in situ*. The majority of these liquid culture studies isolate species after melting and limit their studies to single-species responses to environmental changes. Consequently, studies on microbial communities are limited in number, leading to limited confidence in current estimates of overall sea-ice primary productivity (Vancoppenolle and Tedesco, 2016).

It could be argued that the primary purpose of isolated liquid culture studies is to reduce the number of variables and thus be able to make claims about species-specific behaviour with higher certainty. Relating these claims to sea-ice algae in natural sea-ice algae communities, strongly relies on isolated species behaving in liquid cultures as they do in their natural habitat. There is some evidence for overlap in natural sea-ice algae behaviour and that of algae in isolated liquid cultures. Examples are metabolite production rates (Dawson et al., 2020) and the magnitudes of concentrations of chlorophyll α-normalised chromophoric dissolved organic matter (Li et al., 2019). Discrepancies between the results of these studies and the natural sea-ice communities they were compared to, were noted by the authors: Dawson et al. (2020) attribute the lack of some metabolites and the relatively high concentrations of other metabolites to the lack of diversity in their sample, as well as the environmental differences. Li et al. (2019) experience 4 to 8 times higher bacterial growth rates in their cultures than those they cite for natural sea ice. Furthermore, they caution that most of the overlap between their results and those of natural sea-ice studies may be attributed to other causes.

Relating these studies to natural sea-ice algae behaviour thus requires strong evidence from *in situ* studies. It is difficult to reliably relate such studies to the behaviour of sea-ice algae at times

and locations where relevant studies occur rarely or not at all, such as the marginal sea-ice zone or most of the Southern Ocean in winter. Miller et al. (2015), Ryan et al. (2011), Meiners and Michel (2016), and Vancoppenolle and Tedesco (2016) highlight the need for *in situ* studies due to the lack of data on the behaviour of sea-ice algae in sea ice as opposed to liquid media due to the differences in environmental conditions, community compositions and the relatively short time over which most laboratory studies are performed.

2.6 Post-Extraction Salinity Profile Evolution through Storage

Sea ice, as the biome with the largest global seasonal change, has increasingly received attention from the scientific community over the past 50 years, with some of the earliest published work being on the mechanical properties of sea ice (Weeks and Assur, 1967).

2.6.1 Common storage practices & techniques

Gravity causes brine to drain from coring samples during extraction as well as during the period prior to analysis. In order to limit the degree to which brine drains from sea-ice samples, their typical handling prior to analysis involves storage at temperatures below -20 °C, if they are not immediately analysed (Miller et al., 2015). Miller et al. (2015) suggest storing biogeochemical samples which cannot be analysed within hours of extraction to limit biological growth post-extraction. The analysis of mechanical properties, as recently summarised by Timco and Weeks (2010), and imaging (Golden et al., 2007; Eicken et al., 2000; Galley et al., 2015, and others) of sea ice often necessitates transport of samples to land-based laboratories due to the size, fragility and sensitivity of equipment needed to perform these techniques. Storage durations during transportation may last several weeks to months and additional storage prior to analysis may last several years (Roberts et al., 2009). Currently, land-bound imaging techniques, such as MRI, X-ray and CT-scanning can be used to determine the structure of brine channels and pockets, as well as the location of voids and air pockets within the sea ice.

2.6.2 Issues with current storage practices & techniques

Changes to the temperature of sea ice affect the chemical composition within its brine channels through phase changes and precipitation of various constituents as indicated in Figure 2.1. Densities of the brine and ice change with temperature and pressure build-ups in brine channels and pockets result in the expulsion of brine during sea-ice growth (Griewank and Notz, 2013). Since thermal changes may result in brine expulsion and brine drainage during sea-ice growth, changing sea-ice temperatures for storage may also result in changes to the brine content. The only prevailing study on the effects of core storage on brine content is a theoretical study by Cox and Weeks (1986), where changes to the salinity profile and effects due to time were not considered. Cox and Weeks (1986) investigated the degree to which sea-ice porosity and brine content would change due to brine expulsion, with thermal cycling as the cause of these changes. Table 2.1 shows the results of their theoretical study of changes to brine content as a result of brine expulsion from changing core temperatures from several initial temperatures to -54 °C and the subsequent warming of the sea ice to the initial temperature.

Table 2.1: Changes in porosity and salinity due to brine expulsion as a result of storing samples at –54 °C and then warming them to –10 °C, adapted from Cox and Weeks (1986). S_i denotes the samples initial salinity content, T_i the initial temperature and S_f the salinity after cycling

S_i (PSU)	1	5	10	15
T_i (°C)		S_f (PSU)		
-30	0.97	4.83	9.66	14.49
-20	0.95	4.75	9.51	14.26
-15	0.93	4.66	9.32	13.99
-10	0.91	4.54	9.09	13.63
-8	0.89	4.47	8.95	13.42
-6	0.88	4.38	8.75	13.13
-4	0.85	4.23	8.47	12.70
-2	0.8	3.98	7.96	11 .94

This storage temperature lies well below the upper boundary of the recommendation of Miller et al. (2015) to store cores below –20 °C and should be associated with more extreme initial changes to brine content than higher storage temperatures, since a smaller temperature difference between *in situ* and storage temperature would result in less brine expulsion (Cox and Weeks, 1986). Storing at a higher temperature may lead to loss of brine due to other mechanisms, such as brine drainage, but the extent to which this would occur has not yet been quantified for common storage conditions. Roberts et al. (2009) found that the diffusion of methanesulphonic acid out of sea-ice cores stored at –20 °C for several years was substantial. Within models for desalination, the process of diffusion is discounted as negligible in comparison to the effects of other processes such as brine drainage (Notz and Worster, 2009). Although the experiments of Roberts et al. (2009) do not touch on the salinity of sea ice, their conclusions do suggest that the degree to which diffusion of brine may occur is worth investigating. Beyond the study of Roberts et al. (2009), published experimental work or data on the effects of storage time on sea-ice cores has not been found.

The overall loss of brine is not the only issue storage of cores may cause: Since natural sea ice typically does not have a uniform temperature, the temperature differential between *in situ* and storage conditions will not be uniform. Thus, the degree of brine loss due to lowering the temperature for storage will differ along a stored core. As such, core storage is likely to have an impact on the brine profile. The entries for sea ice with an initial salinity of 10 PSU in Table 2.1 show that sea ice initially at –2 °C would lose 20% of its brine due to cycling to –54 °C, whereas sea ice initially at –4 °C would only lose 13% of its initial brine content. Storing biological cores thus becomes problematic, especially in such studies where bottom-ice algae are under investigation, as they inhabit the warmest part of the sea ice and are thus most likely to be expelled from the sea-ice matrix. This loss of algae is not easily accounted for, as it is unlikely that drainage of algae would be proportional to the drainage of brine due to the sticky nature of the cell walls of many diatom species or other factors, such as the presence of EPS.

2.7 Rationale of Study

Sea-ice algae are an understudied part of the global biosphere and are significant to polar food webs. This lack of research is particularly prevalent in the southern hemisphere and the

marginal sea-ice zone, where long-term *in situ* studies of sea-ice algae are not practical. Studying the behaviour of algae in this environment is of value to biogeochemical and earth system models since their contribution to the global climate is non-trivial. Methods of transportation for the studying of sea-ice algae *ex situ* are currently not well suited to providing earth system models with sufficiently relevant data. Thus, an alternative method of study is required. Logistic limitations, such as the short time available for research at each location, safety concerns during operation on sea ice, and the laboratory space available on ships, emphasize the need for compact methods of transporting sea-ice algae to land facilities, where these limitations are not of concern.

Chapter 3

Research Aim, Objectives, Hypothesis and Key Questions

The aim of this study is to design and construct an environmental chamber for the transportation of living sea-ice algae and compare the performance of this system to existing sea-ice algae transportation methods. The project aim may be sub-divided into the following objectives:

1. To establish whether long-term storage of artificial sea-ice cores at $-20\ ^{\circ}\mathrm{C}$ alters their brine profiles and thus algae profiles.
2. To identify which environmental conditions need to be mimicked at minimum to sustain the life of sea-ice algae.
3. To construct an environmental chamber capable of sustaining and growing sea-ice algae during transportation by providing favourable growth conditions.
4. To obtain sea-ice algae from the Southern Ocean and transport them to land-laboratories at the University of Cape Town alive.
5. To attempt the continued survival of these algae communities in liquid media for a time equal to or longer than time spent in the environmental chambers.
6. To compare overall algae concentrations and community compositions of transported and cultured algae.

Chapter 4

Brine Profile Development due to Core Storage

Storing cores at -54 °C, where the majority of salts have precipitated, is not always possible due to a lack of sufficient storage capacity and logistical and equipment constraints. Besides these limitations it is also not always desirable to store at such low temperatures, especially when storing cores for biological analysis, since it may lead to the destruction of microorganisms due to the increase in osmotic pressure and crystallisation of water in the cells. -20 °C was chosen as the storage temperature for this work. This corresponds to the survivability limit for low-temperature life in sea ice (Thomas, 2002) and the upper limit for storage temperatures recommended by Miller et al. (2015). It also corresponds to the lowest storage temperature available on the SA Agulhas II, the research vessel used for sample collection in Chapter 5.

4.1 Ice Growth Set-Up, Conditions and Analysis

An experiment was designed to investigate potential changes to algae and the deviation in the salinity profile through the storage of cores at the available conditions. Spring first-year sea ice in the Southern Ocean may reach up to 2 metres in depth (NSIDC, 2018). Producing artificial sea ice of this depth was not possible due to the equipment constraints, both in storage and sea-ice growth. Depth and quality of the sea ice were constrained by the largest artificial sea-ice growth tank available at the University of Cape Town, being a 0.8 metre tall (0.65 m liquid height) 500-litre system designed and tested by Hall (2019). Besides the strict height limitation of the tank, the maximal sea-ice depth was further constrained by the need to prevent excessive increases in sea-ice salinity due to changes to the solution salinity (Weeks and Cox, 1974). This motivated the choice to limit sea-ice depth to a maximum of 250 mm.

An artificial seawater solution was prepared with 380 litres of deionised water and AquaForest sea salt (for composition see Figure D.1), with a final solution salinity of 35 PSU. Practical salinity was estimated by first measuring conductivity and temperature with an AZ8303 conductivity meter, calibrated using a $12880 \frac{\mu S}{cm}$ solution. The relationship between these two parameters was then used (based on the Unesco 1978 Practical Salinity Scale (Unesco, 1981)) to solve for practical salinity in Scilab. The code is shown in Appendix E.

Typical sea-ice core extraction may be performed with a corer. But, due to laboratory height constraints, as well as health, safety and equipment preservation concerns, a corer could not be used in conjunction with this tank. Extraction of sea ice from this tank in prior experiments had been performed using an electric reciprocating saw and bent wire, which was used to pull samples out of the tank after cutting them free. This process is time-consuming, dangerous, physically difficult, may damage samples substantially and does not produce sea ice of equal

horizontal dimensions. Deviation in vertical cutting dimensions may make sample extraction impossible, as samples which get wider towards the base cannot be extracted without further trimming of the sample or surrounding sea ice. To circumvent all these issues during extraction, an alternative method was developed: growing sea ice in pipes within the artificial sea-ice tank. Using this method eliminates the need for any cutting during extraction and ensures uniformity in horizontal dimensions between samples.

PVC pipes of 110 mm diameter were cut into 300 mm long pieces. Two 20 mm holes were drilled near the top of each pipe for suspension and extraction purposes. Wires were strung through the holes and the tubes suspended from a metal grid. The metal grid was suspended above the artificial seawater, with the PVC pipes protruding out of the water by 50 mm, as seen in Figure 4.1.A. A fan was installed a metre above and away from the side of the tank, pointed at the water surface and activated. The laboratory was cooled to 0 °C for 24 hours to allow the solution to achieve a near-freezing temperature, upon which the laboratory temperature was lowered to −20 °C and the experiment initiated. After 7 days of freezing, samples were meant to be extracted; however, the utilisation of a fan to improve the rate of heat transfer to the sea-ice surface resulted in uneven freezing, with some edge regions having a thickness of 100 mm and others no ice at all. As such, the fan was deactivated, and the freezing period was extended by 7 days. Following the extension, samples were pulled out of the sea ice in their PVC pipe casing, as shown in Figure 4.1.B, pushed out of their pipe and rested or stored horizontally before segmentation.

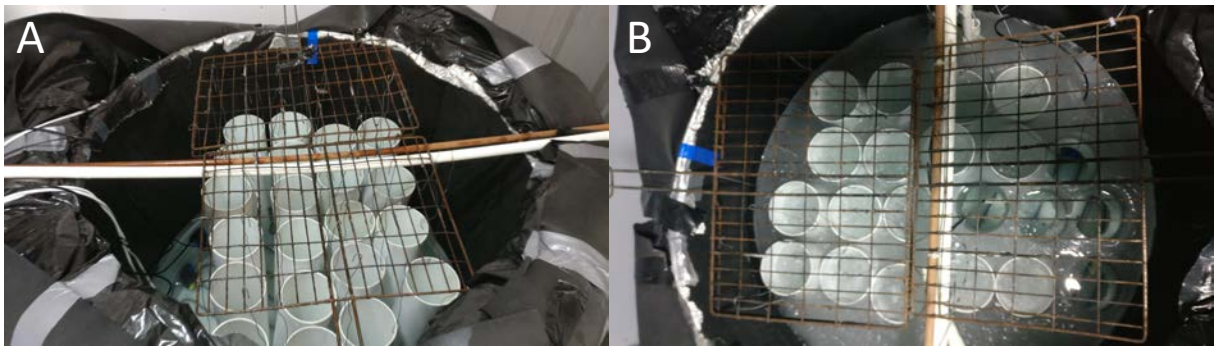

Figure 4.1: (A) 500 litre tank with core extraction pipes prior to freezing (B) extraction of samples from 500 litre tank

3 samples were dedicated to each storage time in order to be able to use t-tests or Mann-Whitney or t-tests with statistical significance. Figure 4.2.A shows the distribution of samples throughout the tank and Figure 4.2.B the respective sample depths. Samples were extracted by inserting a screwdriver through the 20 mm holes near the top of the pipes and pulling the samples out of the sea ice individually. Figure 4.1.B shows the tank after extraction of the first 5 samples.

Samples were stored horizontally at −20 °C for durations of 0 minutes, 15 minutes, 1 day, 3 days, 7 days, 14 days and 35 weeks respectively. All samples were segmented horizontally into 20 mm discs using a stainless-steel bandsaw, melted and their salinity calculated. Samples 1.2 through 2.3 were only extracted after processing of the prior sample was complete, since the processing time required for each sample exceeded their storage time. The deviation in sample depths was not noticed until after segmentation of these samples, resulting in the differences in sample depth distribution between these two samples and all other samples.

The deviation in sample depths is likely to have occurred due to positioning of the fan and the resulting difference in air flow experienced at the surface of the artificial sea ice.

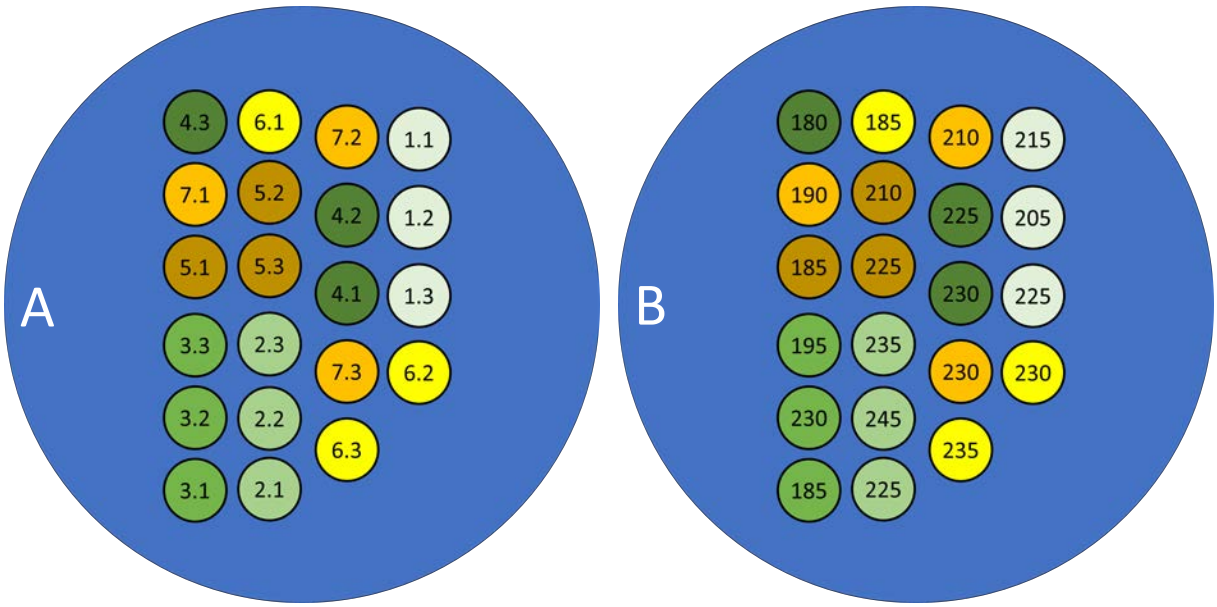

Figure 4.2: Sample layout and vertical dimensions from artificial sea-ice storage experiment with (A) numbered sample locations and (B) artificial sea-ice sample depths in mm

4.2 Brine Profile & Core Shape Development

Upon extraction, all samples had uniformly cylindrical walls with constant diameter. As samples aged, deformities appeared, being so pronounced after 35 weeks that an additional vertical segmentation of these samples was performed before horizontal segmentation. Figure 4.3.A illustrates the deviation from its prior circular cross-section and Figure 4.3.B deviations along the length of this sample.

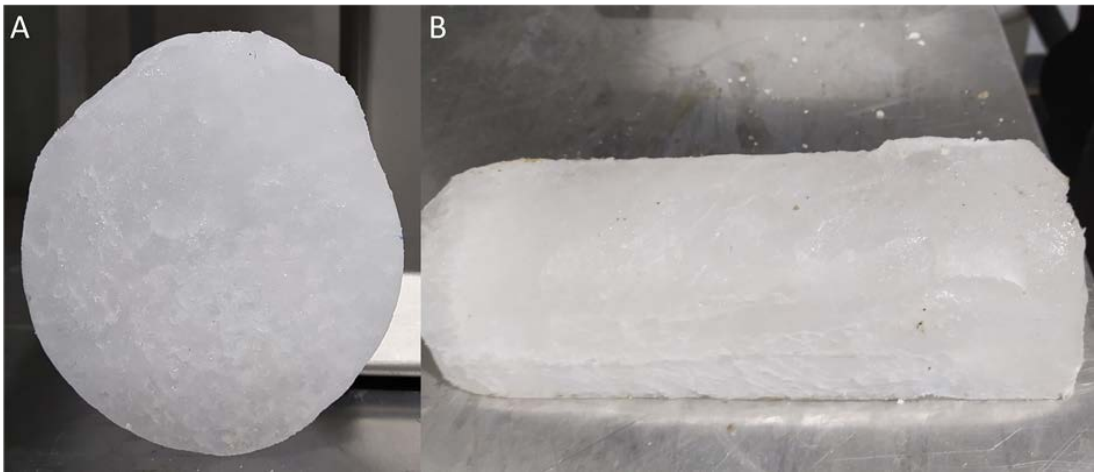

Figure 4.3: (A) Horizontal and (B) vertical core deformation after 35 weeks of storage at −20 °C

The horizontal segmentation intended to determine whether long term storage favours brine profile modification in the direction of gravity. Thus, the gravitational bottom and top (semi-cylinder below and above the center of mass during storage, see Figure 4.3.B) of each core were identified before segmentation by observing areas of significant uniform deformation along the cylindrical wall of each core. Identification of these areas was simple for two of the three cores; however, the second core (see Figure 4.3.A) exhibited two areas of significant deformation, likely caused by contact with a side-wall of the cold storage unit. The larger of the two areas of deformation was assumed to be the gravitational bottom of the core, meaning the side of the core which it rested on during storage.

Figure 4.4 shows all salinity results of the storage experiment, expressed as vertical salinity profiles of averages of each [storage time & depth] group, consisting of 3 samples. The y-axis represents the percentage of the full depth of each vertical core sub-sample instead of real depth, as the depths of the cores varied greatly during the experiment (see Figure 4.2.B). Comparisons of salinities at each real depth would be irrelevant for this study, as the statistical results of comparing the bottom of a short core with the centre of a long core would remove result relevance to profile shape, as well as inflate variance.

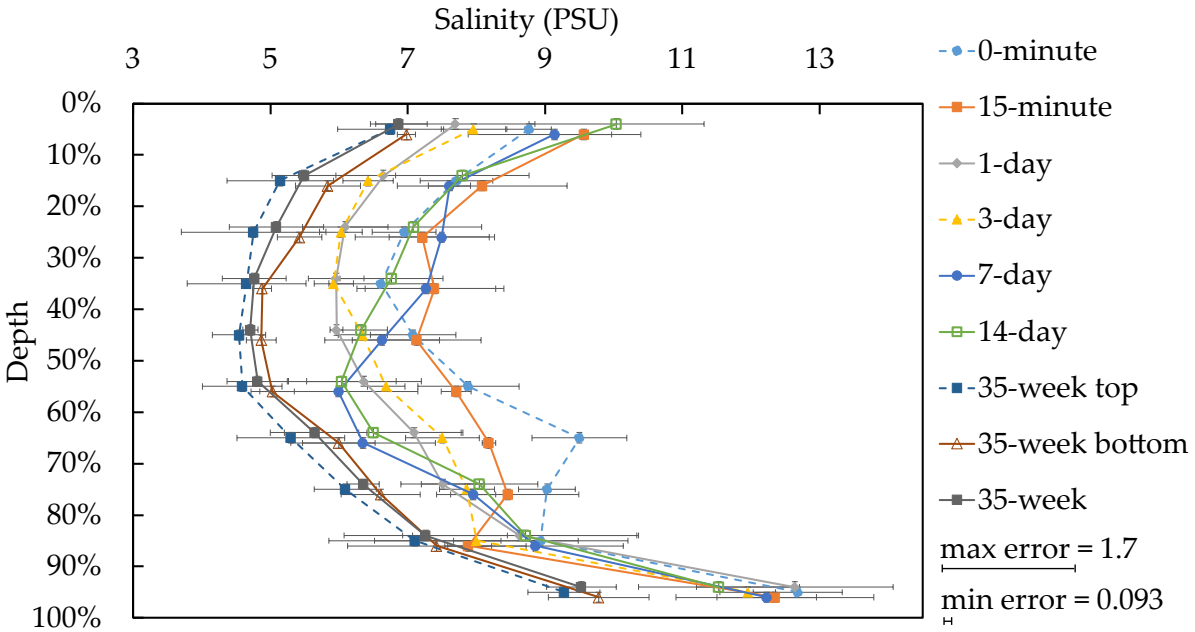

Figure 4.4: Practical salinity profiles of all 3-sample-time-groups. Some data points within each 10% group have been shifted vertically for legibility. These changes do not correspond to actual differences in height

Since each core was cut into 20 mm discs the number of slices per long core exceeded those of shorter cores, the minimum and the maximum number of slices being 9 and 13 slices respectively. To make equal-depth% comparisons of salinities, artificial core sample populations were generated from the data using piecewise polynomial interpolation in Scilab, providing sets of 13 artificial slices per core. The full set of interpolated and raw data is available in Table A.1 and A.2. All error bars in graphs shown in this section represent 1 standard deviation. Shapiro-Wilk tests were performed on all cores and [storage time & depth] data groups. It was found that salinity data was not normally distributed within cores (< 4%) or within [storage time & depth] data groups (< 94%) to an extent where it could be assumed to be normally distributed with sufficient (95%) confidence (see Table B.1 and B.2 for full Shapiro-Wilk results).

Since normal distribution could not be assumed, 1-tailed Mann-Whitney tests were performed on time-grouped data sets.

Figure 4.5 shows the results of 1-tailed Mann-Whitney tests between the interpolated practical salinity results of the immediate segmentation data (blue), and the results of cores stored for a day (orange) and all other storage groups without considering depth. The results show that the probability of salinity not having changed after a day and 35 weeks are $P = .018$ and $P < .001$ respectively. Samples stored for 7 and 14 days showed a likelihood of P>.05 of belonging to the same set as those stored for 0 minutes, due to their great variance and their increased salinity relative to the 3-day storage samples. This, paired with the fact that these samples were gathered exclusively from the outer perimeter and centre of the tank respectively, led to the decision to consider these data as outliers. Comparisons between 1-day storage samples and samples stored for longer periods show to what extent storage alone caused changes to the brine profile, since the brine expulsion due to changes in temperature and pressure considered by Cox and Weeks (1986) would have taken place at this stage.

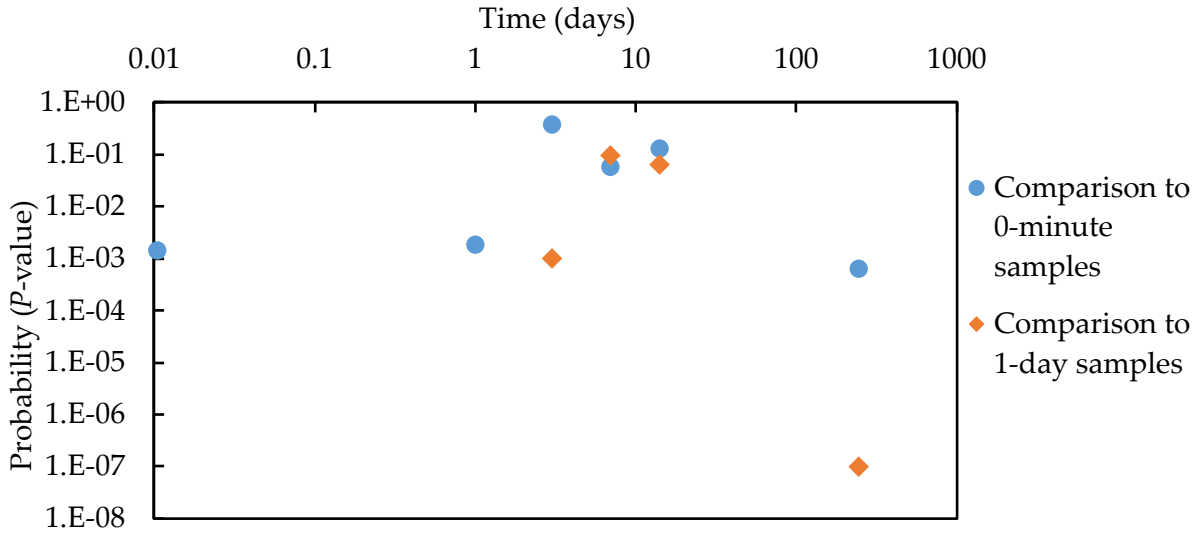

Figure 4.5: 1-tailed Mann-Whitney probabilities of salinity profiles not having changed over time, comparing all results to results from immediate segmentation (blue), and all results to those from cores stored for 1 day (orange)

Figure 4.6 is a reproduction of Figure 4.4 without the outlier samples. Besides the visible reduction of practical salinity over time the degree to which the artefact at 70% depth is expressed is also reduced. Despite the overall practical salinity not changing significantly between 0 minutes and 15 minutes of storage, the degree of change of the artefact expression between them is strongest. A possible explanation for this is the re-distribution of salt owing to internal stresses and pressure changes due to density changes, caused by interstitial salinity changes.

The initial W-shaped salinity profile develops to become a C-shaped salinity profile over time, as is evidenced by the differences in profiles between the 0-minute and 1 day samples shown in Figure 4.6. In addition to the shift in profile, there is clear evidence for desalination of cores. Cores stored for 0 minutes, 1 day and 35 weeks had average bulk salinities of 8.5, 7.5 and 6.1 PSU respectively. It was noticed that large drops of ice and brine formed on the sides of sea-ice cores in the freezer, travelling down the cores as storage time progressed. Drops that accumulated below the cores in ice ponds were not collected and analysed and may account

for the loss in salt. Desalination between cores stored for 0 minutes and 1 day was found to be 11% and between 1 day and 35 week samples the desalination reached 19%.

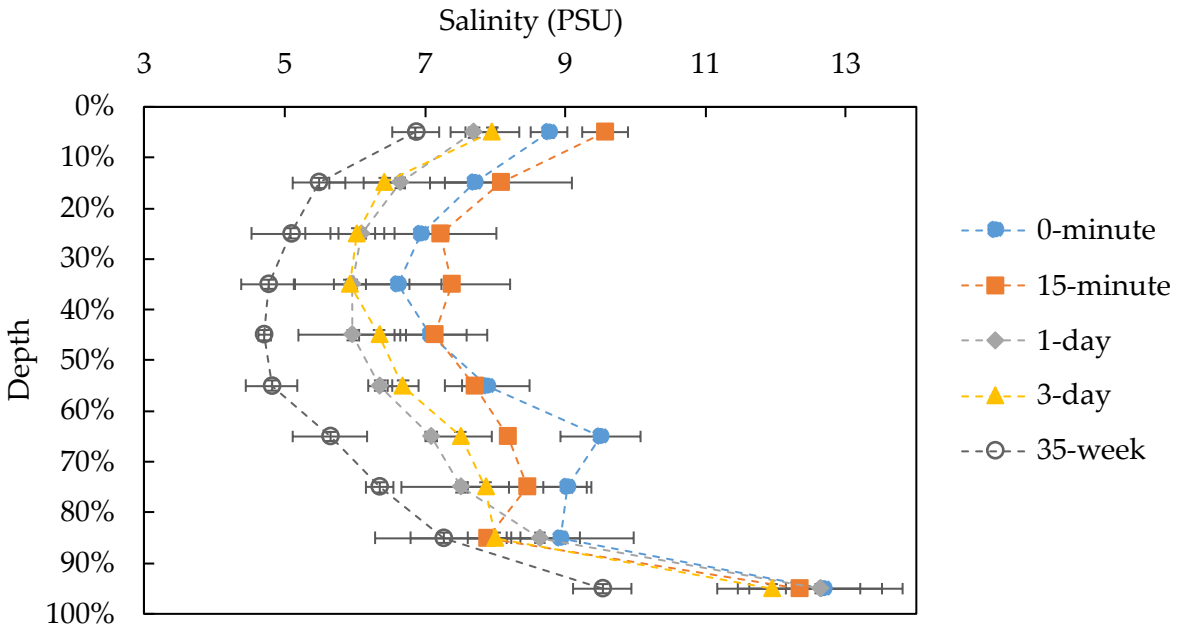

Figure 4.6: Salinity profile development results without outliers

Besides the bulk desalination during storage at -20 °C and the changes to the vertical salinity profile, storage may cause further changes to the salinity profile depending on sample orientation during storage. Figure 4.7 shows the differences between the assumed gravitational top and bottom semi-cylinders of samples stored for 35 weeks. The Shapiro-Wilk test on all of these data reveals that the likelihood of them belonging to the same population is $P = .08$, and thus not low enough to confidently claim that a significant change has occurred.

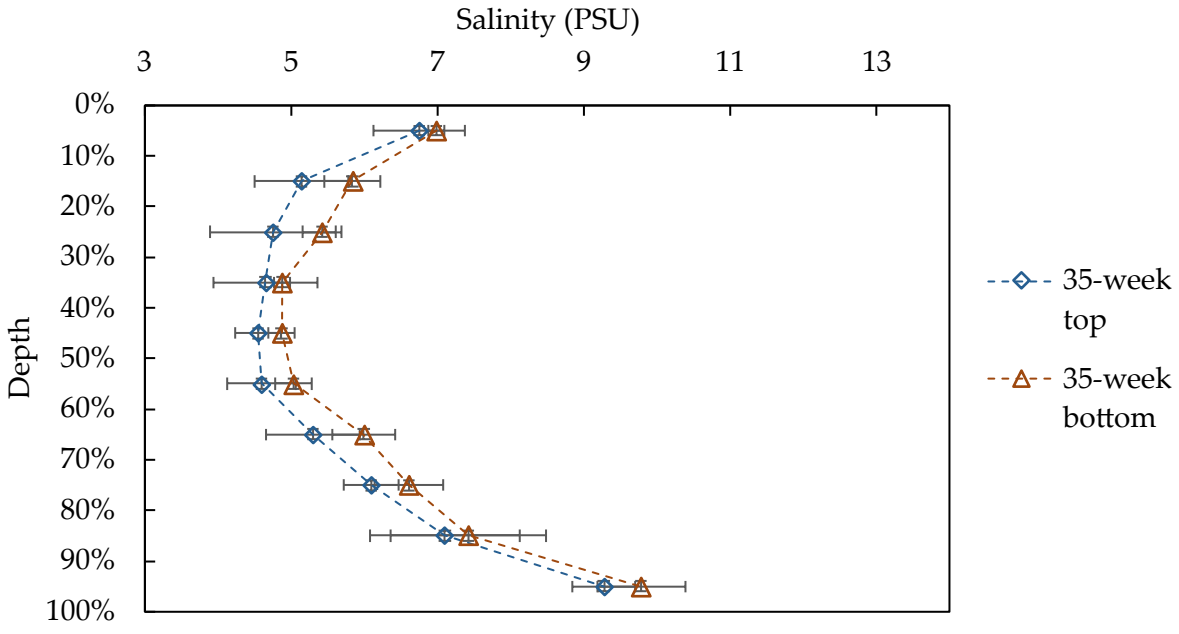

Figure 4.7: Salinity profiles of the vertical top and bottom of samples stored for 35 weeks, where verticality is relative to storage orientation

Visual inspection of one of the 35-week samples yielded two possible storage orientations. The practical salinity results for this core are shown in Figure 4.8 and show no statistically significant difference between the gravitational top and bottom of the core, with a two-tailed Shapiro-Wilk test of their data yielding a probability of $P = .91$ of them belonging to the same group. It is thus likely that the core was misaligned during cutting due to incorrectly assuming its orientation during storage.

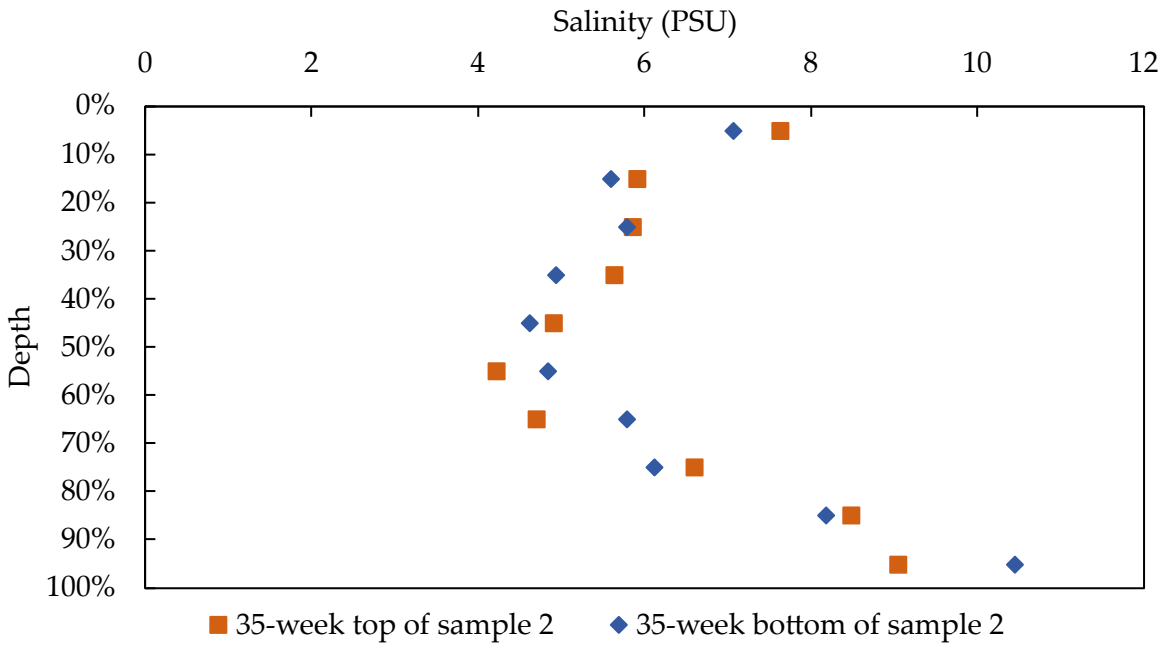

Figure 4.8: Salinity profiles of the proposed outlier of 35-week storage samples

Figure 4.9 represents the non-outlier salinity results of gravitational top and bottom segments of the 35-week storage samples. The Shapiro-Wilk test likelihood of these data belonging to the same group is insignificant ($P = .03$), less than half of the prior probability for the full data set for this storage time and statistically significant enough to report with confidence that storage orientation changes salinity profile changes to samples.

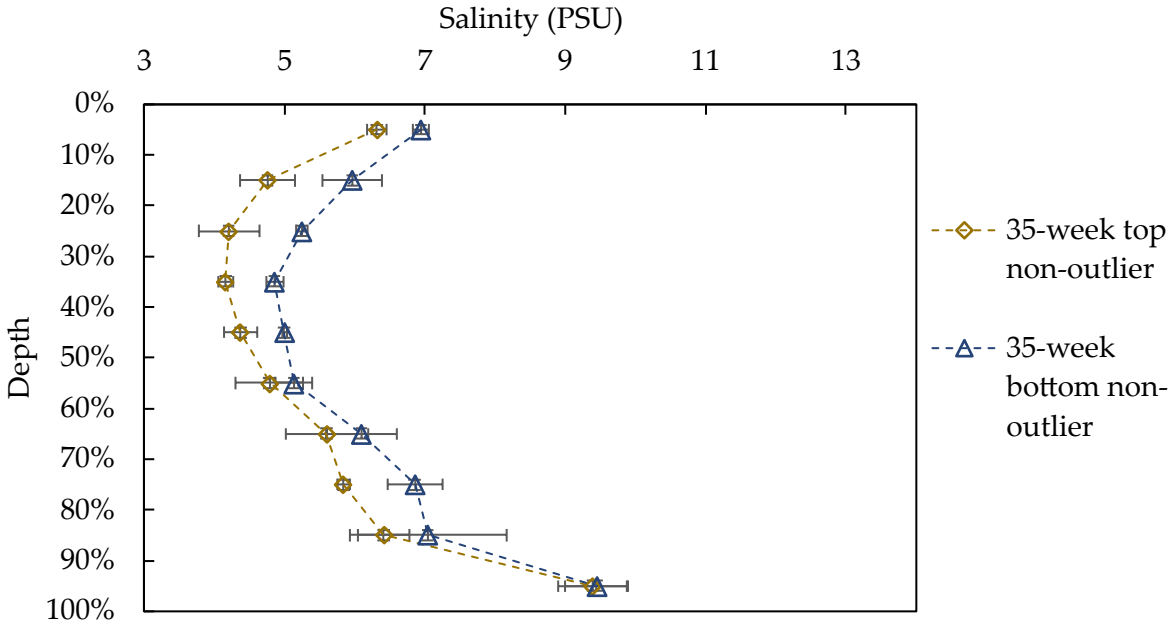

Figure 4.9: Salinity profiles of the top and bottom halves of samples stored for 35 weeks without the outliers

4.3 Discussion of implications of storing sea-ice cores for biogeochemical and other purposes

After a day of storage, cores can be assumed to be approximately at thermal equilibrium. As such, bulk desalination and brine profile changes before this storage time can be attributed to brine expulsion, as expected by Cox and Weeks (1986). Changes to bulk salinity and salinity profiles after a day of storage should not be attributed to thermally induced brine expulsion. Figure 4.5 shows that even 2 days of storage at −20 °C (comparing 3-day storage to 1-day storage) may cause significant changes ($P < .001$) to the salinity profile, with desalination between samples stored for 1 day and 35 weeks reaching 19%. This indicates that the claim of Cox and Weeks (1986), that salinity does not change once the entire core reaches storage temperature, do not apply when storing at this temperature. Since an approximate temperature of −20 °C has been reached after a full day of storage and the conditions for permeability are not met (Golden, Ackley, and Lytle, 1998), it can be assumed that both drainage and brine expulsion no longer function as they do *in situ* (Notz and Worster, 2009). Without the ice-ocean interface, other desalination processes, like salt fractionation and meltwater flushing, are no longer viable. The only process of desalination left from the five commonly suggested processes (Notz and Worster, 2009) is brine diffusion. Thermally driven brine diffusion requires a temperature gradient along the ice, which may still exist in common freezing units due to the constant loss of heat to the space outside of the freezing unit, the localised heat-pumping common to most freezing units and the resulting inevitable minor temperature-cycling required to bring the freezing unit back to the desired temperature. Temperature gradients resulting from this effect would be specific to the freezing unit and conditions, an investigation into which lies outside of the scope of this project.

If diffusion is responsible, then the effects of storage duration on the brine profile, core shape and affiliated core properties would be exacerbated at higher storage temperatures, since brine

volume increases with temperature as shown in Figure 2.1. It would also suggest that storing sea ice at any temperature where brine exists will cause deformation of the brine profile with time. Cox and Weeks (1986) show that storing sea-ice cores at low temperatures causes changes to their bulk salinity, with brine expulsion being as high as 17% for cores which were near melting temperature *in situ* (Table 2.1). This is often the case for cores collected during spring or summer blooms. Desalination between 0 minute and 1 day storage samples can largely be attributed to brine expulsion and amounted to 11% in this work. The difference between this value for desalination and the 17% reported by Cox and Weeks (1986) can be explained by the differences in storage temperatures and the initial temperature profiles between their work and this work. Since temperature profiles were not determined during extraction in this work, an exact comparison of our results with their varying initial temperatures cannot be made.

Storing sea-ice cores at warmer temperatures for prolonged periods, as done by Yan et al. (2020) at -5 °C for three weeks, is likely to cause many changes to the sea-ice community. At -5 °C sea ice is permeable (Golden, Ackley, and Lytle, 1998) and brine drainage alone should cause a substantial loss of biomass, since algae inhabit the brine. This is of particular concern for algae situated at the exposed ends of the cores since they are liable to expulsion, due to the changes in brine channel volumes. The core desalination observed in this experiment suggests that algae inhabiting the centre of the core would also be expelled from cores as storage proceeds. The synergistic detriments of these effects, the lack of a method to supplement nutrients and the potential damage caused by the consistently higher saline environment to bottom-ice algae (Kauko et al., 2018), severely reduce the likelihood of a natural sea-ice community not being altered during transportation. It was concluded that performing a study on these effects with natural sea-ice cores was not a valuable exercise, considering the difficulty with which such cores are acquired.

Chapter 5

Hybrid System Design Considerations and Operating Choices

The hybrid system designed and tested in this section was used to transport sea-ice samples gathered during the SCALE winter and spring cruise of 2019 on the SA Agulhas II from the Southern Ocean to land facilities at the University of Cape Town. Details of the sampling station types, locations and dates are presented in Table 5.1 below, with more specific information on the spring cruise given in Table A.4.

Table 5.1: Ice sampling station types, dates and opening locations (in decimal degrees) of the winter and spring SCALE cruises of 2019

Season	Station type	Date	Latitude	Longitude
Winter	Consolidated	July 27th	-58.1378	-0.00442
	Pancake lifting	July 28th	-56.8018	0.30262
Spring	Consolidated	October 24th	-59.3248	0.066617
	Consolidated	October 24th	-58.9833	0.011883
	Consolidated	October 29th	-59.3645	8.158917
	Consolidated	October 30th	-59.4726	10.88933
	Floe lifting	November 1st	-58.5488	17.93818
	Floe lifting	November 3rd	-58.4491	21.99735

5.1 Proposed Hybrid System

Existing methods of transporting living algae face issues with preserving the community composition. Communities or specific species gathered with these methods require a relatively long adaptation time before use in laboratory experiments. In the case of solid medium transportation, there are also some issues with temperature fluctuations and associated brine and algae loss. To preserve the algal community complexity, improve the survivability during transportation and provide the potential for immediate relevant experimentation, a new transporting system is required: The system needs to be a simple, reliable, affordable, and scalable method of transporting living algae from the Southern Ocean in an environment mimicking their natural habitat. Environmental changes need to be minimised to reduce unwanted changes to the algal community and provide the ability to experiment immediately without the need for algae to adapt to new conditions. This project explores the ability of a hybrid system between the existing liquid and solid medium transport methods to provide the desired effects. The basic functionality of the hybrid system must satisfy control over the key parameters affecting the sea-ice algae environment, namely temperature and the temperature gradient. Control over

temperature alone is insufficient to supply an environment in which algae can grow, as such control over irradiation and access to nutrients is required. Furthermore, since the algae need to be extracted from the system for analysis, considerations towards ease of sampling need to be made.

Figure 5.1 shows the basic set-up for transportation of living algae in the hybrid system, providing control over the sea ice and water temperature. Water temperature below the sea ice is increased by an electrical resistance-based heating cable at the lower tank walls, controlled through a relay with a temperature probe on the inside of the tank. The heating circuit activates once the water temperature reaches the programmed set-point, allowing for control over sea-ice thickness through changes in the freezing point of seawater with changes in salinity. Detailed design choices and reasoning can be found in Appendix C, with pictures of the development of the hybrid system being shown in Figure C.6.

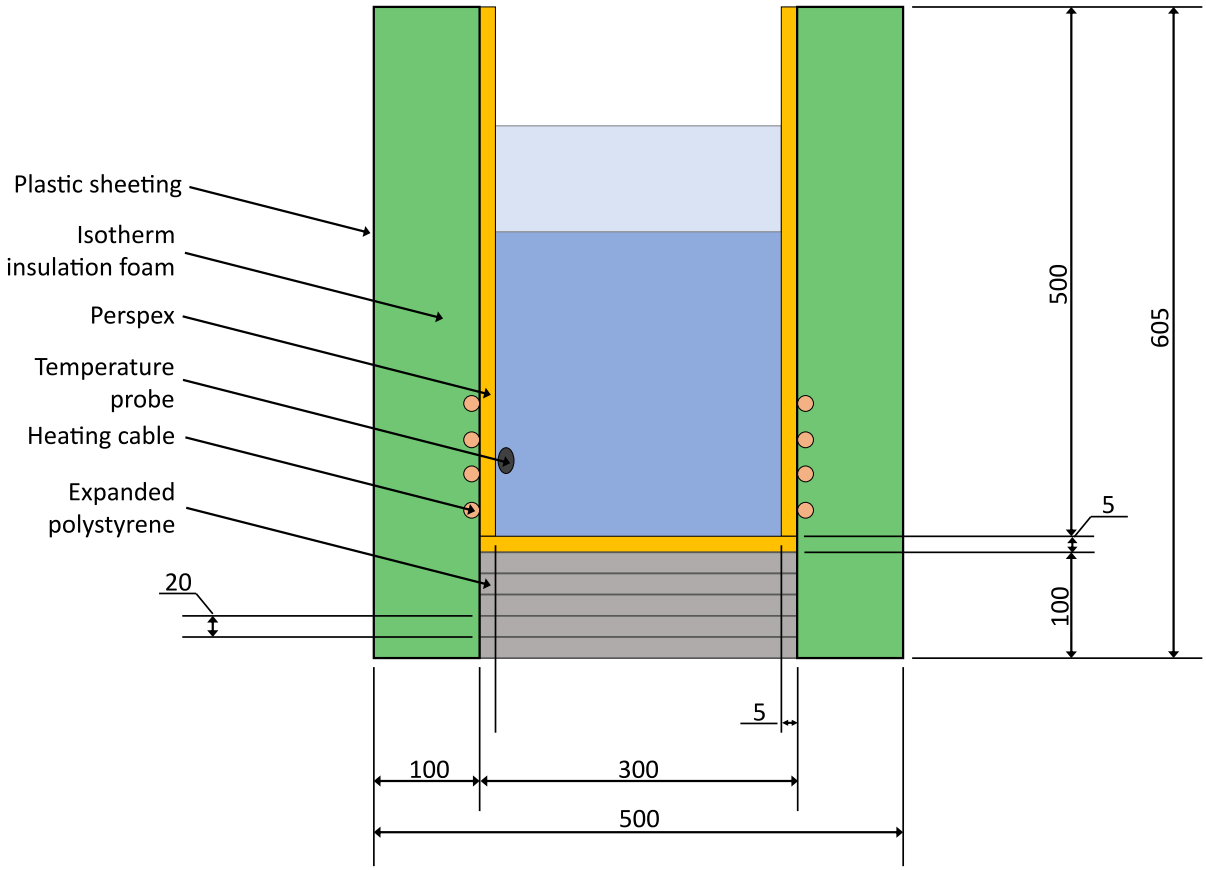

Figure 5.1: Basic hybrid system (all dimensions in mm) to control phase fractions through insulation and heating

Excessive cooling from the sides and bottom of the tank is prevented through insulation and the heating applied at the lower part of the tank, thus allowing for a system in which sea ice exclusively grows from the top and exhibits a vertical temperature profile. The choice of location for the source of heat is twofold: (1) the sides of the tank have 6.66 times greater surface area than the bottom and thus experience more unwanted cooling and (2) heating the sides instead of the bottom supplies superior thermally induced mixing within the water column, allowing for easier access to nutrients for algae interacting with the water column.

5.2 Winter Cruise

The basic tank-system shown in Figure 5.1 was intended to achieve the transport of living sea-ice algae from the marginal sea-ice zone in the Southern Ocean during winter of 2019. Two samples were gathered for this work using a Kovacs Mark II ice corer: The first from on-ice coring from a floe and the second through on-deck coring from pancake ice.

5.2.1 Winter Cruise Set-Up Intent

To increase the number of samples that could be housed within a single tank, a measuring cylinder with an internal diameter of 95 mm and height of 700 mm was introduced into the system, with the intent for it to stand on the floor of the tank as shown in Figure 5.2. Cooled filtered seawater was to be poured into the tank before inserting the cores, and the sea-ice depth to be kept constant through rigorous control over the heating supplied by the temperature control circuit.

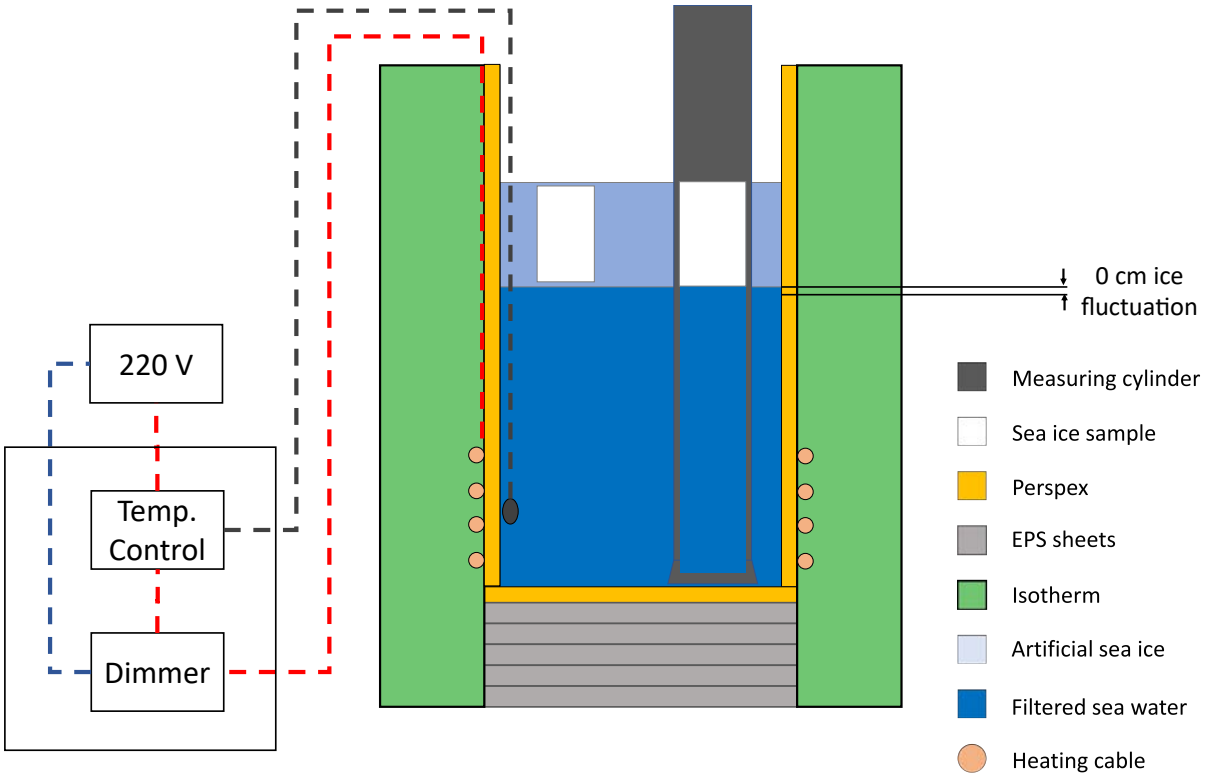

Figure 5.2: Winter intent schematic of tank and control circuit

The set-up shown in Figure 5.2 allows for two samples to be transported: one within the dedicated measuring cylinders and one within the solution to become the surrounding sea ice. Filtered seawater would need to be filled into the tank or tube and cooled down close to freezing before sample integration, with the tank sample being the first to enter the system since the tank's sea ice and seawater are necessary elements for the temperature control of the measuring cylinders. Freezing the measuring cylinder in place would not be possible without counteracting its buoyancy, which was to be achieved by placing a large weight onto the cylinder.

5.2.2 Winter Cruise Execution

On the morning of the day of the first ice station, the tank was filled to a height of 380 mm with filtered seawater which had previously been stored at 5 °C, leaving the measuring cylinder empty. The consolidated core was cut into two 200 mm pieces and both core segments were placed into the solution in the tank. A temperature relay was set to heat the tank once the temperature of the seawater reached -3.0 °C, causing a 200 mm thick sheet of sea ice to form at the laboratory temperature of -10 °C. Due to excessive rocking and rolling of the ship after the inclusion of the first sea-ice sample, securing the measuring cylinder in place was not possible with top weights. Due to this, the measuring cylinder tilted, resulting in uneven freezing of the sample. Cored samples were more uneven than expected in diameter. Consequently, the pancake core could not be introduced into the measuring cylinder without modification and was thus cut into two semi-cylinders. The bottom segments of this core melted within the measuring cylinder.

An effort was made to limit the time between sample extraction, processing, and introduction into the tanks to limit the degree of brine drainage. The time between core extraction and introduction into the tank did not exceed 60 minutes; however, the pancake from which a sample was procured rested on deck for several hours before the sample was extracted. Changes to the environmental temperature (-5 to -24 °C) due to laboratory operations and the need to store cores caused significant fluctuations in the sea-ice thickness during on-ship transportation. Upon arrival at the mobile laboratory land location, the 3-phase power of the laboratory, necessary for the cooling of the laboratory, was successfully connected; however, the single-phase power necessary for tank temperature control was not connected and complete freezing of the system commenced at -20 °C.

The winter cruise set-up showed several shortcomings: Using a measuring cylinder taller than the tank as containment for a sample resulted in the inability to control its buoyancy during freezing of the tanks seawater. The indented graduation marks on the surface of the measuring cylinders prevented movement of the cylinders once the sea-ice sheet formed, resulting in a reduced usable volume due to the rise of the measuring cylinders during freezing as shown in Figure 5.3.

Introducing a sample into the tank's seawater removed the possibility of breaking the sea-ice sheet and changing the misalignment of the cylinder. The sample alignment within the tank could also not be controlled, as its geometry resulted in a 90° vertical tilt of the sample and thus a disturbance of the temperature profile of the sample as it froze into the sea ice, invalidating the sample, since its environment could not be preserved.

Light to the samples was only controlled through laboratory lighting, being switched on for 6 hours daily. The initial period of sample introduction was accompanied by 36 hours of constant lighting, necessary for the use of the laboratory during sea-ice operations. Exposure of the top of the tank resulted in faster acclimatisation of the tank to laboratory temperatures, an undesired effect when the temperature of the laboratory varied greatly during laboratory operations. Additionally, the lack of shielding between the tank contents and laboratory allowed for contamination of the samples from other laboratory operations.

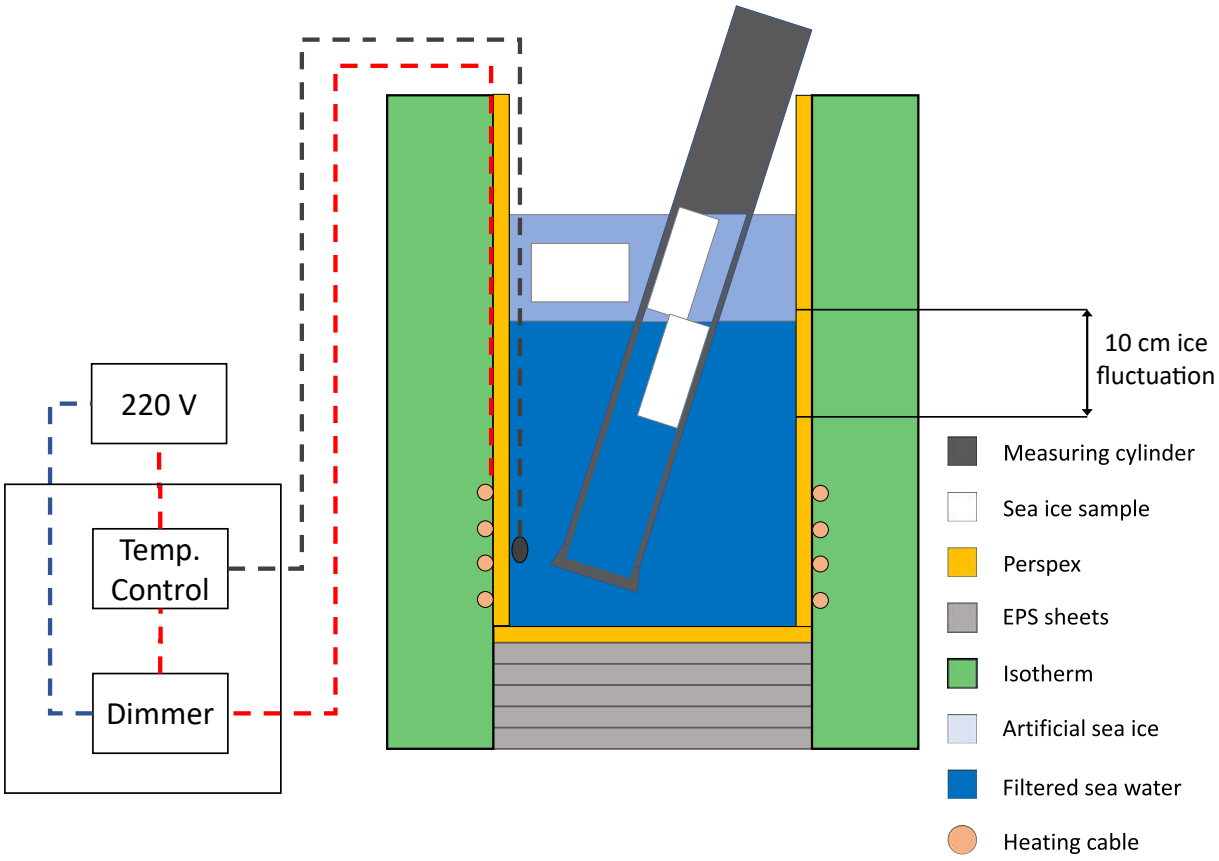

Figure 5.3: Winter reality schematic of tank and control circuit

5.3 Spring Cruise

The spring cruise allowed for a significant increase in the number of samples that could be accrued due to the increased sea-ice contact time. Figure 5.4 shows the spring cruise route and relevant stations, with sea-ice coring stations marked in blue. Four floe samples were cored on-ice at stations marked 1-4 and two cores were obtained through on-deck coring from brash ice in stations marked 5 and 6. Due to the increase in samples an additional tank was constructed for sample housing and transport. Samples 1-3 and 4-6 were transported in tank 1 and 2 respectively. Logistic details of the sea-ice stations are presented in Table A.4.

Snow was not added to the tubes in the tanks, even though it was present on all floes and broken floes before coring, increasing the light received by algae in the tank relative to their natural environment. This choice was made in the interest of consistency since the amount of snow each site would have was not known before sampling. A snow depth exceeding 100 mm (as was present at some sites) could not have been accommodated in the tanks.

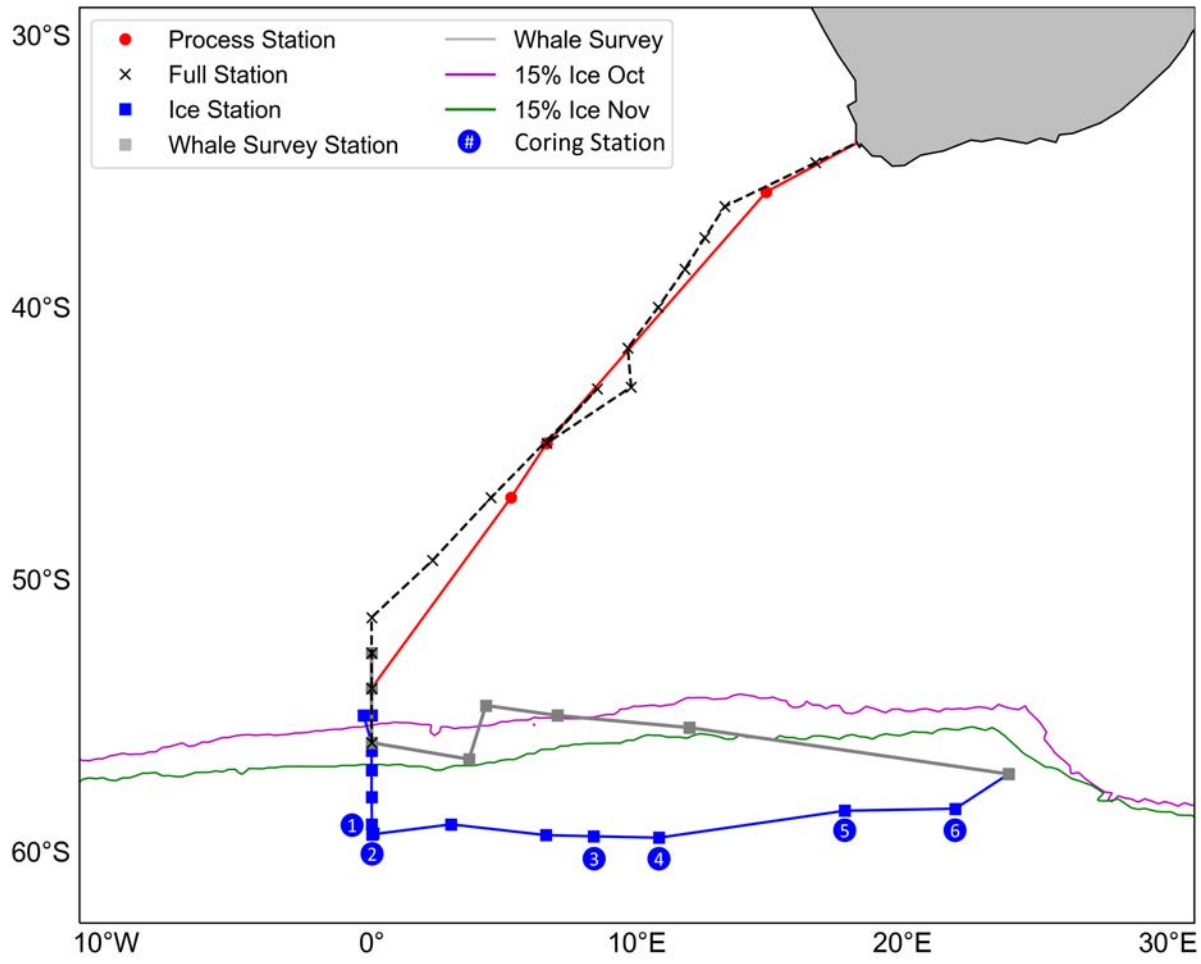

Figure 5.4: Spring cruise route and stations

5.3.1 Spring Cruise Set-Up and Execution

The constituents for a modified f/2 medium (henceforth referred to as nutrient solutions) were prepared on the 25$^{\text{th}}$ of September 2019 according to the recipe outlined by Hallegraeff, Anderson, and Cembella (2004) in Figure A.1 and stored in the dark at 5 °C before, during and after the cruise for use in the tanks and later liquid culturing.

Six polyvinyl chloride tubes with an internal diameter of 110 mm were cut into 400 mm sections and one side sealed with polyvinyl chloride caps and waterproof tape. These tubes replaced the measuring cylinders from the winter cruise set-up as sample isolation chambers, henceforth called hybrid tubes. Due to their height not exceeding 500 mm, weights could be placed onto the hybrid tubes during freezing of the surrounding solution, preventing both vertical and horizontal disturbances to the hybrid tube locations.

Samples obtained through on-ice coring were transported to the laboratory, segmented with a bandsaw and introduced into the tank within 25 minutes of extraction to limit the degree of brine loss and temperature change. Seawater was gathered from holes directly below the cores as outlined in Figure 5.5. Cores were segmented and the core-hole seawater introduced into the relevant hybrid tube as outlined in Figure 5.6. To show the change in algal concentrations before and after transport, each tank sample was vertically bisected. All non-tank sections were

melted in the dark at 5 °C over 3 days, decanted into 50 ml plastic Eppendorf conical tubes and preserved with glutaraldehyde (1% final concentration).

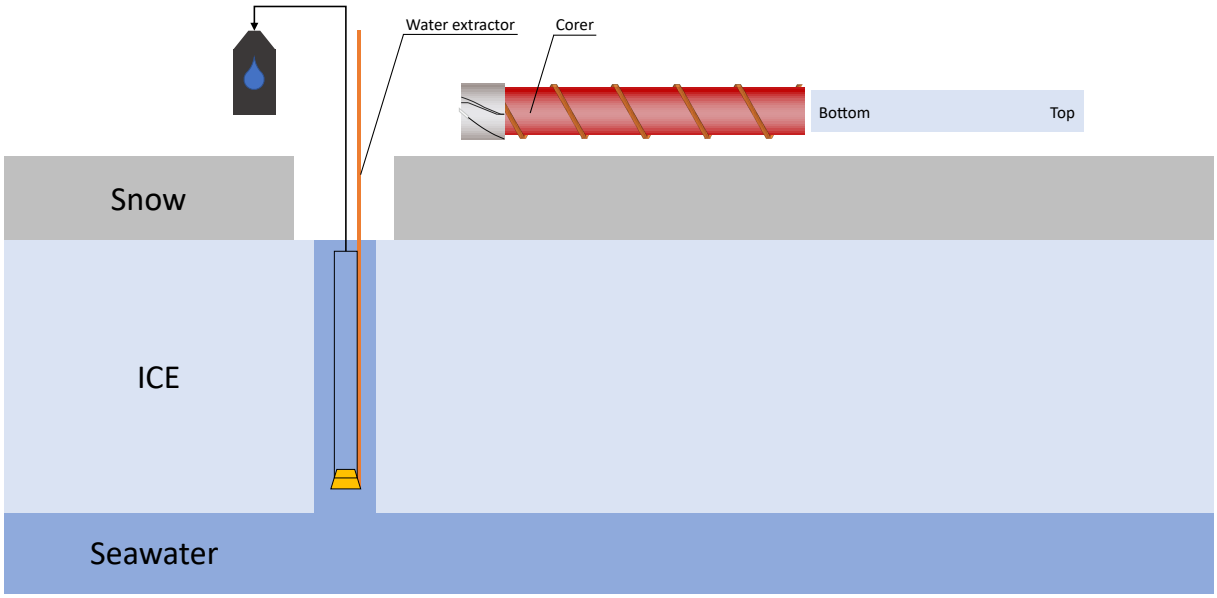

Figure 5.5: On-ice coring procedure

Samples obtained from broken floes were visibly discoloured from high algal content and had a distinct organic odour. Filtered seawater was used as a replacement for core-hole water for these samples. The time between lifting a piece of brash and the introduction of the segmented core pieces into the tank ranged from 7 to 12 minutes. Over the two-week duration of the return trip, the colour of sea ice obtained from broken floe pieces in the tank changed significantly, deepening in its darkness of green during the first three days after introduction into the tank (see Figure A.2), suggesting significant algal growth in the system, and regressing again during the rest of the journey, presumably due to the significant reduction in temperature and consequent reduction in algae occupied brine volume in the upper layer. Observations of the state of the sea ice in the hybrid tubes from the sides or bottom of the tank were not possible due to the opaque nature of the tubes.

A hole was drilled into each sample with an 8 mm drill bit every 7 days and several concentrated nutrient solutions were injected with a milli-Q rinsed pipette, raising the concentrations of nutrients in the system to those outlined by the modified f/2 medium recipe (see Figure A.1) under the assumption that all nutrients were consumed within 7 days. Transferral of nutrient solutions had to occur within 10 seconds of exposing the pipette to the laboratory air, and within 1 second of insertion into the drilled hole, as the nutrient solution froze within the pipette otherwise. Convection through thermally-induced density changes were the only source of circulation besides Brownian motion within the tanks. Studies on the extent of mixing were not performed in this work. As such, the degree to which nutrients were included in the sea ice from the seawater in the tanks is unknown.

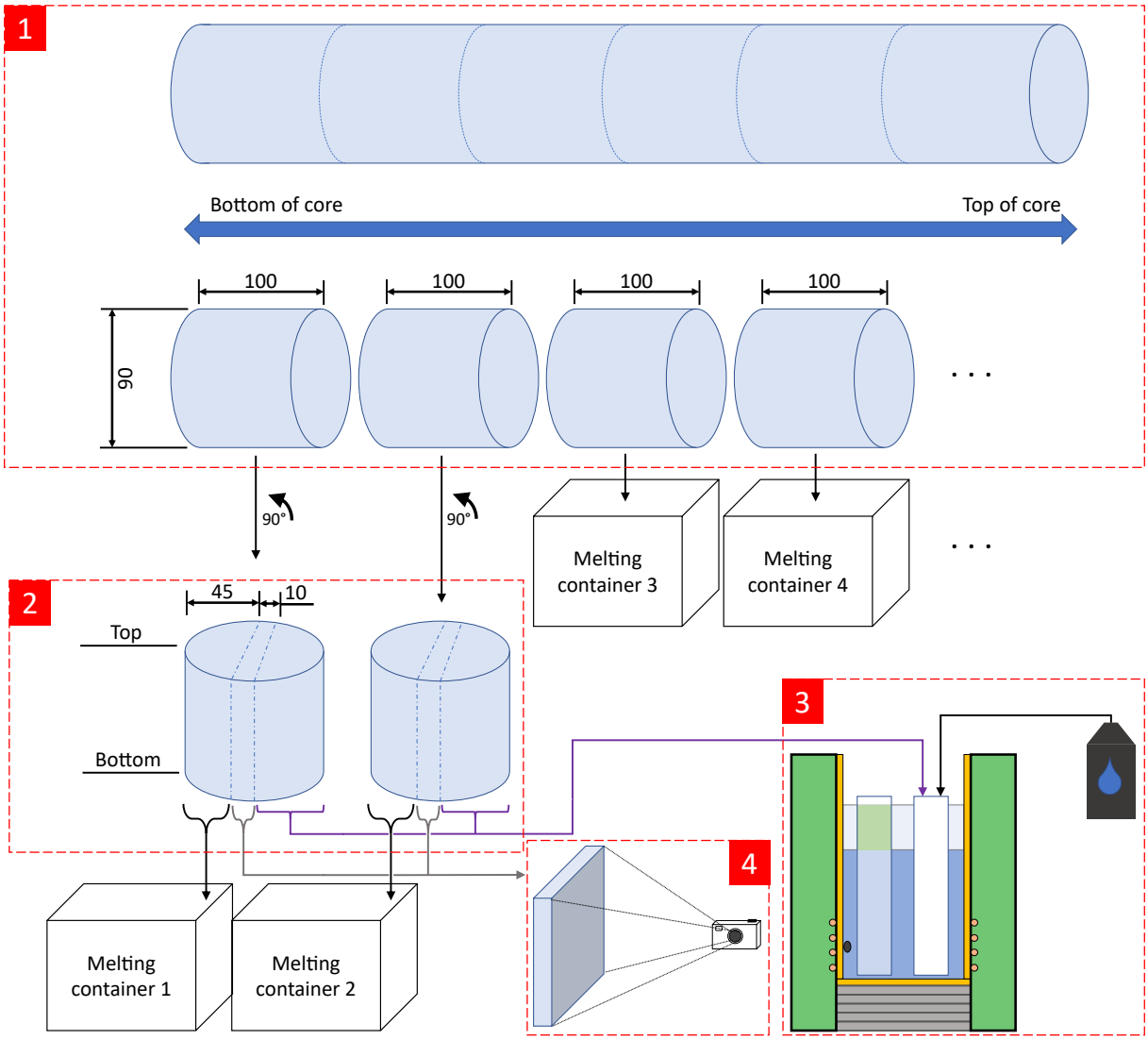

Figure 5.6: Core segmentation and sample dedication procedure

Following the inconsistencies in light conditions during the winter voyage, the LED strip of dedicated growth lights shown in Figure D.2 was procured and adhered to two 400x400 mm timber boards to provide consistent and adequate lighting to samples. This configuration of timber boards and an LED strip provided the necessary controllability over light, as well as improved protection from laboratory activity-related physical impurities and a buffer to laboratory temperature changes due to the semi-isolation of the air between the timber boards and sea-ice surfaces. Thermal fluctuations were particularly reduced in Tank 1, which exhibited observed sea-ice depth fluctuations of no more than 30 mm, as illustrated in Figure 5.7. A 15-minute incremental 24-hour timer was installed to control light exposure duration.

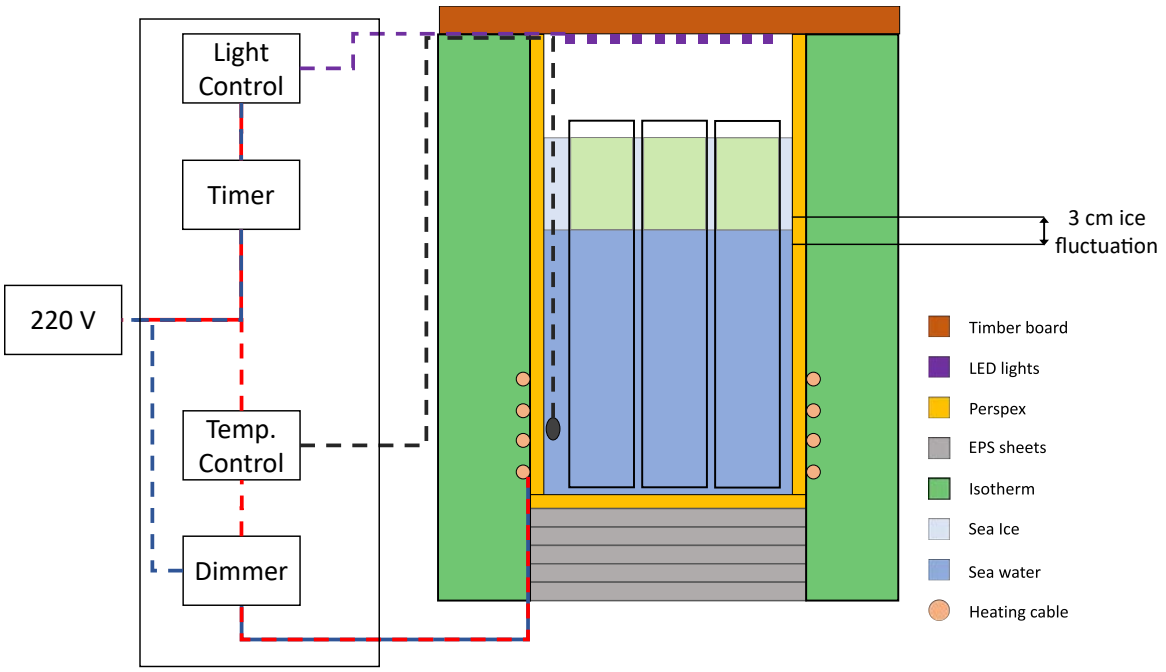

Figure 5.7: Spring cruise tank schematic

Considering the effects sea-ice thickness and snow have on the penetrative ability of light to reach the bottom of sea ice, it can be assumed that bottom-ice algae light conditions differed substantially between sampling locations. Figure 5.8 shows two cases of sea-ice conditions within the sampling time frame, with fully consolidated sea-ice floes, covered in snow and reaching into the horizon (A) and brash ice with little snow and visible algae growth (B). Since each sample was shortened to 100 mm, the light reduction from the sea ice above the incubation segments was not present. The increase in irradiation was not uniform through all samples, as the origin locations, snow cover and initial core lengths differed.

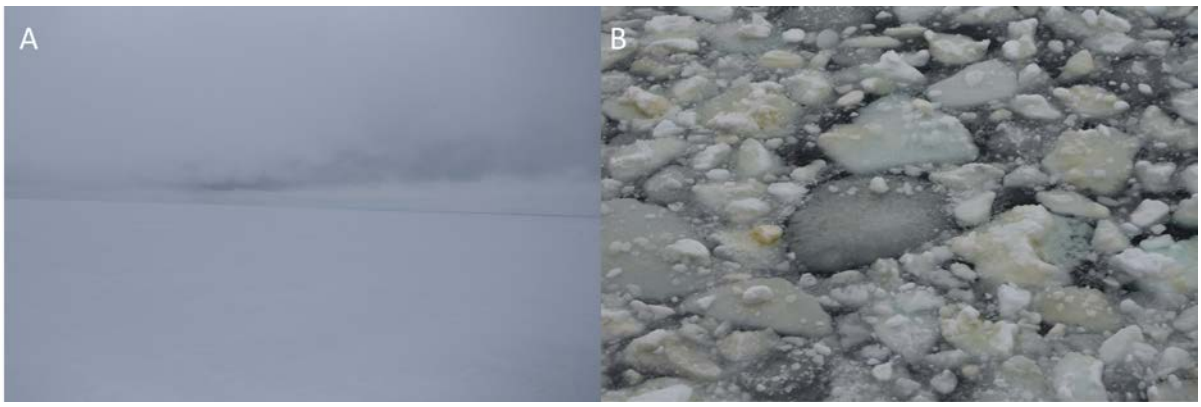

Figure 5.8: Sea-ice conditions on the (A) 24^{th} of October 2019, consolidated sea ice to the horizon and (B) on the 22^{nd} of October 2019, brash ice with visible algae (brown) discolouration floating in the Southern Ocean

In order to fully mimic the light conditions algae experienced at the bottom of their respective floes, an individual light would have had to have been used for each sample to mimic the light

conditions on the day of sampling. Snow from the coring location would have had to have been introduced into the respective hybrid tube in the tank, and a means of absorbing light dependent on the prior sea-ice thickness above the lower segment of each incubation core would have had to have been implemented. The samples gathered from broken floes would have had to have additional lights installed along the depth of the hybrid tube to imitate light reaching the bottom of the broken flow from the side due to changes in the revolution angle of the sun. Each hybrid tube's lights would have had to have been further individualised through dimmers to be able to simulate these changes in revolution angle. The lights of different hybrid tubes would have needed to be on separately timed circuits due to the differences in sunlight duration at the sampling points. All of these provisions would have achieved a light environment similar to that which bottom-ice algae would have had *in situ*; however, it would not have accounted for changes in snow thickness, sea-ice depth, location or weather conditions which natural sea ice and its algae would experience.

Limited by the single light source, single light duration and intensity for both tanks had to be chosen, knowing that the chosen intensity would not be able to imitate the lighting conditions each of the algae experienced *in situ*. The spectrum of the LEDs was not specified by the supplier and was not measured due to a lack of suitable equipment during or after the cruise.

PAR sensors or similar measurement devices were not available during the cruise; consequently, the light intensity of the LED strip was adjusted to be approximately equal to the intensity of natural light available on the ship on the 23^{rd} of October, one day prior to sea-ice sampling, by visual inspection: The timber boards and light strip were taken to the upper aft deck, where light interference from objects above or adjacent was judged to be at a minimum. When the sun reached its zenith a timber board was placed approximately 50 mm from the deck with the lights facing down, casting a shadow onto the deck below it. The lights were switched on and individual LEDs progressively covered with duct tape until the shadow disappeared. The same number of LED lights was covered on the second board, leaving 19 of the LEDs uncovered per timber board. Each LED light provided a light flux of 2.47, totalling the light flux per tank to 47 $\frac{\mu\mathrm{mol}}{\mathrm{m^2 s}}$, assuming equal distribution of light throughout the tanks.

Several models for calculating the amount of time between sunrise and sunset at any given location depending on the time of year exist. Forsythe et al. (1995) found that their modifications, Equation 5.1, 5.2 and 5.3 of the CBM model developed by Schoolfield (1982) are accurate when compared to two other models, achieving errors not exceeding 7 minutes for the duration of a day between 30°S and 60°N.

$$\theta = 0.2163108 + 2\arctan\left(0.9671396\tan\left(0.00860(\mathrm{J}-186)\right)\right) \tag{5.1}$$

$$\phi = \arcsin\left(0.39795\cos\left(\theta\right)\right), \tag{5.2}$$

$$\mathrm{D} = 24 - \frac{24}{\pi}\arccos\left[\frac{\sin\frac{\mathrm{p}\pi}{180} + \sin\frac{\mathrm{L}\pi}{180}\sin\phi}{\cos\frac{\mathrm{L}\pi}{180}\cos\left(\phi\right)}\right] \tag{5.3}$$

"θ" denotes the revolution angle of the sun (in radians), "J" the day of the year, "ϕ" the sun's declination angle (in radians), "L" the latitude, "p" the daylength coefficient (in degrees) and "D" the amount of daylight in hours. Choices in "p" define the sunrise and sunset as described in Table A.9.

Daylight hours at the locations of sampling were estimated in Excel using a model modified by Forsythe et al. (1995), based on the latitudes and dates of sampling. Results ranged from 14.7

to 15.4 hours of daylight with an average of 15.1 hours (full results shown in Table A.10, where sunrise and sunset are defined so that the centre of the sun is even with the horizon.

Several aspects of the system design and lighting resulted in an increase in light availability to algae in the system relative to their natural environment:

1. A lack of snow cover in the tank, resulting in a decrease in albedo
2. The shortening of samples and consequent reduction in light absorbed by the sea ice
3. The unchanging orthogonal angle of the LED lights, lacking the reduction of light through the increase in effective thickness of the sea ice between algae and the surface

Despite the desire to have algae increase in concentration in the tanks, it was thought that the sum of these light-increasing effects would result in a strong shift in the algal community compositions, which was not desired. Thus, the timer was set to run for a time shorter than the average calculated daylight duration, being set at 12 hours of light daily.

Temperature profiles and magnitudes of samples in the tanks differed substantially from those the samples had at extraction. Since snow was not added to the system, the sea-ice surface was not insulated from the air and can be assumed to have remained approximately at the ambient temperature of -10 °C for most of the journey. This was significantly cooler than the measured average temperature of -1.91 °C cores typically had 10 cm above the ice-water interface. During sea-ice operations, use of the mobile polar laboratory increased the ambient laboratory temperature to 0 °C for periods usually not exceeding one hour. Melting of the sea-ice surface within the tanks was not observed during this time, likely due to the heat capacity of the system, insulation around the tanks and the reduction in airflow near the sea-ice surfaces due to the presence of light boards on top of the tanks.

The temperature control unit of Tank 1, housing the first three samples, failed intermittently: The unit would activate the heating circuit at the first breach of the set-point temperature and failed to activate on subsequent set-point temperature breaches. To re-activate the controller, it had to be restarted. Due to safety constraints during the turbulent sections of the journey, this could not always be performed, resulting in maximal sea-ice depth fluctuations of 100 mm in this tank.

Chapter 6

Algal Concentration Development

6.1 Post spring cruise sample processing and handling

Upon conclusion of the spring cruise, both tanks were kept in the polar laboratory at -20 °C with functioning heating and melted at 5 °C from 28^{th} of November to the 2^{nd} of December 2019 in the hybrid tubes. A film of algae was observed in some of the tubes, whilst the water seemed clear. A sterilised glass rod was used to agitate the water until the film was no longer noticeable. Samples 1-4 were decanted into clear 250 ml and samples 5 and 6 into 500 ml glass bottles and their nutrient concentrations re-adjusted. Sub-samples were taken from the excess melt water and preserved using glutaraldehyde in the same concentration as in prior samples.

Figure 6.1: Experimental set-up for liquid incubation of melted spring cruise samples

A continuously flowing air supply was attached to filters with rubber tubing and plastic pipes and fed into the sample culturing bottles, providing gases and agitation. The bottles were partially wrapped with tin foil to reduce the constant influx of light provided by the laboratory, shown in Figure 6.1, leaving approximately the same surface area of the bottles exposed to the light as the upper liquid surface area inside each bottle. The exact light conditions were not measured and control over the light duration or intensity at the source was not possible.

Nutrient adjustments were made to the liquid cultures in the same interval and the same bulk concentration as during the cruise. After 20 days of incubation a noticeable film of algae formed in the incubation bottles of some of the samples, in contrast to the relatively clear water.

Further additions of nutrients followed weekly, and 50 ml samples were taken from each culturing bottle on the 5^{th} of February 2020 and preserved with glutaraldehyde. Microscopy of all preserved samples followed from March to June of 2021.

6.2 Algal quantification methodology

To quantify the community volumes and compositions within samples, cell counting via microscopy was employed. Small volumes of each sample were poured into single-welled microscopy slides. Wells had a diameter of 26 mm and a depth of 5.0 mm, totalling a volume of 2.7 ml per well. Slides were rested for 15 minutes before counting to allow algae to settle, as was necessary due to the depth of the slides. Algae were counted within single transect passes under 400X magnification with an inverted Zeiss Axiovert.A1 microscope using a quicksilver lamp as a light source. The magnification area was measured to be 0.56 mm, bringing the transect volume to 0.073 ml. A microscope-integrated Axiocam 105 color was used to produce images of algae for classification, grouping and documentation purposes. Cell concentrations were calculated by dividing the number of counted cells of each taxonomic group by the transect volume. Raw cell count and concentration data is presented in Table A.5 and A.7.

Sea ice and the underlying seawater were not separated during the melting after transportation. Due to the abundance of algae in the sea ice relative to the water, it was assumed that the growth of algae in the seawater during transportation was negligible and that all growth occurred in the sea ice. As such, concentrations for transport samples were inflated by a factor of 3.5, the ratio of [water and sea-ice volume]:[sea-ice volume].

6.3 Microscopy results and discussion

Algae concentrations ranged from 46 000 to 1 200 000 cells per ml, with a cell length of 10 μm as the lower identification boundary due to magnification limitations. Besides two dinoflagellates, all counted cells were diatoms. As such, reporting of algae will be exclusive to diatoms.

6.3.1 Overall and class level cell concentration development

Figure 6.2 shows the class-specific concentration development of all six samples from the concentrations after coring (labelled "Immediate"), followed by concentrations after 29 to 39 days (depending on the sample) of transportation and melting (labelled "Transport") to the concentrations each sample achieved after the subsequent 56 days of incubation in liquid form (labelled "Culturing"). Overall concentrations can be interpreted from this figure as the sum of pennate and centric concentrations. Differences in between Transport and Immediate concentrations within a numbered sample thus show growth or loss of algae during transportation and differences between Culturing and Transport show growth or loss during liquid culturing.

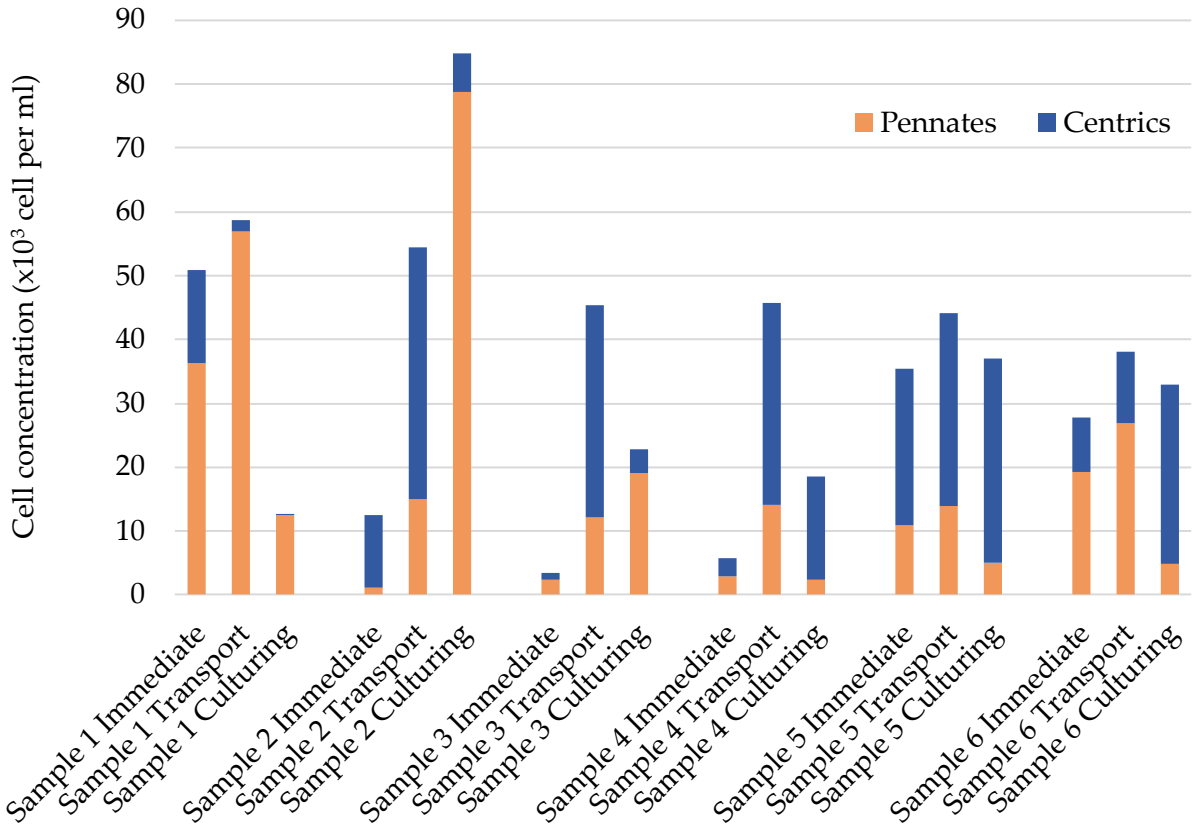

Figure 6.2: Development of diatom concentrations from extraction to liquid culturing at class level, grouped by sample and ordered by incubation time (largest to smallest)

Total and pennate concentrations for all samples increased during transportation, while centric concentrations increased in all but the first sample. Total cell concentrations were reduced after liquid culturing in all but the second sample, while pennate concentrations fell in all but samples 2 and 3, and centric concentrations fell in all but samples 5 and 6. Large differences between the immediate samples were expected in both their overall algae concentrations, as well as the community compositions. Each sample was sourced from a different location as illustrated in Figure 5.4, spanning a sampling distance of 1250 kilometres.

The largest difference in concentrations between immediate core samples is that of samples 1 and 3 with the overall algae concentration of sample 1 being 15 times greater than that of sample 3. A large difference is also observed between the concentrations after culturing when comparing samples 1 and 2, with a factor of 6.8, whereas the difference between cell concentrations after transportation is comparatively small, the maximum factor being 1.5. The fact that the overall cell concentrations of post-transport samples are higher as sample incubation time increases, suggests a trend correlating hybrid system incubation time and cell concentration.

Figure 6.3 plots an empirically derived logistic growth function for overall cell concentrations before and after transportation in the hybrid tanks. Logistic growth is a basic approach when describing growth with limitations, as should be the case in the hybrid tanks due to the limitations of both space and available nutrients.

Logistic growth is described by equation 6.1, where c is the upper population boundary, r

is a growth rate constant and a the determinant in setting the y-intercept, provided that c is constant.

$$\mathrm{y} = \frac{\mathrm{c}}{1 + \mathrm{a} \times \mathrm{e}^{-\mathrm{r} \times \mathrm{x}}} \tag{6.1}$$

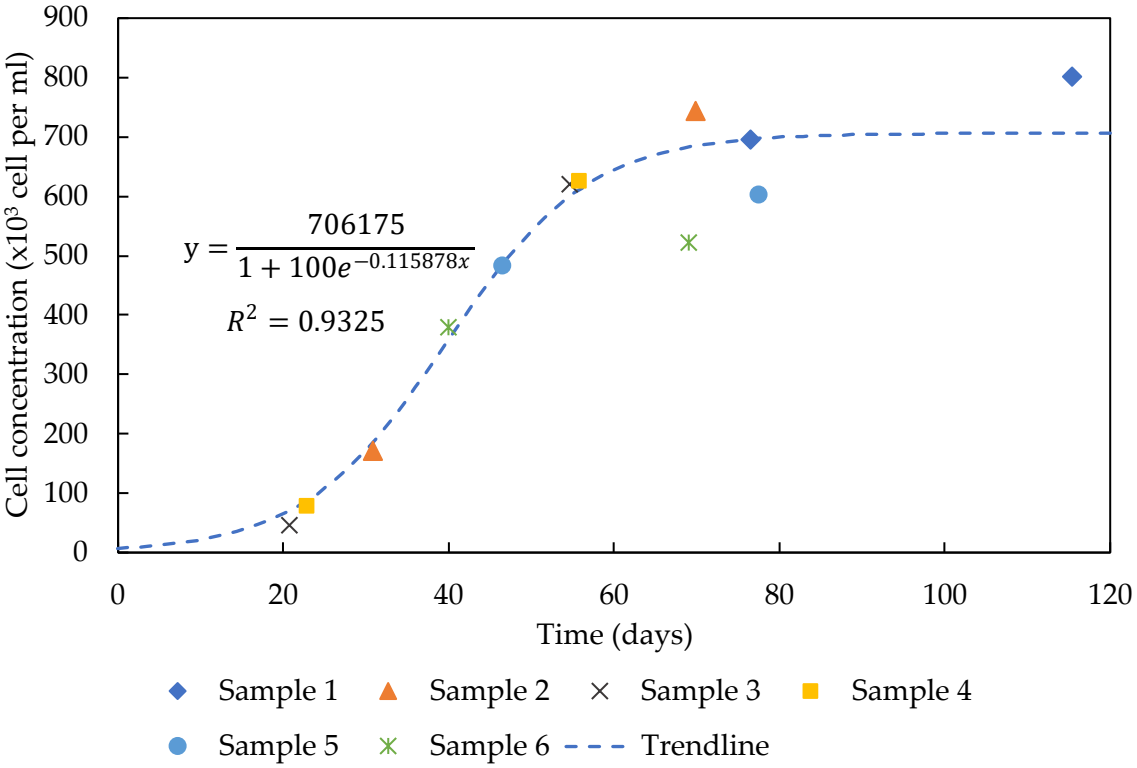

Figure 6.3: Empirically suggested logistic growth trend for cell growth in hybrid system during transportation. Sample numbers correspond to chronological spring sampling dates as seen in Table 5.1

Typically, when attempting to fit data to a function, the variable in x, in our case time, is known. It would be intuitive to assume that the starting time in fitting our data to a curve should be 0; however, the time of formation and thus *in situ* growth of algae is unknown. Growth conditions *in situ* also would not have corresponded to the relatively constant conditions of the hybrid tank and thus the only usable known data are the algae concentrations and incubation time. A single initial starting time should not be assumed, since the initial concentrations were not equal across samples. Instead, the starting time of each sample pair was optimised for, in conjunction with optimisations for the upper population boundary and the growth rate, by minimising the squared error in y of the real data relative to the assumed function. The correlation coefficient R^2 achieved through this optimisation exceeds 0.93 and is a reasonably good fit considering the number of data points; however, as the function under investigation is non-linear, the degree of certainty the R^2 value produces in suggesting goodness of fit may be reduced. Equation 6.1 and the derived parameters $c = 706175 \frac{\mathrm{cells}}{\mathrm{ml}}$ (maximum cell concentration), $r = 0.115878 \frac{1}{\mathrm{day}}$ (growth rate) and $a = 100$ (shift on the x-axis) may be used to roughly estimate the final concentration a sample may achieve in the hybrid tanks. To do this, one would solve for a theoretical starting time with the starting concentration and extrapolate the theoretical end time by adding the incubation time to the theoretical starting time and subsequently calculate the estimate for

the final concentration. Caution is advised when using this equation since growth conditions would need to closely mimic those used in this work for the results to be meaningful.

6.3.2 Dominant taxa

Figure 6.4 shows the five most abundant pennate taxa. The largest fraction of pennates by count were unidentified pennates (Figure 6.4 C) with sizes ranging from 15 to 30 μm. These are not assumed to be the same species nor genera, as sizes, length-to-width ratios and curvatures at the edges varied substantially between cells. Debris of each of the identified pennate taxa was found in at least one of the samples, with the most prevalent debris being that of large *Cylindrotheca closterium* cells. *Cylindrotheca closterium* also exhibited the largest size range of all identified taxa, ranging from 50 to 500 μm in length, as seen when comparing the sizes of the two cells in Figure 6.4 D.

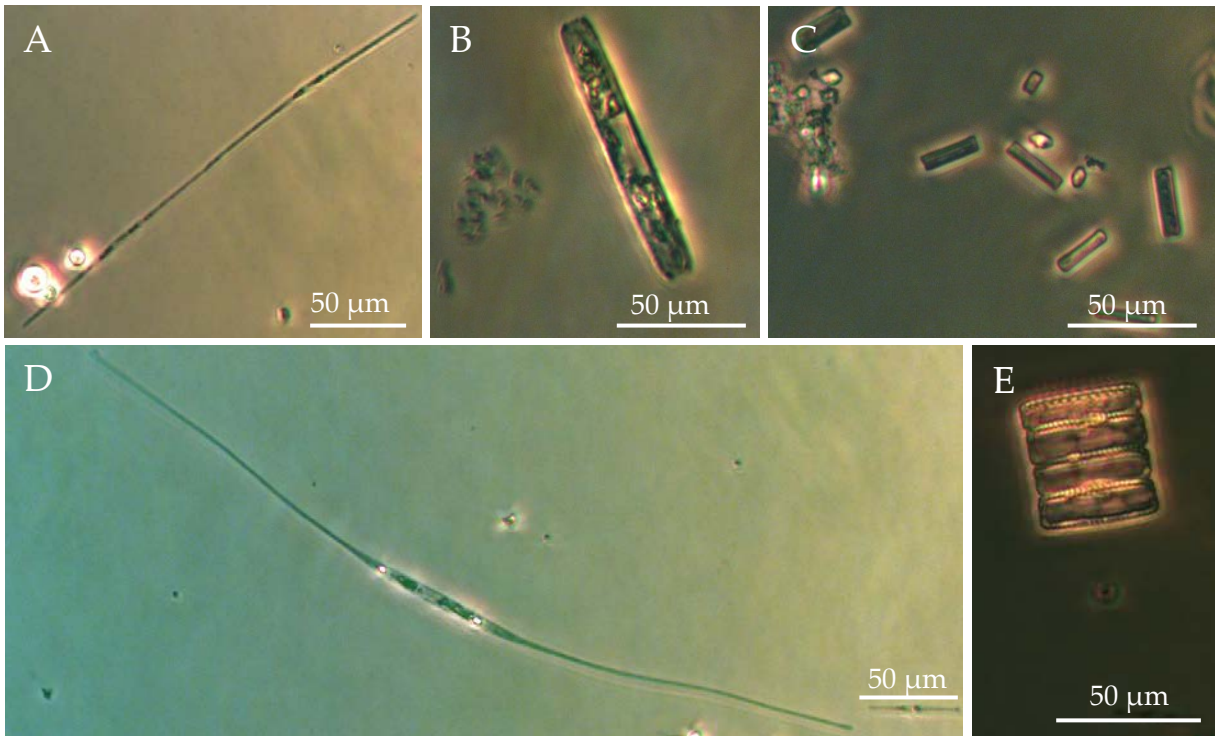

Figure 6.4: Dominant pennates: (A) *Pseudo-nitzschia spp.*, (B) *cf. Navicula spp.*, (C) unidentified pennate diatoms, (D) *Cylindrotheca closterium* and (E) *Fragilariopsis kerguelensis*

Unidentified circular centric diatoms were the dominant centric group in this study (Figure 6.5 C), ranging from 10 to 20 μm in diameter with some outliers (>10) exceeding 50 μm in diameter. Debris of *Odontella* cells was abundant. Conversely, debris of unidentified centrics was not found, despite the overall abundance of unidentified centric cells. Figure 6.5.A was classified as *Dactyliosolen spp.* due to the similarity in shape to *Dactyliosolen phuketensis* and the distinct stripes across its body. This may be an example of *Eucampia* instead; however, the majority of these cells appeared in the same length and individual *Eucampia* cells were not identified in any of the samples.

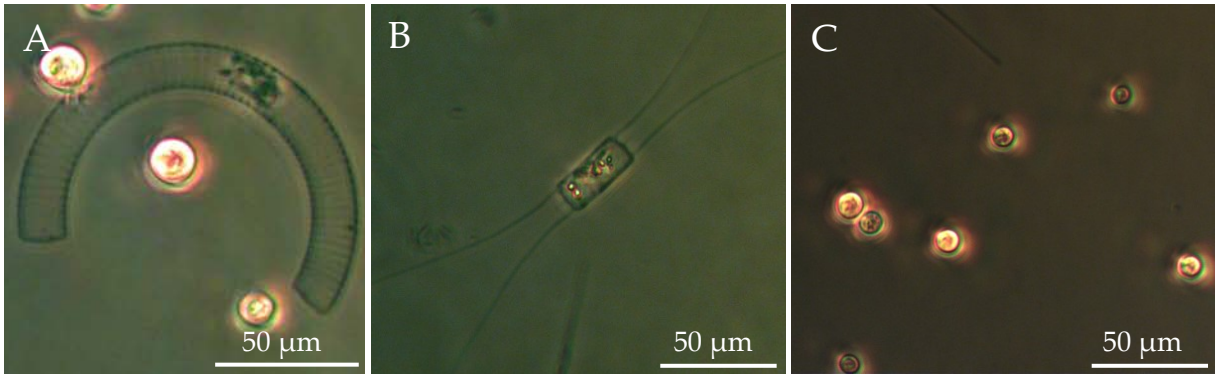

Figure 6.5: Dominant centric diatoms: (A) *Dactyliosolen spp.*, (B) *Odontella spp.* and (C) unidentified centric diatoms

6.3.3 Lower-level concentration developments and community compositions

Distributions of the different taxonomic groups in all samples are shown in Figure 6.6. Several taxonomic groups show trends in growth or reduction through changes from immediate sample concentrations to their transport counterparts. This also applies to changes from transport concentrations to culturing concentrations.

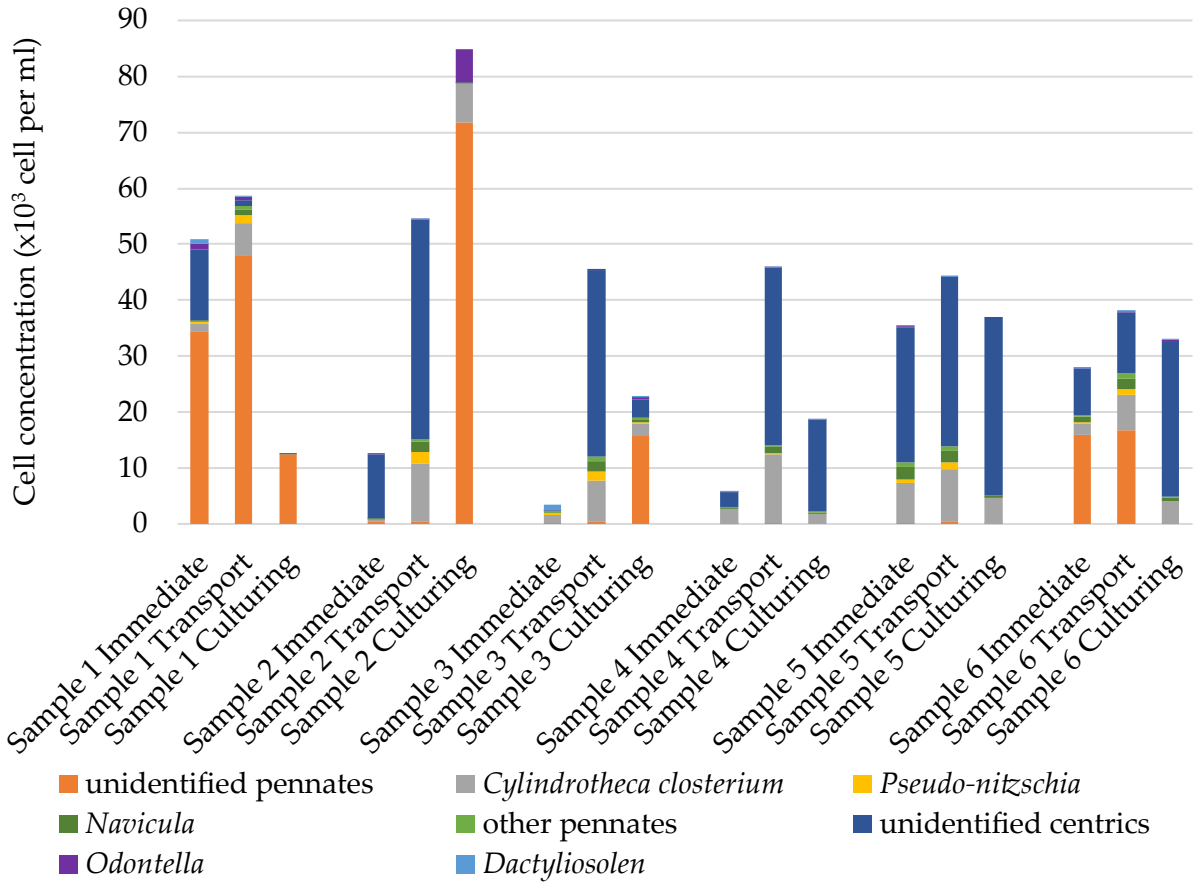

Figure 6.6: Concentration development at lower taxonomic levels from extraction to liquid culturing. Transport concentrations are the result of hybrid system incubation of immediate samples and Culturing concentrations the result of the subsequent culture in fully liquid form

One example of these observed trends is the *Cylindrotheca closterium* concentrations increasing in all samples from their immediate to transport phases, indicating growth during transportation. Similarly, the changes of the same group from transport to culturing indicate a reduction of cell concentrations for all samples during their liquid culture incubation time. Additional examples are *Cylindrotheca* concentrations increasing during transportation for all samples excluding sample 4 and unidentified centrics increasing in concentration during transportation in all but the first sample and decreasing in concentration from transport to culturing in samples 1 through 4.

Shapiro-Wilk tests were performed on all taxonomic groups within their sampling times. Only 24% of all groups were found to be normally distributed with 95% confidence and thus normality could not be assumed. To determine the statistical significance of taxonomic group concentration development trends, one-tailed paired-sample Wilcoxon signed-rank tests were performed, comparing the concentrations of each taxonomic group at a measured time (n=3) to the concentrations of the same group at both other measurement times. The null hypotheses of these tests were that concentrations did not change. Test results are listed in Table 6.1. Results deemed significant are paired with $*$ for ease of reading, where $*$ signifies $P<.05$.

Table 6.1: One tailed paired-sample Wilcoxon signed-rank tests comparing taxonomic group concentrations at different time points with n=3 for each group. The average change and direction of change are also shown, with negative growth denoting a reduction in algae concentration

	Change during transportation		Change during liquid culturing		Overall change	
Taxonomic group	growth (%)	*P*-value	growth (%)	*P*-value	growth (%)	*P*-value
unidentified pennates	110	.031*	4900	.422	4500	.422
Cylindrotheca closterium	590	.016*	-63	.016*	230	.500
Pseudo-nitzschia sp.	1200	.016*	-80	.016*	-69	.047*
Navicula	1000	.031*	-83	.016*	-25	.109
other identified pennates	230	.016*	-69	.016*	16	.422
unidentified centric	20000	.078	-28	.219	2000	.281
Odontella	77	.281	3800	.500	2500	.340
Dactyliosolen spp.	32	.422	-76	.219	-25	.063
pennates	370	.016*	29	.281	1300	.500
centrics	770	.078	-27	.219	150	.281

The average growth of all taxonomic groups was positive during transportation, with most of these changes being statistically significant (barring *Odontella* and *Dactyliosolen spp.*). Conversely, the only statistically significant growth results during liquid culturing show reductions in algae concentrations. Wilcoxon tests comparing immediate to culturing concentrations showed statistically significant reductions in Culindrotheca, Pseudo-nitzschia sp. and Navicula concentrations (Table 6.1. The inclusion of the taxonomic group "other pennates" is included for the sake of completeness, but there is no strong justification to assume that the subgroups within it should behave similarly considering their diversity. The only commonality this group shares, them being pennates, is better covered by the full analysis of all algae of this

group within this work, which only exhibit significance in their change during transportation ($P = .016$).

There is strong evidence for significant changes to cell concentrations in the hybrid system during transportation. These changes are beneficial in that they promote the survival of algae, but potentially detrimental to experiments intending to investigate community reactions closely related to the original colony. Provided there is an increase in cell concentrations and an unchanged community distribution, this concern can be largely alleviated and may even benefit such studies, since samples of higher concentration may be diluted with appropriate salt and nutrient additions to provide a community of equal concentrations to the original colony. Figure 6.7 shows the taxonomic group distributions, illustrating the fractional change of taxonomic groups through intra-sample comparisons of the fractions at different time points for each sample.

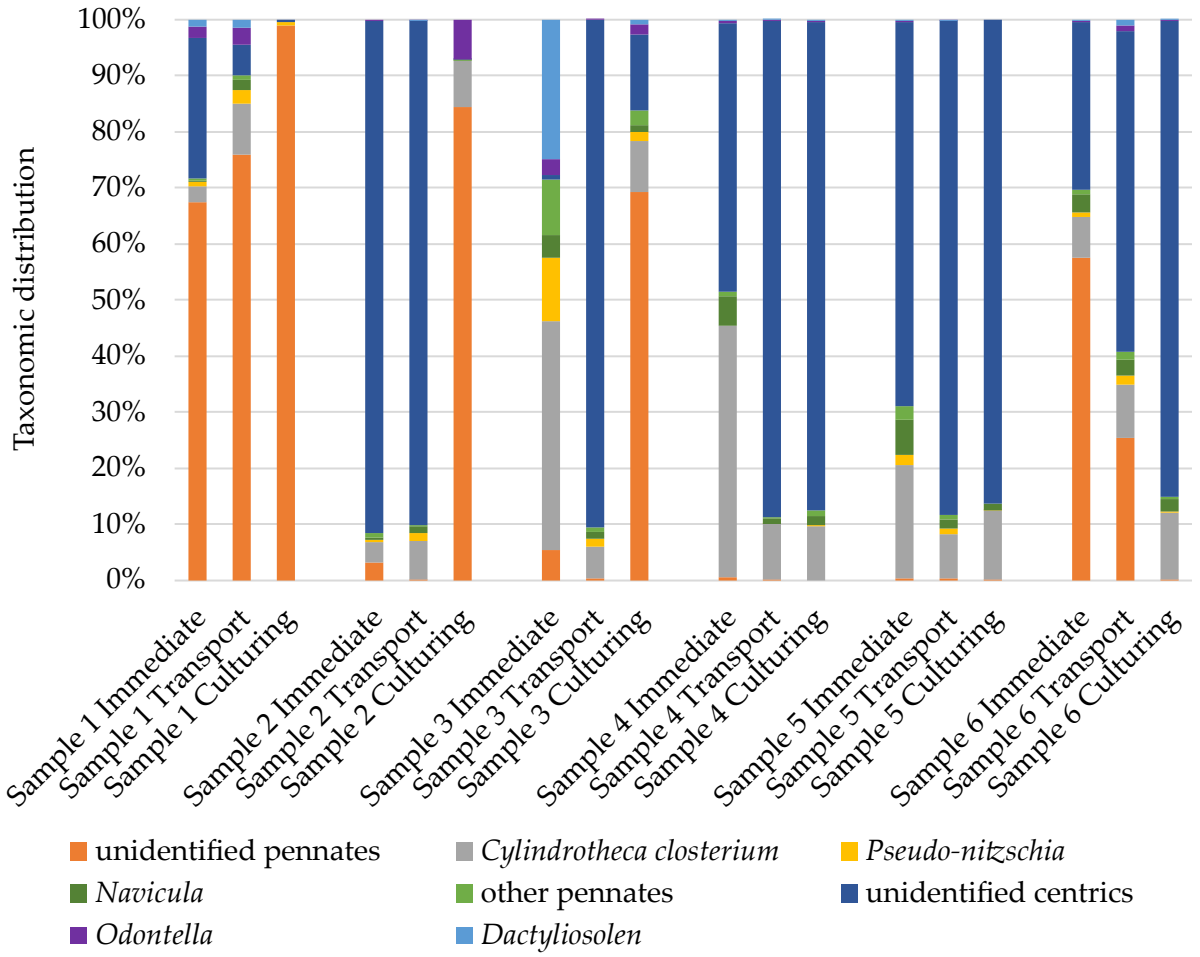

Figure 6.7: Taxonomic distributions within samples

Similarities between some of the taxonomic groups were observed when comparing each "transport" cell distribution to the corresponding "immediate" cell distribution. For example, unidentified pennates remained comparatively constant, as did unidentified centric diatoms. The sample with the largest degree of variation is sample 3, though this is likely largely due to the low initial concentration of algae in this sample. A substantial degree of change is expected following the more than 11-fold increase in concentration this sample experienced. Changes from

"transport" to "culturing" are far more evident particularly in samples 2 and 3. Here the concentration of unidentified pennates increased from less than 1% of the overall concentrations to dominating their respective samples by making up 84% and 69% of their "culturing" sample cell concentrations respectively. Sample 6 exhibits the opposite change, with the concentration of unidentified pennates changing from 44% to 0.12% between transport and culturing.

Figure 6.8 shows the Bray-Curtis Dissimilarity indices for the intra-sample changes to community distributions comparing transport to immediate (blue) and liquid culture distributions (orange), as well as the immediate and liquid culture distributions (grey). In all but samples 1 and 4, the dissimilarity caused by liquid culturing exceeds that caused by transportation. Since a lower measure in dissimilarity indicates a lower degree of change between two samples, the Bray-Curtis Dissimilarity indices support the claim that community changes during transportation in the hybrid system are lower than those accrued during liquid culturing.

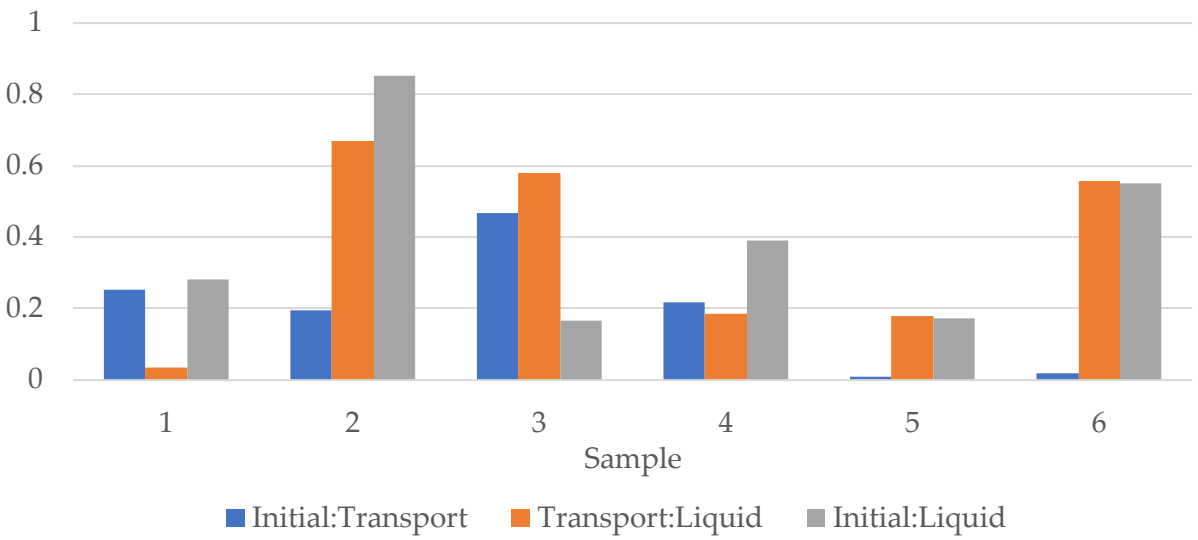

Figure 6.8: Bray-Curtis Dissimilarity indices for fractional intra-sample development of sea ice algae community distributions with (blue) the development from initial to post-transport concentrations, (orange) post-transport to post-liquid culture development and (grey) the overall development

6.4 Discussion of differences between the impacts of the hybrid and liquid culturing systems

The substantial differences in both the initial concentrations and community compositions throughout all samples can be attributed to the differences in the history of their origin sea ice and the differences in origin locations. Although these differences are thus substantiated, they do prevent group trend identification with statistical significance to a large extent, being the most likely cause for deviation from normality.

6.4.1 Relevance of sample storage duration for this study

Long-term storage of fixed algae samples is not advised, since samples tend to change over time even if they have been treated with preservatives such as Lugols solution (Williams, Beckett, and Maxwell, 2016), formaldehyde or glutaraldehyde (Marie, Rigaut-Jalabert, and Vaulot, 2014). Which exact fixative is best for preservation depends on the species being preserved (Marie, Rigaut-Jalabert, and Vaulot, 2014). Naik and Anil (2017) investigated the effects of preservation time and temperature on the cell counts of *Tetraselmis indica* and discovered no significant changes at 5 °C after 6 months (their longest storage time) besides an initial loss observed at all storage times.

Due to logistical constraints, equipment availability and later the onset of the Covid-19 pandemic, samples could not be analysed until March of 2021. The samples in this work were preserved for a longer period (17-19 months) than the 6-month duration investigation by Naik and Anil (2017). Exact effects of glutaraldehyde on the individual species in this work are unknown; however, all samples were stored for a similar duration and at the same conditions. Thus, the effects of long-term storage on the relative taxonomic algae group concentrations should have been equal in all samples.

6.4.2 Reasons for algal concentration declines from hybrid to liquid treatments

Samples were incubated for different periods, ranging from 29 to 39 days in the hybrid system to 56 days in liquid incubation. Changes which the differences in incubation time might have caused are not accounted for in the comparisons of concentrations of the two treatments. Besides the differences in incubation time, differences in lighting (intensity and spectrum) between hybrid and liquid incubation are undesirable parameters. The effects of both of these differences in conditions were not quantified. The results of Andersson (2015) suggest that following the melting of sea ice, an acclimatisation period is necessary before algae adapt to the new conditions, so the longer incubation time in liquid culture loses significance. Algae were adapted to higher light levels than they would have been subject to *in situ* due to the removal of snow and other factors. It is thus unlikely that their change to the liquid culture environment would have caused additional photodamage to them. The light intensity available during liquid culturing was not less than that during transportation and provided constantly. Thus, growth limitations due to a lack of light are also not likely.

Sugie et al. (2020) found significant evidence (P<.001) that an increase in temperature and dissolved carbon dioxide in a fully liquid environment leads to growth of *Cylindrotheca Closterium* and *Pseudo-nitzschia* diatoms. These two species significantly (P=.016) decreased in concentration during liquid culturing in this work despite an increase in temperature and carbon dioxide availability during this treatment. Due to the long melting duration (24 h) and the large volume of seawater in the tank, it is unlikely that osmotic stress caused noticeable damage to algae (Campbell et al., 2019). Assuming that the decrease in concentrations was not caused by osmotic stress during the melting procedure, leaves only the phase change as a possible reason for these decreases.

Two possible reasons for preferred growth in the hybrid system relative to liquid culturing are the concentration of nutrients in brine channels (Meiners and Michel, 2016) and the increased surface area provided by brine channels. Algae were found to prefer the surfaces of the hybrid tubes after melting and the incubation bottles during liquid culturing. Krembs, Gradinger, and

Spindler (2000) estimate an internal brine channel surface area of 0.6 to $4\frac{\mathrm{m}^2}{\mathrm{kg}}$ for a temperature range of -2 to -6 °C within sea ice. Assuming $1\frac{\mathrm{m}^2}{\mathrm{kg}}$ of sea ice for the hybrid system due to the larger temperature range, results in a surface area to liquid ratio of $0.3\frac{\mathrm{m}^2}{\mathrm{l}}$, whilst the incubation bottles offer approximately a fifth of that at $0.06\frac{\mathrm{m}^2}{\mathrm{l}}$. With algae being so locally concentrated, nutrient availability may have become an issue during liquid culturing. Since the algae films in the incubation bottles were visible to the naked eye, the films likely consisted of several layers of cells of algae. As such, algae closer to the bottle walls would have had less access to nutrients and the penetrative ability of nutrients into the algae film may have limited growth.

6.4.3 Drivers for changes in taxonomic distributions

Neither the hybrid system nor liquid culturing resulted in an unchanged taxonomic distribution from the prior state of the algae communities. The degree of change from *in situ* algae communities to those after transportation are attributable mostly to the change in light conditions and increased nutrient availability. Increased irradiance levels may have caused to very light-sensitive algae species (Mangoni et al., 2009), whilst less sensitive species may have experienced growth due to the disappearance of light limitations. The changes to taxonomic distributions during hybrid transportation are not as significant as those during liquid culture when comparing their Bray-Curtis Dissimilarity indices. This is not surprising when considering the degree of environmental change from the hybrid system to liquid culturing (temperature, salinity, surface area, the decrease in bulk nutrient concentrations, etc.). The change from the hybrid system to liquid culturing is a closer simulation of phytoplankton bloom conditions after melting rather than a simulation of the sea-ice environment.

Chapter 7

Overall Discussion

In situ studies on algal responses to environmental changes are difficult to perform (Arrigo, 2016), expensive (Miller et al., 2015), and logistically limited (Cimoli et al., 2017). Laboratory experiments with cultures of algae obtained from sea ice offer many advantages in terms of costs, ease of access and control over environmental conditions. The current state of knowledge on sea-ice algae requires more studies of algal behaviour in sea ice and the behaviour of complex communities (Vancoppenolle and Tedesco, 2016), especially for primary production models (Meiners and Michel, 2016) and greater earth system models (Vancoppenolle et al., 2013). But, the majority of these studies eliminate community complexity and change the algae environment drastically by extracting them from their sea-ice matrix through melting (Ryan et al., 2011). The process of obtaining algae from the sea-ice matrix through melting may cause damage to some algae species (Ryan et al., 2011). The aim of this dissertation was to design and evaluate an alternative sea-ice algae transportation method to existing methods. The alternative transportation method was intended to reduce the degree to which environmental conditions change from the *in situ* to the *ex situ* state, relative to existing transport methods, in an attempt to provide a method of transport which allows results of laboratory studies on sea ice to be more relevant to *in situ* sea-ice algae than they currently are.

As a means of transportation of living sea-ice algae, transport in solid medium is likely to perform poorly due to the required low temperature and potential negative effects low temperature may have on individual algae. Temperatures above -5 °C should not be considered since they would allow for permeability of the sea ice during transportation (Golden, Ackley, and Lytle, 1998) and would thus subject the sea ice to continuous drainage during the journey. Temperatures below this are likely to cause changes to the sea-ice algae community, particularly bottom-ice algae due to the increased salinity (Kauko et al., 2018). The need to cool sea-ice cores quickly after extraction to limit brine drainage for this method puts an additional strain on algae, since they are subjected to osmotic stress due to the change in temperature. Depending on the duration of transportation the issues observed in the storage experiment may become an additional detriment to this method of transportation. Besides these changes during transportation, the need to cool cores may result in an additional alteration of the sea-ice algae community due to the expulsion of brine when sea-ice temperatures are reduced. The results of this study show that the initial brine expulsion when storing at -20 °C may cause brine expulsion of up to 11% and a further brine loss of 19% was noted for cores stored for 35 weeks. These changes in brine concentration may substantially affect the concentrations of sea-ice algae species inhabiting brine channels depending on their location within the sample. Previous studies on sea-ice cores where the brine content and brine profile are non-trivial to the results of the study may have larger errors associated with them than previously expected if their samples were stored under similar conditions and for similar or longer durations than in this study. Changes such as those seen in Figure 4.3 would not be noticed on sea-ice cores drawn with a corer, since coring typically results in a rough surface and any deformations caused by storage are likely to be attributed to the coring procedure. This may be one of the reasons why the

topic of core storage has received so little attention. Due to the small sample size, single storage temperature and issues with artificial sea-ice growth, the results of this study do not suffice to produce a model for the relationship between storage temperature, time and desalination.

The designed hybrid system simulated general sea-ice algae environmental conditions such as temperature, irradiance, salinity, and nutrient availability to the required degree. Hybrid transport was effective in achieving the goal of keeping algae alive, since bulk algae concentrations did not decrease during transportation, with 6 of the identified taxa increasing in concentration with statistical significance (Table 6.1). Conversely, liquid incubation was detrimental to algae survival in this study since algae concentrations decreased on average during this treatment, with the decrease of 4 of the identified taxa being statistically significant (Table 6.1). Moreover, hybrid transport was more effective in preserving the community composition than liquid incubation, as shown by the Bray-Curtis Dissimilarity indices in Figure 6.8. There are several possible explanations for the preferred growth of algae in the hybrid system over the liquid system, such as increased localised nutrient concentrations and greater surface availability. Changes in algae concentrations during transport and liquid incubation were expected, due to the design of the systems encouraging growth by removing nutrient and light limitations. Larger algae communities are preferable when wanting to use these algae for future experiments, especially those experiments which take place in artificial sea ice, since adaptation time, the likelihood of loss of algal biomass and community complexity due to a failure to adapt to new experimental conditions, are likely to be lower. The hybrid system addresses the issues raised by Miller et al. (2015), Ryan et al. (2011), Meiners and Michel (2016), and Vancoppenolle and Tedesco (2016) on the need for *in situ* studies due to differences in environmental conditions between *in situ* studies and common laboratory studies by more closely simulating the *in situ* conditions. The need for sample collection persists and full control over environmental conditions is not possible in the current hybrid system. Despite this, the logistical and economic benefits of the hybrid system make it a good alternative to *in situ* studies when these are not logistically possible and provides a better environment to perform sea-ice experiments than the liquid culture alternative. The hybrid system in its current form can be used to simulate consolidated sea-ice conditions. Studies on the formation of free-floating sea ice are not possible with the current configuration due to spatial constraints and the lack of methods to introduce turbulence into the system. As such, the hybrid system is more appropriate for simulating Arctic sea-ice than sea-ice in the marginal sea-ice zone of the Southern Ocean.

Chapter 8

Conclusions & Recommendations

8.1 Conclusions

This study aimed to design and evaluate an alternative transport method of living sea-ice algae to existing methods in order to improve the degree to which laboratory studies of sea-ice algae may be relevant to conclusions regarding their *in situ* counterparts. The overall conclusions of this work were:

- Storing cores at -20 °C does not suffice to prevent changes to the brine profile, bulk salinity and consequently many properties of the stored sea ice, even after a constant storage temperature is achieved throughout the core.
  - Besides changing brine profiles, storage at -20 °C also results in deformation of the core shape, which has implications for studies in the broader sea-ice field, such as those on the mechanical properties of sea ice.
  - Since the salinity profile and content of cores change during long-term storage at low temperatures, such as -20 °C in this work, it is unlikely that the algae profile and concentrations in such cores can be preserved.
- The objective to bring living algae communities from the Southern Ocean to the University of Cape Town was achieved through transport in the hybrid system.
  - Appropriate light, temperature, salinity and nutrient conditions were identified to be the main requirements for sea-ice algae survival. Algae concentrations increased for all samples during transportation.
  - A potential limit to the overall cell capacity and associated logistic growth function of the hybrid system, regardless of cell type, was determined ($R^2 = 0.93$). The cause of this possible cell capacity limit was not determined, and the limit is likely to change if environmental conditions were to be changed.
  - Taxonomic group distributions remained relatively constant when comparing the initial and the post-transportation concentrations, indicating that all algae were able to adapt to changes from the *in situ* environment, such as the higher light availability due to the removal of snow.
  - The hybrid system offers the possibility to perform experimentation with sea-ice algae in an artificial sea-ice environment, mimicking consolidated sea-ice conditions.
- The hybrid system caused overall preferable changes to algae community compositions and concentrations than those caused by the liquid incubation in this work.

- Liquid incubation in this work resulted in reductions in overall algae concentrations for 5 out of 6 samples from their post-transportation to their post-liquid incubation states. There is strong evidence ($P = .016$) for reductions in concentrations of 3 of the 5 well-identified taxa during liquid culturing.
- Hybrid incubation caused weaker changes to taxonomic group distributions than liquid incubation. It is possible that the lower degree of environmental change from *in situ* conditions to hybrid conditions relative to those caused by melting for liquid incubation causes a lower degree of change to the taxonomic group distribution of the algae community.

8.2 Recommendations

Storing sea-ice cores for prolonged periods at -20 °C is not recommended when the brine content and profile are non-trivial to the study and future studies on stored cores should report the storage temperature and time. Furthermore, storage of algae in cores should be avoided where possible when the survival of algae and the preservation of the algae community is of concern. A more detailed investigation into the effects of storage on the brine content and profile of sea-ice cores within the range of common storage temperatures would be valuable to the sea-ice community at large.

The hybrid system designed in this work was successful in growing the algae community; however, differences between the hybrid and *in situ* environments existed and caused algae communities to change during transport in this system. As such, changes to the hybrid system and surrounding methods are recommended to improve the degree of environmental simulation when it is desirable to preserve these:

- Dedicated lights could be installed for each sub-sample and measurements or location specific estimates of *in situ* light conditions made to change individual light settings. The spectral output of lights used in future hybrid systems should correspond to the wavelengths required by sea-ice algae. Snow could be gathered before coring and introduced into hybrid tubes after core segments are frozen in place.
- Measurements of the community composition and production rates could be made during transportation to provide necessary information for dedicated nutrient compositions and feeding schedules. The community composition could be determined on ship using microscopy with the same segmentation and sample dedication procedure as in this work. Sample specific $(\text{chlorophyll } a) : (\text{algal biomass})$ ratios could be determined and production rates estimated using Normalised Difference Index measurements.
- Sea-ice depth control in the system was acceptable, but fluctuations in sea-ice depth was noticeable and could be improved if seawater were to be circulated and more sensitive temperature controllers were to be used. Methods of circulation within the sample tubes should thus be investigated, as these could also improve the liquid exchange between brine channels and seawater in the system. The height of the hybrid system could be increased to increase the maximum allowable sample height and reduce the sensitivity to seawater heating rate fluctuations.

Beyond the use of the hybrid system for transport without significant impact on algae communities, it may also be used for immediate experimentation with collected sea-ice algae. Using

the system in this way would be preferable when changes to the environmental conditions prior to experimentation are to be minimised. In this capacity the hybrid system provides the opportunity for controlled long-term studies on the responses of algae on environmental changes without the adaption time otherwise required for laboratory sea-ice algae studies.

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Appendix A

Additional Figures and Tables

Table A.1: Interpolated salinity values for artificial sea-ice cores, with salinity in PSU. Sample numbers can be correlated to those in Figure 4.2

Sample		Depth									
		0%	11%	22%	33%	44%	56%	67%	78%	89%	100%
0- minute	11	8.45	7.13	6.42	5.78	6.46	7.73	9.74	9.37	8.36	12.10
	12	8.75	7.85	7.15	6.81	7.06	7.23	8.72	8.57	8.06	12.54
	13	9.10	8.15	7.27	7.25	7.71	8.68	10.04	9.15	10.39	13.38
15- minute	21	9.13	6.82	6.29	6.77	6.18	7.90	8.28	8.44	7.79	12.47
	22	9.93	8.15	7.12	6.85	7.15	7.47	8.09	9.50	8.26	13.72
	23	9.64	9.29	8.23	8.55	8.05	7.76	8.17	7.43	7.60	10.85
1-day	31	8.51	7.18	6.51	6.34	5.86	5.53	6.31	8.09	10.53	13.82
	32	8.21	7.52	6.38	5.99	6.02	6.35	7.43	7.58	7.22	11.04
	33	6.37	5.23	5.38	5.54	6.02	7.20	7.54	6.87	8.15	13.05
3-day	41	7.43	6.30	6.10	5.99	6.73	6.70	7.41	7.69	7.04	11.51
	42	8.31	6.14	5.69	5.61	6.02	6.95	8.09	7.58	7.25	12.03
	43	8.14	6.83	6.30	6.17	6.28	6.40	7.02	8.32	9.71	12.30
7-day	51	8.13	7.69	6.61	6.11	5.75	4.92	5.61	8.18	9.87	11.70
	52	8.74	7.87	7.96	7.84	6.73	5.85	5.89	7.57	7.43	11.93
	53	10.55	7.27	7.91	7.86	7.40	7.21	7.56	8.10	9.29	13.05
14-day	61	11.24	8.92	8.03	7.19	6.13	5.18	5.10	7.13	10.54	10.73
	62	10.18	7.28	6.05	5.90	6.07	6.20	7.67	8.21	8.27	10.99
	63	8.70	7.18	7.18	7.20	6.76	6.74	6.74	8.80	7.34	12.87
35-week top	71	6.18	5.14	4.63	4.27	4.13	4.30	5.01	5.92	6.78	9.88
	72	7.63	5.92	5.86	5.64	4.91	4.22	4.70	6.61	8.48	9.05
	73	6.45	4.37	3.77	4.04	4.60	5.26	6.18	5.75	6.04	8.90
35-week bottom	81	7.06	6.39	5.33	4.98	5.03	4.87	5.60	7.24	8.16	9.90
	82	7.06	5.60	5.79	4.93	4.62	4.84	5.79	6.12	8.18	10.45
	83	6.83	5.53	5.16	4.73	4.96	5.40	6.59	6.47	5.92	9.00
35-week average	91	6.62	5.76	4.98	4.63	4.58	4.59	5.30	6.58	7.47	9.89
	92	7.34	5.76	5.82	5.29	4.76	4.53	5.25	6.36	8.33	9.75
	93	6.64	4.95	4.46	4.39	4.78	5.33	6.39	6.11	5.98	8.95

Table A.2: Raw salinity data (PSU) for artificial sea-ice cores,continued in Table A.3. Sample numbers can be correlated to those in Figure 4.2

		Depth (cm)						
Sample		0	2	4	6	8	10	12
0- minute	11	8.45	7.13	6.42	5.78	6.46	7.73	9.74
	12	8.75	7.92	7.30	6.78	7.02	7.06	7.66
	13	9.10	8.43	7.37	7.32	7.26	7.79	8.57
15- minute	21	9.13	7.04	6.39	6.38	6.78	6.21	7.78
	22	9.93	8.36	7.74	6.88	6.85	7.00	7.46
	23	9.64	9.57	8.37	8.36	8.55	7.97	7.68
1-day	31	8.51	7.06	6.44	6.24	5.58	5.87	7.58
	32	8.21	7.52	6.38	5.99	6.02	6.35	7.43
	33	6.37	5.21	5.44	5.63	6.55	7.67	6.94
3-day	41	7.43	6.32	6.21	5.81	6.58	6.71	6.86
	42	8.31	6.24	5.75	5.56	5.86	6.35	7.49
	43	8.14	6.73	6.24	6.19	6.35	6.66	8.01
7-day	51	8.13	7.56	6.41	6.02	5.38	4.95	7.61
	52	8.74	7.89	7.92	7.97	7.30	6.09	5.81
	53	10.55	7.40	7.58	7.99	7.86	7.58	7.12
14-day	61	11.24	8.76	7.85	6.80	5.62	4.95	6.37
	62	10.18	7.49	6.19	5.86	6.03	6.10	6.56
	63	8.70	7.33	7.04	7.32	7.07	6.74	6.74
35-week top	71	6.18	5.05	4.54	4.18	4.18	4.65	5.75
	72	7.63	5.92	5.86	5.64	4.91	4.22	4.70
	73	6.45	4.50	3.81	3.88	4.43	4.84	5.67
35-week bottom	81	7.06	6.25	5.15	5.00	4.97	5.09	6.91
	82	7.06	5.60	5.79	4.93	4.62	4.84	5.79
	83	6.83	5.59	5.26	4.78	4.83	5.15	5.76
35-week average	91	6.62	5.65	4.85	4.59	4.58	4.87	6.33
	92	7.34	5.76	5.82	5.29	4.76	4.53	5.25
	93	6.64	5.04	4.54	4.33	4.63	5.00	5.72

Table A.3: Raw salinity data (PSU) for artificial sea-ice cores,continued from Table A.3. Sample numbers can be correlated to those in Figure 4.2

		Depth (cm)					
Sample		14	16	18	20	22	24
0- minute	11	9.37	8.36	12.10			
	12	9.04	8.31	8.20	12.54		
	13	9.71	10.05	8.76	11.14	13.38	
15- minute	21	8.13	8.61	8.10	7.99	12.47	
	22	7.47	8.09	9.42	8.97	8.38	13.72
	23	8.46	7.32	7.68	7.68	10.85	
1-day	31	10.18	13.82				
	32	7.58	7.22	11.04			
	33	7.82	13.05				
3-day	41	7.65	7.55	7.12	11.51		
	42	8.15	7.36	7.41	12.03		
	43	9.50	12.30				
7-day	51	9.68	11.70				
	52	6.19	7.79	7.45	11.93		
	53	7.25	7.56	8.91	6.68	11.32	13.05
14-day	61	10.18	10.73				
	62	8.12	8.13	8.39	10.99		
	63	6.71	7.42	9.25	6.92	12.87	
35-week top	71	6.61	9.88				
	72	6.61	8.48	9.05			
	73	6.23	5.63	6.20	8.90		
35-week bottom	81	8.04	9.90				
	82	6.12	8.18	10.45			
	83	6.84	6.25	6.00	9.00		
35-week average	91	7.32	9.89				
	92	6.36	8.33	9.75			
	93	6.54	5.94	6.10	8.95		

Table A.4: Additional information on cores collected during the SCALE spring cruise of 2019 in the marginal sea-ice zone of the Southern Ocean

Station number	1	2	3	4	5	6
Station type	Consolidated	Consolidated	Consolidated	Consolidated	Floe lifting	Floe lifting
Date	2019/10/24	2019/10/24	2019/10/29	2019/10/30	2019/11/01	2019/11/03
Opening time	12:35	19:45	12:31	11:26	13:16	11:33
Closing time	16:07	01:22	15:41	16:24	18:20	13:23
Time of extraction	12:50	09:20	10:05	11:25		
Time of processing	13:15	09:35	10:25	11:40		
Processing duration	00:25	00:15	00:20	00:15	00:07	00:15
Opening latitude	-59.3248	-58.9833	-59.3645	-59.4726	-58.5488	-58.4491
Closing latitude	-59.3078	-58.9608	-59.3309	-59.4672	-58.5432	-58.4263
Opening longitude	0.0666	0.0119	8.1589	10.8893	17.9382	21.9974
Closing longitude	0.0915	0.0473	8.1822	10.9394	17.9735	21.9917
Core depth (mm)	800	800	700	800	300	600
Temperature (°C)	-1.87	-2.074	-1.806	-1.875	-1.564	-1.46

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L1 Medium

Guillard and Hargraves (1993) - please see note at the bottom of this page

This enriched seawater medium is based upon f/2 medium (Guillard and Ryther 1962) but has additional trace metals. It is a general-purpose marine medium for growing coastal algae.

To prepare, begin with 950 mL of filtered natural seawater. Add the quantity of each component as indicated below, and then bring the final volume to 1 liter using filtered natural seawater. The trace element solution and vitamin solutions are given below. Autoclave. Final pH should be 8.0 to 8.2.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
$NaNO_3$	75.00 g L^{-1} dH_2O	1 mL	8.82×10^{-4} M
$NaH_2PO_4 \cdot H_2O$	5.00 g L^{-1} dH_2O	1 mL	3.62×10^{-5} M
$Na_2SiO_3 \cdot 9\ H_2O$	30.00 g L^{-1} dH_2O	1 mL	1.06×10^{-4} M
trace element solution	(see recipe below)	1 mL	---
vitamin solution	(see recipe below)	0.5mL	---

L1 Trace Element Solution

To 950 mL dH_2O add the following components and bring final volume to 1 liter with dH_2O. Autoclave.

Figure A.1: L1 algal medium recipe for enriched seawater based on f/2 medium (Hallegraeff, Anderson, and Cembella, 2004)

Figure A.2: Final spring cruise sample after three days of incubation in the hybrid tank

Table A.5: Microscopy cell count data for all taxonomic groups for samples 1 - 3. The individual samples are split into **I**mmediate, **T**ransport and **C**ulturing

	Sample 1			Sample 2			Sample 3		
Taxonomic group	I	T	C	I	T	C	I	T	C
unidentified pennates	2505	1002	909	28	6	5241	13	7	1156
Cylindrotheca	105	120	0	34	218	515	100	155	151
Pseudo-nitzschia sp.	29	31	6	3	44	0	28	36	27
Navicula	6	23	0	4	38	2	10	35	21
Fragilariopsis kerguelensis	15	12	0	8	7	4	16	16	11
Haslea sp.	0	0	0	0	0	2	0	0	8
pennate spp. (oval - stripes)	0	1	0	0	0	0	6	1	14
pennate spp. (oval - rim)	1	0	0	0	1	3	2	2	0
pennate spp. (leaf - center line & stripes)	0	0	0	0	0	0	0	0	5
star conglommerate of pennates	0	0	0	0	0	0	0	0	3
unidentified centrics	932	72	4	835	2873	1	2	2434	227
Odontella	76	38	0	3	2	439	7	2	30
Dactyliosolen	48	20	0	0	8	0	61	0	15
Pennates	2661	1189	915	77	314	5767	175	252	1396
Centrics	1056	130	4	838	2883	440	70	2436	272
Total algae count	3717	1319	919	915	3197	6207	245	2688	1668

Table A.6: Microscopy cell count data for all taxonomic groups for samples 4 - 6. The individual samples are split into **I**mmediate, **T**ransport and **C**ulturing.

	Sample 4			Sample 5			Sample 6		
Taxonomic group	I	T	C	I	T	C	I	T	C
unidentified pennates	2	3	0	10	7	3	1171	350	3
Cylindrotheca	189	259	131	523	197	332	147	134	288
Pseudo-nitzschia sp.	0	1	3	43	28	3	14	21	4
Navicula	22	27	21	166	40	32	69	38	53
Fragilariopsis kerguelensis	4	3	7	32	17	0	11	12	7
Haslea sp.	0	0	0	24	0	0	0	0	0
pennate spp. (oval - stripes)	0	0	0	4	0	0	2	1	1
pennate spp. (oval - rim)	0	0	0	1	1	0	0	4	0
pennate spp. (leaf - center line & stripes)	0	2	0	0	2	0	0	3	0
star conglommerate of pennates	0	0	7	0	0	0	0	0	0
unidentified centrics	201	2309	1183	1775	2200	2335	611	793	2042
Odontella	2	3	4	3	2	0	2	14	5
Dactyliosolen	1	3	3	7	6	0	6	14	3
Pennates	217	295	169	803	292	370	1414	563	356
Centrics	204	2315	1190	1785	2208	2335	619	821	2050
Total algae count	421	2610	1359	2588	2500	2705	2033	1384	2406

Table A.7: Algae cell concentration data extrapolated from Table A.5, samples 1 - 3. The individual samples are split into **I**mmediate, **T**ransport and **C**ulturing

	Sample 1			Sample 2			Sample 3		
Taxonomic group	I	T	C	I	T	C	I	T	C
unidentified pennates	34256	47959	12431	383	287	71672	178	335	15809
Cylindrotheca	1436	5744	0	465	10434	7043	1368	7419	2065
Pseudo-nitzschia sp.	397	1484	82	41	2106	0	383	1723	369
Navicula	82	1101	0	55	1819	27	137	1675	287
Fragilariopsis kerguelensis	205	574	0	109	335	55	219	766	150
Haslea sp.	0	0	0	0	0	27	0	0	109
pennate spp. (oval - stripes)	0	48	0	0	0	0	82	48	191
pennate spp. (oval - rim)	14	0	0	0	48	41	27	96	0
pennate spp. (leaf - center line & stripes)	0	0	0	0	0	0	0	0	68
star conglommerate of pennates	0	0	0	0	0	0	0	0	41
unidentified centrics	12745	985	55	11419	39289	14	27	33285	3104
Odontella	1039	520	0	41	27	6003	96	27	410
Dactyliosolen	656	274	0	0	109	0	834	0	205
Pennates	36390	56909	12513	1053	15029	78865	2393	12062	19091
Centrics	14441	1778	55	11460	39426	6017	957	33313	3720
Total algae concentration	50831	58687	12568	12513	54455	84882	3350	45374	22810

Table A.8: Algae cell concentration data extrapolated from Table A.6, samples 4 - 6. The individual samples are split into **I**mmediate, **T**ransport and **C**ulturing

	Sample 4			Sample 5			Sample 6		
Taxonomic group	I	T	C	I	T	C	I	T	C
unidentified pennates	27	144	0	137	335	41	16014	16752	41
Cylindrotheca	2585	12397	1791	7152	9429	4540	2010	6414	3938
Pseudo-nitzschia sp.	0	48	41	588	1340	41	191	1005	55
Navicula	301	1292	287	2270	1915	438	944	1819	725
Fragilariopsis kerguelensis	55	144	96	438	814	0	150	574	96
Haslea sp.	0	0	0	328	0	0	0	0	0
pennate spp. (oval - stripes)	0	0	0	55	0	0	27	48	14
pennate spp. (oval - rim)	0	0	0	14	48	0	0	191	0
pennate spp. (leaf - center line & stripes)	0	96	0	0	96	0	0	144	0
star conglommerate of pennates	0	0	96	0	0	0	0	0	0
unidentified centrics	2749	31576	16178	24274	30085	31932	8356	10844	27925
Odontella	27	41	55	41	27	0	27	191	68
Dactyliosolen	14	41	41	96	82	0	82	191	41
Pennates	2968	14120	2311	10981	13976	5060	19337	26947	4868
Centrics	2790	31658	16274	24410	30195	31932	8465	11227	28034
Total algae concentration	5757	45778	18585	35391	44171	36991	27802	38174	32903

Table A.9: Daylength definitions defined by the position of the sun with respect to the horizon, copied from Forsythe et al. (1995)

	Daylength definition (with and without twilight)	p (degrees)
1	Sunrise/Sunset is when the centre of the sun is even with the horizon	0.0
2	Sunrise/Sunset is when the top of the sun is even with the horizon	0.26667
3	Sunrise/Sunset is when the top of the sun is apparently even with horizon (US government definition)	0.8333[a]
4	With civil twilight	6.0
5	With nautical twilight	12.0
6	With astronomical twilight	18.0

[a] This value is the summation of the radius of the sun (in degrees as seen from Earth) plus the adopted value for the refraction of the light through the atmosphere of 34 minutes (USNO, 1992)

Table A.10: Results of using Equation 5.3 to estimate daylight hours at sampling sites according to latitudes and the sampling date

Sample	1	2	3	4	5	6
Day of year	297	297	302	303	305	307
Revolution angle	2.094	2.094	2.181	2.198	2.233	2.268
Declination angle	-0.200	-0.200	-0.230	-0.236	-0.247	-0.258
Latitude	-59.195	-59.244	-59.592	-59.565	-58.791	-58.697
p	0	0	0	0	0	0
Daylight hours	14.652	14.657	15.135	15.219	15.283	15.435

Appendix B

Statistical test results

Table B.1: Shapiro-Wilk tests for intra-core salinity normality (n=10). Normality is assigned if it is achieved with 95% confidence

Sample	W-stat	*P*-value	normal
11	.943	.585	yes
12	.761	.005	no
13	.877	.120	no
21	.842	.046	no
22	.787	.010	no
23	.888	.163	no
31	.824	.029	no
32	.811	.020	no
33	.758	.005	no
41	.712	.001	no
42	.822	.026	no
43	.803	.016	no
51	.928	.427	no
52	.845	.050	no
53	.762	.005	no
61	.922	.370	no
62	.879	.128	no
63	.671	<.001	no
71	.807	.018	no
72	.937	.518	no
73	.909	.274	no
81	.883	.142	no
82	.844	.049	no
83	.872	.104	no
91	.844	.049	no
92	.894	.189	no
93	.877	.121	no

Table B.2: Shapiro-Wilk tests for inter-core salinity normality, comparing interpolated cores slices at the same depth from the same segmentation time (n=3). Normality is assigned if it is achieved with 95% confidence

	Depth	0%	11%	22%	33%	44%	56%	67%	78%	89%	100%
0-minute	W-stat	.998	.946	.855	.947	.999	.969	.909	.933	.842	.968
	P-value	.911	.553	.253	.555	.950	.660	.416	.499	.220	.656
	normal	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
15-minute	W-stat	.976	.998	.993	.782	.999	.958	.989	>.999	.941	.995
	P-value	.706	.919	.840	.073	.954	.605	.798	.979	.529	.860
	normal	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
1-day	W-stat	.853	.859	.835	.994	.774	>.999	.814	.991	.939	.939
	P-value	.247	.265	.201	.853	.053	.988	.149	.816	.525	.523
	normal	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
3-day	W-stat	.894	.912	.965	.959	.975	.997	.974	.860	.809	.967
	P-value	.367	.426	.641	.611	.699	.893	.692	.268	.137	.652
	normal	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
7-day	W-stat	.925	.946	.776	.757	.989	.988	.854	.847	.917	.876
	P-value	.470	.552	.059	.016	.799	.792	.252	.234	.443	.314
	normal	yes	yes	yes	no	yes	yes	yes	yes	yes	yes
14-day	W-stat	.991	.791	.994	.759	.815	.970	.975	.973	.946	.839
	P-value	.818	.094	.846	.020	.151	.669	.696	.685	.550	.213
	normal	yes	yes	yes	no	yes	yes	yes	yes	yes	yes
35-week top	W-stat	.882	>.999	.990	.854	.985	.812	.896	.894	.952	.859
	P-value	.331	.990	.811	.251	.769	.144	.373	.365	.576	.264
	normal	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
35-week bottom	W-stat	.768	.804	.938	.881	.867	.794	.890	.954	.757	.981
	P-value	.041	.124	.518	.327	.286	.101	.355	.587	.015	.739
	normal	no	yes	yes	yes	yes	yes	yes	yes	no	yes
35-week both	W-stat	.770	.754	.982	.931	.811	.806	.786	.998	.976	.857
	P-value	.044	.008	.742	.493	.142	.129	.081	.918	.706	.260
	normal	no	no	yes	yes	yes	yes	yes	yes	yes	yes

Appendix C

Basic Hybrid System Design

C.1 Algal Growth Tank Design

Using a small sea-ice growth tank has several advantages and disadvantages relative to artificial sea-ice growth in larger tanks. The smaller size lowers the material requirements for the tank, insulation, heating, and maximal growth solution volume, reducing both the capital and operational costs. Due to the smaller exposed air-water surface area the likelihood of contamination is reduced and can be further reduced if a lid is used to close the tank.

C.1.1 Tank geometry and materials

A cylindrical design was chosen as the base geometry in order to circumvent the risk of failure of wall seams at sea-ice height with sea-ice expansion at low temperatures. It was decided that the walls of the tank should be clear in order to allow for visual inspection of the solution during freezing. Determining sea-ice depth visually is an additional benefit made possible by having clear tank walls. Perspex was chosen over glass as the material of construction for the tank walls due to its superior flexibility and the resulting lowered risk of wall fracture due to sea-ice expansion.

A 1000 mm tall Perspex tube, with outside and inside diameters of 300 and 290 mm respectively, was procured and cut into two 500 mm tall tubes. Two 5 mm thick square pieces of Perspex were cut into disks of 305 mm diameter and chemically bonded to a tube respectively. The tanks are shown in Figure C.1

Figure C.1: Perspex tanks after bonding with bottom plates

Figure C.2: Water- and forced convection proofing of Isotherm jacket

C.1.2 Insulation and heating

The wall insulation had to be malleable, in order to accommodate for monitoring equipment outside of the tank such as cameras. It needed to have a high thermal resistivity and be hydrophobic since the cooling of the laboratory would cause moisture to build up on the insulation jacket. Isotherm, a polyester product made out of recycled PET bottles, fulfils these requirements and has a moisture absorption capacity of 2% weight per weight (Brits Nonwoven (Pty)Ltd, 2015).

The floor insulation needed to be a rigid, water repellent material due to the high likelihood of spillage during the transfer of water and mixing with salt, as well as the sea-ice extraction. As such, expanded polystyrene was chosen as the material of construction. The thickness of both the bottom insulation plate and the insulation jacket were chosen to be 100 mm based on the design by Nomura, Yoshikawa-Inoue, and Toyota (2006). 20 mm thick expanded polystyrene sheets were cut into disks of 300 mm diameter using heated wire and bonded into 100 mm thick stacks. 100 mm thick Isotherm was cut to a height of 600 mm and a length of 1570 mm. Figure C.2 shows how plastic sheeting was wrapped around the Isotherm and heat sealed to further reduce the risk of moisture absorption and the infiltration of cold air into the jacket and light into the tanks through their walls.

In order to confirm the suitability of the insulation materials and their thickness, the relative cooling through the insulation and several sea-ice depths was calculated. It was assumed that there was no fouling at the tank walls and that heat transfer is at steady state and that the convective heat transfer coefficients of air and the saline water are equal at all surfaces. Free convection is assumed inside the tank and all outer surfaces. All outer surfaces were assumed to have a temperature of -10 °C and the inner tank walls to have a temperature of -2.0 °C. It is assumed that the expanded polystyrene insulation is in direct contact with air from below. Sharqawy, Lienhard, and Zubair (2010) reports the following properties for saline water: a density of $1028 \frac{\text{kg}}{\text{m}^3}$, a viscosity of $1906 \frac{\text{kg}}{\text{m.s}}$ and a heat capacity of $3990.1 \frac{\text{J}}{\text{kg.K}}$ at 0 °C and 35 grams

of salt per kilogram of seawater. They report a thermal conductivity of $0.57\frac{\mathrm{W}}{\mathrm{m.K}}$ and a thermal expansion coefficient of $14.3\frac{1}{\mathrm{K}}$ at 10 °C and 30 grams of salt per kilogram of solution.

C.1.2.0.1 Thermal properties

- Brits Nonwoven (Pty)Ltd (2015) reports a thermal resistivity of $1.81\frac{\mathrm{K.m^2}}{\mathrm{W}}$ for 100 mm thick Isotherm insulation at standard temperature and pressure. As such, the thermal conductivity of Isotherm lies at $\frac{0.1\ \mathrm{m}}{1.81\frac{\mathrm{K.m^2}}{\mathrm{W}}} = 0.0552\frac{\mathrm{W}}{\mathrm{m.K}}$
- Perspex Distribution (2018) reports the typical thermal conductivity of Perspex to be $0.21\frac{\mathrm{W}}{\mathrm{m.K}}$ at standard temperature and pressure.
- Zwiebel (2014) reports the typical thermal conductivity of M-class expanded polystyrene to be $0.0349\frac{\mathrm{W}}{\mathrm{m.K}}$ at 0 °C average material temperature and standard pressure with a decreasing thermal conductivity as temperature decreases.
- Pringle, Trodahl, and Haskell (2006) report the thermal conductivity of sea ice to lie at 2.14 ± 0.11 and $2.09 \pm 0.11\frac{\mathrm{W}}{\mathrm{m.K}}$ for the top 10 cm and the remainder of first-year sea ice respectively at the relevant temperatures, pressures and salinities.
- Tera Analysis Ltd. (2020) reports a natural heat transfer coefficient of $2.90\frac{\mathrm{W}}{\mathrm{m^2.K}}$ for air at –20 °C on a 500 mm tall vertical plane, corresponding to the heat transfer occurring on the outside of the insulation jacket.
- Tera Analysis Ltd. (2020) reports a natural heat transfer coefficient of $2.40\frac{\mathrm{W}}{\mathrm{m^2.K}}$ for air at -20 °C affecting a horizontal plane with an area of $0.06605\mathrm{m}^2$ and a perimeter of $0.9111\mathrm{m}$ in a downward fashion, corresponding to the heat transfer occurring at the air-polystyrene interface.
- Tera Analysis Ltd. (2020) reports a natural heat transfer coefficient of $4.90\frac{\mathrm{W}}{\mathrm{m^2.K}}$ for air at –20 °C affecting a horizontal plane with an area of $0.06605\mathrm{m}^2$ and a perimeter of $0.9111\mathrm{m}$ in an upward fashion, corresponding to the heat transfer occurring at the air-ice interface.
- Tera Analysis Ltd. (2020) reports a natural heat transfer coefficient of $47.1\frac{\mathrm{W}}{\mathrm{m^2.K}}$ for a saline solution at –1.8 °C on a 500 mm tall vertical plane, corresponding to the heat transfer occurring at the tank wall.
- Tera Analysis Ltd. (2020) reports a natural heat transfer coefficient of $30.2\frac{\mathrm{W}}{\mathrm{m^2.K}}$ for a saline solution at –1.8 °C affecting a horizontal plane with an area of $0.06605\ \mathrm{m}^2$ and a perimeter of $0.9111\ \mathrm{m}$ in a downward fashion, corresponding to the heat transfer occurring at the air-polystyrene interface.

$$\mathrm{q} = \mathrm{U.A.}\Delta\mathrm{T} \tag{C.1}$$

The heat transferred through a medium (q) is the product of the overall heat transfer coefficient (U), the surface area of the medium (A) and the temperature differential of the temperatures on either side of the medium (ΔT).

$$\frac{1}{\mathrm{U}} = \frac{1}{\mathrm{h_1}} + \sum \frac{\mathrm{s_n}}{\mathrm{k_n}} + \frac{1}{\mathrm{h_2}} \tag{C.2}$$

For heat transfer through a media with equal surface areas on each side "U" is calculated through Equation C.2 below, with "h_1" and "h_2" being the heat transfer coefficient on fluids on either side of the combined media and "s_n" and "k_n" being the thickness and heat transfer coefficient of each layer within the combined medium.

$$\frac{1}{U} = \frac{D_3}{D_1.h_{in}} + \frac{D_3.\ln\frac{D_2}{D_1}}{2.k_{Perspex}} + \frac{D_3.\ln\frac{D_3}{D_2}}{2.k_{Isotherm}} + \frac{1}{h_{air}} \tag{C.3}$$

Radial heat loss from the tank is described by Equation C.3, with "U" being the overall heat transfer coefficient for the area as in Equation C.1, "h_{fluid}" and "h_{air}" being the heat transfer coefficient of the fluid within the tank and the air outside respectively and $k_{perspex}$ and $k_{isotherm}$ being the heat transfer coefficients of the Perspex tank wall and Isotherm insulation jacket respectively. The "D_{1-3}" denote the various diameters as illustrated in Figure C.3.

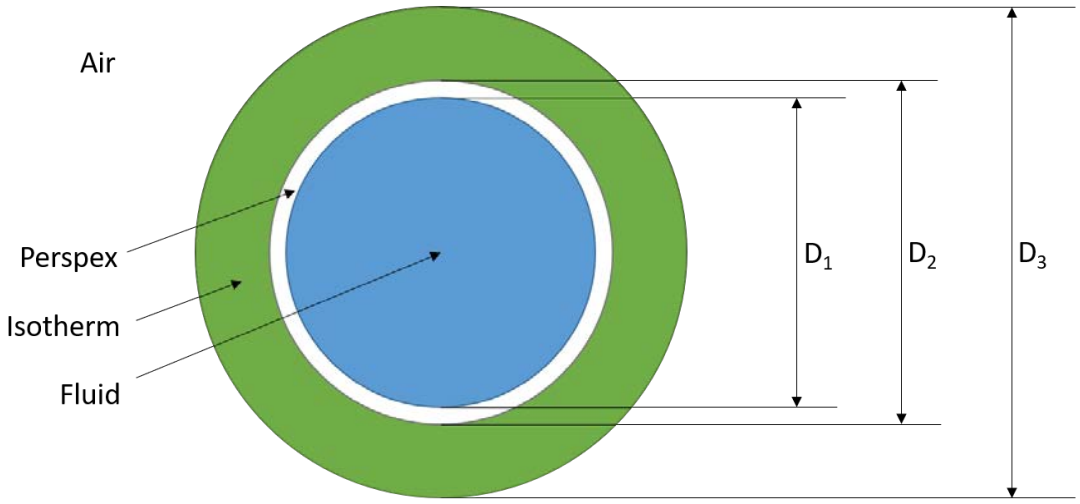


Figure C.3: Top-down cross section of the tank, illustrating the individual variables of Equation C.3

Table C.1: Variations in the heat transfer in Watt into a tank with varying sea-ice thicknesses.

Ice depth	$q_{horizontal}$	q_{upward}	$q_{downward}$	$\frac{q_{downward}}{q_{tot}}$
10 mm	3.57	0.360	5.36	0.576
20 mm	3.47	0.360	5.23	0.577
50 mm	3.19	0.360	4.93	0.581
100 mm	2.73	0.360	4.50	0.593
200 mm	1.81	0.360	3.83	0.638

Without insulation, approximately 23% of all transferred heat would be transferred out of the tank from the top. With insulation, the ratio of cooling occurring from the top is at least two times greater, as can be seen from Table C.1, with approximately 60% of heat being transferred out of the top of the tank under the previously listed assumptions. Although the insulation improved the degree to which the tank set-up mimics sea-ice growth, cooling through the sides

and bottom resulted in supercooling and platelet formation within the solution as shown in Figure C.4.

In order to prevent supercooling and unwanted sea-ice formation at the vessel walls, heating cable was coiled around the bottom 100 mm of the tanks. In order to control the rate of heating, a dimmer was installed into the heating circuit.

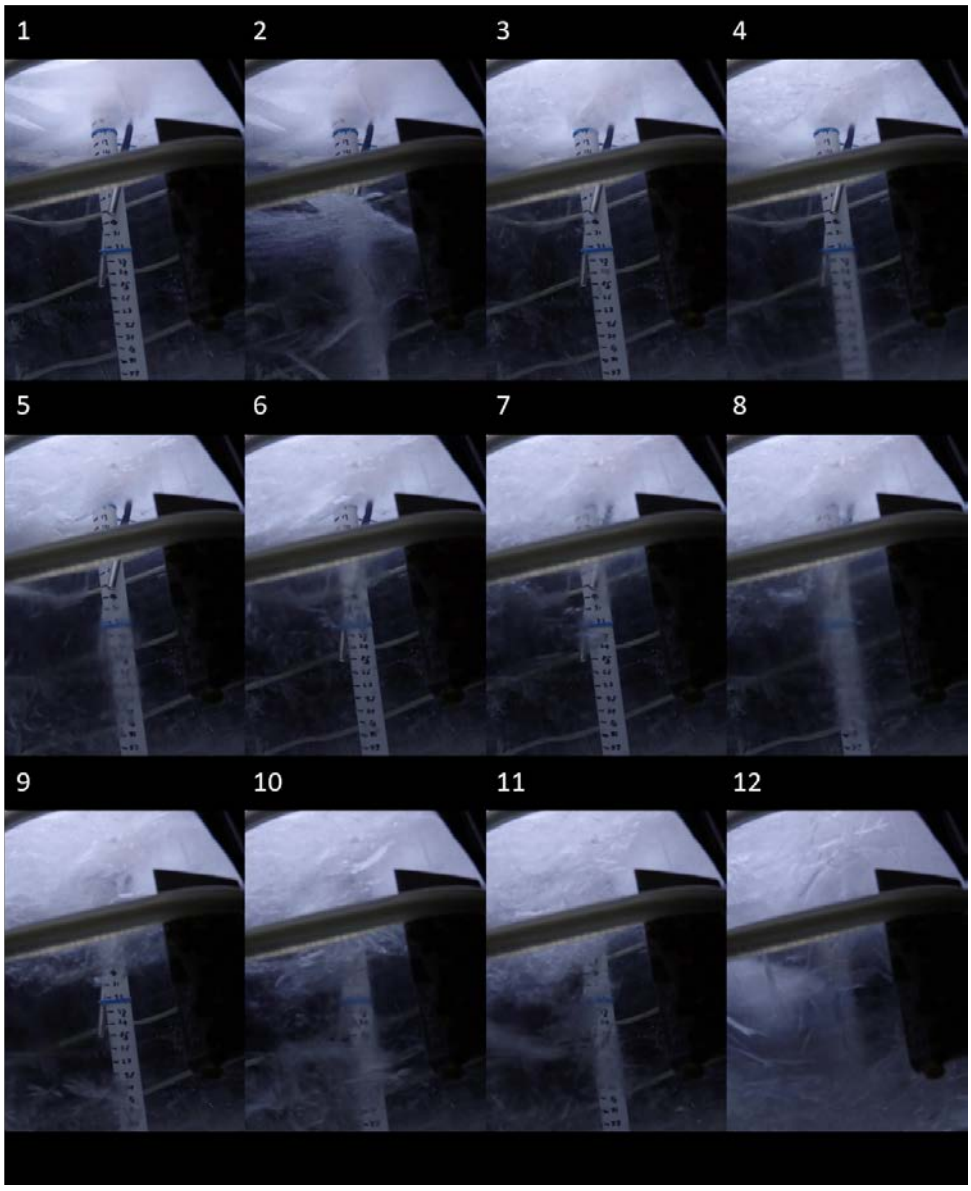


Figure C.4: Supercooling during sea-ice formation without side-heating, leading to platelet formation below the sea-ice sub-surface

Attempts to control heating solely with a dimmer severely impacted sea-ice growth and resulted in irreplicable results. As such, a temperature controller was introduced into the heating circuit. Since the salinity of the solution below the sea ice increases as the sea ice grows thicker, so does the freezing point. Thus, the final sea ice thickness is predetermined through the temperature set in the temperature controller, provided that an experiment is not terminated prematurely and that the rate of heating set by the dimmer exceeds the rate of cooling. Figure C.5 shows a tank with insulation jacket and heating cable from above prior to a freezing

experiment.

Figure C.5: Tank with closed insulation and heating cable

C.1.3 Agitation

In light of the need of fluid motion during experiments which investigate the rate of diatom uptake into sea ice, two methods of agitation were tested: magnetic and propeller driven agitation. Due to the geometry and size of the tanks, agitation mimicking Antarctic conditions, namely strong wave motion during the formation stages prior to consolidation, is not possible. The circular geometry results in tall waves at the edges and small waves at the centre, with the diameter of the tank limiting the maximum wavelength to a degree that makes these waves incomparable to those of the Southern Ocean. As such, current motion is the only feasible mode of agitation in circular tanks of the given size.

Propeller agitation was tested using a "4000 L/H Aquarium Fish Tank 360 Water Wave Maker" (Build Property (Pty) Ltd., 2020). The wave pump was installed at a height of 150 mm from the bottom and the lowest available pump rate and continuous flow selected. Pumping caused significant ripples on the water surface and particulates introduced into the tank were agitated vigorously. The pump was deemed too strong for algal growth experiments due to the high likelihood of algae incurring physical damage through the degree of agitation and the sharp blades of the pump.

Magnetic stirring was tested using a H4000-SE magnetic stirring plate and a 30 x 6 mm and 50 x 8 mm stirrer bar respectively. Small plastic particles were introduced into the artificial seawater solution and a range of rotational speeds tested with both stirrer bars. The larger bar showed good agitation over a wide range, reaching the level of agitation of the pump above at the highest rotations and generating a vortex in the centre of the tank. At high and low speeds, the stirrer bar was not resistant to slight leaning of the tank, leaving the effective operational area and needed to be manoeuvred back to the centre of the tank. The small stirrer bar only achieved acceptable levels of agitation at the highest rotational speeds and was highly susceptible to leaning at these, frequently leaving the effective area of operation without external disturbances and not re-entering it without external assistance.

C.1.4 System development images

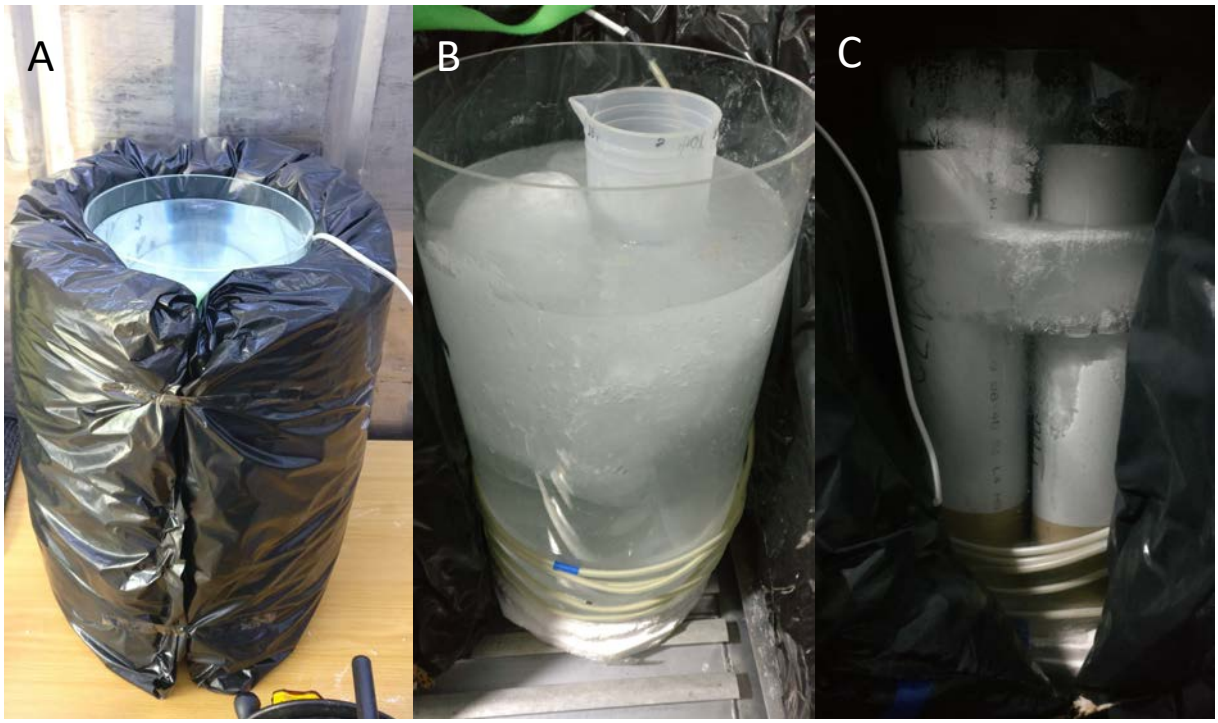


Figure C.6: Pictures of the hybrid system showing the (A) basic hybrid system with closed insulation (B) winter hybrid system with open insulation and (C) spring hybrid system with open insulation while lights are off.

Appendix D

Equipment and data sheets

ICP TEST RESULTS

Sample	304216
KH	8.0
Salinity	33

Unwanted heavy metals

Element	Analysis	Recommended values
Al	0.0019	0,00 - 0,01 mg/l
As	0.00	0,00 - 0,003 mg/l
Cd	0.00	0,00 - 0,0002 mg/l
Cu	0.00	0,00 - 0,012 mg/l
Hg	0.00	0,00 mg/l
La	0.00	0,00 mg/l
Pb	0.00	0,00 mg/l
Sb	0.00	0,00 - 0,0005 mg/l
Sc	0.00	0,00 mg/l
Se	0.00	0,00 - 0,0015 mg/l
Sn	0.00	0,00 - 0,001 mg/l
Ti	0.00	0,00 - 0,01 mg/l
W	0.00	0,00 mg/l

Li-Group

Element	Analysis	Recommended values
Li	0.0200	0,15 - 0,20 mg/l
Mo	0.0006	0,0045 - 0,012 mg/l
Ni	0.00	0,00 - 0,01 mg/l

Fe-Group

Element	Analysis	Recommended values
Co	0.00	0,00 - 0,0006 mg/l
Cr	0.00	0,00 - 0,0004 mg/l
Fe	0.0495	0,00 - 0,006 mg/l

Nutrient Group

Element	Analysis	Recommended values
P	0.0010	0,00 - 6,00 mg/l
PO4	0.00	0,00 - 0,04 mg/l

Macro Elements

Element	Analysis	Recommended values
B	3.56	4,05 - 5,00 mg/l
Br	60.1	55,00 - 74,00 mg/l
Ca	360	380 - 460 mg/l
K	362	360 - 420 mg/l
Mg	1219	1188 - 1460 mg/l
Na	10472	9720 - 11880 mg/l
S	779	810 - 990 mg/l
Sr	0.00	7,20 - 8,80 mg/l

I-Group

Element	Analysis	Recommended values
I	0.00	0,055 - 0,07 mg/l
Mn	0.0121	0,00 - 0,0022 mg/l
V	0.00	0,00 - 0,0025 mg/l
Zn	0.0046	0,00 - 0,007 mg/l

Ba-Group

Element	Analysis	Recommended values
Ba	0.0029	0,00 - 0,04 mg/l
Be	0.00	0,00 mg/l

Si-Group

Element	Analysis	Recommended values
Si	0.0897	0,02 - 2,90 mg/l

Figure D.1: Salt composition for aquaforest artificial sea salt, batch no. 304216 (Aquaforest, 2019)

Figure D.2: LED strip (Bright Star Lighting, 2019)

Appendix E

Scilab code for salinity calculations

```
//Practical Salinity Calculator
//Valid for 2 < Sp < 42
//and -2C < T < 35C
//https://salinometry.com/pss-78/

////////////////////////////////////////////////////
filepath='C:\Users\user-pc\Documents\Uni\Master\Data\Composition\Salinity Data\'
name='October2020'
extension='.txt'
////////////////////////////////////////////////////
// Importing raw data
filename=filepath + name + extension
//'C:\Users\Mark\Documents\Uni\Master\Data\Composition\Salinity
Data\20C10psu.txt'
fid = mopen(filename, 'r');
done_yet = 0
i=0
while (done_yet == 0)
   i=i+1
   [num_read, Names(i), Cond(i), Temp(i)] = mfscanf(fid, "%s %f %f")
   if (num_read <= 0)
      done_yet = 1;
   end
end
mclose(fid)
////////////////////////////////////////////////
//Constants for PSS-78
a=[0.008;-0.1692;25.3851;14.0941;-7.0261;2.7081]
b=[0.0005;-0.0056;-0.0066;-0.0375;0.0636;-0.014]
c=[6.766097E-01;2.00564E-02;1.104259E-04;-6.9698E-07;1.0031E-09;0]
d=[0;3.426E-02;4.464E-04;4.215E-01;-3.107E-03;0]
e=[0;2.07E-05;-6.37E-10;3.989E-15;0;0]

Cref=42.914  //Conductivity of 35 PSU seawater at 15 C and 0 barg in mS/cm
p=0        //Pressure difference in dbar
k=0.0162
////////////////////////////////////////////////
```

Figure E.1: Scilab code for determining practical salinity using temperature and conductivity

```
n=length(Cond)
for j=linspace(1,n,n)
   C=Cond(j)
   T=Temp(j)
   R=C/Cref
for i=linspace(0,4,5)
   rti(i+1)=c(i+1)*T^i
   epi(i+1)=e(i+1)*p^i //Is 1 when pressure difference is 0
end
rt=sum(rti)
Rp=1+sum(epi(2:4))/(1+d(2)*T+d(3)*T^2+R*(d(4)+d(5)*T))
Rt=R/(Rp*rt)
airt(1)=a(1)
birt(1)=b(1)
for i=linspace(1,5,5)
   airt(i+1)=a(i+1)*Rt^(i/2)
   birt(i+1)=b(i+1)*Rt^(i/2)
end
Sp(j)=(sum(airt)+sum(birt)*(T-15)/(1+k*(T-15)))
end
////////////////////////////////////////////////
writetable=1
if writetable==1 then
   printf('%10s %14s %13s %10s \n','Sample','Conductivity','Temperature','Salinity')
   printf('%10s %14s %13s %10s \n','','[mS/cm]','[Celsius]','[PSU]')
   printf('%10s %14.2f %13.2f %10.2f \n',Names,Cond,Temp,Sp)
end
```

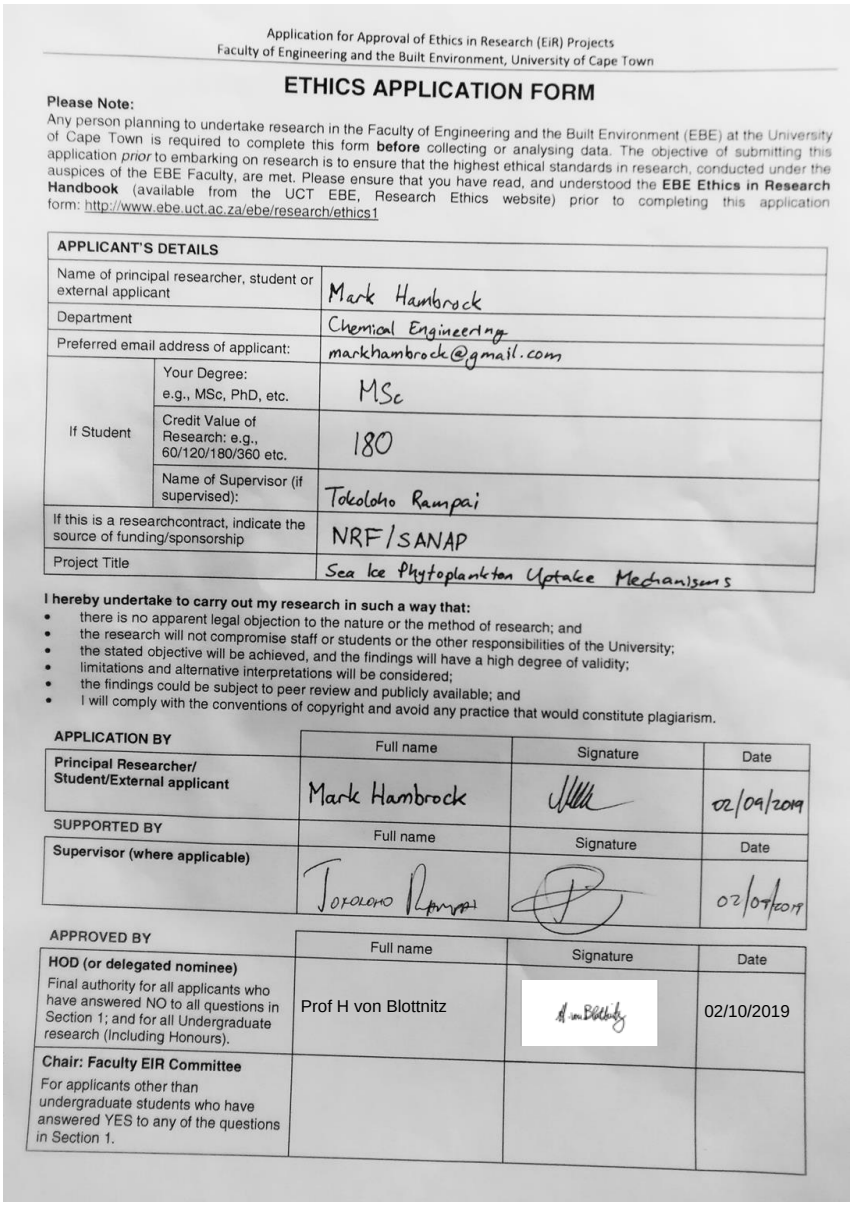
Figure E.2: Continued scilab code for determining practical salinity using temperature and conductivity

The code above (Figure E.1 and E.2) accepts data files (.txt format) where data is presented in columns, with data identifiers being presented in the first, measured conductivities in millisiemens per centimetre in the second and temperatures in °C in the last column. The code uses the Practical Salinity Scale 1978 (PSS-78) (Unesco, 1981) to estimate practical salinity using temperature, conductivity, and the pressure difference between a reference sample and the samples under investigation. It is assumed that hydrostatic pressure is negligible since all measurements are made at atmospheric pressure, equivocal to the measurement of the reference conductivity. Data is printed in a table copying the input table with an additional column at the end containing the practical salinity results. All equations and equation parameters, as well as the reference salinity data were obtained from Unesco (1981).

Appendix F

Ethics Approval

All of the UCT ethics requirements and guidelines have been considered. It has been concluded that no ethical considerations apply to this work.



Application for Approval of Ethics in Research (EiR) Projects
Faculty of Engineering and the Built Environment, University of Cape Town

ETHICS APPLICATION FORM

Please Note:
Any person planning to undertake research in the Faculty of Engineering and the Built Environment (EBE) at the University of Cape Town is required to complete this form **before** collecting or analysing data. The objective of submitting this application *prior* to embarking on research is to ensure that the highest ethical standards in research, conducted under the auspices of the EBE Faculty, are met. Please ensure that you have read, and understood the **EBE Ethics in Research Handbook** (available from the UCT EBE, Research Ethics website) prior to completing this application form: http://www.ebe.uct.ac.za/ebe/research/ethics1

APPLICANT'S DETAILS		
Name of principal researcher, student or external applicant		Mark Hambrock
Department		Chemical Engineering
Preferred email address of applicant:		markhambrock@gmail.com
If Student	Your Degree: e.g., MSc, PhD, etc.	MSc
	Credit Value of Research: e.g., 60/120/180/360 etc.	180
	Name of Supervisor (if supervised):	Tokoloho Rampai
If this is a researchcontract, indicate the source of funding/sponsorship		NRF/SANAP
Project Title		Sea Ice Phytoplankton Uptake Mechanisms

I hereby undertake to carry out my research in such a way that:

- there is no apparent legal objection to the nature or the method of research; and
- the research will not compromise staff or students or the other responsibilities of the University;
- the stated objective will be achieved, and the findings will have a high degree of validity;
- limitations and alternative interpretations will be considered;
- the findings could be subject to peer review and publicly available; and
- I will comply with the conventions of copyright and avoid any practice that would constitute plagiarism.

APPLICATION BY	Full name	Signature	Date
Principal Researcher/ Student/External applicant	Mark Hambrock		02/09/2019
SUPPORTED BY	**Full name**	**Signature**	**Date**
Supervisor (where applicable)	Tokoloho Rampai		02/09/2019

APPROVED BY	Full name	Signature	Date
HOD (or delegated nominee) *Final authority for all applicants who have answered NO to all questions in Section 1; and for all Undergraduate research (Including Honours).*	Prof H von Blottnitz		02/10/2019
Chair: Faculty EIR Committee *For applicants other than undergraduate students who have answered YES to any of the questions in Section 1.*			

Appendix G

Health, Safety & Environmental Considerations

To minimise risks to people and the environment important hazards related to this project and responses to these hazards have been identified:

G.1 Health

Experiments will often be carried out at -20 °C, with sea-ice testing being carried out at -10 °C. Personal protective equipment (PPE) should include a cold-room jacket and gloves as well as rubber-soled shoes and long pants. A lack of PPE will increase the likelihood of cold-related illnesses in affected laboratory personnel.

G.2 Safety

G.2.1 Cold-room hazards

The container forming the cold-room environment separates the laboratory from its surroundings with double-layered metal walls and doors which lock from the outside. Accidental confinement of any personnel in the cold-room may, through deprivation of heat, water and air, lead to panic attacks, frostbite and potentially death. The container is largely sound-proof, increasing the hazard. Accidental confinement might occur through negligence upon locking the container and parking of cars in front of the container doors during experiments and analysis.

Accidental closing of the doors can be prevented by locking the seal handles in place during experiments and analysis. Accidental confinement through parked cars can be prevented by forbidding parking in the path of the container doors.

G.2.2 Mechanical hazards

The heavy machinery used during sea-ice segmentation described in Section 4.1 may lead to injury or loss of limbs if they are operated incorrectly. PPE for coring and segmentation should include laboratory glasses and heavy-duty gloves.

G.2.3 Chemical hazards

Deionised water and marine salt form the two constituents of the freezing solution. Neither pose a significant chemical hazard, salt being an eye irritant. Chemicals used in cleaning the laboratory equipment, such as hydrochloric acid, are a larger hazard. Appropriate PPE is to be worn when working with hazardous cleaning agents.

G.3 Environment

Due to the water shortage in the province of South Africa where experiments were performed, the amount of water used in experimentation should be limited. As such the freezing solution should be recycled after experimental runs where possible. A volume adjustment with deionised water followed by a salinity measurement and adjustment with marine salt reduce the overall water consumption to a minimum.