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Factors influencing sea-ice algal abundance, community composition and distribution in the marginal ice zone of the Southern Ocean.

By

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Declaration

I, Simone De Villiers Louw, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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Signed   **Date**

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List of Abbreviations

Antarctic Bottom Waters	AABW
Antarctic Circumpolar Current	ACC
Circumpolar Deep Water	CDW
Dissolved Organic Carbon	DOC
Dissolved Organic Matter	DOM
Dissolved Organic Nitrate	DON
Extracellular Polymeric Substances	EPS
Filtered Sea Water	FSW
Marginal Ice Zone	MIZ
Organic Compatible Solutes	OCS
Particulate Organic Matter	POM
Polar Front	PF
Scanning Electron Microscopy	SEM
Seasonal Sea-Ice Zone	SSIZ
Southern Boundary of the ACC	SBACC
Southern Ocean	SO

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Abstract

Microalgae and bacteria living within Antarctic sea-ice play a fundamental role in polar ocean biogeochemistry and are thought to be responsible for approximately 25 % of the primary production in sea-ice covered regions of the Southern Ocean. Yet, a paucity in temporal and spatial measurements in the marginal ice zone (MIZ) during winter means that the understanding of sea-ice algal dynamics are poorly constrained, leaving large uncertainties in parameters driving Southern Ocean food-web variability. Therefore, this work aims to clarify the understanding of relationships between biochemical parameters and overwintering sea-ice algae within the MIZ during two austral winter campaigns in the Southern-Indian and Southern-Atlantic MIZ within 2017 and 2019 respectively. Data was collected for phytoplankton abundance, biomass, species description and nutrients concentrations. Two methods of sea-ice algae extraction were used to analyse and thus highlight any effect sea-ice processing may have on species community composition and abundance. Results revealed the presence of an abundant functional sea-ice algae community residing within sea-ice microhabitats during 2017, as opposed to much lower concentrations in 2019. Disparities between the two winter campaigns were likely owed to the passing cyclonic storms in 2017, facilitating the proliferation of sea-ice algae, to biomass' values above 8 µg/L in the sea-ice interior. Comparison between methods of sea-ice algae extraction suggest the possible underestimation in dinoflagellate abundance during the 2017 winter campaign owed to direct melting of sea-ice samples. Yet, similar dominant taxa (genera: *Fragilariopsis* spp*., Pseudo-nitzschia* spp*.,* Coscinodiscus spp. and *Chaetoceros* spp.) and size classes (>2.7 µm) were observed in both cruises. Additionally, a significantly higher sea-ice algae biomass (<0.5 µg/L) was observed when compared to surrounding surface water (>4µg/L). This highlights the ecological significance of the austral sympagic MIZ during winter, providing a vital environment for overwintering sea-ice algae communities, independent from pelagic phytoplankton species.

General introduction

Sea-ice ecosystems are considered one of the largest biomes on earth, occupying approximately 7 % of Earth's surface at its largest extent during winter (McMinn et al., 2010a). These ecosystems are considered regionally important with regards to local biogeochemical processes, while hosting a variety of microbial communities. Sea-ice also plays a pertinent role in ocean-atmosphere CO_2 gaseous exchanges, and as such is regarded as an essential constituent and indicator for climatic variations (Vancoppenolle et al., 2009). Sea-ice enables CO_2 gaseous exchange with the atmosphere through the presence of brine channels (**Figure 1. 1**) formed during sea-ice formation (Moreau et al., 2015). Dissolved material, salt, and inorganic carbon are concurrently concentrated in brine channels and excluded from the crystalline lattice of the sea-ice structure (Moreau et al., 2015). The magnitude at which sea-ice sequesters CO_2 is yet to be quantified (Moreau et al., 2015), while future research is needed to clarify the associated control that sea-ice algae communities may have on the transport of CO_2 to surrounding sea-ice interfaces (Rysgaard et al., 2011). With increased input of anthropogenic CO_2 in the last decade, the concern is raised regarding the diminishing of the sea-ice extent of both the northern and southern hemisphere polar regions.

The circumpolar sea-ice cover encircling the Antarctic continent has become a prevalent topic regarding its significant role in global oceanic processes and regulating the Earth's climate. The sea-ice cover forms part of the global ice-albedo feedback system cooling the polar surface waters and expelling brine from the sea-ice sheet (Meiners et al., 2016). Dense brine water ultimately sinks and generates the Antarctic Bottom Waters that form part of the global thermohaline circulation (Meiners et al., 2016). The global thermohaline circulation, also known as the conveyer belt, does not only regulate global climate temperature but transports dissolved organic matter, nutrients to surface waters and provides essential oxygen to the deep-sea anaerobic organism (Kaiser et al., 2005). The sea-ice cover has been varying in extent over the last 30 years, increasing marginally since satellite efforts were initiated in 1979 (Constable et al., 2014). However, a decrease in sea-ice extent is suggested between 1950's and 1970's austral summer months (October to April) (Constable et al., 2014). Conversely, on recent examination of the last 40 year satellite records, Antarctic sea-ice extent shows an increase until the year 2014 (Parkinson, 2019). Thereafter, precipitous loss of sea-ice extent is seen, even more so compared to the Arctic (Parkinson, 2019). More apparent changes observed in the Southern Ocean (SO) are considered to be surface warming, a poleward shift in Antarctic fronts, and regional changes in sea-ice extent (Constable et al., 2014). The latter being more variable, where regions such as the Antarctic Peninsula are experiencing rising atmospheric temperatures, warming of the upper 300 m waters by 0.6 °C, a shortened (90 days) ice-covered season and the loss of its perennial ice future (Mendes et al., 2018). Changes in sea-ice conditions in specific Antarctic regions will greatly influence the vertical distribution of pack ice algae communities, in return, regional biogeochemical fluxes could be adversely effected (van Leeuwe et al., 2018; Meiners., et al 2012).

Winter sea-ice algae dynamics (composition, abundance, community distribution, and relationship between sea-ice interfaces), specifically within the seasonally ice-covered regions of the SO Marginal Ice Zone (MIZ) is poorly documented (Edwards et al., 1998). The contribution of sea-ice algae to polar marine biogeochemistry is difficult to quantify due to the large variability in production and composition of sea-ice algae, varying between orders of magnitude across the vertical sea-ice profile (Arrigo, 2017). Scientific field observation and data sets over complete annual seasons are sparse making precise quantifications of biochemical trends within the MIZ challenging with data collection particularly bias towards summer month (Gruber et al., 2019; Sambrotto et al., 2003). However, relatively new technology such as biogeochemical Argo floats measuring parameters such a nitrate, salinity, temperature and chlorophyll have provided the opportunity to understand the fate of phytoplankton in the Southern Ocean, with the potential to record vertical profiles during polar nights and ice covered regions (Moreau et al. 2020). The expected increase in anthropogenic induced environmental variability pertaining to sea-ice chemistry, physical properties, and extent will modify existing sympagic (ice-associated) food webs, consequently altering the fate (timing and magnitude) of organic matter released from sea-ice. To both identify and quantify ice algae dynamics during winter, specifically in the South-Atlantic and Indian Ocean MIZ, will add significant insight into current and expected trends associated to the SO food web, sea-ice algae CO_2 sequestering efficiency, and primary production.

According to the Palmer Long-Term Ecological Research (LTER) project, the timing of the ice retreat and advance alters various ecological processes such as the biochemical cycling, species distribution, food type and availability thereof (Massom & Stammerjohn, 2010). How thermodynamic changes will affect the microbial community living within the sea-ice environment is therefore particularly significant. Sympagic environments are generally described as productive habitats particularly in spring and summer where primary production derived from sea-ice may range between two and 24 % or in some instance till approximately 60 % of the total primary production measured within sea-ice covered marine regions (Arrigo, 2017; McMinn et al., 2010a). Primary production within the coastal region of Antarctic is predominantly determined by the presence of sea-ice where 55 % of the primary production occurs when sea-ice is present (McMinn et al., 2010b). However, a recent modelling study suggest that primary production from sea-ice algae to the overall primary production of the Southern Ocean is said to be a mere 1 %, but is nonetheless considered crucial for Antarctic organisms such as Antarctic krill (Meiners et al., 2016). Primary production is anticipated to decline within the coastal region of the Antarctic Peninsula due to the onslaught of climate change (McMinn et al., 2010a). It is further noted that the earlier melt of sea-ice will contribute to the early arrival of phytoplankton spring blooms which already become nutrient deprived during summer, consequently causing their earlier demise (McMinn et al., 2010a).

It is important to note that each sea-ice algae functional group plays a pertinent role in global biochemical cycling. To identify species assemblage in sea-ice is therefore important in understanding how each species contributes to these regionally significant biochemical cycles. A case study conducted over several regions around the west and the east Antarctic by van Leeuwe et al., (2018) suggested that four functional groups may be defined and characterized according to their role in biochemical cycling. The first two groups termed pennate and centric diatoms were described as likely important contributors to carbon fluxes due to frequent high algal biomass and high carbon concentrations within cells, respectively. Autotrophic flagellates including dinoflagellates were determined to be important producers of dimethyl sulphide, and lastly, heterotrophic protists including dinoflagellates and ciliates were found to be vital during low light conditions (van Leeuwe et al. 2018). If microbial assemblages undergo a taxa-shift due to changing brine chemistry, effects will occur on both biochemical and ecological scales.

Numerous studies have been undertaken to characterise SO sea-ice microbiota (Lizotte, 2001; Garrison, 1991; Horner, 1985), yet large uncertainty still remains concerning ecological significance, methods of extraction, species descriptions, presence of sea-ice biota functional groups, and estimates in sea-ice algae biomass. Variability in these parameters are particularly exacerbated during austral winter in the MIZ. Therefore, this study aims to understand some of these drivers responsible for variances observed in the MIZ, through investigating biochemical variables over two winters in the Southern-Atlantic and Indian Ocean MIZ within 2019 and 2017 respectively. In Chapter 2 and 3 sea-ice algae taxonomic composition and abundance in both sea-ice and underlying surface water were examined with emphasis on the type of sea-ice (frazil, pancake, and consolidated sea-ice), and microhabitat (top, interior, and bottom) biota was found in. In Chapter 2 sea-ice algae community composition and biomass were compared to sympagic physicochemical properties (temperature, nutrients, and salinity) to elucidate any drivers responsible for variances in sea-ice algae community structure. In Chapter 4 two methods of sea-ice algae extraction were investigated to attempt to account for any significant influence that melting method might have on species composition and biomass.

Chapter 1

Literature review

1.1 Physical description of the Southern Ocean

The SO has a large geographical range divided into four natural zones as the Atlantic, Indian, West Pacific and East Pacific, each separated by features such as the Antarctic Peninsula, Kerguelen Plateau, Macquarie Ridge, island chains (the Scotia Arc), and seamounts respectively (Constable et al., 2014; Talley et al., 2011). The Antarctic continent is considered as the SO southern-most boundary while the northern "political" boundary according to the Antarctic treaty is limited to 60 °S, however the SO dominant circumpolar current influences physical oceanic properties far beyond this latitudinal point (Talley et al., 2011). The SO is further depicted into zones and fronts in order to relate physical processes to regional biochemical trends (Pollard et al., 2002). In this study, main fronts and zones are classified according to Orsi et al., (1995). Several other fronts are also identified, however these propagate over time in a meandering and splitting fashion (Post et al., 2014).

The Antarctic Circumpolar Current (ACC) dominates the general circulation of the SO and is a continual attribute of the Polar Front and Sub-Antarctic Front (Orsi et al., 1995). The ACC is considered the largest flowing current system in the world with a volume transport of 134-165 Sv, following a persistent eastward direction around the Antarctic continent (Post et al., 2014; Talley et al., 2011; Bargagli, 2005). The ACC plays a fundamental role in global processes such as the thermohaline circulation through its vital connection to the Indian, Pacific, and Atlantic basins, also transporting heat, nutrients, CO_2 and other water constituents around the globe (Post et al., 2014). On a regional scale, ACC ventilates the SO interior through the overturning of water masses (Post et al., 2014). The ACC biochemical composition is largely known as a low chlorophyll, a high nutrient region where iron is considered the limiting factor (Post et al., 2014). The ACC does, however, acquire iron when the ACC circulates over surrounding plateaus (Post et al., 2014). Both study areas of the project were located south of the ACC following the subpolar regime of the SO defined by cyclonic cells, as well as wind and buoyancy driven flows (Orsi et al., 1995). The subpolar region furthest from the Antarctic continent consists of clockwise cyclonic cells of recirculating water which include the Ross Sea and Weddell Sea gyres (Orsi et al., 1995). The subpolar region is considered from the Antarctic continental margin up to 65 °S containing persistent easterly winds (Orsi et al., 1995). The easterly winds are likely to be very strong due to the left ward deflection of katabolic winds associated to the continent and ice sheet, driving the flow of the Antarctic Coastal Current westward (Bargagli, 2005). Due to the Ekman transport deflecting the easterlies of the Antarctic Coastal Current in a southerly direction and the ACC westerlies in a northerly direction, a region of divergence occasionally known as the Antarctic divergence (having some relation to the Southern Boundary of the ACC (SBACC) is generated (Bargagli, 2005; Orsi et al., 1995).

Therefore, the Antarctic divergence serves as a boundary between Antarctic easterlies and westerlies influenced by Ekman driven currents which further propagates as a function of Ekman transport either north or south, modified by the atmospheric interface and Antarctic Surface Water (Post et al., 2014).

The Antarctic seasonal ice-covered region is termed the seasonal sea-ice zone (SSIZ) which include the SBACC and interacts with the ACC, Antarctic Divergence, and Antarctic Coastal Current (Massom & Stammerjohn, 2010; Robinson & Brink, 2006). This region grows annually from land fast ice adjacent to the Antarctic coastline during autumn (Talley et al., 2011). In late summer the SSIZ will detach, however, can remain and form multiyear ice (Talley et al., 2011). Subsequent flow and deformation of detached seasonal sea-ice are driven by the regional wind regime and ocean currents. (Talley et al., 2011; Massom & Stammerjohn, 2010). According to data collected from the National Snow and Ice Data Centre, the annual mean sea-ice cover alongside the Antarctic continent primarily propagates in a westerly direction corresponding to the cyclonic flow of the Weddell and Ross Sea gyres (Talley et al., 2011). East of this region the sea-ice propagates following the general wind and circulation present in the ACC (Talley et al., 2011). Processes involved once the sea-ice begins to propagate include the Coriolis force, sea surface tilt, and internal stress (Massom & Stammerjohn, 2010). Sea-ice motion responds rapidly to changes in wind velocity during cyclonic or anti-cyclonic conditions, while ice cover thickness, production, and concentration are subsequently modified (Massom & Stammerjohn, 2010). Ecological processes in the SSIZ are also primarily governed by the wave-ice interface where water flows onto the surface of sea-ice floes supplying nutrient and biological matter to sea-ice environment, concomitantly removing snow (Massom & Stammerjohn, 2010).

The MIZ surrounding the Antarctic can be described as a region of transition coupled to wave-ice interaction relating to pack ice and floating ice floes (Smith et al., 2011). In the MIZ, sea-ice transforms from consolidated inner pack ice to open water, serving as a physical buffer against wave action for inner pack ice (Strong et al., 2017). Wave action can penetrate the MIZ pack ice for hundreds of kilometres, generating rounded ice floes throughout the region (Stroeve et al., 2016). If the sea-ice floes are small, they are generally quite mobile and can propagate over large spatial regions resulting in large variability of the ice floe's physical and biochemical properties in confined areas (Stroeve et al., 2016). MIZ sea-ice dynamics are also influenced by irradiance, surface mixing, stratification, and the formation of polynyas (Stroeve et al., 2016; Constable et al., 2014). Polynyas around the Antarctic are particularly influenced by strong easterly katabolic winds where newly formed ice is forced away from the Antarctic continent, resulting in the increased formation of sea-ice at the ice edge (Stroeve et al., 2016). Additionally, the MIZ has strong implication for surrounding environment altering biophysical water properties, meteorological processes, and atmospheric boundary layer conditions (Strong et al., 2017). The MIZ extent and variability also serve as an important denominator in habitat partitioning for biota such as the Antarctic minke whales (Strong et al., 2017).

1.2 Sea-ice dynamics

Sea-ice is influenced by multiple physical processes determining biochemical variables present within the sea-ice structure, such as temperature which ultimately determines the mechanism of formation and type of ice structure present (Scott et al., 1994; Cota et al., 1991). Sea-ice dynamics differ in each sector of the SO largely dependent on the current, latitudinal range, and topography (Post et al., 2014). For example, sea-ice within the east Antarctic from about 30 to 160 °E occurs in a narrow and mobile band compared to the West Antarctica which has large ice sheets containing embayment's where prominent hydrographic features (cyclonic gyres) influence the dispersal and motion of sea-ice (Post et al., 2014).

Sea-ice types can be broadly categorized as land-fast and pack ice, each with disparate porosity, thickness, brine capacity, and salt concentrations (Post et al., 2014; Cota et al., 1991). The dominant ice type found in the SO is pack ice (frazil- dominated sea-ice) which is generally considered first-year ice due to the melting of most sea-ice in the first subsequent summer season. An exception to this is the western Weddell Sea and Bellingshausen Amundsen Sea-ice shelf which contains thick multiyear ice (Torstensson et al., 2018; Talley et al., 2011; Allison et al., 1993). Pack ice within the SO grows rather slowly and steadily reaching its maximum extent during the austral spring usually in September at a latitude of approximately 60 to 65 °S (Post et al., 2014; Talley et al., 2011). Pack ice of the SO generally has a thickness between 0.5 and 1 m compared to fast ice or multiyear ice which has a thickness between 1 and 3 m (Robinson & Brink, 2006). Rapid decay of ice is observed as the seasons progress, but usually in December reaching a minimum of sea-ice cover in March (Post et al., 2014).

Salinity and temperature of surface water in the SO Antarctic zone is approximately 34 PSU with temperature above -1.91 °C (Fripiat et al., 2017; Haecky et al., 1998). Minor super-cooling of the surface waters results into the formation of fresh water ice crystals excluding salt from the ice structure forming a thin surface sea-ice layer, concurrently creating a thin high density brine solution underneath the ice (Bargagli, 2005; Haecky et al., 1998). In calm conditions the thin sea-ice layer is termed nilas, and it grows as water molecules bind to the ice-surface under freezing conditions (Naumann et al., 2012; Allison et al., 1993). As the thin sea-ice continuously interacts with surrounding surface water, higher density brine solution is forced into the sea-ice structure through convection overturning (Fripiat et al., 2017; Eicken, 2003). This is caused by the presence of a downward temperature gradient in the ice and a upward brine gradient below the ice (Fripiat et al., 2017). Simultaneously, brine channels and pores are formed in the sea-ice structure as a function of ambient ice temperature (Gleitz & Thomas, 1992). Ahead of the sea-ice interface, a layer of continuously supercool water persists due to the higher rate (one order of magnitude) of heat transport from the surface water compared to the influx of ions (salt) expelled from sea-ice, creating a region of lamellar and cellular composition ice usually termed the skeletal layer of fully developed columnar sea-ice (Eicken, 2003). This

layer, in conjunction with the sea-ice interface, governs the exchange of heat and salt (including bulk salinity of growing sea-ice) between sea-ice and surface waters (Mock & Thomas, 2005; Haecky et al., 1998). These processes are usually responsible for the microstructure and porosity of sea-ice (Mock & Thomas, 2005).

During turbulent conditions in the SO particularly at the MIZ, the formation of frazil ice crystals in the form of quiaxed and isotropic grains (millimetre sizes) are observed (Ackley & Sullivan, 1994). This is vastly different from columnar ice in the form of anisotropic crystals (centimetre dimensions) grains shaped during quiescent conditions (Ackley & Sullivan, 1994). Congelation of frazil crystals forced by a strong wave and wind action develops into a sludge-like ice structure at the surface waters termed grease ice (Bargagli, 2005; Ackley & Sullivan, 1994). Grease ice is composed of individual crystals clumped together as frazil ice (Naumann et al., 2012). Growing grease ice forms into a semi-consolidated frazil-dominated ice sheet (Bargagli, 2005). When ice formation exceeds 30 to 40 % and freezing temperatures continue, a complete consolidated frazil-dominated ice sheet forms (Bargagli, 2005). Due to the close proximity of the advancing ice sheet to the open ocean, wave fields pass through the ice cover as a result of the surface-wind interface and this forms pancake ice (**Figure 1. 1**) (Ackley & Weeks, 1990). Pancake ice forms when the grease layer or suspended crystals aggregate at the surface, resulting in loosely aggregated discs, initiating the pancake life cycle (Kaiser et al., 2005; Ackley & Weeks, 1990). The transformation from grease ice to pancake ice has also been linked to a critical solid fraction (Naumann et al., 2012). However, when the influence of wind and wave fields are no longer permeable or sufficient, the pancake life cycle will stop and congelation ice will form in unaffected pack ice regions (Ackley & Weeks, 1990).

Figure 1. 1: Pancake ice in the MIZ of the SO at -56° 80'
178" S; 00° 30' 262" E in austral winter July 2019.

Pancake ice is usually 10 cm thick, but can reach a thickness of 20 to 50 cm (generally observed in the Weddell Sea sector) extending several meters across, forming super pancakes (Petrich & Eicken, 2016; Kaiser et al., 2005). Larger pancake structures are associated to the annual growth of the Antarctic pack ice during late summer or early autumn resulting into a consolidated ice sheet of large ice floes (Bargagli, 2005; Kaiser et

al., 2005; Gleitz & Thomas, 1992). Large floes accumulate snow on the surface that form features such as rubble ice (Petrich & Eicken, 2016). Ice floes could further thicken and widen through congelation ice growth (Kaiser et al., 2005). Although the ice floes appear to have lost their pancake origin, core samples depict consolidated ice as individual pancakes stacked on top of one another (Petrich & Eicken, 2016). Mechanical forces responsible for the formation of ice floes have been reported to be thermodynamic growth and welding of pancake ice structures in the MIZ (Atlantic sector) (Alberello et al., 2019). However, this study was conducted during anomalous climatic circumstances (storm conditions) that could have increased existing sea-ice thickness through rafting and ridge formation, consequently having a significant impact on sea-ice biological communities (Massom & Stammerjohn, 2010). Cave-like gaps also form in pack ice during these storm conditions providing an essential habitat for krill and overwintering larvae (Massom & Stammerjohn, 2010).

During the early stages of grease ice formation, inoculation of particulate organic matter (POM) from the water below are scavenged and concentrated. This includes sea-ice algae, organic waste, and bacteria (Kaiser et al., 2005; Lizotte, 2001). The sympagic environment provides an effective pore system where increased light due to stabilised ice structure is available for captured biota, while the opposite will be observed when the season progresses (Gleitz & Thomas, 1992). It has also been observed that sea-ice algae communities can reach a biomass three to four times higher than that of the surrounding pelagic waters (Post et al., 2014). As sea-ice grows, dissolved material is initially higher in brine channels than the surrounding seawater. However after a few days, desalination of the sea-ice occurs (Fripiat et al., 2017). Mechanisms controlling desalination of sea-ice are considered to be the migration of brine pockets, brine expulsion, and gravity drainage (Eicken, 2003). Gravity drainage is the most significant regulating process in the desalination process, often occurring after flooding and during cold time periods (Vancoppenolle et al., 2010). The latter is largely associated with the size and interconnectivity of sea-ice pores (Eicken, 2003). If permeability of sea-ice is high or the vertical gradient is unstable (caused by increased density and salinity), gravity drainage or convective overturning is also observed (Meiners & Michel, 2016; Vancoppenolle et al., 2010). This allows for the exchange of cold saline water with warmer less saline water from lower sea-ice layers (Eicken, 2003). Therefore, it is reasonable to assume that the ice structure is permeable and the brine pores coalesce into a brine network when the brine volume of sea-ice is measured above 5 % (Fripiat et al., 2014; Vancoppenolle et al., 2010). However, brine expulsion could also occur during density build-up of brine solution within brine pockets, flowing into sea-ice micro-pore network (Eicken, 2003). The microstructure evolution of sea-ice is therefore a function of temperature (Eicken, 2003). Sea-ice pores elongate and interconnect as temperature increases until the percolation threshold at a given salinity is reached (Eicken, 2003). This plays a fundamental role regulating the transport ability, permeability, and thermal conductivity of sea-ice (Eicken, 2003).

The formation rate of sea-ice also influences the biomass of algae found within its structure since a faster rate of formation (usually observed by frazil ice) can entrap a phytoplankton bloom for instance, present within a localised boundary, creating varying concentration of biomass (may be over a few orders of magnitude) and ice structures within a few kilometres (McMinn et al., 2010a). This is markedly different from the continuous ice sheet formed due to conductive heat loss along the temperature gradient of dense columnar crystalline structure of land fast congelation ice. Congelation ice rejects POM during sea-ice formation (Scott et al., 1994). However, findings from Clarke & Ackley (1984), revealed that congelation ice could have two orders of magnitude higher chlorophyll a concentrations than that of frazil dominated ice.

1.3 Relationship between sea-ice dynamics and sea-ice algae

The relationship between sea-ice dynamics (seasonal melt, extent, and the timing thereof) and phytoplankton biomass within confined regions have shown varying results in a number of studies listed in Robinson & Brink (2006). Contrasting results are likely owed to hypotheses concluded over specific regions, where sea-ice advance is secondary to the hydrodynamic circulation and bathymetric features within the region (Robinson & Brink, 2006). For example, coastal regions are generally described as productive, and as such, productivity may be transported via the circulation of gyres and upwelling events into open waters from the coast of Antarctica (Robinson & Brink, 2006). However, the most productive region surrounding the Antarctic continent is considered south of the SBACC when the coastal current and pack ice is at its widest (Robinson & Brink, 2006). Here, high productivity is owed to enhanced surface water nutrients, melting ice, and reduced wind stress (Robinson & Brink, 2006). Spatial distribution of the high productivity is also latitudinally aligned with the sea-ice extent, however concomitantly influenced by the northward extension of the coastal current found at the west side of nearby gyres (Robinson & Brink, 2006). Consideration should also be given regarding the retention of production in the gyre as well as further transport within the ACC. This process may also be enhanced through the northward extension of the MIZ in non-gyre regions of East Antarctica due to wind stress curl as well sea-ice formation (Robinson & Brink, 2006).

The increased frequency in strong waves associated to the south-ward shift of storm tracks are anticipated having significant influence on both sea-ice and pelagic environments, especially during summer and autumn (Massom & Stammerjohn, 2010). Favourable effects for the SO pelagic and sea-ice ecosystem are highlighted as the increased formation of frazil ice and upwelling related primary production, induced by stronger winds (Massom & Stammerjohn, 2010). Therefore, strong winds (> 40 knots) frequently observed during winter storm conditions within the Weddell Sea and at the advancing ice edge (58 °S, 6 °E) are regarded as an important driver for pack ice dynamics (Ackley & Weeks, 1990). For example, the newly deposited thin snow cover can rapidly shift in hours and cause snow loading on an ice sheet that can reach a thickness of 1 m (Ackley & Weeks, 1990). At this thickness the sea-ice is depressed due to the weight of the snow, causing

surface flooding (Ackley & Weeks, 1990). As a result, sub-zero atmospheric temperatures consolidate the deposited snow-ice layer, while remaining snow may be shifted with strong winds (Ackley & Weeks, 1990). This process is repeated throughout the region (Ackley & Weeks, 1990).

1.4 Relationship between biochemistry of sea-ice and sea-ice algae

Nutrients are readily available in surface waters south of the Polar Front (PF) for phytoplankton and sea-ice algae, due to upwelled Circumpolar deep water (CDW) (Robinson & Brink, 2006). CDW continuously mix in surface water south of the Polar Front (PF) due to brine rejection as the sea-ice cover advances in autumn (Robinson & Brink, 2006). During sea-ice formation solutes or macro nutrients conservatively move with salinity as indicated by theoretical dilution lines resembling surface seawater ratios (Meiners & Michel, 2016). Thereafter, nutrient concentration is anticipated to fluctuate due to boundary processes either enriching or depleting nutrient pools independently from salinity (Meiners & Michel, 2016). C-shaped vertical profiles have often been observed in Antarctic sea-ice for the distribution of inorganic nutrients (phosphate, nitrite, nitrate and silicic acid) (Castrisios et al., 2018; Torstensson et al., 2018). Temperature and salinity profiles of Antarctic pack ice also generally show similar C/S and I/ʕ -shaped profiles (Fripiat et al., 2017). These profile shapes are often a function of dominating physical processes as well as seasons such as sea-ice melt in summer and desalination processes during cold ice growth (Torstensson et al., 2018; Fripiat et al., 2017).

Bulk macro nutrients recognized as dissolved inorganic nitrate, nitrite, ammonium, phosphate and silicic acid are usually low in sea-ice when compared to surrounding seawater (Meiners & Michel, 2016). Low macro nutrient concentrations can either be due to external input or depletion via a prolific biological community (Meiners & Michel, 2016). Low macronutrient concentrations in sea-ice (particularly nitrate and silicic acid) are detrimental for the survival of sea-ice algae communities, especially for diatom cell growth (Meiners & Michel, 2016). Several studies have found no evidence for complex nitrogen cycling in sea-ice, therefore indicative of an inactive microbial community (Meiners & Michel, 2016). However, recent studies have suggested that sea-ice nitrification could account for approximately 70 % of nitrogen assimilation in spring pack ice, during which nitrate is regenerated within the sea-ice as the season progress (Meiners & Michel, 2016). Available nitrogen could then be used for the synthetization of biomass or as an energy supply for cell growth (Fripiat et al., 2017).

The presence of a prolific biological community can further alter the chemistry of the surrounding sympagic environment through increased photosynthesis. Constituents derived from primary production and a high alga standing stocks modify brine channel chemistry through exhausting CO_2 concentrations, creating a excess of dissolved oxygen, and a more alkaline environment (reaching a pH value of 10) (Thomas & Dieckmann, 2002). Other conditions expected are increased ammonium increased dissolved POM, and low

nutrient concentrations (Thomas & Dieckmann, 2002). Decline of organic matter and differences in sequestration ratios of nitrogen, phosphorus , and carbon depends largely on the size, life history, assemblage and abundance of each sea-ice alga community (Lyon & Mock, 2014). Changes in sea-ice biochemistry contributed by high sea-ice algae biomass governs their species community compositions to some extent. These include changes in the sea-ice brine chemistry such as available nutrient, salt concentrations, and pH (Arrigo & Thomas, 2004). For example, the SIPEX-2 study (east Antarctica pack ice) revealed that disparate sea-ice micro habitats utilised different forms of nitrogen. Bottom communities (usually having the highest biomass) showed preference for newly formed nitrate, whereas internal communities showed a higher uptake of ammonium (considered part of the regeneration microbial loop) (Meiners et al., 2016).

Primary production associated with autotrophic species is related to complex processes involved in photosynthesis such as electron transport, harvesting of light, and carbon fixation (Arrigo, 2017). Most significant environmental conditions affecting these processes are salinity, light, and available nutrients (Arrigo, 2017). Environmental conditions such as pH, CO_2 concentration, and the amount of UV radiation are usually not investigated due to their limited effect (Arrigo, 2017). However, increased input of anthropogenic CO_2 in the atmosphere has serious implications for marine ecosystems specifically in polar regions or high latitudes where adverse effects are likely to be experienced more (Torstensson et al., 2015). Colder water in these regions facilitates greater solubility of CO_2, along with the decrease seawater pH (ocean acidification) (Torstensson et al., 2015). Effects of elevated pH are expected in various SO Antarctic zones, such as the Antarctic Peninsula where increased warming of the upper 300 m waters is anticipated (Horner et al., 1992). Seawater pH is projected to decrease from 8.05 to <7.8 with increased seasonal variability by the year 2100 in McMurdo Sound Bay (McMinn et al., 2014). Adverse effects caused by elevated pH could include changes in seawater and ice carbonate speciation as well as Antarctic alga dynamics (primary production, sea-ice algae community structure, and rigidity of sympagic algae cell physiology) (McMinn et al., 2014). To consider effects of increased pCO_2 on ice algae dynamics, long term acclimation should to be studied. However, no study has been undertaken for longer than one month on this aspect (Torstensson et al., 2015). Nonetheless, Horner et al. (1992) found that increased pCO_2 within the sea-ice environment could give preference to larger diatom species subsequently altering species composition in sea-ice. Additionally, experimental studies have shown enhanced (20 %) sea-ice algae growth at the surface sea-ice layer when exposed to increased CO_2 at a constant pH (McMinn et al., 2014). When the pH value decreased (increase H^+) from 8 to <7.2, growth rate decreased by 50 % through changing photosynthetic efficiency (McMinn et al. 2014). Although, it is important to note that studies of cultured algae do not show ecological laws contained by natural communities where more complex species diversity, community structure, functional relationships, and the probability of species occurrence are displayed (Torstensson et al., 2018; Zaoli et al., 2017).

1.5 Relationship between sea-ice and sea-ice algae

Mechanisms responsible for the capturing of sea-ice biota during ice formation are explained by local physical processes such as wave fields concentrating cells through pump action and small scale circulations such as Langmuir cells including suspended biota from pelagic waters (Ugalde, 2015). During sea-ice formation, exchange and accumulation of cells are owed to migration of riffles (velocities measured between 0.3 and 0.11 ms⁻¹) and increased water fluxes at the sea-ice-surface interface (Mock & Thomas, 2005). Additionally, bending of sea-ice cover during strong swells located at the outer edge of the pack ice (including the MIZ) may also have an important role in enhancing cell concentrations through percolation pumping (Eicken, 1992). The outer pack conditions may also favour the incorporation of adhesive cells due to effective retainment in the pore system (Eicken, 1992). Inclusion of adhesive cells is likely to be a function of individual frazil crystal sizes which is usually millimetres to a few centimetres in diameter, thus smaller cells are more likely to be retained (Eicken, 1992). It is also worth noting that raphe containing species such as pennate diatoms and foraminifera might be favoured during frazil ice formation due to cells being "inherently sticky" (Eicken, 1992). An experiment described in Eicken (1992) revealed that ice algae cells released from the sea-ice into the water column aggregated, whereas pelagic species did not.

The biodiversity of sea-ice biota after initial sea-ice formation is likely to resemble that of the water they were recruited inform. Thereafter dominance of species such as psychrophilic bacteria are likely to develop. Species that reside in sea-ice (specifically diatoms) increase after the ice age (Arrigo, 2017). Microhabitats or niches in sea-ice provide algae or sea-ice biota with defined conditions within each layer according to species preference (Horner et al., 1992). Algae communities are categorized according to three main segments: surface, interior, and bottom ice layers (**Figure 1. 2**) (Horner et al., 1992). Microalgae species are usually most abundant in the bottom 20 cm and freeboard layer due to newly stabilized conditions and close proximity to underlying surface water containing essential nutrients for algae growth (Arrigo, 2017; Meiners & Michel, 2017). However, variations relating to abundance of positioned communities are observed over time and sea-ice type, as observed with comparatively different dominant communities in land fast and pack ice, respectively (Bargagli, 2015). For example, interior sea-ice communities are described as an important characteristic of Antarctic pack ice (Arrigo, 2017). Although physicochemical conditions within the brine channels could reach 173 PSU and temperatures below -16 ° C which are great enough to conceal measurable metabolic activity of sea-ice algae (Arrigo, 2017; Bargagli, 2005; Allison et al., 1993)
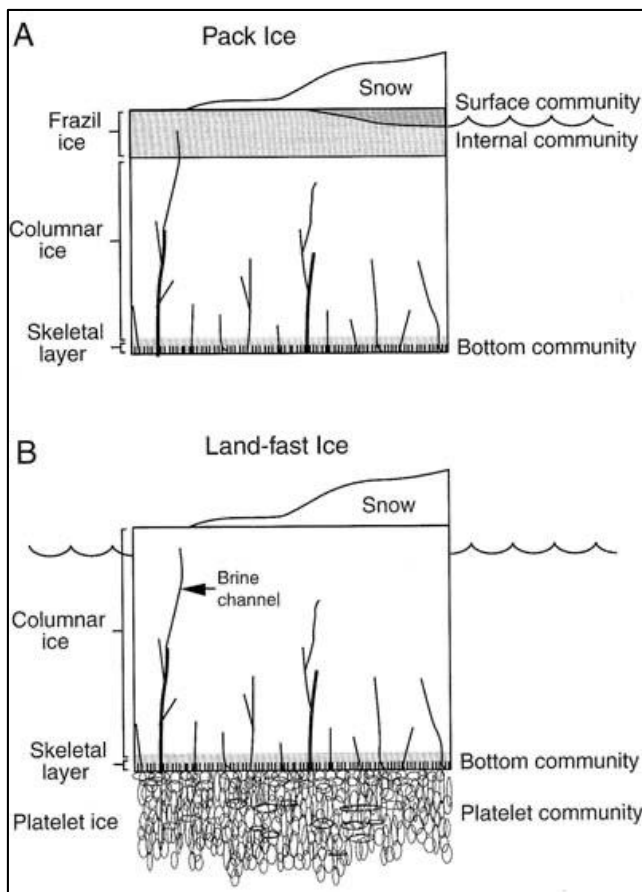
Figure 1. 2 Schematic comparison between pack ice (A) and land fast-ice (B) sea-ice algae communities according to microhabitats (depth layers) (Arrigo & Thomas, 2004).

In pack ice, high sea-ice algae biomass is commonly observed near the ice-surface interface with a general order of one magnitude higher in photosynthetic efficiency when compared to surrounding algae communities (Bargagli, 2015). Abundant surface communities are largely contributed by pressure ridge formation and ice-floe break up (Ackley & Sullivan, 1994). During pressure ridge formation, ice build-up occurs on one of the two ice structures causing a break at the ridge points, consequently flooding one of the ridges, resulting in a down-casting effect (Ackley & Sullivan, 1994). This process is replicated throughout the MIZ where large floes up to kilometres wide break up into smaller floes, creating surface saline pools where high biomass algae blooms proliferate (Massom & Stammerjohn, 2010; Ackley & Sullivan, 1994).

The primary factor limiting growth of pack ice communities particularly surface and interior microhabitats is nutrients, especially the micro nutrient iron (Massom & Stammerjohn, 2010; Arrigo & Thomas, 2004; Arrigo, 2017). However, nutrients are entrained in sufficient concentrations for sea-ice alga growth through various physical processes that affect large regions of the Antarctic pack ice, such as surface flooding and snow loading (Meiners et al., 2017; Massom & Stammerjohn, 2010). These processes are also considered essential for the accumulation of sea-ice algae cells (Eicken, 2003). Surface flooding concentrates sea-ice algae and nutrients in sea-ice through the lateral flow of water from sea-ice cracks, ridges, and brine inclusions (Eicken,

2003), while excessive snow loading and sea-ice rafting can also prompt surface flooding (Bargagli, 2005; Arrigo & Thomas, 2004). However, flooding induced by snow loading can only occur when snow density is approximately one quarter to a half of the sea-ice floe thickness (Ackley & Sullivan, 1994). Snow loading does not only alter the micro structure of sea-ice, but also plays a pivotal role in sea-ice algae growth and primary production (Massom & Stammerjohn, 2010). At first, snow loading may restrict sea-ice algae growth through light restrictions, but as the winter season progresses, it could prevent photo inhibition for shade adapted sea-ice algae species, prevent interior sea-ice melting, and reduce the loss of bottom ice through physical processes such as melting or sublimation (Eicken, 2003). Snow loading can also assist in optical advantages through increased photosynthetically-available radiation (Massom & Stammerjohn, 2010).

Brine channel morphology, particularly surface area, diameter, temperature, and ice texture plays a pertinent role in structuring predator-prey interactions and microorganism abundance (Krembs et al., 2000). For example, Krembs et al., (2000) found that surface area covered by micro-organisms (i.e. algae) range between six and 41 % at -2 °C. As the temperature increased from -2 °C, algae coverage increased due to surface area reduction (Krembs et al., 2000). Narrow brine channels of ≤ 200 µm in diameter could also provide a refuge site for sea-ice microbial communities (Krembs et al., 2000). However, predators such as the Arctic foraminifer possess extreme physiological flexibility enabling them access to microbial communities residing in narrow brine channels (Krembs et al., 2000). Other studies have found evidence of Antarctic copepod such as *Drescheriella glacialis* and *Stephos longipes* consuming a sea-ice diet consisted of sea-ice algae production and sea-ice (Arndt & Swadling, 2006).

1.6 Physiological adaptations of sea-ice algae against challenging sympagic environment

Sea-ice algae in the MIZ of the SO occupy an environment that displays a significant seasonal shift in the advancing and receding of the Antarctic ice sheet of approximately 16 x 10⁶ km² annually, occupying a region of 19 million km² at its maximum extent in September (Robinson & Brink, 2006; Gleitz & Thomas, 1992). This is one of the largest changes in physical properties on earth (Robinson & Brink, 2006). The sympagic environment also presents variable challenges for sea-ice algae such as extreme low temperature (sub-zero values) and intermittent availability of light during seasonal shifts due to increase in day lengths, snow coverage, and ice thickness (Petrou et al., 2010). Despite the presence of strong gradients of salinity, space, light, and temperature frequently observed in the ice, a propensity to adapt to these environmental challenges are required by the cells of the Antarctic microbial ice communities within the brine channels, particularly diatoms (Petrou et al., 2010; Horner et al., 1992). Sympagic environments rapidly transition between ice and water in a matter of days to hours making physiochemical acclimations necessary for sea-ice algae survival (Thomas & Dieckmann, 2002). Changes in brine channel temperature, even with the slightest shift from one to 2 °C have significant implication for both the sea-ice algae community as well as

the surrounding sea-ice microstructure (Mock & Thomas, 2005). In spite of this, in situ culture studies have shown that diatom species can continue photosynthetic activity below -8 °C and 110 PSU (Castrisios et al., 2018). Species of *Chaetoceros* can even grow at temperatures below -16 ° C and salinities of above 196 PSU (Castrisios et al., 2018). Phototrophs in sea-ice ecosystems especially in the Arctic are likely to form cysts or transition into dormant life stages during rapid transitional conditions, conversely to sea-ice phototrophs of the Antarctic that does not usually conform to these strategies except for a few diatom genera and dinoflagellate species (Kennedy et al., 2019; Martin et al., 2012). Instead, metabolic and physiological adaptations are found in Antarctic species (Kennedy et al., 2019). The abundant psychrophilic Antarctic-diatom species, *Fragilariopsis cylindrus* makes use of an ancient metabolic strategy referred to as dark metabolism (Kennedy et al., 2019). This is an active process where cells maintain some degree of functional and structural organization of photosynthetic capacity and apparatus so that when re-illumination occurs, photosynthesis may almost instantaneously commence (Kennedy et al., 2019).

The adhesion of diatoms to various structures such as the sea-ice lattice is an active process based on synthetisation of proteins, glycoprotein (i.e. EPS), and other metabolic components (Cooksey & Wigglesworth-Cooksey, 1995). The initial adhesion response of diatoms to surfaces via secretion of EPS is still unclear. However, EPS macromolecules in the form of ice-binding proteins (IBP) are suggested as an characteristic feature of psychrophilic diatoms species (Janech et al., 2006). IBP serve as a multipurpose protein which function as a bonding agent and cryoprotectant for Antarctic diatoms (Janech et al., 2006). They contribute to the success of diatom species in sea-ice and at freezing temperatures (Janech et al., 2006). IPB counteract the growth of larger ice crystals through a pitting effect, causing deformities in the ice crystalline structures (Janech et al., 2006). Therefore, the extracellular secretion of the antifreeze IBP prevent the recrystallization of sea-ice and subsequent damage to diatom cell membranes (Janech et al., 2006). IPB are not only found in Arctic and Antarctic diatoms but in other organisms occupying cold and icy environments, such as bacteria, fungi, fish, and plants (Janech et al., 2006). IBP function as an ice recrystallization inhibitor for a range of different marine species.

1.7 Ecological role of sea-ice algae

To identify sea-ice algae as a functional group (living as a community, dependant on the sea-ice habitat) rather than circumstantial (temporarily residing as an assemblage), species metabolism and abundance have been compared to the surrounding pelagic community (Lizotte, 2003). Although, to define species metabolism and other biological functions, taxonomy and ecological role is often used to differentiate if species residing within the sea-ice habitat are in fact, a functional group (Lizotte, 2003). Taxonomy of sea-ice biota in the SO is largely dominated by protists (single-celled eukaryotes) specifically diatoms since these communities are considered prolific within the community structure (Lizotte, 2003). Diatoms have been

described in detail in sea-ice in both the Antarctic and Arctic (showing a wide range of species diversity of 200 and 100, respectively), due to their easily preserved cell walls (frustules) comprised of silicon (Lizotte, 2003). Other protists usually observed in the sea-ice are dinoflagellates, amoebas, and ciliates, the latter being the most diverse non-algae group (Lizotte, 2003). Sea-ice biota are further defined as a functional group according to their ecological role which is generally characterised by species feeding strategies (Lizotte, 2003). Sea-ice protist groups are predominantly photoautotrophic, capturing energy from light via the process of photosynthesis (Lizotte, 2003). Therefore, diatoms are generally most abundant, whereas flagellate algal groups (dinoflagellates, prymnesiophytes, and cryptophytes) are more likely to show mixotrophic feeding strategies (phagotrophic, phototrophic, and osmotrophic) (Vonnahme et al., 2020). Different feeding strategies are often advantageous over disparate spatial and temporal scales. For example, mixotrophic nodes are advantageous when inorganic nutrients are limited and light restriction persists (Vonnahme et al., 2020). The latter describes an environment usually observed during winter conditions when heavy snow accumulation persists (Ackley & Weeks, 1990).

Algae species that dominate throughout the annual seasonal cycle in Antarctic waters have likely adapted a flexible life strategy, while undergoing extreme morphological plasticity due to continuous ephemeral conditions (residing between the brine channels in winter sea-ice and in pelagic waters during ice melt in summer) (Ligowski et al., 2012). Pennate species are the most abundant micro algae in sea-ice, comprising up to 90 % of the standing stock of ice flora in austral summer and frequently reaching 300 mg/m³ chlorophyll a in sea-ice (Petrou et al., 2011; Ugalde, 2015). Species of pennate genera *Nitzschia*, *Fragilariopsis,* and *Navicula* are generally observed forming both long chains or large unicellular cells (>20 µm), while blooms of pennate species have been observed in all key habitats of sea-ice (Arrigo, 2017). Centric diatoms are also found in sea-ice assemblages, though common under specified conditions such as the entrainment of *Chaetoceros* blooms in newly formed ice during autumn (Arrigo, 2017). The species of *Chaetoceros* can be found as single cells or as colonies, categorised by distinctive appendages protruding from their rectangular cells. The appendages are called setae or spines (Ligowski et al., 2012). These hollow appendage are a morphological advantage for planktonic species (increase buoyancy, nutrient uptake, and to fend off grazers) (Kooistra et al., 2010), therefore peculiar to find in high abundance and even growing in sea-ice environments (Ligowski et al., 2012). Usually the inclusion of large diatom cells, long chains, and setae-bearing species are excluded, broken, and restricted in sea-ice. However, *Chaetoceros dichaeta* a common species associated to the sympagic Antarctic environment, undergoes morphological adaptation of its setae, spacing between adjoining cells, and the presence of a extended protrusion from each valve centre (rimoportula). Additionally, in *C. dichaeta* cultures, the setae bend both inwards and outwards in a single chain, while all setae are confined inside the valve margin (Ligowski et al., 2012). This has been previously recorded as anomalies in this planktonic species, but has now been suggested as a competitive advantage for species residing within confined spaces such as sea-ice brine channels (Ligowski et al., 2012). It is clearly evident that the external

environment influences the setae orientation in *C. dichaeta* when compared to other diatom species such as *Corethron pennatum*, in which the spines are arranged at 45 ° angles from the cell body (Ligowski et al., 2012).

1.8 Southern Ocean food web

SO sea-ice ecosystems contain a microbial assemblage of algae, protozoans, and bacteria which serves as the primary energy source for Antarctic organisms (Andreoli et al., 2000). Primary production derived from microbial assemblages, specifically ice algae and phytoplankton, conforms with the surrounding sea-ice conditions which ultimately controls both the magnitude and state at which food sources are exported to higher trophic levels (Christensen et al., 2017). Predatory marine organisms graze on protozoans and small crustacea underneath ice floes, facilitating the transfer of carbon derived from phytoplankton and ice algae species (Kohlbach et al., 2018). The Antarctic region further displays strong variability of temporal and spatial distribution of primary production over each annual cycle due to the ephemeral state of the SSIZ, providing the Antarctic food web with a limited available time for biological processes such as grazing and reproduction (Kohlbach et al., 2018). The Antarctic krill *Euphausia superba* is regarded as one of the Antarctic's invaluable primary food sources considering its ecological importance in providing a large magnitude of dietary carbon to fish, marine mammals, and seabirds (Kohlbach et al., 2017). With the majority of predictions based on simulations suggesting the reduction of sea-ice extent and thickness in the following decades over a large area, *E. superba* stocks may be under increased grazing pressure within the SO (Arrigo, 2017; Kohlbach et al., 2017). Since krill spawns during the austral summer (December to March), subsequent larvae are highly dependent on ice algae as their primary food source during winter as they graze under ice floes, feed on cells released due to brine drainage or ice breakup. While the primary prey for crustacean zooplankton is usually considered to be pelagic phytoplankton, when biomass declines to become insufficient to sustain krill larvae during winter (Stewart & Fritsen, 2004), sea-ice algae becomes important for krill in its furcilia larvae stage. This has further been highlighted by higher abundance of larvae underneath ice floes compared to pelagic waters and decreased abundance associated with receding ice in winter (Kohlbach et al., 2017).

During periods of low primary production (as expected during winter or as a result of climate change induced alterations) some Antarctic grazers are able to survive through alternate biochemical, physical, and behavioural advantages (Kolbach et al., 2018). The euphausiid *Thysanoessa macrura* may gain a competitive advantage due to lipid deposits (such as wax esters and triacylglycerols) reserved for winter months ensuring limited dependency on sea-ice algae primary production during winter (Kolbach et al., 2018). Other copepod species will undergo diapause moving into deeper water layers, while the copepod *Calanus propinquus* is considered to be a winter active species (Kolbach et al., 2018). Therefore, severe alteration in the SO food web and carbon flux can be expected if modifications in community structure are observed at low abundant

species (Kolbach et al., 2018). The significance of sea-ice algae's role in the synthetisation and exportation of carbon to the pelagic environment and higher trophic levels has yet to be quantified since large spatial and temporal variability exists (Kohlbach et al., 2018). It is suggested that if the sea-ice extent retreats by 50 %, an 86 % reduction of primary production derived from sea-ice algae will follow (Kohlbach et al., 2018).

1.9 Seeding of phytoplankton blooms

Sea-ice in the SO gives effect to a two-phase ecosystem, depicted through the annual exchange of organisms and material between the pelagic and sympagic environment (Lizotte, 2001; Priddle et al., 1996). This includes the capturing of overwintering resting spores and cells while accommodating a natural progression of sea-ice algae species succession (Lizotte, 2001). Seeding of phytoplankton blooms within the MIZ forms part of this system. The seeding of phytoplankton communities from sea-ice algae could contribute as much as 40 % to the annual primary production south of the Antarctic convergence zone (Kang & Fryxell, 1993). The seeding effect can be described as the inoculation of algae cells in the pelagic waters, thereby influencing the magnitude and species composition of pelagic phytoplankton assemblages (Haecky et al., 1998). In sea-ice covered regions such as the Antarctic, Bering Sea, and the Canadian Arctic, the seeding effect is found to occur through comparable taxonomic assemblages developing prior and following ice break up in the pelagic and sympagic environments (Haecky et al., 1998). However, Haecky et al., (1998) highlighted that sea-ice algae could actually be dead when inoculated into pelagic waters from sea-ice, which inhibits seeding. Additionally, if sinking rate is estimated too high , probability for ice algae cells to remain suspended in the water column is unlikely, and ice algae cell concentrations would be insufficient for seeding (Haecky et al., 1998). However, seasonal blooms are frequently observed within the MIZ surface waters where low density and high nutrient water dominate during spring (Edwards et al., 1998). Initial optimal conditions for bloom development in the MIZ is described by the "Sullivan model" as vertical stabilization of the water column once sea-ice melts or retreats, and wind strength is reduced (Quéguiner et al., 1997). The stabilized water column in conjunction with newly available light stimulates surface phytoplankton blooms (Stroeve et al., 2016). Seasonal blooms provide pelagic and terrestrial marine organisms from higher trophic food levels with food necessary for survival. These include zooplankton, fish, mammals and endemic Antarctic bird species during the breeding season (Stroeve et al., 2016). For example, the snow petrel, *Pagodroma nivea* has been found to increase foraging in the MIZ and ice edge during the breeding season, while breeding success was correlated to increased sea-ice extent in the previous winter (Stroeve et al., 2016). The increase in sea-ice extent in winter is further related to increased krill biomass, providing abundant resources for breeding birds such as *P. nivea* in the following summer (Stroeve et al., 2016).

1.10 References

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Chapter 2

Southern-Indian Ocean sea-ice algae species composition, abundance, and associated physicochemical environment during the austral winter of 2017.

2.1 Introduction

The Southern Ocean (SO) is considered to be an ecoregion with a significant role in global climate regulation (Chapman et al., 2020). The SO sea-ice plays a central role in the global overturning circulation through the generation of Antarctic Bottom Waters, where melting sea-ice forms dense shelf water, and subsequently sinks. The Antarctic Bottom Waters functions as a global heat and is postulated to be a CO2 sink (Ohshima et al., 2013). Furthermore, the SO is responsible for 40 % of atmospheric CO2 uptake, while 10 % is sequestered via the biological pump (Deppeler & Davidson, 2017). SO sea-ice algae forms part of the biological pump via the seeding of sea-ice edge blooms in the MIZ (Yoshida et al., 2020; Szymanski & Gradinger, 2016). These blooms contribute approximately 50% of the total MIZ primary production in the SO (Yoshida et al. 2020). However, it is still largely debated which sympagic or pelagic phytoplankton species are responsible (Yoshida et al., 2020). Apart from the well-known contributions of phytoplankton and sympagic algae to regional biochemical possesses (Nair et al. 2015; Kang et al., 2001), they also serve as a primary energy source for higher trophic food levels such as zooplankton and fish (Kohlbach et al., 2018). The transference of carbon to higher trophic-level pelagic organisms is especially important when pelagic primary production is assumed to be close to zero in winter (Kohlbach et al., 2018).

The Antarctic sea-ice is a heterogenous environment having an ephemeral state of physical properties (Yoshida et al., 2020; McMinn, 2017). Sea-ice algae aggregated within this environment are exposed to extreme fluctuations of light, temperature, nutrients, pH, and gas (CO2 and O2) (McMinn, 2017). These abiotic factors influence sea-ice algae photosynthetic efficiency, production, and physiology. However, the combined effect of the latter is not well described during melt and freezing processes (McMinn, 2017). The MIZ is therefore an ideal environment to identify which sea-ice algae species can withstand abiotic co-stressors in both sea-ice and the pelagic environment, either as phytoplankton cells or resting spores.

SO winter sea-ice dynamics significantly influence air-sea flux processes, which are important for estimating future climate change trends (Tison et al., 2020). However, current SO winter sea-ice biogeochemical and physical properties are under sampled (Tison et al., 2020; Sambrotto et al., 2003) and phytoplankton community response (photo physiology and biomass) to changing winter sea-ice morphology is sparsely documented (Torstensson et al., 2018). Sampling of SO provinces especially during winter is logistical challenging, resulting in a paucity of information that covers successive seasons (Torstensson et al., 2018;

Sambrotto et al., 2003; Brierly & Thomas, 2002). The harsh winter climate renders the SO inaccessible and costly for field sampling. Insufficient data collected over SO provinces results in the erroneous representation of ecological communities and their significance e.g., phytoplankton assemblages (Chapman et al., 2020; Bouman et al., 2012).

Each diverse bioregion surrounding the Antarctic are impacted by discrete environmental stressors associated with climate change (Deppeler & Davidson, 2017). The short and medium-term effect of climate change on the sea-ice extent will likely have considerable influence on trophic food web interactions and release of organic material during seasonal sea-ice melt (Brierly & Thomas, 2002). Similar to the sea-ice extent trends observed in the SO, the Weddell Sea sector showed an increase in sea-ice extent up until 2014 (Parkinson, 2019). Thereafter, a rapid decrease was reported between 2015 and 2018, reaching a near-minimum in 1999 (Parkinson, 2019). The importance to understand ecosystem functioning during this period and location could provide useful information in community response to declining sea-ice extents, particularly if these trends are predicted for the future.

The net-effect of climate induced stressors is predicted to impact phytoplankton community productivity and composition in most bioregions (Deppeler & Davidson, 2017). As a result, it is reasonable to anticipate a change in SO carbon export, biochemistry, and trophic food web structure (Deppeler & Davidson, 2017). The eco-physiological response of phytoplankton to environmental change is taxon specific and includes the ability to adapt (Lacour et al., 2017). It is therefore important for us to understand how climate change will influence the response of phytoplankton taxa in sympagic and pelagic ecosystems. In this study we will be investigating the taxonomic composition and abundance of sea-ice algae and surrounding phytoplankton in the SO MIZ, Southern-Indian Ocean sector at 65°S, 30°E during the austral winter of 2017. The sympagic physicochemical environment (nutrients, temperature, and salinity) were investigated in order to attempt to account for any significant influences on sea-ice algae community structure and abundance.

2.2 Methodology

2.2.1 Study area

Sea-ice samples were collected during the austral winter of July 2017 in the MIZ of the Southern-Indian Ocean sector at $65°$S, $30°$E (**Figure 2. 1**). Two surface water samples (at <3 m depth) were collected near (~5 m) the sea-ice samples. The MIZ edge consisting of 30 % ice cover was reached at 61.7 °S as defined by De Jong et al., (2018). The MIZ was covered with first year ice consisting of pancake ice floes approximately 40 cm in depth. The MIZ was an unconsolidated ice sheet consisting of 50 % pancake floes between the size of 2.3 and 4 m in diameter, with frazil ice filling the interstitial gaps (Vichi et al., 2019). Three individual pancake ice samples were collected using sampling apparatus designed by the Engineering Department at UCT. The ice samples were approximately 1 m in diameter and were collected at night to avoid light exposure. Two of the ice samples were cut into three vertical and three horizontal segments using an electric saw. Each segment consist of three cubes of 10 cm^3. The third pancake was divided into four vertical and four horizontal segments, containing four cubes with 10 cm^3 dimension. Each cube was directly melted in the dark at room temperature and used for chlorophyll a analysis, phytoplankton identification, and nutrient analysis.
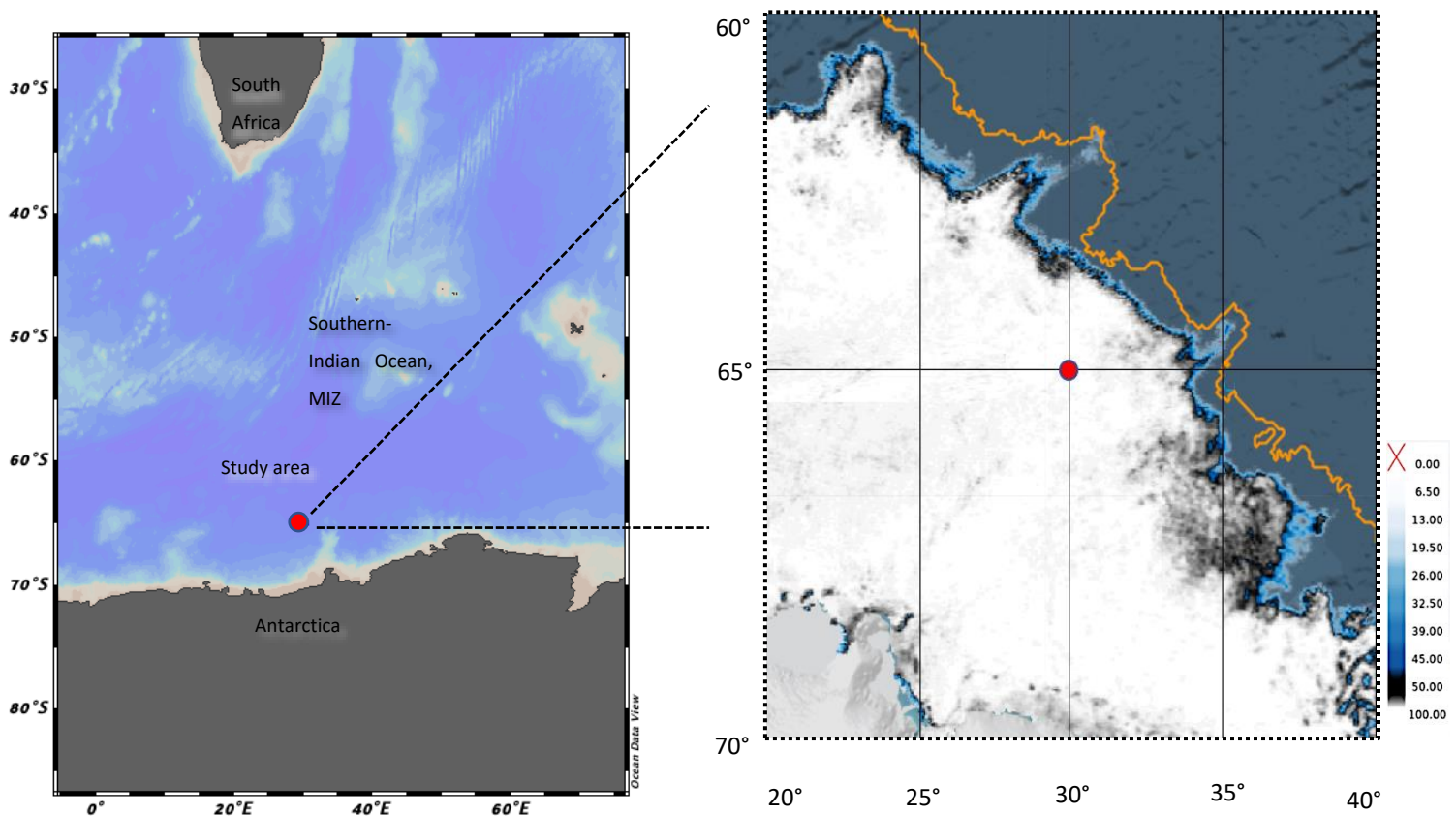
Figure 2. 1: Area of interest, **Left**: Sampling location of sea-ice in the Southern-Atlantic Ocean, marginal ice zone. (red dots). **Right**: Sample locations showing average sea-ice concentration and median sea-ice extent (orange line) for the 3rd of July 2017 in the Southern Ocean (map extracted from meereisportal.de).

2.2.2 Sea-ice algae species composition

Sea-ice samples used for algae identification were directly melted at room temperature in the dark to prevent algae growth and osmotic stress. Melted samples (400 mL) were preserved with 2.5 mL of Lugol's iodine solution (2 % final concentration) and stored in amber bottles at low room temperature (20 °C), away from direct sunlight on board the SA Agulhas II. At the Cape Peninsula University of Technology ecotoxicology laboratory, the sea-ice samples were settled using the Utermöhl method (Utermöhl, 1931). Twenty mL of sample was left to settle onto a coverslip for a period of 24 hours. Phytoplankton species were counted and identified using an inverted light microscope (Olympus CKX41) at a magnification of 200x.

Forty mL of three melted sea-ice samples containing the most diverse species assemblages were analysed using Scanning Electron Microscopy (SEM). Samples were filtered onto a Whatman 0.2 μm Nuclepore Track-Etch Membrane filter and left to dry for ~12 hours in a petri dish. These were transported to the Iziko South African Museum Marine Biology Department for analysis on a Hitachi T4000 Plus Table-top SEM). Images were viewed at a voltage of 5 kV using Backscatter Electrons.

Identification of taxa from light microscopy and SEM was carried out using Scott & Marchant, (2005). A total of 48 sea-ice algae taxa were identified, but due to limitation in microscope resolution, some could not be reliably discriminated to species level. Therefore, the following algae groups were characterised to genus level; *Fragilariopsis* spp., *Pseudo-Nitzschia* spp., and *Nitzschia* spp..

2.2.3 Chlorophyll a analysis

Sea-ice and surface seawater samples used for chlorophyll a analysis were analysed on board the SA Agulhas II. Samples of melted sea-ice and collected surface seawater (of approximately 100 and 500 mL respectively) was immediately filtered onto a single Whatman 0.3 μm and 2.7 μm filters, respectively. Filters were extracted using 8 mL of 90% acetone, and left for a period of 24 hours at -20°C. The extracted sample was poured into a cuvette before placing it in a Turner Designs Trilogy fluorometer, measuring raw chlorophyll a concentration through the non-acidifying method (Welschmeyer, 1994). The fluorometer was calibrated with a Sigma-ldrich® chlorophyll a analytical standard (*Anacystis nidulans*) (Weir et al., 2020). Size fractionated chlorophyll a were utilised to classify sea-ice algae cells according to cell sizes termed pico-plankton and large algae cells. Large algae cells were classified as the cell fraction greater than 2.7 μm. Pico-plankton were calculated by subtracting the chlorophyll of the large algae cells (>2.7 μm, i.e. micro and nano-plankton) from the total chlorophyll (>0.3 μm).

2.2.5 Standard spectrophotometric nutrient analysis

Standard spectrophotometric analysis was done to determine the nutrient concentrations of each segment of pancake ice and ice core (Grasshoff et al., 1983). Approximately 50 mL of each sample was used for nitrate, nitrite, silicate, and phosphate analysis (Weir et al., 2020). Additionally, 50 mL of 16 sea-ice samples were collected for ammonium analysis. All samples collected for nutrient analysis were filtered immediately after melting using a 0.2 µm syringe filter and frozen at -20°C. Samples were analysed at the Marine Biogeochemistry Laboratory at the University of Cape Town. A Latchet Quick-Chem Flow Injection Analyzer was used to determine nitrate, nitrite, and silicate concentrations under methods 31-107-04-1-C (minimum detection limit = 0.12 µmol NO_3^-/L) and 31-144-27-2-A (minimum detection limit = 0.1 µmol SiO_4^{4-}/L). Nitrite and phosphate were determined using the colorimetric methods described by Grasshoff et al. (1983). Accuracy of nitrate, nitrite, silicate, and phosphate concentration deviated at approximately ± 0.18 µM, ± 0.04 µM, ± 0.08 µM, and ± 0.11 µM, respectively. The minimum detection limit was 0.1 µM, 0.2 µM, 0.05 µM, and 0.05 µM, respectively. All references to total nitrogen as N were calculated by adding NO_3^-+NO_2^-. Note that NH_4 concentration were not added to total nitrogen (N) due to lower number of sample collected. To account for the effects of fluid transport in sea-ice i.e. nutrient concentrations at seawater salinity, salinity-normalised nutrient concentrations were calculated according to methods outlined by Fripiat et al. 2017. Ammonium concentrations were measured according to the fluorometric method of Holmes et al. (1999) using a Turner Designs Trilogy fluorometer equipped with a UV module. Precision was ± 0.05 µM and the detection limit was <0.05 µM. The matrix effect (ME) was calculated using the standard addition method (Saxberg and Kowalski 1979) and an ME correction, typically ≤5%, was applied to all samples (Taylor et al. 2007).

2.2.7 Salinity and Temperature analysis

Temperature was recorded immediately after sea-ice collection by inserting an electrical thermometer into the centre of each individual pancake ice cube. After each individual sea-ice cube was melted , an Autosal salinometer was used to measure salinity according to standard procedures listed in Guildline Instruments (1981) and UNESCO (1978). Brine volume was computed using temperature and salinity measurements according to methods outlined in Cox & Weeks (1983).

2.2.8 Statistical analysis

Species community data were analysed using univariate and multivariate statistical analysis. Multivariate analysis was done using the PRIMER v6 statistical package together with the additional package, PERMA-NOVA Plus (Anderson et al., 2008; Clarke & Gorley, 2006). Data were fourth root transformed and converted

to a similarity matrix using the Bray-Curtis similarity coefficient. A one way permutational multivariate analysis of variance (PERMANOVA) was used to test the homogeneity of variance ($p<0.05$) of the sea-ice algae (including all protist functional groups) community structure, and physicochemical (nutrients) parameters according to sea-ice depth layers, discrete pancake samples, and depth integrated samples. The similar percentage routine (SIMPER) using Bray-Curtis distance was used to identify species contributing the most towards (dis)similarity in community composition across depth layers (shown as species contribution and average abundance). Standard diversity indices were calculated: the Shannon Wiener diversity index H'= Σ¡ p¡(logp¡) (1) by sea-ice depth layer (Hayek and Buzas 2013); and species evenness (Pielou's J) was determined using H' /H' max = H'logS (2) where H' max is the maximum possible value of Shannon diversity (log S) (Clarke & Warwick, 2001). Species richness (S) was determined using Margalef d = (S -l)/logN (3), where N is number of individual cells (Clarke & Warwick, 2001). A hierarchical clustering analysis and non-metric multidimensional scale plot (MDS) were used to represent a proximity matrix as a configuration plot in order to indicate the similarity of species community assemblages relative to their position (Clarke & Warwick, 2001). MDS plots illustrate "natural algae groupings" according to sea-ice depth layer and individual samples. Similarly, a principal component analysis (PCA) was used to emphasize variation and distribution of nutrients using Euclidean distance. A Distance Based Linear Model (DistelM) was used to explain the relationship between biological variation and nutrients (nitrate, nitrite, phosphate, and silicate) using the Permanova plus package. The DistelM partitioned the variation of biological data according to a multiple regression model (based on parameters; depth-layer and sample) using the R^2 function as a stepwise procedure.

The IBM SPSS 26 statistics package was used to derive relationships between sea-ice algae community abundance and physicochemical sympagic variables. To test homogeneity of variances between sea-ice samples, a multivariate analysis by combined dependent variables was used. No significant difference between variables was identified considering Pillai's trace p-value. The Shapiro Wilkinson normality test was used to verify the equality of variances of sea-ice algae abundance in sea-ice samples. A Spearman's rank correlation coefficient was computed to determine the strength of the monotonic relationship between environmental variables and sea-ice protist functional groups. Significance levels for all statistical tests were taken to be at $p<0.05$.

2.3 Results

2.3.1 Physicochemical environment

All three pancake samples showed a brine volume above 5 % at each sample depth (**Figure 2. 2**). Salinity was low across all sea-ice pancake samples, ranging between 2 and 6 PSU, showing no distinct trend with depth (isohaline profile) (**Figure 2. 3**). Sea-ice temperature was recorded between -1 and -2 °C, also showing an isothermal profile (**Figure 2. 4**). Average nutrient concentrations: nitrate (NO_3^-), silicate ($Si(OH)_4$), nitrite (NO_2), ammonium (NH_4^+) and phosphate (PO_4^{3-}) were below 42 μM across all sea ice samples (**Figure 2. 5** and **Figure 2. 6**). Nitrate and silicate were considerably higher (between 29 and 42 μM, **Figure 2. 5**) compared to phosphate, nitrite, and ammonium concentration, which only reached a maximum concentration close to 8 μM (**Figure 2. 6**). Nitrogen (N: nitrate + nitrite) and silicate (Si) were significantly correlated ($p < 0.05$, $R^2 = 0.7$), and mostly remained constant at N:Si molar ratio of 1:1 throughout all sea-ice samples (**Figure 2. 7**). Nitrogen (N: nitrate + nitrite): Phosphate (N:P) ratios were highly variable ranging between 0 and 40 with an average of 28:1, with the exception of two samples reaching much higher ratios above 120 (**Figure 2. 8**). No correlation ($R^2 = 0.005$) was observed between N and P concentrations (**Figure 2. 8**). Surrounding surface water nitrogen compounds (ammonium ~ 0.7 μM, nitrate ~ 26.91 μM, and nitrite ~ 0.23 μM) were notably lower when compared to sea-ice concentrations (**Figure 2. 5** and **Figure 2. 6**). In particularly, nitrate concentrations were approximately 5 μM higher in almost all sea-ice depth layers with the exception of pancake 3s' top sea-ice layer (**Figure 2. 5**). Conversely, silicate concentrations were higher in surface water (46.72 μM), when compared to sea ice concentrations, and phosphate concentration were similar between surrounding surface water and sea-ice concentrations (**Figure 2. 5** and **Figure 2. 6**).

Salinity and nutrients were generally not found to be significantly correlated, between different sea-ice pancake samples or at different depths. Ammonium, however, increased significantly with decreasing salinity in pancake 2. According to the two-way PERMANOVA procedure, nutrient concentrations were grouped significantly differently ($p(perm) < 0.001$) according to individual pancake samples, but not depth. The Principal Component Analysis (PCA) for nutrients further motivated this grouping (**Figure 2. 9**). Pancake 1 and 3 total variation was largely attributed to above-average concentrations of nitrate and silicate (PC1, 48.8 %) compared to pancake 2 (**Figure 2. 9**). Phosphate made a large contribution to the ordination distribution of pancake 2, however marginal since the PC 2 only explains 26.4 % of the ordination.

2.3.2 Sea-ice algae biomass

Average total biomass (shown as total chlorophyll >0.3 μm) of sea-ice algae was high, peaking in the interior of all sea-ice samples (**Figure 2. 10**). Large-sea-ice algae (>2.7 μm) dominated over pico-sea-ice algae for all depth layers in the pancake sea-ice samples. Sea-ice algae biomass was found to be significantly ($p < 0.05$)

higher than that of the surrounding surface water (<5 m) with an average of 2.3 µg/L. Significant differences (p<0.05) between individual pancake samples and between top and interior depth layers were also found. All size fractions of chlorophyll a concentration was significantly higher in the interior layers, but these were lowest in pancake sample 2.
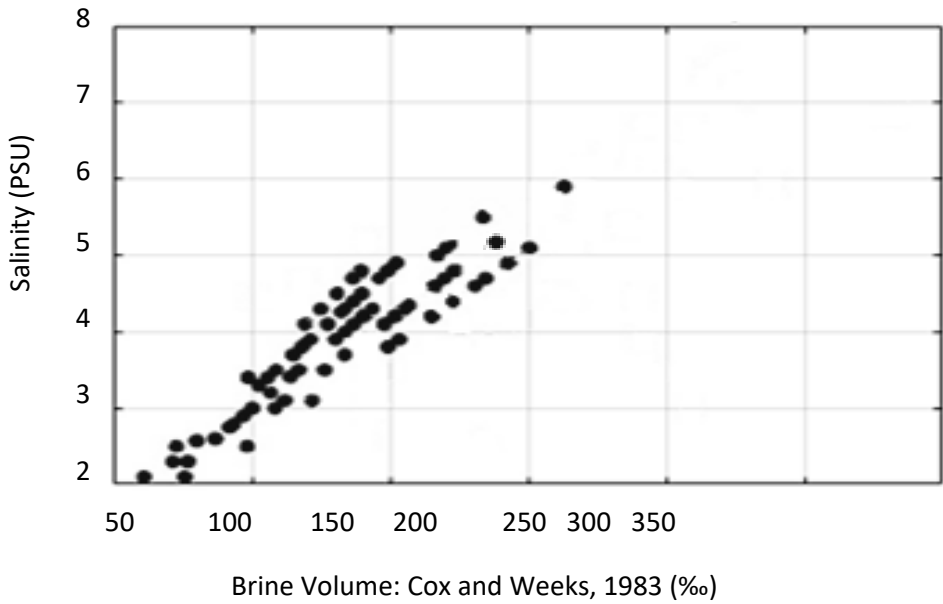
Figure 2. 2 Brine volume and salinity of pancake sea-ice samples.
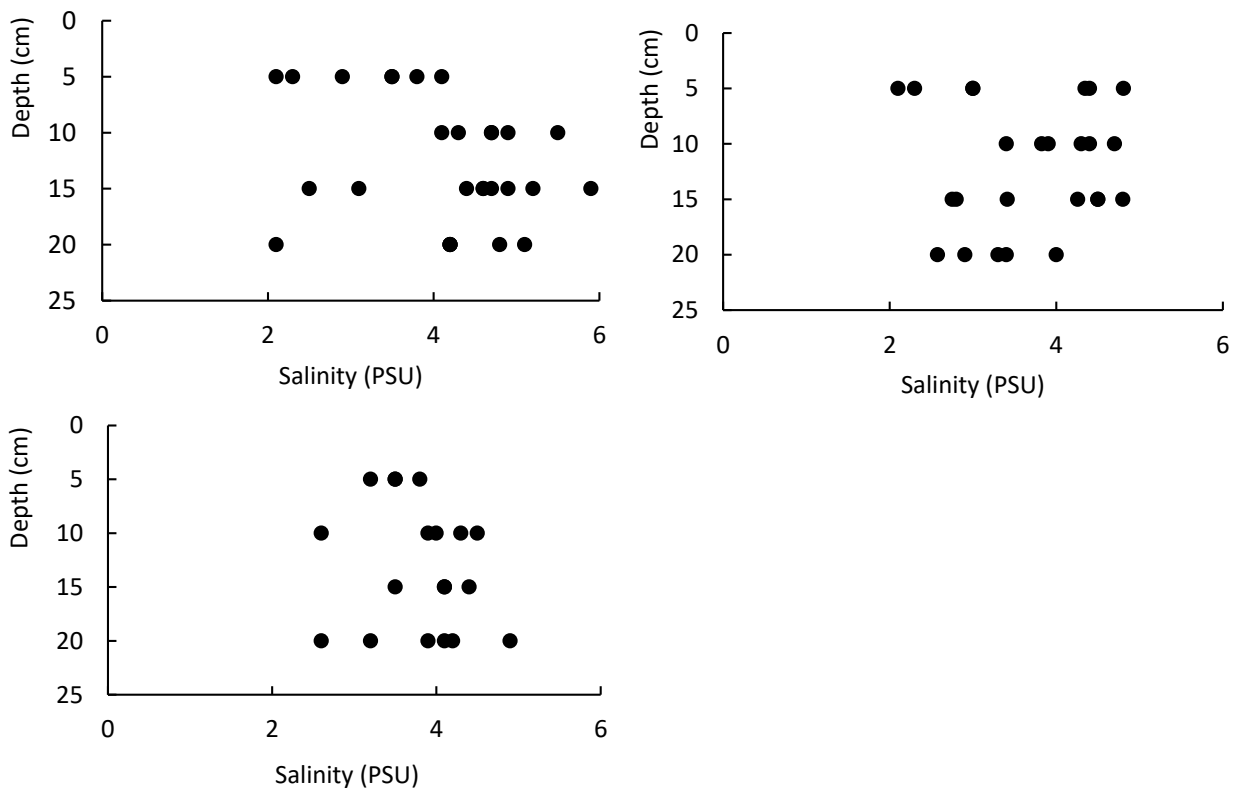
Figure 2. 3 Vertical salinity profiles (PSU) of pancake sea-ice samples.
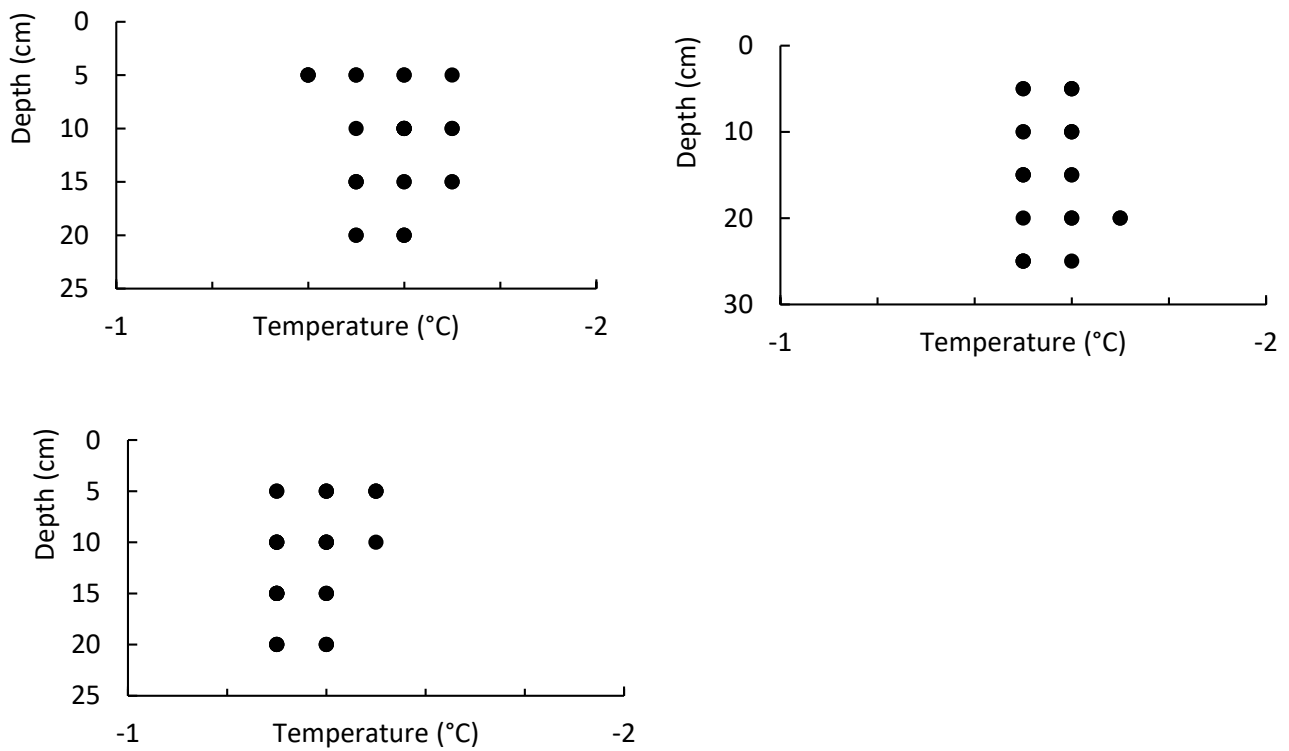
Figure 2. 4 Vertical temperature profiles (°C) of pancake sea-ice samples.
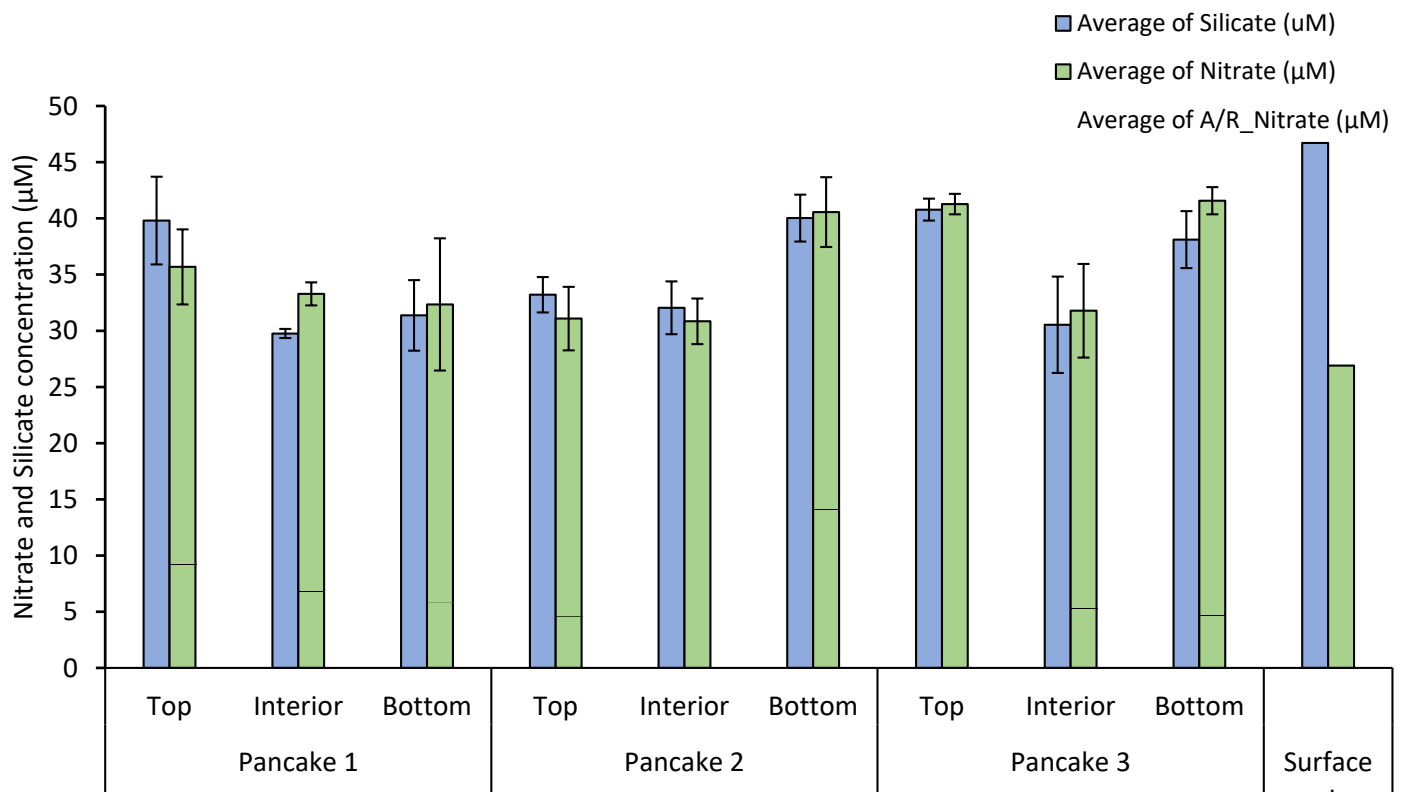
Figure 2. 5: Average nutrient (nitrate and silicate) concentration (µM) of pancake sea-ice. Vertical bars denote ± Standard Error (SE) of the mean.
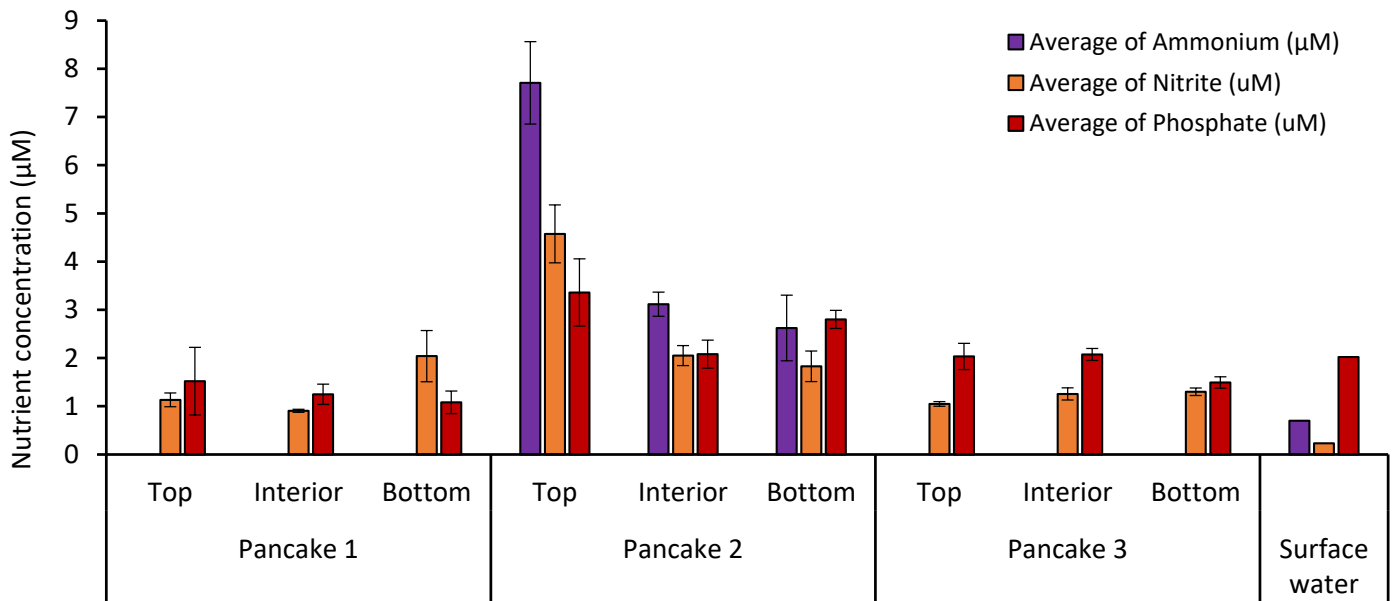
Figure 2. 6: Average nutrient (nitrite, ammonium, and phosphate) concentration (μM) across pancake sea-ice samples according to depth layers. Vertical bars denote ± SE of the mean.
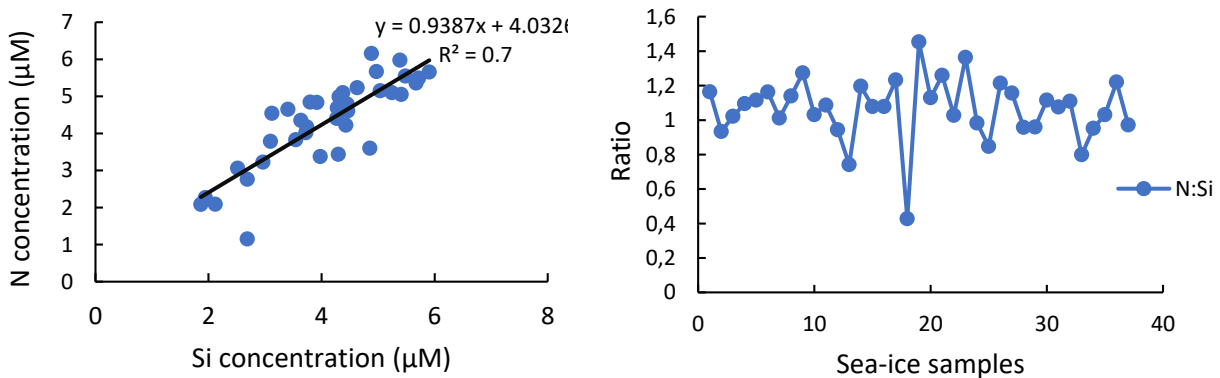
Figure 2. 7: Nitrogen (N) and Silicate (Si) (μM/L) (a) molar ratio and (b) relationship ratio across pancake sea-ice samples.
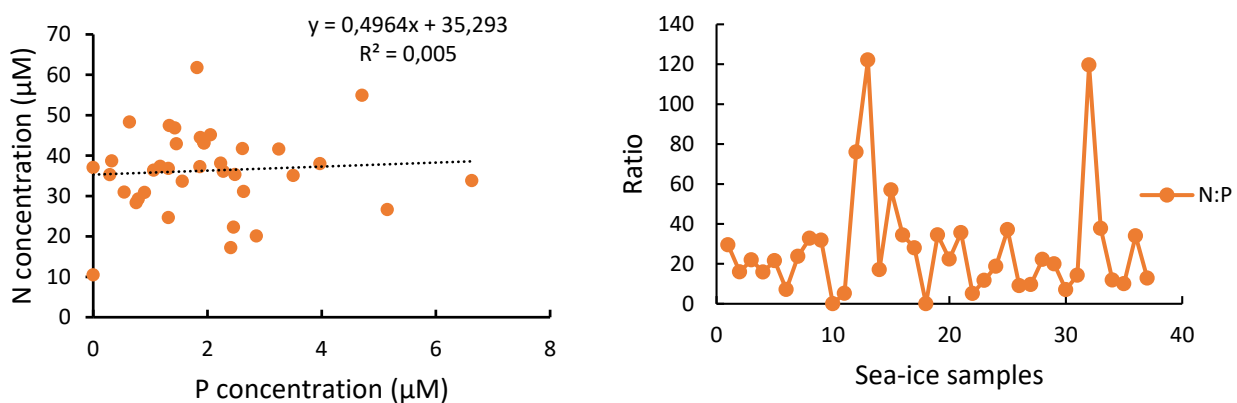
Figure 2. 8: Nitrogen (N) and phosphate (P) (μM/L) (a) molar ratio and (b) relationship ratio across pancake sea-ice samples.

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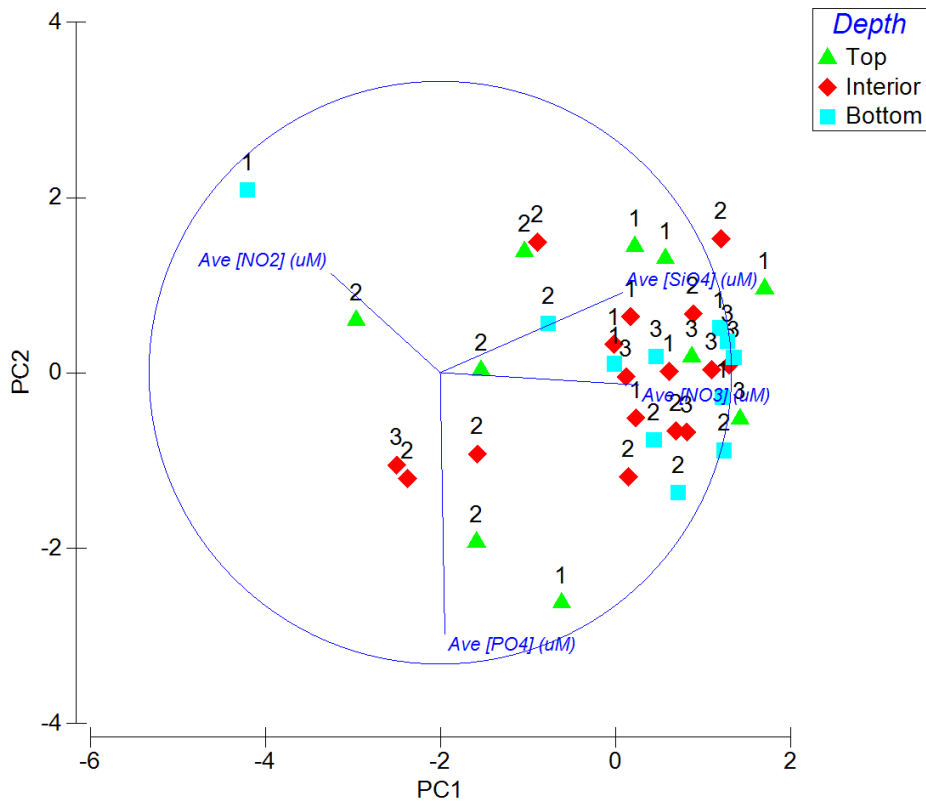
Figure 2. 9: PCA of nutrient concentrations in pancake sea-ice samples. Number indicates pancake sample (1, 2 or 3).
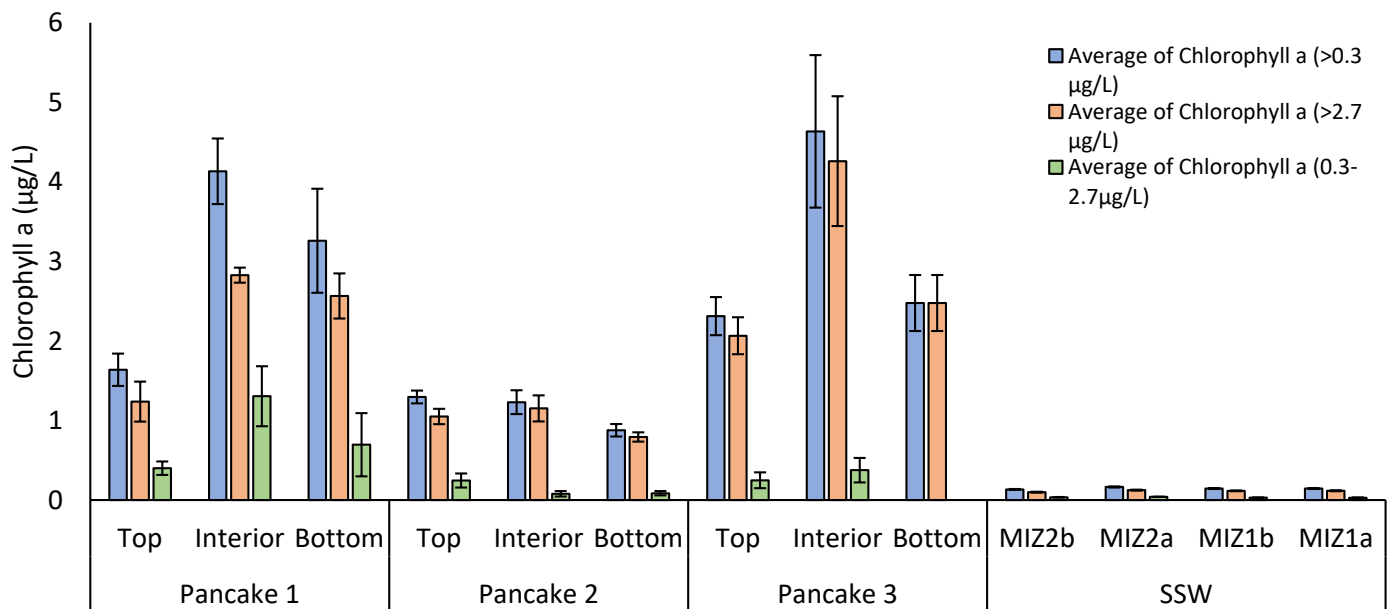
Figure 2. 10: Average chlorophyll a (μg/L) of pancake sea-ice samples according to depth layer and in surface seawaters (SSW) associated with the pancake ice. Vertical bars denote ± SE of the mean.

2.3.3 Biological community structure

Sea-ice algae cell abundance was significantly (p<0.01) higher in sea-ice (6076—14730 cells/mL), compared to surrounding surface seawater (23 - 473 cells/mL) (

Table 2. 1). Sea-ice algae were generally concentrated in the interior. Diatoms represented the dominant functional group in sea-ice and the surrounding surface water (

Figure 2. 11). Pennate diatoms dominated all layers, often exceeding concentrations of 600 cells/mL. Centric diatom concentrations were much lower than that of pennate diatoms, however still considerably higher compared to other primary protist groups, exceeding 26 cells/mL (**Figure 2. 12**) in all sea-ice depth layers. Sea-ice centric diatoms cell concentration was markedly lower when compared to surrounding surface water (

Figure 2. 11). Conversely, sea-ice pennate diatoms cell concentration was notably higher when compared to surrounding surface water (

Figure 2. 11). Protist groups; ciliates, silicoflagellates, and coccolithophores showed higher average abundance than dinoflagellates (**Figure 2. 12**). The protist community (excluding diatoms) showed no distinct concentration pattern according to depth layer (**Figure 2. 12**). However, coccolithophores were generally much higher than other protist groups, and mostly concentrated within the bottom layers. Conversely, in surrounding surface water, the dinoflagellate cell concentration was much higher compared to other sea-ice protist groups (ciliates, silicoflagellate, coccolithophores), the latter either being absent or very low (2 cells/mL) (

Table 2. 1 and **Figure 2. 12**).

The sea-ice protist community (

Table 2. 1) showed a clear dominance of the diatom genus Fragilariopsis, which gave the highest average abundance across all depth layers (**Table 2. 2**). Additionally, it was responsible for most similarity between community structure in the top (20 %), interior (22 %) and bottom (25 %) layers. Other dominant pennate diatoms found across all depth layers were identified as Pseudo-nitzschia spp. and Proboscia spp. Dominant centric diatom species identified were Chaetoceros spp., Chaetoceros dichaeta, and Coscinodiscus spp. mostly showing high species contribution across all depth layers. Interestingly, markedly lower abundance of Dactyliosolen antarcticus was observed in the top layer, whereas Corethron spp. contributed above 90 % to (dis)similarity in this layer opposed to interior and bottom layers (**Table 2. 2**). However, low average dissimilarity (37 %) between depths were found indicating that the species responsible for dissimilarity in each respective depth layer were largely similar.

No clear pattern was observed for diversity indices (species richness and diversity) in sea-ice samples (**Table 2. 3**). However, species were mostly unevenly distributed (Pielou J<0.4) throughout all depth layers, with the

exception of pancake 3' top layer (Pielou J<0.66). Therefore, it can be suggested that dominant species were present in each respective sea-ice depth layer. Surrounding surface water diversity indices differed considerably from the sea-ice samples. However, due to low replicate samples of surrounding surface water no conclusive result can be drawn from **Table 2. 3**. Nevertheless, it should be noted that the number of species found in the water was within a similar range to that of the sea-ice samples (10-23) (**Table 2. 3**).

Significant differences ($p < 0.001$) between discrete samples, depths, and depth integrated samples were observed in species community structure using PERMANOVA. Conversely, no significant difference between species abundance was found using MANOVA (Pillai's trace p-value). The MDS analysis showed distinct groupings between community structure according to depth and pancake sea-ice sample (**Figure 2. 13**). Distinctive groups were formed where top zones are generally closely correlated, while interior and bottom layers are mostly grouped together. Groupings are in agreement with significant differences among depth layers considering that no significant difference was found between the bottom and interior layer of pancake samples. Note that the surface seawater sample was markedly different according to the MDS at one of the sampling locations (MIZ 2), however similar at another location (MIZ 1) (**Figure 2. 13**).
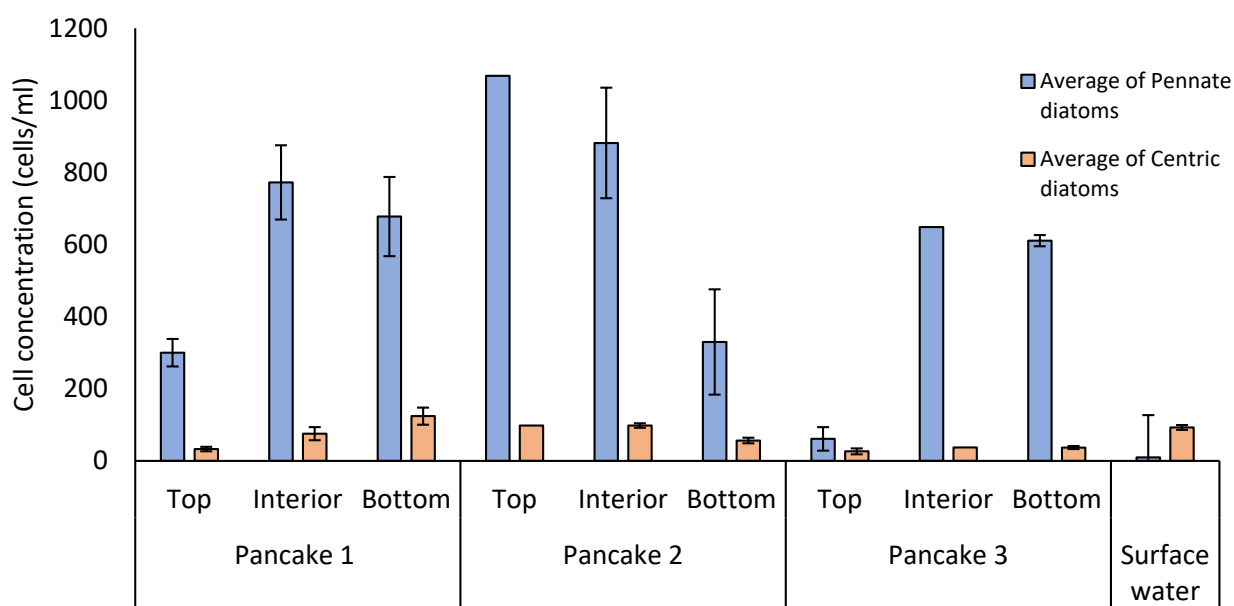
Figure 2. 11: Average diatom cell concentration (cell/mL) across pancake sea-ice samples according to depth. Vertical bars denote ± SE of the mean.
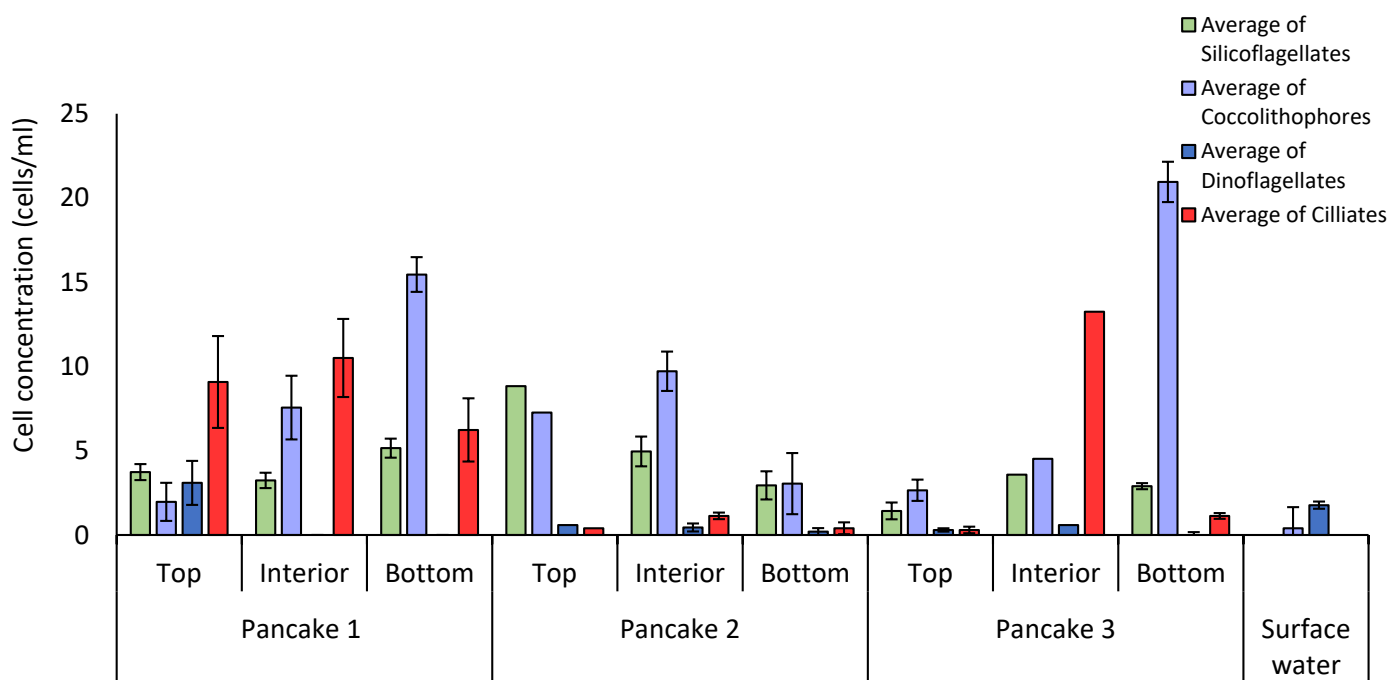
Figure 2. 12: Average cell concentration (cells/mL) of protist groups in pancake sea-ice samples. Vertical bars denote ± SE of the mean.

Table 2. 1: Protists recorded in pancake sea-ice and surface water samples (MIZ 1 & MIZ 2). Total abundance (cells/ml) is listed of taxa grouped according to phylum.

Protist community classification	Pancake 1	Pancake 2	Pancake 3	MIZ 1	MIZ 2
Actinopoda	*0*	*1*	*0*	*0*	*0*
Phaeodaria	*0*	*1*	*0*	*0*	*0*
Phaeogromia spp.	*0*	*1*	*0*	*0*	*0*
Challengeridae	*0*	*1*	*0*	*0*	*0*
Protocystis bicornuta	*0*	*1*	*0*	*0*	*0*
Ochrophyta	*8329*	*14730*	*6076*	*23*	*473*
Bacillariophyceae	*8329*	*14730*	*6076*	*23*	*473*
Biddulphiales	*1263*	*1808*	*669*	*6*	*171*
Asterolampraceae	*12*	*14*	*2*	*0*	*0*
Asteromphalus hookeri	*0*	*0*	*1*	*0*	*0*
Asteromphalus hyalinus	*0*	*8*	*1*	*0*	*0*

Asteromphalus spp.	5	2	1	0	0
Eucampia antarcticus	8	4	0	0	0
Chaetoceracea	**863**	**1219**	**281**	**3**	**39**
Chaetoceros atlanticus bulbous	8	19	9	0	0
Chaetoceros convolutus	0	22	0	0	0
Chaetoceros criophilus	38	100	4	0	10
Chaetoceros dichaeta	157	161	102	0	0
Chaetoceros spp.	659	918	165	3	29
Coscinodiscaceae	**143**	**136**	**125**	**0**	**10**
Coscinodiscus spp. & *Thalassiosira* spp.	143	136	125	0	10
Heliopeltaceae	**2**	**0**	**0**	**0**	**0**
Actinoptychus senarius	2	0	0	0	0
Hemidiscaceae	**0**	**2**	**0**	**0**	**0**
Actinocyclus spp.	0	2	0	0	0
Leptocylindraceae	**31**	**30**	**14**	**0**	**0**
Corethron spp.	8	21	7	0	0
Leptocylindrus danicus	23	9	7	0	0
Rhizosoleniacceae	**145**	**223**	**124**	**1**	**10**
Dactyliosolen antarcticus	96	126	54	1	6
Guinardia spp.	8	20	9	0	0
Proboscia spp.	37	75	59	0	4
Rhizosolenia spp.	4	3	2	0	0
Unknown centrics	**67**	**184**	**122**	**1**	**112**
Bacillariales	*7052*	*12892*	*5386*	*17*	*300*
Bacillariaceae	**6859**	**12615**	**5119**	**17**	**298**
Cylindrotheca closterium	18	36	2	1	8
Fragilariopsis kerguelensis	52	246	103	0	6
Fragilariopsis spp. (excluding *F. kerguelensis*)	6656	12016	4892	15	277
Nitzschia spp.	0	12	0	0	0
Pseudo-nitzschia delicatissima	38	4	72	0	0
Pseudo-nitzschia spp.	95	302	49	1	8
Fragilariaceae	**1**	**23**	**0**	**0**	**0**
Thalassiothrix antarctica	1	23	0	0	0
Naviculacceae	**28**	**75**	**50**	**0**	**2**
Membraneis challengeri	0	2	0	0	0
Navicula glaciei	2	0	7	0	0
Navicula spp.	26	42	38	0	2
Pleurosigma spp.	1	0	5	0	0
Unknown species 1	0	31	0	0	0
Unknown pennates	**164**	**179**	**216**	**0**	**0**
unknown diatoms	14	29	22	0	2
Ciliophora	**103**	**12**	**59**	**1**	**0**
Spirotrichea	**0**	**2**	**0**	**0**	**0**
Tintinnida	0	2	0	0	0

Xystonellidae	*0*	*2*	*0*	*0*	*0*
Cymatocylis spp.	*0*	*2*	*0*	*0*	*0*
Unknown ciliates	**103**	**11**	**59**	**1**	**0**
Haptophyta	**100**	**119**	**112**	**0**	**0**
Haptophyceae	*100*	*119*	*112*	*0*	*0*
Coccolithophores	*100*	*119*	*112*	*0*	*0*
Unknown coccolithophores	**100**	**119**	**112**	**0**	**0**
Ochrophyta	**48**	**87**	**32**	**1**	**0**
Dictyochophyceae	*48*	*87*	*32*	*1*	*0*
Dictyochales	*48*	*87*	*32*	*1*	*0*
Dictyochaceae	**48**	**87**	**32**	**1**	**0**
Dictyocha speculum	*47*	*84*	*32*	*1*	*0*
Unknown silicoflagellates	*2*	*3*	*0*	*0*	*0*
Pyrrophyta	**12**	**7**	**4**	**4**	**0**
Dinophyceae	*12*	*7*	*4*	*4*	*0*
Dinophysales	*11*	*7*	*4*	*0*	*0*
Amphisoleniaceae	**1**	**0**	**0**	**0**	**0**
Dinophysis antarctica	*1*	*0*	*0*	*0*	*0*
Gymnodiniales	*1*	*0*	*0*	*0*	*0*
Gymnodiniaceae	**1**	**0**	**0**	**0**	**0**
Gymnodinium spp.	*1*	*0*	*0*	*0*	*0*
Peridinales	*1*	*0*	*0*	*0*	*0*
Protoperidiniaceae	**1**	**0**	**0**	**0**	**0**
Protoperidinium spp.	*1*	*0*	*0*	*0*	*0*
Unknown dinoflagellates	**10**	**7**	**4**	**4**	**0**
Sarcomastigophora	**2**	**2**	**0**	**0**	**0**
Granuloreticulosea	*2*	*2*	*0*	*0*	*0*
Foraminifera	*2*	*2*	*0*	*0*	*0*
Unknown foraminifers	**2**	**2**	**0**	**0**	**0**

Table 2. 2: Similarity (% calculated using SIMPER) of sea-ice algae species which contribute the most towards similarity across depth layers. Average dissimilarity across all groups is 36.6 %.

	Top		Interior		Bottom	
	Av.Abund (cells/ml)	Contrib (%)	Av.Abund (cells/ml)	Contrib (%)	Av.Abund (cells/ml)	Contrib (%)
Fragilariopsis spp.	3.99	20.46	5.09	22.5	4.63	25.78
Unknown small pennates	1.67	10.06	1.83	7.54	1.71	7.14
Proboscia spp.	1.18	6.22	1.45	6.2	1.08	3.75

Species						
Pseudo-nitzschia spp.	1.29	4.04	1.67	6.36	1.71	10.27
Fragilariopsis pseudonana	1.17	3.52	1.74	6.72	0.89*	1.49*
Corethron spp.	0.79	3.42	0.68*	1.66*	0.35*	0.39*
Chaetoceros spp.	2.01	9.76	2.57	10.78	2.36	12.1
Coscinodiscus spp. &. *Thalassiosira* spp. & *Porosira* spp. (discoid centrics)	1.64	9.74	1.73	7.8	1.78	9.53
Unknown small centrics	1.38	8.17	1.43	5.26	0.87	2.49
Chaetoceros dichaeta	1.35	6.67	1.35	4.59	1.72	6.71
Dactyliosolen antarcticus	1.11	3.79	1.6	6.81	1.56	8.4

Species below 90% contribution shown as *

Table 2. 3: Average diversity indices across pancake sea-ice samples and surrounding surface water.

Pancake	Depth	Diversity indices			
		No. of species	Abundance (cells/ml)	Margalef d	Pielou J'
1	Top	16.0	360.8	2.5	0.34
	Interior	16.8	886.5	2.3	0.27
	Bottom	15.5	838.0	2.2	0.38
2	Top	22.8	1223.5	3.1	0.32
	Interior	17.3	1027.8	2.4	0.32
	Bottom	14.3	405.8	2.3	0.38
3	Top	10.8	96.5	2.2	0.66
	Interior	16.5	732.0	2.4	0.30
	Bottom	15.5	691.5	2.2	0.29
	MIZ1 (water)	16.0	30.0	4.4	0.66
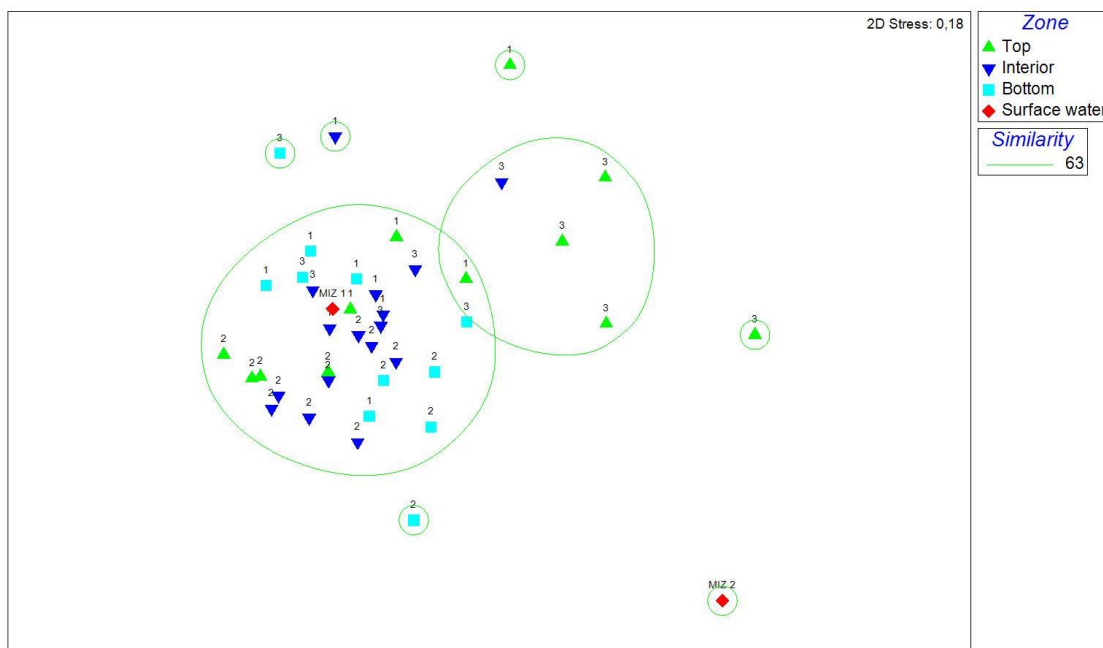
Figure 2. 13: MDS of sea-ice and surrounding surface water protist community structure, showing 63 % similarity clusters. Data was fourth root transformed, using the Bray Curtis similarity resemblance matrix's'.

2.3.4 Relationship between biochemical sympagic environment and algae community structure

Several significant relationships ($p<0.05$) were identified between nutrients and sea-ice algae biomass (chlorophyll a) when pancake ice floes were tested irrespective of sea-ice depth layers. Overall, pico sea-ice algae (0.3-2.7 μm) showed a significant ($p<0.05$) negative correlation with nitrate, whereas large sea-ice algae (2.7 μm) showed a significant ($p<0.05$) negative correlation with phosphate. When sea-ice depth layers were individually tested, additional significant relationships between nutrients and chlorophyll a was observed (**Table 2. 4**). A significant relationship within the top layer of sea-ice samples were found between silicate and large sea-ice algae cell. The interior layer showed a significant ($p<0.05$) negative correlation between nutrients (nitrate and silicate concentrations) and pico-sea-ice algae biomass, as well as nitrite and total chlorophyll a concentration. The bottom layer also showed a positive correlation between pico-sea-ice algae ($p<0.05$) and nitrite concentrations.

Total sea-ice algae, pennate diatom, and silicoflagellate abundance (cells/mL) increased significantly ($p<0.05$) with decreasing silicate, nitrate, and increasing nitrite concentrations within the top layer of all sea-ice samples (**Table 2. 5**). The interior layer was characterized by the significant positive correlation ($p<0.05$) between ciliate and silicoflagellate abundance and phosphate concentrations. The ciliate group also showed a significant ($p<0.05$) negative correlation with nitrite values. The bottom layer showed significant ($p<0.05$)

negative relationships between centric diatoms and nitrite values. Additionally, ciliates had a significantly (p<0.05) strong positive relationship with silicate concentrations for the bottom layer in all sea-ice samples.

The relationship between nutrients and sea-ice algae was analyzed using a Distance Linear Model output (DistelM, **Table 2. 6**). Independently tested nutrients: nitrite, nitrate, and silicate influenced the total variation of the biological community significantly (p<0.05) (marginal test, **Table 2. 6**). However, when cumulative effects (sequential test) of nutrients were analyzed, nitrate and nitrite were the only variables with a significant impact on the total biological variation (**Table 2. 6**). In spite of this, nutrient concentrations do not explain the total variation of the biological community structure very well (16 %) (**Figure 2. 14**). When the DistelM was tested exclusively according to protist functional groups (pennate diatoms, centric diatoms, dinoflagellates, coccolithophore, silica flagellates, and ciliates), total variation explained by nutrients increased marginally (1.5 %) (**Table 2. 6**) However, only silicate was identified to contribute significantly (p<0.05) to the total biological variation (**Table 2. 6**).

Table 2. 4: Relationship between sympagic nutrients and sea-ice algae biomass according to depth layer (Spearman rank correlation coefficient).

		Total Chl a (>0.3 µm)	Chl a (>2.7 µm)	Chl a (0.3-2.7 µm)
Top	(NO_3^-) µM	0,33	0,34	-0,24
	$(SiO_4)^{4-}$ µM	0.55	0.60	-0.24
	$(NO^-)_2$ µM	-0.03	0.13	-0.20
	$(PO^{-3})_4$ µM	-0.47	-0.32	-0.10
Interior	(NO_3^-) µM	-0.19	-0.07	-0.72*
	$(SiO_4)^{4-}$ µM	-0.42	-0.31	-0.75*
	$(NO^-)_2$ µM	-0.54*	-0.48	-0.47
	$(PO^{-3})_4$ µM	-0.12	-0.08	-0.07
Bottom	(NO_3^-) µM	0.18	0.20	-0.31
	$(SiO_4)^{4-}$ µM	-0.12	-0.11	-0.24
	$(NO^-)_2$ µM	-0.15	-0.12	0.64*
	$(PO^{-3})_4$ µM	-0.51	-0.53	-0.35

Significance shown at p <0.05. marked as *

Table 2. 5: Relationship between sea-ice algae cell abundance and biochemical parameters according to depth layer (significance shown as p<0.05).

		NO₃⁻ (μM)	(SiO₄)⁴⁻ (μM)	NO₂⁻ (μM)	(PO₄)³⁻ (μM)
Top	Total protist cells	-0.88*	-0.72*	0.71*	0,18
	Dinoflagellates	-0.42	-0.45	0.01	0.01
	Pennate diatoms	-0.89*	-0.79	0.72*	0.18
	Centric diatoms	-0.60	-0.45	0.71*	0.27
	Silicoflagellates	-0.73*	-0.66*	0.66*	-0.16
	Coccolithophores	-0.37	-0.09	0.48	0.24
	Ciliates	0.13	0.17	-0.33	0.10
Interior					
	Total protist cells	-0.39	-0.44	0.17	0.37
	Dinoflagellates	-0.03	0.25	0.24	-0.20
	Pennate diatoms	-0.39	-0.42	0.15	0.27
	Centric diatoms	-0.06	-0.03	0.26	0.45
	Silicoflagellates	-0.26	-0.35	0.14	0.53*
	Coccolithophores	-0.06	0.10	0.10	0.18
	Ciliates	0.17	-0.08	-0.50*	-0.03
Bottom					
	Total protist cells	-0.22	0.14	-0.25	-0.47
	Dinoflagellates	0.47	0.10	0.40	-0.10
	Pennate diatoms	-0.37	0.42	0.06	-0.59
	Centric diatoms	0.25	-0.59	-0.72*	-0.22
	Silicoflagellates	0.19	0.04	-0.16	0.02
	Coccolithophores	-0.19	0.53	0.15	-0.39
	Ciliates	-0.07	0.76*	0.60	-0.22

Significance shown at p <0.05. marked as *

Table 2. 6: Distance linear model results showing how well nutrient concentrations explain biological community using the R^2 function.

	Sea-ice algae		Protists	
	Marginal test	Sequential tests	Marginal test	Sequential tests
	P	P	P	P
(NO₃⁻) μM	0.05*	0.04*	0.09	0.09

$(SiO_4)^{4-}$ µM	0.02*	0.22	0.01*	0.06
$(NO^-)_2$ µM	0.02*	0.02*	0.11	0.10
$(PO^{-3})_4$ µM	0.27	0.23	0.67	0,88

Significance shown at $p < 0.05$, marked as *
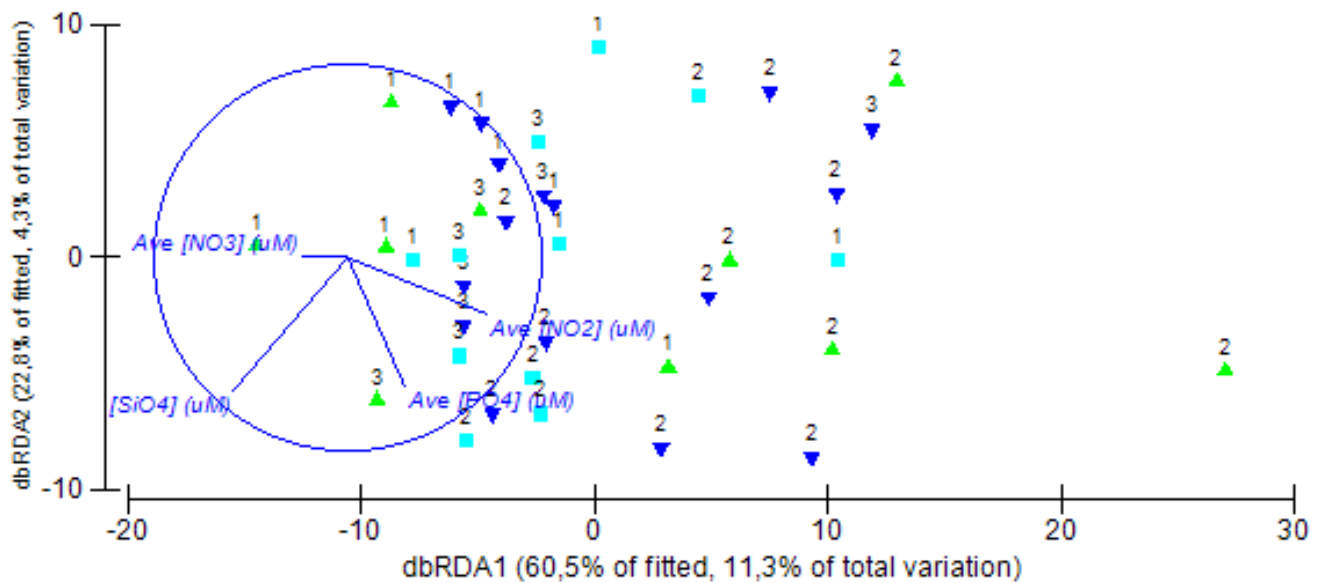
Figure 2. 14: Distance linear model (DistelM) of the relationship between nutrient concentrations and sea-ice algae biological community variation. Data was fourth root transformed, using the Bray Curtis similarity resemblance matrix's'. Note that the numbers indicate pancake samples (1, 2, and 3), and symbols show depth layers (top ▲, interior ▼, and bottom ■).

2.4 Discussion

2.4.1 Biochemical environment

Sea-ice brine volume fraction is a determinant of sea-ice permeability which in turn controls the mobility of biogeochemical fluxes (Tison et al., 2020). All sea-ice samples in this study were considered permeable, given the >5% brine volume threshold (**Figure 2. 2**), according to the percolation theory (Golden et al., 1998).

Therefore, the physical sea-ice structure is characterized by a coalesced brine network where seawater (brine) can move freely within the sea-ice samples (Fripiat et al., 2014). The surface area of brine pockets and autotrophic biomass is somewhat consistent at a brine fraction between 3 and 8 % (Becquevort et al., 2009). Brine volume fractions above 8% allow for a higher accumulation of biomass (Becquevort et al., 2009). However, fluid flow throughout the sea-ice could vary according to depth due to different growth stages experienced by natural sea-ice (Lieblappen et al., 2018).

Pancake floes collected at all three sampling sites were no more than 1 m in diameter which indicates pancake growth via the frazil "life cycle", as opposed to welding shown by pancake floes of larger sizes (>4 m) (Alberello et al., 2019). Pancake ice floes formed via the frazil life cycle are characterised by a decoupled brine channel system, with varying channel sizes not following likely trends associated with depth layers in sea-ice (Lieblappen et al., 2018). Modelling studies have shown new characteristic behavior related to frazil sea-ice brine morphology where brine channels rapidly join, split, or remain in place, while consisting of more brine branches when compared to columnar sea-ice types (Lieblappen et al., 2018). Therefore, it is possible that the frazil sea-ice life cycle could account for large differences in biochemical properties with respect to the sea-ice depth profile found in this study, since accumulation of organic material follows the uneven distribution of brine channels and pockets.

Steep nutrient gradients were not observed in this study (**Figure 2. 5**, and **Figure 2. 6**), while salinity showed a homogenous profile with no clear distinction between depths (**Figure 2. 3**). An isohaline profile is usually characteristic of melting sea-ice and snow loading compared to a C-shape profile characteristic of growing sea-ice (Torstensson et al., 2018; Tison et al., 2020). In this study, the homogenous isohaline profile is likely due to the permeability of the sea-ice structure and cyclonic storm conditions causing flooding of the sea-ice surface during the sampling period (1-4 July 2017). During this study's sampling period, in situ observation of several cyclones and an explosive extratropical cyclone crossing the South Atlantic MIZ was recorded (Vichi et al., 2019). These generated extreme regional variability concerning MIZ sea-ice behavior and morphology (Vichi et al., 2019). The likely implications from such extreme weather events includes extensive meridional heat advection onto sea-ice, intensified sea-ice drift, ice edge deformation, and far-reaching waves causing a persistent unconsolidated sea-ice sheet (Vichi et al., 2019). These conditions were likely to have a considerable effect on the fluid flow, biogeochemical properties, and microstructure of sea-ice, especially at the ocean-atmosphere interface. It should be noted that collection methodology of pancake sea-ice samples could have resulted in significant brine drainage, subsequently modifying the sea-ice salinity and biochemical profile. Furthermore, it is postulated that brine behavior largely regulates the inorganic nutrient pool in sea-ice (Vancoppenolle et al., 2010). This is highlighted by brine convection in the skeletal layer and similar nutrient and salinity vertical profiles often observed in newly formed sea-ice (Vancoppenolle et al., 2010). Salinity and nutrients (nitrite, nitrate, phosphate, and silicate) were not significantly correlated between

different sea-ice pancake samples or at different depths. Salinity and nutrient profiles often become dissociated after ice ages or over complete seasons due to a prolific biological community (Fripiat et al., 2017; Dieckmann et al., 1991). Therefore, it is likely that sea-ice samples consisted of an active sympagic biological community across all pancake sea-ice samples.

This study's findings reveal a higher accumulation of algal cells (abundance and biomass) within sea-ice samples when compared to surrounding surface seawater. This could suggest that some sea-ice algae species were actively growing or accumulated in sea-ice during the winter sampling period. Although cell concentration cannot solely corroborate the active growth or productivity of the sea-ice algae, N:Si ratios at a constant 1:1 across most sea-ice samples, and significant positive correlation ($p < 0.05$, R=71) between the latter, is evidence of active biochemical exchange during or prior to this study's sampling period. The N:Si ratio is largely dependent on diatom abundance (McMinn et al., 2000). Diatoms consume nitrate and silicate at a ratio of 1:1, when favorable environmental conditions persist, e.g. Fe-replete waters (Weir et al., 2020; Hutchins & Bruland, 1998; Takeda, 1998). The SO sea-ice environment has been depicted as a biogeochemically active reservoir for iron (Lannuzel et al., 2016). Therefore, the sea-ice algal community were likely not subjected to low iron concentration limiting primary production as commonly observed for phytoplankton communities in the SO surface waters (Kaufmann et al., 2009). When sea-ice nutrient data were compared to the surrounding surface water, all measured DIN compounds (ammonium, nitrate, and nitrite) were higher in sea-ice. The latter may be indicative of active regeneration via the detritus based microbial food web or assimilation by sea-ice algae (Fripiat et al., 2017; Fripiat et al., 2015). Surrounding surface water DIN concentration were similar to typical values associated to the SO and corresponded to similar accumulation concentrations found in sea-ice brine (Thomas & Dieckman, 2002). For example, sea-ice brine nitrate and ammonium concentrations of 121 and 48 μM has been found, respectively. The latter were likely attributed by cell lysis, algal mortality, inefficient grazing, and nitrogen remineralisation (Günther et al., 1999; Arrigo et al., 1995). Conversely, silicate concentrations were higher in surface water (47 μM) when compared to sea-ice concentrations (between 30 and 41 μM). A slightly depleted silicate pool were likely a result of the large alga standing stock (dominated by diatoms) using silicate to construct cell frustules (Thomas & Dieckman, 2002).

The accumulation of ammonium in sea-ice (>2 μM) and the observed decline of concentrations (top to bottom) were likely controlled by remineralisation and assimilation processes. Assimilation of ammonium decrease with decrease light availability due to light dependent primary production (sea-ice algae), conversely to remineralisation of ammonium via the microbial loop, not constrained by light (Fripiat et al., 2017). Therefore, increased ammonium in the top sea-ice layers were likely supplemental to remineralisation. Additionally, it is thought that ammonium concentration <1 μM inhibit the assimilation of nitrate (Priscu & Sullivan, 1998). However, since all sea-ice samples showed a ammonium concentration >2

µM, accumulation of nitrate (>5 µM) via assimilation were not inhibited in most sea-ice layers. Additionally, high nitrate concentrations have commonly been linked to regeneration processes associated to the microbial food chain (Roukaerts et al., 2016), and remineralization from nitrification (Fripiat et al. 2014). The latter has been shown to contribute to 70 % of nitrate found in Antarctic spring sea-ice. Furthermore, Fripiat et al. (2015) has revealed that dissolved inorganic nitrogen (DIN) (nitrate, nitrite, ammonia) are assimilated and regenerated by Antarctic pack-ice algae, either using DIN as an energy resource or to produce biomass. Similarly, results of this study provide evidence for the regeneration and/or assimilation of nutrients by sea-ice algae to produce biomass, particularly favoring the growth of large diatoms in austral winter pack ice. This is contradictory to what has been predicted for the early-winter sea-ice microbial community (Fripiat et al., 2017).

The N:P ratios recorded in this study were largely at a ratio higher than that of seawater (>16:1) (Redfield et al., 1963) at 28:1 (average). High N:P ratios found in this study could be contributed by N-fixing organisms such as cyanobacteria (Quan & Falkowski, 2009). Competition between non-nitrogen fixing species and nitrogen fixing species is said to control the oceanic N:P ratio (Klausmeier et al., 2008; Redfield 1958). This has not been suggested for polar sympagic studies until recently. The cyanobacteria specie, *Atelocyanobacterium thalassa* has been correlated to substantial nitrogen fixation in Antarctic waters, particularly in ice covered regions (Shiozaki et al., 2020). Instead, flexibility surrounding the "fixed" Redfield ratio has been encouraged since global patterns became more apparent within specified oceanic regions showing constant deviations from the so-called standard (Matsumoto et al., 2020). The latter emphasised by considerable variability in nitrate, phosphate and silicate consumption with the introduction of Fe in Fe-limited environments such as the SO (Takeda, 1998). It has been shown that with the introduction of iron (5-40 µM), N:P and C:P ratios can increase (De Baar et al., 1997). In addition, large variability in N:P ratios in this study could be associated to species-specific metabolic requirements, physiological response, life history, cell size, and distribution in sea-ice (Matsumoto et al., 2020; Lyon & Mock, 2014; Weber & Deusch, 2010; Klausmeier et al., 2008). However, remineralization and release of phosphate from POM is more rapid when compared to nitrogen compounds (Burkhardt et al., 2014), while easily adsorbed by metal organic complexes particles (Fripiat et al., 2017). Additionally, similar concentration was observed between sea-ice and surrounding surface concentrations. Therefore, this study cannot confidently elucidate the relationship between phosphate and sea-ice biochemical cycling via the microbial loop or primary production.

2.4.2 Size fractionation of sea-ice algae 4.2 Size fractionation of sea-ice algae

This study shows a clear dominance of diatoms within the protist community, primarily by large cell size fraction (>2.7 µm), in both sea-ice and surrounding surface water. A large concentration of diatoms in the surface water could well be explained by the occurrence of 'flushing events', i.e., brine drainage, which are

found to be common in Antarctic waters (Lizotte, 2003). The abundance of large diatoms, particularly EPS-producing pennates, have been observed in newly formed sea-ice, owing to favorable accumulation via scavenging during frazil ice formation (van Leewe et al., 2018). The dominance of larger cells could also be characteristic of a more established sea-ice community (Tison et al., 2020). Greater contributions from nano (2-20 µm) phytoplankton cells in seawater and bottom sea-ice in the MIZ and polar frontal zone have been postulated by Detmer & Bathmann (1997), suggesting a lower importance and dominance (cell abundance) of autotrophic pico-sea-ice algae. Likewise, pico-sea-ice algae concentrations (0.3-2.7 µg/L) were low in underlying surface waters. A shift in sea-ice algae size fractions and species community structure could be anticipated with increasing climate change associated parameters, such as an increase in pCO2 (Hancock et al., 2018). The impacts across the plankton community in the MIZ during early winter are unlikely to be felt uniformly; with larger species less tolerant of increases in pCO2 concentrations, and so at greater risk of being adversely affected.

2.4.3 Microhabitats

A high abundance and biomass of sea-ice algae was observed throughout the sea-ice depth profile. This is commonly observed in Antarctic pack ice, where biological communities are distributed throughout the depth profile (Torstensson et al., 2018). However, sea-ice algae community structure was found to be significantly different between individual samples and depth layers. The latter is indicative of established microhabitats in sea-ice. Sea-ice algae reside within microhabitats according to species-specific preferences, therefore disparate taxa are expected according to depth layers (top, interior, and bottom) (Horner et al., 1992). However, dominance of pennate taxa throughout the sea-ice depth profile is commonly observed since species are able to freely migrate within brine channels, especially specialized species from the Naviculoid genera (van Leeuwe et al., 2018).

Top

Pack-ice algae community studies, specifically surface and interior assemblages, are largely descriptive and sparse making it challenging to describe in situ community dynamics and trends (Legendre et al., 1991). However, the surface layer of pack-ice has been shown to contain high sea-ice algae biomass due to communities residing within the infiltration layers (Lizotte, 2003). The infiltration layer is located at the ice-surface interface, characterized by snow loading and rafting of pancake floes causing flooding of the sea-ice surface (Arrigo & Thomas, 2004). Yet, in this study, biomass was generally the lowest in the top layers of the

pancake. Sea-ice algae growth was likely restricted in this layer due to the passing of explosive cyclone across the study area exposing the upper column of sea-ice to abiotic stressors (Lizotte, 2003). Additionally, small (<1 m) pancake floes collected were likely submerged during sampling period, allowing the exchange (loss or gain) of biochemical properties between surface sea-ice and seawater. Therefore, significant differences between species community structure between the top layer and lower layers (interior and bottom) could be explained by the continued depression of sea-ice due to synoptic conditions. At the same time, disparate habitats created were likely responsible for species accumulating in varied abundance throughout the depth profile. This is emphasized with the species Corethron spp. and Dactyliosolen antarcticus showing a large and minor contribution, respectively, to community structure in the top and lower layers.

Interior

Higher average total biomass and abundance was observed at the interior of all sea-ice samples, compared to top and bottom layers. Dense interior community are usually a product of the heterogenous structure of Antarctic pack-ice, after sea-ice age, refreeze, and surface flooding (van Leewe et al., 2018; Ugalde et al., 2016). Similar to the (2017) PIPERS study, a dense (high biomass) interior community in the MIZ of the Ross Sea was found during winter (Tison et al., 2020). However, the interior community described by Horner et al. (l992) characterize this study's interior layer best, since similar sea-ice algae biomass (<10 ug/L) and community assemblage (consisting of diatoms, dinoflagellates, ciliates, and copepods) was found (Horner et al., 1992). Mixed interior communities of pennate diatom (36 %) and flagellate groups (54 %) have been previously recorded in Antarctic pack-ice, however a lesser contribution from the centric genera (4 %) to community abundance was observed when compared to this study (van Leeuwe et al., 2018). Furthermore, a significant relationship between ciliates and nutrients (decrease nitrite and increase phosphate) in this layer suggest the regeneration of the macronutrient phosphate. Regeneration of macronutrients has often been described in sea-ice, controlled by sympagic biota such as bacteria, foraminifera and other protozoa including ciliates (Dieckmann et al., 1991).

Bottom

A significant negative correlation between increasing pico-sea-ice algae cells and decreasing nitrite concentration was observed in all sea-ice bottom layers. In contrast to this study's findings, sea-ice bottom-layer plankton communities have previously shown a preference for newly formed nitrate (Meiners et al. 2016). Autotrophic pico-sea-ice algae of Antarctic waters usually depicts a community of small flagellates, specifically from the class Prasinophyceae, and less commonly from the cyanobacteria phyla (Detmer & Bathmann, 1997). However, cyanobacteria are more likely to explain significant negative correlation in this

study since they are able to exclusively consume either nitrate, nitrite, or ammonia for growth (Maeda, 1998). However, Koh et al. (2012) postulates that cyanobacteria play an insignificant role in Antarctic sea-ice (fast ice).

A higher accumulation of coccolithophores is observed in the bottom layer compared to other protozoa groups (excluding Bacillariophyceae). Species succession from a diatom- to coccolithophore-dominant community is usually anticipated according to the popular framework described in Margalef's mandala (Margalef, 1978). This seminal study prescribed various environmental requirements for successful growth such as nutrients and light; however, recent efforts have highlighted coexistence of coccolithophores and diatoms within the global oceans, and distribution in various biochemical niches in the SO (Nissen et al. 2018). Concomitantly, coccolithophores are primarily found in northern sub-Antarctic waters and usually not observed further south (Trull et al., 2018), though in recent decades a poleward shift has been suggested (Nissen et al., 2018), specifically for the major pelagic calcifyer *Emiliania huxleyi* (Winter et al., 2014). Therefore, the sea-ice environment, specifically the bottom layers of pancake ice, could provide a unique opportunity for coexistence in the SO where physicochemical requirements for growth are met for both functional groups to proliferate simultaneously. This is highlighted by findings of Nissen et al. (2018), showing significant control of the top-down effect concerning diatom and coccolithophore abundance.

2.4.4 Surrounding surface water and sea-ice

This study found that sea-ice algae biomass was significantly higher in sea-ice than the surrounding surface water. Similar low surface water (<0.1 µg/L), high sea-ice (>1 µg/L) concentrations were observed in the PIPERS study within the MIZ of the central and western Ross Sea (CRS and WRS) during early winter (Tison et al., 2020). A chlorophyll a concentration above 1 µg/L is usually considered a bloom threshold in the open ocean. For this reason, this study highlights the extreme biomass that sea-ice retained during the winter, considering that sea-ice biomass reached as much as 5 µg/L in the sea-ice interior. It is therefore reasonable to hypothesize that sea-ice algae were growing within the pancake sea-ice during austral winter and were likely seeded from the surrounding surface water. This is emphasized by a lack of significant difference between sea-ice and the surrounding surface seawater for both species community structure and the number of species found. Strong overlap in species community assemblage between pack-ice and water column is often observed and could be interpreted as the seeding effect from the surrounding water through scavenging (Szymanski & Gradinger, 2016; Brierly & Thomas, 2002; Kristiansen et al., 1998). However, disparate findings within specified temporal and spatial periods have been observed, baring no significant similarities in community structure between sea-ice and surface water (Garrison et al., 1987). Therefore, seeding effect between sympagic and pelagic ecosystems should be investigated according to season and

region concomitantly. For this reason, the site of this study (Fig 1) could be hypothesized as a closely coupled system during winter, similar to the Weddell Sea pack ice during late summer (Garrison et al., 1987).

2.4.5 Relationship between biochemical parameters and sea-ice algae abundance according to depth layer

Low nitrite and phosphate concentrations in this study did not show any limiting effect on sea-ice algae community, since high N:P ratios were maintained alongside high average total biomass (<0.9 µg/L) in all sea-ice samples. However, when nitrogen and phosphorus is at a greater concentration than is needed for algae growth, N:P ratio rules are not valid to any further extent (Kim et al., 2007). Moreover, the Distance Linear model (DistelM) revealed that the nutrients measured did not explain total variation of sea-ice algae community structure (Pyrrophyta and Bacillariophyceae) (only 16 %). However, sea-ice algae community structure was best explained by nitrate and nitrite concentrations. Nutrients are not the only factors influencing phytoplankton growth. A suite of trace metals could be used when macro-nutrients do not explain biological variation (Viljoen, 2018). Measured nutrient parameters explained biological variation marginally better (1.5%) when other protist groups (silicoflagellates, ciliates, and coccolithophores) were included, whereas, silica explained variation of protist community best. This could be contributed by the increased abundance of the silicoflagellate group relying on silicate for the construction of their skeletal lattice or heterotrophic feeding strategy, ingesting silica-rich species (McCartney & Loper, 1989).

2.4.6 Community structure

Pennate diatoms were the most dominant taxa in all sea-ice and surface water samples (van Leewe et al., 2018; Ugalde et al., 2016; Lizotte, 2001). They often dominate in seasonal and ephemeral conditions of the SIZ, and diatom sea-ice community of which classes Bacillariaceae abundance are usually highest (Torstensson et al., 2015b; Eiken, 1992; Garrison, 1991). High abundance of pennate species is likely owed to inherent cell stickiness and raphe, favoring incorporation during frazil ice formation and ability to position itself in vertical ice profile according to available light and nutrients (Eiken, 1992). Dominant taxa (Table 2) of this study were similar to Ugalde (2015, East Antarctic pack ice); Meiners et al. (2011, East Antarctic sea-ice); Tison et al. (2020, Ross Sea sea-ice) and to Gomi et al. (2010 surface mixed layer of the eastern Indian Sector of the SO). Although these studies were sampled over disparate temporal and spatial scales, it is evident that similar dominant phytoplankton taxa can be found in both surface water and sea-ice in east Antarctica.

Fragilariopsis spp., was found to be the most dominant genus across all depth layers. Species of the genus Fragilaria are often found dominating the sea-ice environment (Krell et al., 2008), especially pack ice (Lizotte, 2001), and open waters of Antarctic marginal ice-edge zones (Kang & Frexyll, 1992). Some species in this class are considered model psychrophilic types, considering adaptation to extremely low temperatures and high

brine salinities (Krell et al., 2008). Success of species such as F. curta and F. cylindrus is further attributed by small cell size, short chain length, and low settling rates (Leventer et al., 2008). These traits are similar to primary character traits usually underlined for pioneer species (Lizotte, 2003). Other dominant pennate taxa were recorded as Pseudo-nitzschia spp. and Proboscia spp. The success of these species was likely due to their ability to employ overwintering growth strategies, allowing for continual growth during winter conditions (high stress environment) due to cells remaining vacuolated and containing no lipids (Leventer et al., 2008).

Pennate diatoms have generally been found to exist more abundantly than centric diatoms in sea-ice, however a limited number of studies have observed the opposite (Clarke & Ackley 1983; Watanabe, 1982; Ackley et al., 1979; Bunt & Wood, 1963). Centric diatoms were found to be abundant in this study since contribution to community assemblage exceeded the threshold of 10 %. Therefore, centric diatoms could serve as a more pertinent feature in the MIZ sea-ice algae community, on account of their notable contribution to carbon fluxes (van Leeuwen et al., 2018). Dominant centric diatom species identified were *Chaetoceros* spp., *Chaetoceros dichaeta*., and *Coscinodiscus* spp. Common strategies employed by overwintering Antarctic diatoms species are cold and seasonal (winter) growth stages to allow for the increase silicification of algae cell wall (Leventer et al., 2008). A heavier silicified cell wall is likely to protect algae cells from increased grazing pressure during austral winter (Leventer et al., 2008). Dominant centric species *Dactyliosolen antarcticus* and *Corethron* spp. were variable in their contribution at each depth layer. D. antarcticus spp. was markedly lower or sparsely distributed within the top layers compared to the interior and bottom layers. This is likely due to the relatively young age of the top layer of sea-ice since Dactyliosolen species that have been found to accumulate in sea-ice but excluded after unfavorable conditions persist or after ice age (Gleitz & Thomas, 1993; Lizotte, 2001). Conversely, *Corethron* spp. only showed high abundance in the top layer of sea-ice, compared other dominant centric taxa found. This could be owed to physical sea-ice structure properties and species having dissimilar fine structure compared to other dominant centric diatoms in this layer (Fryxell & Hasle, 1971). *Corethron criophilims'* protruding barbed and clawed spines shows no similarity with labiate processes or tubuli and setae of other centric diatoms (Coscinodiscus spp.) and the Chaetoceros genera, respectively (Fryxell & Hasle, 1971). Additionally, spines of the Corethron genera are inherently developed compared to that of Chaetoceros dichaeta setae which is morphologically determined according to external environment (Ligowski et al., 2011). It is well established that C. dichaeta is one of the dominant types of diatoms species found both in the SO waters and bottom ice communities, significantly contributing to total algae biomass (Assmy et al., 2008; Ligowski et al., 2011). Although the long protruding setae of C. dichaeta is ideal for a planktonic lifestyle, continued high abundance in sea-ice has been observed (Ligowski et al., 2011). However, extreme morphological plasticity has been adapted by this overwintering algae species (Ligowski et al., 2011), changing both shape and orientation of seta as well as

aperture length between neighboring cells. Consequently, occupancy space needed in brine network is reduced (Ligowski et al., 2011).

In this study, low average abundance of dinoflagellates in all sea-ice samples compared to other protist groups (ciliates, silicoflagellate, and coccolithophores) was identified. This was in contrast to Torstensson et al. (2015a) findings where bottom sea-ice communities were dominated by the dinoflagellate algae community (Amundsen and Ross Seas). Dominance of dinoflagellate communities were likely contributed by ice thickness and increase growth under low light conditions (Torstensson et al., 2015a). Low dinoflagellate abundance observed in this study could therefore be explained by low average ice thickness (approx. 0.6 m) when compared to high ice thickness of Torstensson et al. (2015a) (ice thickness range between 0.69-1.92 m). It is important to note that sea-ice processing methods such as thawing used in this study could have led to an under-sampling of dinoflagellate species, due to their sensitive cell structure (Torstensson et al., 2015b). Also, challenges faced with light microscopy identification of common gymnodinoid dinoflagellate species could contribute to the recorded levels of abundance (Torstensson et al., 2015b).

2.5 Conclusion

The results of this study have provided evidence for the active growth of sea-ice algae during austral winter (July 2017) in the Southern-Indian MIZ of the SO. This is similar to findings observed during winter in the Weddell pack ice (2017, PIPERS) and Ross Sea (2013, AWECS). During this study's sampling time, synoptic-scale weather events (explosive cyclone) and pancake life cycle had considerable control over the physical

sea-ice structure and physicochemical properties. Relationships ($p<0.05$) observed between sea-ice protist functional groups and macronutrients, as well as nutrient stoichiometry characterised the winter sea-ice environment as a coupled system, which suggest the presence of an active sea-ice algae community showing regenerated production. Contradicting to the findings of Fripiat et al. (2017), which hypothesised that sea-ice algae is not active during winter in Antarctic sea-ice. Moreover, pennate diatoms largely dominated community structure from the *Fragilariopsis*, *Proboscia*, *Pseudo-nitzschia* genera, alongside some centric diatoms such as Coscinodiscus and Chaetoceros genera. Distinct communities according to the sea-ice depth profile was observed revealing elevated cell concentrations within the sea-ice interior layer. However, this study could only partially (16%) explain total biological variation, since measured parameters (nutrients) did not adequately account for distribution and composition of sea-ice algae. For this reason, it is important to measure all drivers effecting sea-ice algae physiology such as trace metals, EPS, osmolytes, and other POM. Further work is now needed to identify what seasonal drivers are responsible for biological variation and how climate change will impact winter sea-ice algae communities (distribution, assemblage, and abundance).

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Chapter 3

Southern-Atlantic Ocean sea-ice algae species composition, biomass, and abundance during the austral winter of 2019.

3.1 Introduction

The annual growth of the Antarctic sea-ice is the most conspicuous physical phenomenon on earth, reaching an extent larger than the Antarctic continent itself, while occupying 8 % of the Southern Hemisphere during winter (Brierly & Thomas, 2002). Therefore, it is apparent that this ephemeral environment serves as a closely coupled system with surrounding biotic and abiotic variables of ocean, ice, and atmosphere (Jeffries & Weeks, 1992). Yet, it is often misrepresented in climatic models as a barrier preventing the exchange of biogeochemical substances. This is largely due to the sporadic distribution of data accumulated over disparate temporal and spatial scales (Meiners et al., 2017; Meiners et al., 2012). However, this problem has recently been alleviated with rigorous sea-ice chlorophyll a sampling in the SO (Tedesco & Vichi, 2014). Dense ice-associated (sympagic) microbial communities have significant control over interchangeable biogeochemical properties. For this reason, accurate representation of microbial sea-ice community dynamics is essential in climate models. Furthermore, sea-ice biota has both significant control in regional and global processes considering that the sea-ice environment forms part of the global thermohaline circulation, biological pump, and sea-ice albedo feedback, while providing organic material to higher trophic organisms during winter seasons when SO pelagic resources are limited (McMinn, 2017; Wilson et al., 2015). Additionally, exudates from marine phytoplankton in the ocean surface microlayer could serve as an important source of ice-nucleating particles, particularly in remote regions such as the SO (Wilson et al., 2015).

All physical sea-ice properties have significant control over sympagic microbial communities especially ice-algae biomass (Meiners et al., 2017; Brierly & Thomas, 2002). Therefore, understanding processes involved during sea-ice formation, decay, and growth is required to elucidate SO sympagic ecology (Brierly & Thomas, 2002). Unlike the congealed ice sheet formed in the Arctic and close to the Antarctic continental shelf (fast ice), the SO ice edge zone is characterized by disparate processes regulating the formation of sea-ice. Strong wind-wave action and turbulence prevent the formation of ice crystals into a continuous consolidated sheet. Instead, frazil ice crystals form at the ocean-atmosphere interface and is subsequently suspended at depth (Doble et al., 2003). Suspended crystals entrap organic material from the water column, concomitantly scavenging microbial organisms including phytoplankton (Thomas, 2012). The temperature gradient at the ocean-atmosphere consolidates crystals into larger sea-ice structures termed pancake ice. Pancake ice floes are initially a few centimeters in diameter, however as wave action becomes less prominent, larger discs form of approximately 5 m in diameter. The latter process is ascribed to the pack ice formation in the MIZ of the Weddell Sea region, also known as a relatively fast ice growth process. This is especially significant since a continuous sheet can originate from the pancake life cycle even in the presence of high ocean heat fluxes. Consolidation of a continuous ice sheet is usually only observed somewhat 270 km from the ice edge. The

growth rate of sea-ice is subsequently reduced after a consolidated ice sheet has formed, following the slow growth life cycle of congelation ice growing no more than four cm (Doble et al., 2003).

The sea-ice environment hosts a diverse microbial community (Krebs et al., 1987), where fluctuations in abiotic parameters induced by climate change have significant control over community structure and abundance. To elucidate how potential climate variation will affect the ecological role of sea-ice microbial communities, the examination of phytoplankton (sea-ice algae) community structure has been motivated (Nunes et al., 2019). Each phytoplankton functional group has distinct biogeochemical and physical controls by the surrounding environment, and therefore its corresponding ecological role differs significantly (van Leeuwe et al., 2018). For example, haptophytes and dinoflagellate synthesize dimethyl sulfoniopropionate (DMSP), which is later transformed to dimethyl sulphide (DMS) (Nunes et al., 2019). DMS is a well-known semi volatile organic aerosol, having significant influence on the earth's radiative properties when found as sulphate (SO_4^{-2}) particles in the atmosphere (Stefels et al., 2007). Sulphate serves as condensation nuclei for water vapor (Stefels et al., 2007), therefore involved in cloud formation. In addition, the diatom functional group are able to modify physical sea-ice properties (pore morphogenesis) via the production of extra polymeric substance (EPS) (Krembs et al., 2011). EPS increases habitability of environment for sea-ice diatoms, concomitantly functioning as a cryoprotectant and osmoprotectant (Krembs et al., 2011). Additionally, EPS is a significant source of biogenic aerosol and supports aggregation of organic material in sea-ice (Underwood et al., 2019).

Ideally, in situ measurements should provide reliable statistical relationships between physical sea-ice properties and depth-integrated chlorophyll so that satellite efforts measuring ice thickness, snow depth, and irradiance could provide more accurate spatial parameterization of SO productivity and biomass (Meiners et al., 2017). This would be an extreme feat for climate model predictions considering large spatial heterogeneity that currently exists in the ice-algae biomass distribution of the SO (Forrest et al., 2019).

The objectives of this study were to characterize the the SO MIZ sea-ice algae community structure and abundance in different sea ice types, and to compare the latter to the underlying pelagic phytoplankton community structure and abundance.

3.2 Methodology

3.2.1 Study area

3.2.2 Sample collection and processing

Usually maximum sea-ice extent is expected between 55 and 60 °S in the Weddell and Indian Sector of the SO, similar to this study sampling site (Lizotte, 2001). This has a great effect on receiving solar radiation and day length for primary production in the SO (Lizotte, 2001). A total of 16 sea-ice samples were collected from four sampling sites within the South East Atlantic section MIZ of the SO during the austral winter, July 2019 (28th to 31st) on board the SA Agulhas II (**Figure 3. 1**).

Two sea-ice cores were collected from five discrete semi-consolidated pancake ice structures within a 5 m radius at 56 80 178 °S, 0 30 262 °E (MIZ 1 N). Semi-consolidated pancake ice cores have larger contributions of granular ice, and a lesser contribution of columnar ice, and are referred to as pancake ice in this chapter. An additional two consolidate pancake ice cores were collected from 58 13 783 °S, 0 00 442 °E (MIZ 3). Consolidated sea ice cores are defined as having larger contributions of columnar ice, and a lesser contribution of granular ice, and are referred to as consolidated ice in this chapter. All sea-ice cores were transported in black plastic bags to avoid light exposure and prevent further photosynthesis. Sea-ice cores averaged approximately 60 cm in depth, and were cut with an electrical band saw into 10 cm depth sections. Three sections (top, interior, and bottom) from each core was used for biochemical analyses. Sea-ice cores were melted at 4 °C for approximately 8 hours in filtered seawater (FSW, 0.3 μm) using a dilution ratio of 1:8 (dilution ratio extracted from Campbell et al., 2019) for each 10 cm^2 section. Samples were stirred every hour to homogenise them and prevent rapid warming. However, one core from each sampling site (MIZ 3 and MIZ N) was melted with no buffer medium (direct melting) at room temperature for a period of approximately 12 hours in the dark.

Frazil sea-ice samples (FS) of a slushy-like consistency were collected between semi-consolidated pancake ice at -57° 00' 07" S°; 00° 31' 03" E (MIZ S) and - 57° 34' 503" S; 0° 00' 28" E (MIZ 2), respectively. A frazil sampler was used for the collection of frazil ice and surrounding water, and separated using a mesh sheet. One FS was collected at each of two sample stations, and subsequently divided in half, resulting in a two replicate FS (FS and rFS) per sampling site. All samples were melted at room temperature (20 °C) in the dark to prevent further photosynthesis. 500 g of FS were melted in 500 ml of filtered seawater (0.3 μm GF/F) of a salinity between 33 and 34.5 PSU which coincided with surface salinity measurements at all sample sites. Replication of in situ environmental conditions, specifically salinity, can be used as a buffering method, to provide the most accurate biological results (Campbell et al., 2019; Ryan et al., 2004). 400 g of rFS were then melted without the use of a buffer medium.

Sea-ice samples were then examined for sea-ice algae using light microscopy and the chlorophyll a concentration measured. Note that chlorophyll a was not measured for two of the frazil sea-ice samples

because the volume after direct melting was not sufficient. Additionally, large algae cell fractions (<20 μm) for frazil ice samples were not measured due to inconsistencies found in results. For further clarification on methodology and statistical methods used, refer to Chapter 2 Section 2.2. Note that multivariate analysis concerning biological data varied slightly. The MDS analysis used in Chapter 2 to explain the relationships between data points could not adequately explain multivariate data (sea-ice algae abundance) presented in this Chapter (3). Therefore, to improve the strength of association between multivariate points (sea-ice abundance) and hypothesised group (location), a CAP analysis was performed. This results revealed that samples from different sampling location were grouped separately - the first axis (CAP 1) showed a canonical correlation of $\delta_1 = 0.82$, whereas the second axis showed a weaker correlation of $\delta_2 = 0.64$.
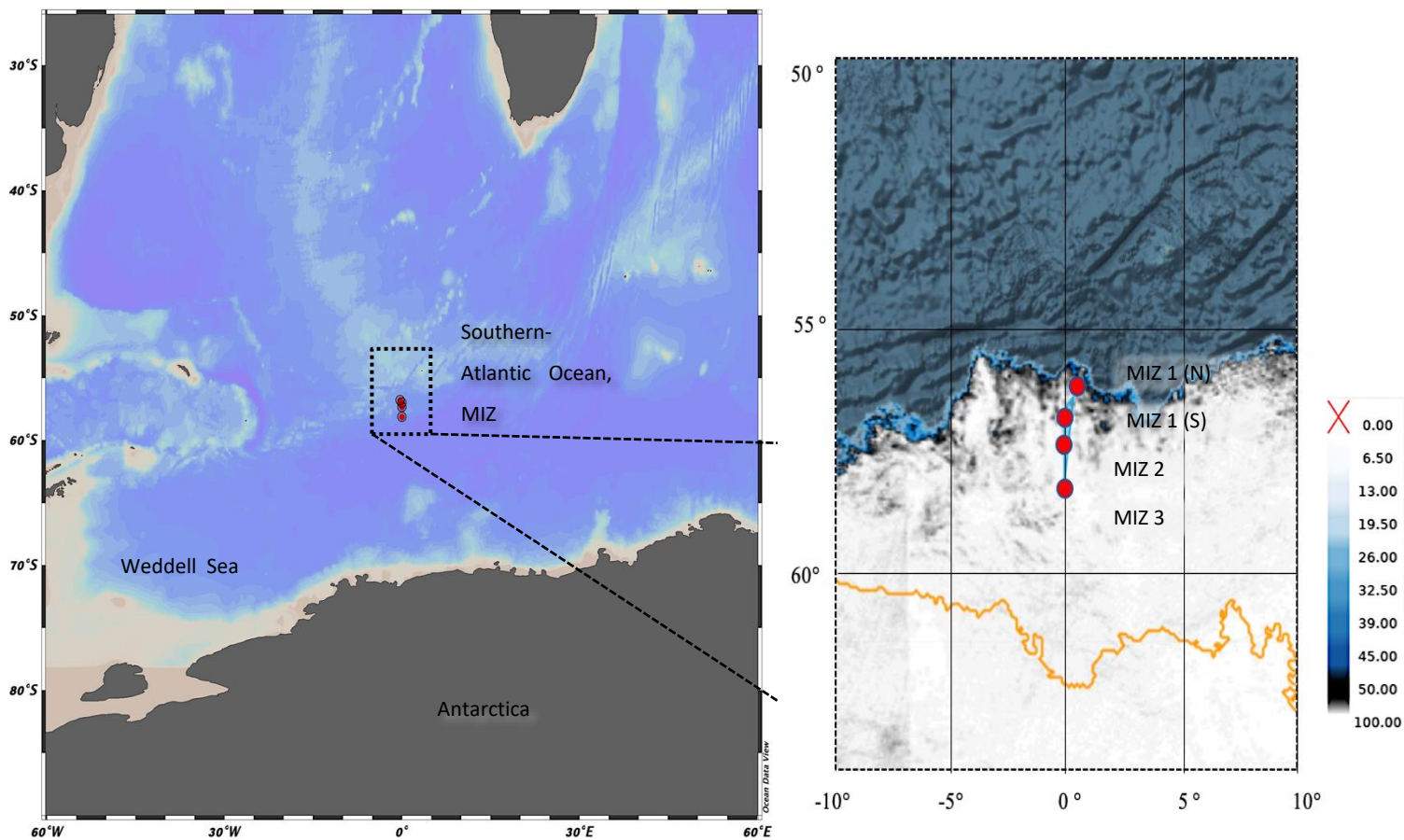
Figure 3. 1: Area of interest, **Left:** Sampling locations of sea-ice in the Southern-Atlantic Ocean, marginal ice zone (red dots). **Right:** Sample locations showing average sea-ice concentration and median sea-ice extent (orange line) for the 28th of July 2019 in the Southern Ocean (map extracted from meereisportal.de).

3.3 Results

Average total sea-ice biomass (chlorophyll a, >0.3 μm) was low, fluctuating between approximately 0.25 and 0.9 μg/L (**Figure 3. 2**). In almost all sea-ice and surface water samples no difference between total biomass and that in cells of larger size fractions (>2.7 μm) was found, revealing that most algae were between 2.7 and

20 μm in size. Biomass of all size fractions increased top-down for each sea-ice type, with the highest average biomass in bottom layers (**Figure 3. 2**). Biomass of large algae cells (shown as >20 μm) was below 0.2 μg/L for all sea-ice and surface water samples. Furthermore, maximum average biomass (>2.7 μg/L) was recorded for frazil ice samples, showing similar high concentrations to bottom consolidated sea-ice layers (**Figure 3. 2**). Most conspicuously, biomass of surrounding surface water was significantly ($p<0.05$) lower when compared to sea-ice samples (frazil and sea-ice) (**Figure 3. 2**).

The sea-ice algae community was dominated by the class Bacillariophyceae , and the order Pennales i.e. pennate diatoms showing highest abundance across all sea-ice types and depth layers (**Table 3. 1**). Average pennate (54 and 220 cells/ml) and centric cell concentrations (9 and 52 cells/ml) were considerably higher than dinoflagellates and other protist groups (**Figure 3. 3** & **Figure 3. 4**). The latter only reaching a maximum average cell concentration of 22 and 8 cells/ml, respectively (**Figure 3. 3** & **Figure 3. 4**). No particularly trend was observed for protist functional groups according to sea-ice type and depth layer (**Figure 3. 3**), although abundance of all protist functional groups was significantly ($p<0.05$) higher in sea-ice when compared to surrounding surface water (**Figure 3. 3** & **Figure 3. 4**).

Highest numbers of individual cells and species were found within the bottom layer of sea-ice cores as opposed to the interior sea-ice depth layer (**Table 3. 2**). Vertical distribution of sea-ice algae community was largely even, except for the bottom sea-ice layers (Pielou's J = 0.68) and even less so for frazil ice samples (J = 0.56) (**Table 3. 2**). Diversity of sea-ice cores (consolidated) and frazil ice collected from MIZ 3 and MIZ 1 S was much higher (>3.1) compared to sea-ice cores (pancake ice) collected from the MIZ 1 N location (<2.1) (**Table 3. 2**). Number of individual species and diversity of surface seawater samples was similar to sea-ice samples. Conversely, seawater samples showed a far lower average cell abundance (11 cells/ml) compared to sea-ice samples (>91).

The CAP analysis shows a clear separation between surface seawater and sea-ice samples. It revealed that the community distribution was more similar between locations MIZ 3 and MIZ 1 S consisting of consolidate sea-ice and frazil ice compared to pancake sea-ice cores at station MIZ 1 N (**Figure 3. 5**). Additionally, no distinct groupings according to depth layer was observed (**Figure 3. 5**). This corresponds with findings from statistical analysis using the PERMANOVA procedure where a significant difference in species community structure and abundance were only found between sea-ice types. Conversely, no significant differences between sea-ice depth layers were found and between surface water and frazil ice samples (MIZ S). Dissimilarity of protist community structure between sampling locations (sea-ice type) was large (>57.7 %), but less (8 %) between sampling location MIZ 3 and MIZ1 S (**Table 3. 3**). MIZ N was largely dominated by pennate diatoms (78 %), specifically from *Fragilariopsis*, while a minor contribution came from centric diatoms (10 %) of the *Coscinodiscus* genus (6.8 %) (**Table 3. 3**). MIZ 3 pennate diatoms contributed the most

towards similarity (55.93 %), specifically dominated by *Fragilariopsis* spp., *Pseudo-nitzschia* spp., and *Cylindrotheca closterium*. (**Table 3. 3**). Centric diatoms contributed 16 %, attributed by *Leptocylindrus* spp*.,* *Guinardia* spp., and *Chaetoceros* spp. (**Table 3. 3**). Additionally, unknown dinoflagellates contributed almost 10 % (**Table 3. 3**). In contrast to sampling stations MIZ 3 and MIZ N, high contributions from dinoflagellates (17.73 %), coccolithophores (4.8 %), and ciliates (15.47 %) were found at MIZ 1 S (**Table 3. 3**). Pennate diatoms also showed high percentage contribution at this site (34.34 %) showing similar dominant species comparable to MIZ 3 (**Table 3. 3**). Centric diatoms contributed the least at this station (11.59 %) of which the *Coscinodiscus* genus was the most dominant (**Table 3. 3**).
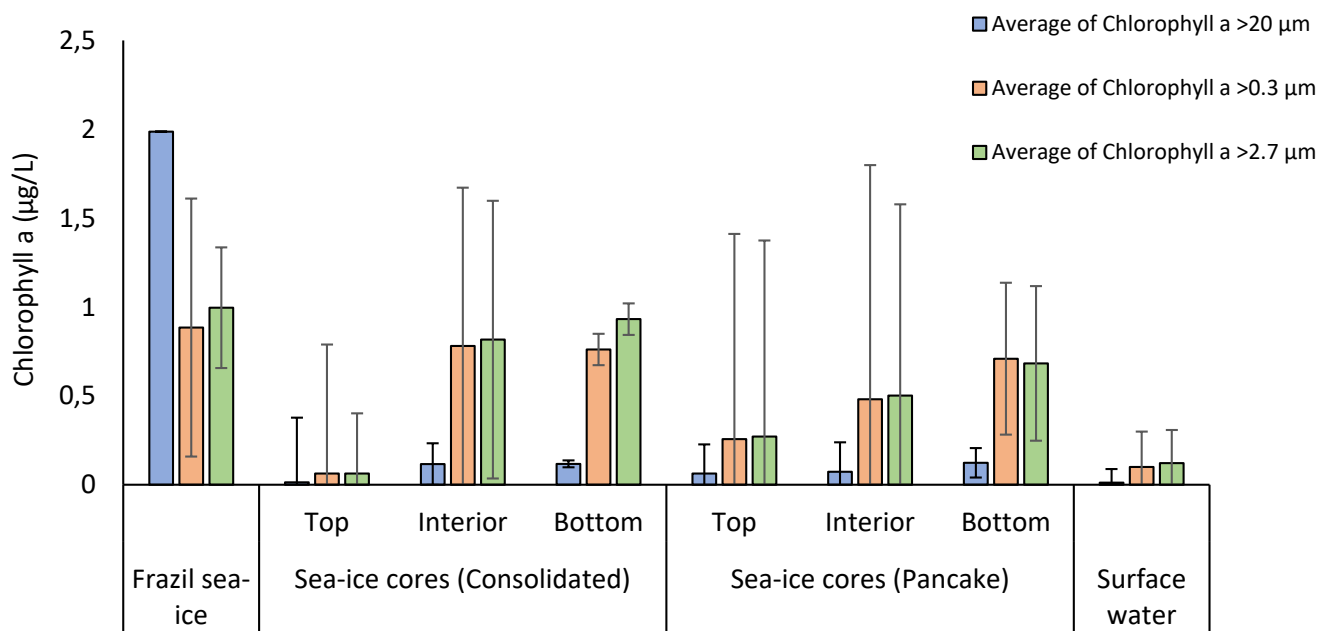
Figure 3. 2: Chlorophyll a (µg/L) across pancake sea-ice samples according to sea-ice type and depth layer. Vertical bars denote standard deviation (+/-SD) of the mean.

Table 3. 1: Sea-ice protists species list according to sea-ice type, showing total abundance (cells/ml) according to phylum.

Protist community classification	Pancake ice (Consolidated)	Pancake ice	Frazil ice
Actinopoda	0.8	0	0.04
Phaeodaria	0.8	0	0.04

Phaeogromia	0.8	0	0.04
Challengeridae	0.8	0	0.04
Protocystis bicornuta	0.8	0	0.04
Ochrophyta	**883.2**	**1736.8**	**626.5**
Bacillariophyceae	**883.2**	**1736.8**	**626.5**
Biddulphiales	**160.2**	**309.0**	**110.8**
Asterolampraceae	1.6	22	4.7
Asteromphalus spp.	0	0	0
Asteromphalus hookeri	1.6	6.3	0
Asteromphalus hyalinus	0	0	1.6
Eucampia antarcticus var. antarctica	0	15.7	3.1
Chaetoceracea	16.5	15.4	17.2
Chaetoceros atlanticus bulbous	0.8	0	0
Chaetoceros brevis	0	0.1	0
Chaetoceros concarnicavs	0.8	0.4	0
Chaetoceros criophilus	0	0	4.7
Chaetoceros dichaeta	1.6	3.1	4.7
Unknown Chaetoceros spp.	13.3	11.8	7.8
Coscinodiscaceae	27.5	88.0	12.6
Coscinodiscus spp.	18.1	80.6	12.6
Coscinodiscus radiata	9.4	7.5	0.1
Heliopeltaceae	0	0	0.8
Actinoptychus senarius	0	0	0.8
Hemidiscaceae	3.9	0	0
Actinocyclus spp.	3.9	0	0
Leptocylindraceae	32.2	66.0	7.9
Corethron spp.	2.4	0	2.4
Leptocylindrus danicus	29.8	66.0	5.5
Rhizosoleniacceae	57.3	41.0	54.2
Dactyliosolen antarcticus	8.6	21.0	2.4
Guinardia spp*.*	44.0	12.2	0
Proboscia spp.	1.57	0.4	8.6
Rhizosolenia spp.	3.14	7.5	43.2
Thalassiosiraceae	4.71	22.8	0
Porosira sp.	4.71	22.8	0
Unknown centrics	16.5	53.9	13.3
Bacillariales	690.9	1381.4	504.8
Cylindrotheca closterium	28.3	27.5	0
Fragilariopsis cylindrus	364.2	1001.7	388.6

Fragilariopsis kerguelensis	22.8	16.2	5.5
Nitzschia spp.	0	7.1	3.9
Pseudo-nitzschia spp.	62.0	24.7	33.0
Pseudo-nitzschia delicatissima	0	12.6	0
Pseudo-nitzschia pungens	0.8	0	0
Fragilariaceae	36.1	10.6	11.8
Thalassiothrix antarctica	36.1	10.6	11.8
Naviculacceae	14.9	63.6	5.5
Membraneis challengeri	0.8	15.7	0
Navicula spp.	3.9	41.6	3.1
Navicula glaciei	3.1	6.3	2.4
Pleurosigma spp.	7.1	0	0
Unknown pennates	161.7	217.5	56.5
Unknown diatoms	32.2	46.315	11.0
Chlorophyta	0	19.4	24.3
Chlorophyceae	0	19.4	24.3
Sphaeropleales	0	19.4	24.3
Radiococcaceae	0	19.4	24.3
Radiococcus spp.	0	19.4	24.3
Choanozoa	14.2	6.28	0
Choanoflagellatea	14.2	6.28	0
Unknown specie 1.	14.2	6.28	0
Ciliophora	22.0	33.4	14.9
Spirotrichea	0.8	3.2	0
Tintinnida	0.8	3.2	0
Xystonellidae,	0.8	3.2	0
Cymatocylis spp.	0.8	3.2	0
Unknown ciliates	21.2	30.2	14.9
Haptophyta	10.99	64.8	8.6
Haptophyceae	10.99	64.8	8.6
Coccolithophores	10.99	64.8	8.6
Unknown *coccolithophores*	10.99	64.8	8.6
Ochrophyta	18.84	44.4	2.4
Chrysophyceae	0	26.7	0
Ochromonadales	0	26.7	0
Dinobryaceae	0	26.7	0
Dinobryon spp.	0	26.7	0
Dictyochophyceae	18.8	17.7	2.4
Dictyochales	18.8	17.7	2.4

Dictyochaceae	16.5	11.4	0.8
Dictyocha speculum	16.5	11.4	0.8
Unknown silicoflagellates	2.4	6.3	1.6
Pyrrophyta	71.4	29.4	27.5
Dinophyceae	71.4	29.4	27.5
Dinophysales	4.7	0	0
Amphisoleniaceae	4.7	0	0
Dinophysis antarctica	4.7	0	0
Gymnodiniales	0.8	1.6	3.9
Gymnodiniaceae	0.8	1.6	3.9
Gymnodinium spp.	0	1.6	3.9
Torodinium spp.	0.8	0	0
Peridinales	22.0	0	0
Kolkwitziellaceae	22.0	0	0
Preperidinium spp.	22.0	0	0
Protoperidiniaceae	0	0	0
Protoperidinium spp.	0	0	0
Prorocentrales	0	0.8	0
Prorocentraceae	0	0.8	0
Prorocentrum spp.	0	0.8	0
Unknown dinoflagellates	44.0	27.1	23.6
Sarcomastigophora	3.1	0	0
Granuloreticulosea	3.1	0	0
Foraminifera	3.1	0	0
Unknown foraminifers	3.1	0	
			0
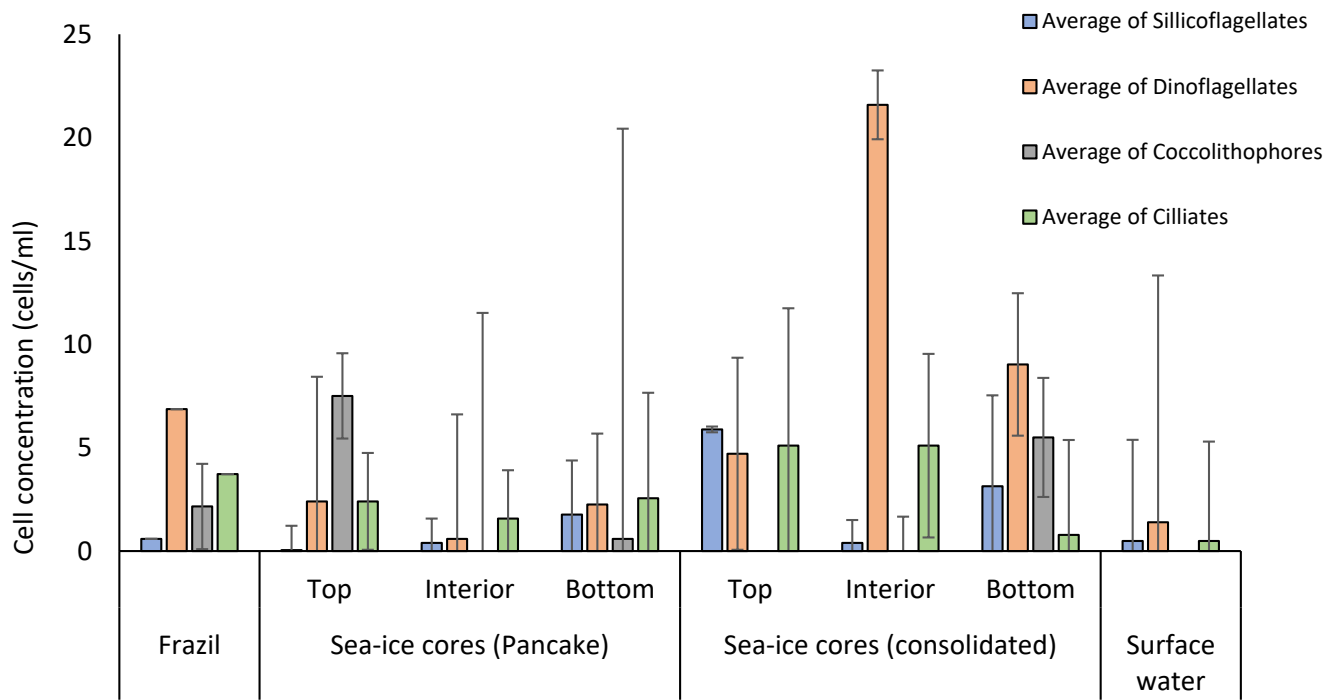
Figure 3. 3: Average protist functional groups' cell concentration (cells/ml) across sea-ice samples according to depth. Vertical bars denote +/-SD of the mean.
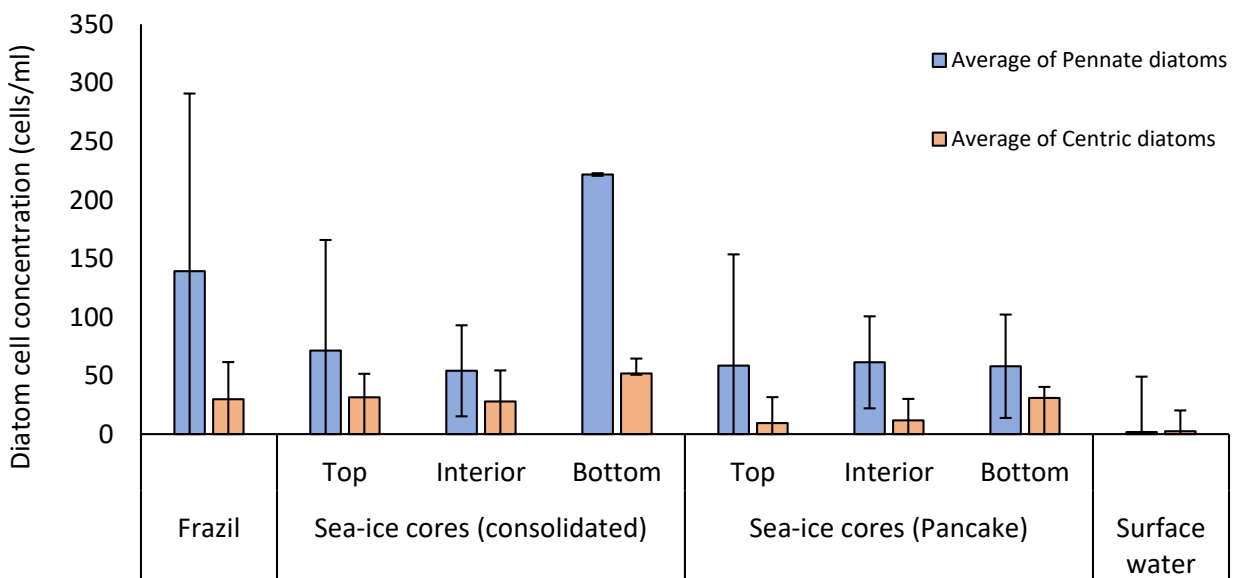
Figure 3. 4: Average diatom cell concentration (cells/ml) across sea-ice samples according to depth. Vertical bars denote +/-SD of the mean.

Table 3. 2: Average diversity indices in pancake sea-ice samples and surrounding surface water.

Sea-ice type	Depth layer	No. of species	No. of individual cells	Margalef d	Pielou J'
Sea-ice core (Consolidated)	Top	18.50	124.00	3.58	0.80
	Interior	16.00	119.00	3.14	0.88
	Bottom	19.50	302.00	3.25	0.68
Sea-ice cores (Pancake)	Top	7.71	92.14	1.53	0.72
	Interior	7.00	91.29	1.33	0.72
	Bottom	10.86	136.00	2.02	0.68
Frazil sea-ice	n/a	17.25	191.25	3.14	0.56
Surface water	n/a	11	12	2.24	1

Figure 3. 5: CAP analysis indicating the strength of association between algae abundance and the hypothesis of group difference at the sampling area. First axis with a canonical correlation of δ1 = 0.82, second axis with a canonical correlation of δ1 = 0.64.

Table 3. 3: Similar percentage (SIMPER) of sea-ice protist species contributing the most towards similarity in the MIZ according to sea-ice type (location). Significant difference between groupings found: p(perm)= 0.008.

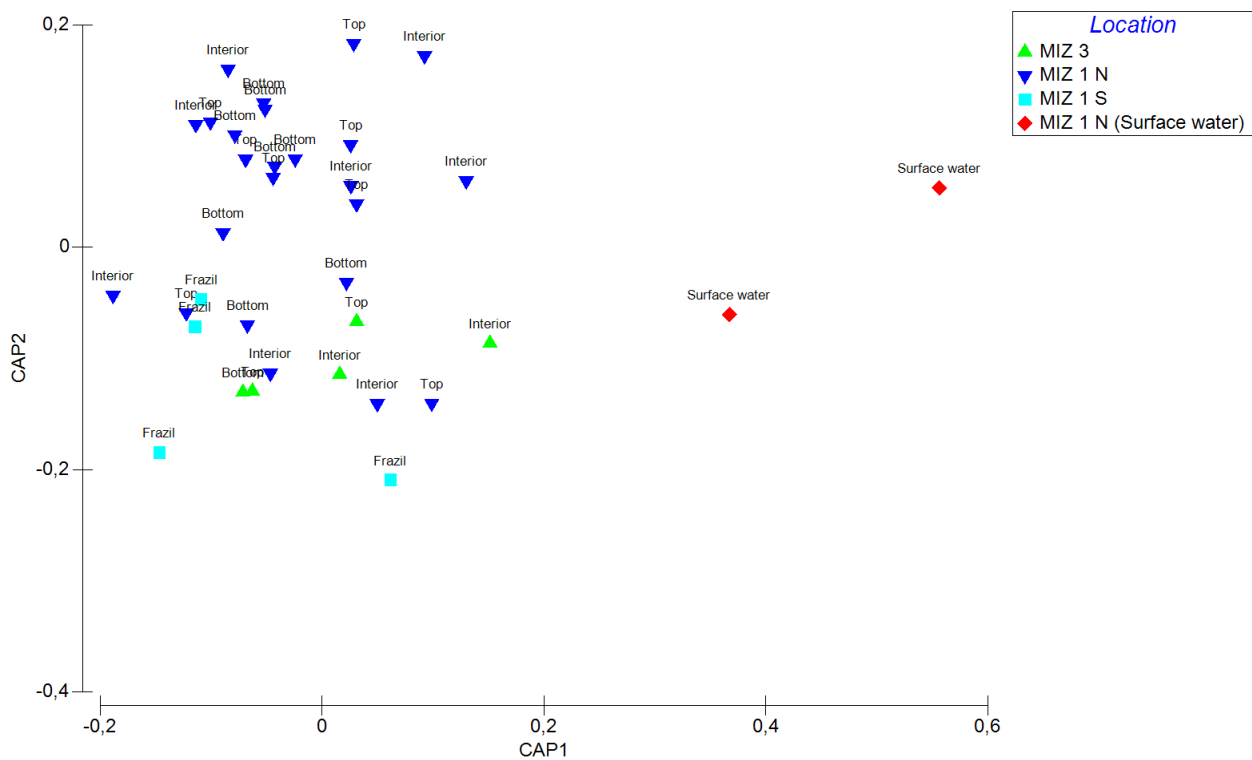| Sea-ice cores (consolidated) MIZ 3		Sea-ice cores (pancake) MIZ 1 N		Frazil sea-ice MIZ 1 S	
Species	Contrib %	Species	Contrib %	Species	Contrib %
Unknown pennates	19.3	*Fragilariopsis* spp.	55.5	Unknown dinoflagellates	16.15
Fragilariopsis spp.	18.2	Unknown pennates	21.05	Unknown ciliates	15.47
Unknown dinoflagellates	10.12	*Coscinodiscus* spp.	6.81	Unknown pennates	15.15
Pseudo-nitzschia spp.	8.94	Unknown centrics	2.2	*Fragilariopsis* spp.	12.68
Leptocylindrus danicus	6.65	*Navicula* spp.	1.95	Unknown diatoms	6.13
Unknown diatoms	6.17	*Leptocylindrus danicus*	1.71	*Coscinodiscus* spp.	5.55
Cylindrotheca closterium	4.93	Unknown dinoflagellates	1.58	*Pseudo-nitzschia* spp.	5.05
Guinardia spp.	3.91			Unknown coccolithophores	4.8
Chaetoceros spp.	3.34			Unknown centrics	4.46
Fragilariopsis kerguelensis	2.86			*Leptocylindrus danicus*	1.58
Unknown ciliates	2.46			*Gymnodinium* spp.	1.58
Coscinodiscus spp.	1.87			*Fragilariopsis kerguelensis*	1.46
Navicula sp.	1.7				
Total contribution (%)	**90.45**		**90.8**		**90.06**

* Dissimilarity between stations MIZ 3 & MIZ 1 N= 66.04, MIZ 3 & MIZ 1 S= 57.71, and MIZ 1 N & MIZ 1 S= 66.67.

3.4 Discussion

This study is located in the Southern-Atlantic MIZ section of the SO bordering on the latitudinal range of the Weddell Sea sector (Figure 3.1). The pancake life cycle is described as the dominant sea-ice formation process in this area, where sea-ice thickness ranges between 0.5 and 0.6 m (Dieckmann et al., 1991; Ackley & Sullivan, 1994, this study). The dominance of a frazil/granular ice-type was anticipated, since floes within this region have been estimated to consist of between 50 and 90 % frazil ice composite (Wilchinsky et al., 2015). The frazil ice lifecycle is responsible for the scavenging of phytoplankton cells suspended in the water column into the sea-ice matrixes (Ewart et al., 2013; Brierly & Thomas, 2002; Clarke & Ackley, 1983). This often explains similarities between sea-ice and surrounding pelagic plankton taxon (Brierly & Thomas, 2002), and possibly the variation in community structure between different sea-ice types.

3.4.2 Sea-ice algae biomass

Average protist biomass was low in the sea ice (< 0.9 µg/L) yet slightly higher than mean biomass of comparable winter cruises of AWECS (2013) in the Weddell Sea and PIPERS (2017) in the Ross sea sector (mean ice thickness 0.79 and 0.87, respectively). However, values were not as high as the spring WedIce cruise in the northwestern Weddell Sea which saw concentrations as high as 16 µg/L, dominated by cells <10 µm size fraction (Peeken et al., 2020). However, the WedIce study is less comparable to this study, since sea ice thickness was much greater in that study – mostly consisting of second year ice. Therefore, this study's low total biomass concentrations could be correlated to the relatively "young" sea-ice age, considering that ice advancement during autumn has been increasingly delayed in both the Weddell Sea and Ross Sea region, having severe implications for the accumulation of sympagic algae (Tison et al., 2020). Additionally, a low ice-algae biomass after initial frazil ice formation could indicate a pelagically seeded heterotrophic ice algae community (Vonnahme et al., 2020). This is postulated, since no significant difference was found between frazil-ice and surface water algae (phytoplankton) communities.

The dominance of large algae cells (between 2.7 and 20 µm) was found in all sea-ice samples, while the greatest accumulation of biomass (average 2.7 µg/L) was observed in frazil ice samples (>2 µg/L) when compared to pancake ice and surface water (<0.2 µg/L). Frazil ice preferential uptake of diatoms, particularly pennate diatoms, is evident when their cell concentration in frazil ice (150 cell/ml) is compared to surrounding that in surface water (<5 cell/ml). This corroborates previous records, revealing the preferential uptake of algae cells of larger cell sizes and specifically diatoms in both the Arctic and Antarctic polar waters (Gradinger & Ikävalko, 1998). Therefore, the importance of frazil ice as an architype environment for initial algae accumulation is emphasised in this study, since biomass reached concentrations usually noted during

bloom conditions (1 mg/m^3) (Lizotte, 2001). However, accumulation of sea-ice algae did not increase from initial frazil ice cell concentration when compared to other sea-ice types.

Average sea-ice algae biomass was significantly higher when compared to the surrounding surface water. Similar to findings of this study's (in 2017) Southern-Indian MIZ, and (2017) PIPERS study in the Ross Sea during early winter. Algae cells incorporated into sea-ice have often been recorded growing in sea-ice brine matrixes, reaching a biomass a few orders of magnitude higher than the surrounding surface water (Post et al., 2014). Survival of algae cells in sea-ice is intrinsically linked to salinity, nutrients, light, and the sea-ice physical structural properties which significantly differ according to distinct sea-ice types (Meiners et al., 2012 ; Andreoli et al., 2000; Krebs et al., 1987).

Higher biomass and abundance was recorded in sea-ice bottom layers, and frazil ice samples ($p<0.05$) when compared to top and interior layers of pancake ice and consolidated sea-ice. Low abundance of larger species and species with spines such as *Chaetoceros* spp., have been correlated to the reduced size of pores and brine channels that limit growth in sea-ice during winter (Ligowski et al., 2012). Therefore, it is hypothesized that the pore size of brine channels at the interior and top layers in this study likely limited or excluded the incorporation of large cells as sea-ice continuously coagulated from initial frazil ice formation (Gleitz & Thomas, 1993). Conversely, during the winter (2013) SIPEX study (East Antarctic pack ice), physical properties related to dynamic (rafting and ridging of floes) and thermodynamic (sea-ice growth) processes, specifically related to sea-ice type (granular, columnar, and frazil ice) did not explain biochemical variations well (Lannuzel et al., 2016). Therefore, other constituents such as EPS and light restrictions are considered contributing to the biochemical variations observed in this studies' (Lannuzel et al., 2016).

3.4.3 Species community structure

Diatoms, particularly pennate diatoms were considerably more abundant than dinoflagellates and other protist groups. This is generally anticipated for Antarctic pack ice algae (Ugalde et al., 2016; Ryan et al., 2006; Lizotte, 2003). Pennate diatom success in the sympagic environment is likely due to inherent benthic strategies employed, and sea-ice formation processes favoring raphe containing species (Ligowski et al., 2012; Eiken, 1992). Additionally, species of *Fragilariopsis* were most abundant throughout all sea-ice and surface water samples. Similar dominant sea-ice algae taxa were observed in this study when compared to the 2017 Southern-Indian Ocean MIZ sea-ice algae (Chapter 2). Like the 2017 sea-ice algae community (Chapter 2), centric diatoms were abundant in all sea-ice samples, highlighting their dominance in marginal ice-edge zones (Horner, 1985). Dominant centric genera differed between sea-ice types in this study. Species abundance of *Leptocylindrus* spp., *Guinardia* spp., and *Chaetoceros* spp. were higher at MIZ 3 (consolidated ice) when compared to frazil and pancake ice samples in which *Coscinodiscus* spp. cells were the most

abundant. Dominance of species in disparate sea-ice types could be correlated to numerous variables: sea-ice physical structure, species morphological plasticity, efficacy to adapt, or presence owed to seeding from underling pelagic water during the sampling period (Lacour et al., 2016; Szymanski & Gradinger, 2016; Ligowski et al., 2012). It should be noted that some cells observed could have been in a dormant state therefore not actively reproducing in the sea-ice (Syvertsen, 1985). (Refer to Chapter 2 Section 2.4.6 for a more detailed report concerning species success owed to physiological traits in sea-ice environments). However, to differentiate between fluctuating abundance and species presence, biochemical variables such as EPS, nutrients and trace macronutrients (iron) could be investigated to resolve uncertainties.

In this study species assemblage and abundance differed significantly ($p(perm) < 0.05$) between sea-ice types, (an average community dissimilarity >57.7 %). Therefore, dominant species could infer environmental limitation or physiological trade-offs required for survival in contrasting sea-ice environments. For example, dominant species within the frazil ice samples were a combination of dinoflagellates, coccolithophores, and ciliates). The frazil ice algae community most likely reflected the initial winter pelagic phytoplankton community, since no significant difference was found between frazil ice and surface water community structure. Likewise, a flagellate-dominant pelagic community has been previously reported in discrete regions surrounding the Antarctic (Peeken et al., 2020) while a diatom-dominated pelagic community was reported in the summer (Takao et al., 2020; Viljoen et al., 2019; Rozema et al., 2016). It is postulated that the transition between large diatom cells and flagellates is owed to the reduction in sea-ice extent, increased stratification, reduced salinities and higher seawater temperature (Biggs et al., 2019). However, changes in phytoplankton regimes (e.g. shifting to a dinoflagellate dominant community) remain to be confirmed due to large number of functional groups (Biggs et al., 2019). It is further hypothesized that sea-ice type (frazil, pancake, or brash ice) can have considerably control over pre-bloom summer phytoplankton community composition and cell size (Biggs et al., 2019). Therefore, the dominant sea-ice type during the "pre-bloom season" could have major implications for structuring the trophic food web in the SO pelagic and sympagic environment.

Community structure in this study showed more similarity between newly formed sea-ice (frazil) and older consolidated ice communities, when compared to pancake ice ones. Similar abundant species; *Fragilariopsis* spp., *Pseudo nitzschia* spp., and *Cylindrotheca closterium* were recorded. These species have been recorded as abundant in both sea-ice and water in Cape Hallett, Bransfield strait, and the Weddell Sea MIZ (Almandoz et al., 2007; Ryan et al., 2006; Kang et al., 2001). However, similar community structures between contrasting sea-ice types are somewhat unusual since frazil ice and consolidated sea-ice originate from different sea-ice growth processes. Consolidated sea-ice forms from slower columnar growth, rejecting organic particulate matter whereas frazil ice formation has a fast growth cycle concomitantly "scavenging" organic particles (Clarke & Ackley, 1983). Therefore, the congelation of newly formed (frazil) ice onto old ice either through

fusing on the top or bottom of the sea-ice floes and rafting of different pancakes could contribute to pancake floes containing disparate physical properties (Doble et al., 2003). This corroborates the findings of Doble et al., (2003) where several pancake ice-types in the Weddell Sea were composed of incongruent sea-ice types (columnar and frazil ice) and displayed deposition at different temporal scales ("new" and "old"). Additionally, average frazil ice biomass and community diversity (Margalef d) was similar between the bottom layer of consolidated pancake ice cores, frazil ice, and surface water. Usually diversity varies with season, geographical location, and ice age. Ice increases in complexity with age, and as a result a more diverse fauna and flora community composition is expected in sea ice (Hop et al., 2020). Therefore, fusing as well as season and geographic distribution is more likely to elucidate diversity and similarities between the two sites, since sampling locations between frazil ice and consolidated ice were in closer proximity when compared to the pancake ice sampling site (MIZ N).

No distinct microhabitats were observed in consolidated and pancake ice samples - species were largely uniformly distributed throughout the ice profile. This suggests that no single species defined individual sea-ice communities of the different layers (Garrison, 1991). Conversely, formation of microhabitats is considered a characteristic feature of Antarctic pack ice revealing habitat preferences of sea-ice algae assemblages in the depth layers i.e. surface, interior, and bottom (Meiners et al., 2012). Lack of microhabitats observed in this study could suggest that the sea-ice algae community was recently established due to the relatively young ice age. This is supported since the highest accumulation of species, abundance, and biomass was found within the bottom layer of sea-ice while sea-ice samples were considered relatively thick (6 m). Usually when sea-ice algae are concentrated within specified layers, sea-ice age could be associated with the distribution of biomass (or abundance). For example, interior and surface communities are commonly observed in older ice or when physical processes such as excessive snow loading, surface flooding, melting, and refreezing occurs (Ugalde et al., 2016). However, dense bottom communities exist when sea-ice is relatively young and thick (between 0.4 and 1 m), respectively (Meiners et al., 2012).

3.5 Conclusion

The results of this study have shown that scavenging via the frazil lifecycle is an important biological driver during winter in the Southern-Atlantic MIZ. This was highlighted by the high accumulation of algae cells in frazil ice, and similar taxa found within the underlying surface water. However, it is questionable if initial accumulation of algae cells have any influence or correlation to sea-ice algae community residing in older more consolidated pancake ice. Regardless, sea-ice algae biomass was higher in all sea-ice samples when compared to underlying surface water (< 6 m), supporting the hypotheses that concentration rather than production is a more significant feature of the SO MIZ during winter (Lizotte, 2003). It was evident that sea-ice algae species from the order *Bacillariales* (*Fragilariopsis*, *Pseudo-nitzschia*, and *Navicula* spp.) and *Biddulphiales* (*Leptocylindrus* and *Coscinodiscus* spp.) was dominant. Conversely, the pelagic phytoplankton community were dominated by flagellate species. However, this study could not corroborate what caused the presence or absence of sea-ice algae species and associated community variations according to disparate depth layers and sea-ice types. Therefore, biogeochemical parameters and physical sea-ice properties are required to elucidate winter sea-ice algae community trends. Further work is now needed to understand what drivers influence algae accumulation according sea-ice types, since long-term climate trends predict the change in dominant Antarctic sea-ice type (first year ice over second year ice) in the following decade. This will adversely affect what sympagic biota will proliferate in sea-ice as illustrated in this study.

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Chapter 4

The study of common melting procedures used to determine for the abundance and community structure of Antarctic sea-ice algae.

4.1 Introduction

Microalgae and bacteria living within sea-ice play a fundamental role in Antarctic biogeochemistry (Torstensson et al., 2019). Approximately 25 % of the primary production in sea-ice covered regions of the Southern Ocean (SO) can be attributed to microalgal and bacterial species (Torstensson et al., 2019). Antarctic sea-ice is often characterised by having steep gradients regarding temperature, salinity, and light attenuation (Krell et al., 2007). Temperature values can range between 0 °C and -15 °C for melting sea-ice and winter sea-ice, respectively (Torstensson et al., 2019). Comparatively, Antarctic sea-ice salinity typically varies between 5 and 70 PSU which can be three times that of the surrounding surface waters (Torstensson et al., 2019; Thomas & Dieckmann, 2002), and values of up to 173 PSU have been recorded in McMurdo Sound Bay (Arrigo, 2017). With such extreme variation in physical conditions, dehydration is therefore a major stressor for micro algae population survival during austral autumn and winter months (Thomas & Dieckmann, 2002). Although it has been observed that sea-ice algae are able to photosynthesise and grow in psychrophilic brine conditions, their chances of survival are mostly enhanced when corresponding osmoregulation can take place (Torstensson et al., 2019). Conversely, when sea-ice melts in spring and hypothermic conditions persist, salinities can drop close to that of fresh water (Thomas & Dieckmann, 2002). Additionally, photosynthetic stress is said to be higher when sea-ice algae is released into hyposaline surface water compared to freezing conditions experienced during incorporation of algae cells into sea-ice matrix (Ralph et al., 2007).

Salinity and other ephemeral sea-ice conditions are usually combated by sea-ice algae through decomposing, accumulating, synthesising, and releasing a wide range of organic compatible solutes (OCS) (Thomas & Dieckmann, 2002; Krell et al., 2007). OCS have various functions in microorganism cells and are highly soluble organic molecules (Torstensson et al., 2019; Krell et al., 2007). However**,** **w**hen OCS accumulate in microorganisms in water limited environments, they usually function as osmolytes (Torstensson et al., 2019). Therefore, OCS can also be considered as a synonym for organic osmolytes (Krell et al., 2007). When biological processes associated with the regulation of OCS are forced or inhibited, sea-ice algae respond through a change in community structure and abundance. It is therefore important to investigate the response of Antarctic sea-ice algae to processing, when osmolyte production may be affected, such as when sea-ice is melted in order to study the protist communities.

4.1.1 Osmoregulation

Osmoregulation is the process organisms used to maintain a constant internal osmotic potential (Kirst, 1990). Turgor regulation is the adjustment of cellular osmotic potential to maintain a constant turgor pressure. This terminology is preferred when investigating microalgae under osmotic stress (Kirst, 1990). Physiological and biochemical alterations of cell organisation during salt stress can also be described using the terms osmoacclimation, osmotic adjustment or osmoadaptation (genetic modification) (Kirst, 1990). Sea-ice algae can be effected by a change in salinity in three primary ways: [1.]Disturbance of cellular water potential (osmotic stress), [2.] ionic stress, and [3.] cellular ionic ratios controlled by a selective permeable membranes (Kirst, 1990).

Osmoacclimation is regulated by a feedback mechanism, described as a two phase system including a detector and effector. This system allows an efflux or influx of water following the osmotic gradient, and osmotic adjustment occurs through regulation of the cellular concentration of organic osmolytes and ions (Kirst, 1990). The first phase of osmoacclimation is a fast (5-10 second) transient process, passively dependent on the physicochemical alga cell membrane properties such as permeability and elasticity (Kirst, 1990). The second phase is slower (40-120 min), and is controlled through metabolic function by adjusting the osmotic potential (Kirst, 1990). Thus, when there is a change in inorganic ion concentration in the external environment (brine in the case of sea-ice flowing over semi permeable cellular membrane), cellular homeostasis is disturbed (Krell et al., 2007). Sea-ice algae experience disturbance in cellular homeostasis or osmotic stress during various natural conditions in the SO. These include [1.] incorporation of algae cells during sea-ice formation [2.] seasonal sea-ice melt, and [3.] diurnal fluxes in physicochemical brine environment (Martin & McMinn, 2018; Krell et al., 2007).

4.1.2 Osmolytes

Micro algae are able to tolerate osmotic stress through the regulation of osmolytes to achieve osmotic balance (Thomas & Dieckmann, 2002). Osmolytes are either accumulated and synthesised during hypersaline conditions or released and decomposed during hyposaline conditions (Thomas & Dieckmann, 2002). Osmotic balance is therefore the equal distribution of osmolyte concentrations between algae cells and the surrounding sympagic environment (brine) (Thomas & Dieckmann, 2002). Osmotic pressure is not only relieved by osmolytes but by means of inorganic ions and compatible solutes (Thomas & Dieckmann, 2002). Ionic composition of micro algae cell and vacuole sap varies according to species. Potassium (K^+), sodium (Na^+), chlorine (Cl^-) and sulphate (SO_4^{2-}) are considered the primary ions usually involved during salt stress (Kirst, 1990). Compatible solutes include, sugars, polyols, and amino acids, which can also aid as a cryoprotectant (Lyon & Mock, 2014). Some sea-ice algae can be more acclimated to osmotic stress through

morphological adaptation, control of osmolytes, and OCS (Mikkelsen & Witkowski, 2010). For example, OCS such as glycine betaine (GBT), is commonly recorded in high concentrations in sea-ice diatoms during salinity changes (Torstensson et al., 2019). GBT is synthesised in most taxonomic groups of microorganisms and is considered the most abundant compatible molecule on the planet (Torstensson et al., 2019). The rate of uptake or release of GBT, choline, and intracellular pools during sea-ice melt is still largely unclear, but could have implications for the physiological and ecological fate of sea-ice algae (Torstensson et al., 2019).

4.1.3 Sea-ice processing

It is difficult to separate the sea-ice matrix from the brine medium when processing ice samples. This is problematic because sea-ice algae live within the brine network (Garrison & Buck, 1986). A variety of artificial sea-ice melting procedures have been used to extract sea-ice algae so that the community structure may be determined. An inaccurate representation of the algal community may result due to osmotic shock during the melting method. Osmotic shock occurs during salinity shifts from brine medium to melt samples, indicated by a hypertonic change in salinity values of 3 to 8 % (Garrison & Buck, 1986).

To accurately measure the communities living in Antarctic sea-ice, consideration should be given to melting procedures during processing. For example, greater species diversity has been measured in brine drained directly from ice floes, compared with that measured in processed (melted) sea-ice samples (Garrison & Buck, 1986). Species with a more rigid structure, such as silicified organisms (including diatoms and archaeomonads) may be over-represented in protist community assemblages compared to ciliates and flagellates (Garrison & Buck, 1986). This could be because these organisms are less prone to cell lysis from osmotic shock. The cell concentration and ecological role of ciliates and flagellates in the SO may have been severely underestimated (Garrison & Buck, 1986). It follows that direct drainage from pore waters should be used rather than melted sea-ice samples.

This study aims to identify the effect of two contrasting sea-ice melting methods commonly used when studying the algal communities of sea-ice cores in polar regions. In particular, the effect of melting methods on the measured sea-ice algae community structure and abundance in the SO MIZ during austral winter 2019 was used (**Figure 3. 1**).

4.2 Methodology

4.2.1 Sample collection

A total of six sea sea-ice samples were collected from four sampling sites in the MIZ during austral winter, July 2019 on board the SA Agulhas II (**Figure 3. 1**).

Frazil sea-ice samples (FS) of a slushy-like consistency were collected between semi-consolidated pancake ice at -57° 00' 007" S; 00° 31' 300" E and - 57° 34' 503" S; 00° 00' 28" E, respectively. A frazil sampler was used for the collection of frazil ice and surrounding water, and separated using a mesh sheet. One FS were collected at two sample stations, and subsequently divided in half, resulting in a replicate FS (rFS) per sampling site. All FS and rFS was melted at room temperature (20 °C) in the dark to prevent further photosynthetic processes. 500 g of FS were melted in 500 ml of filtered seawater (0.3 μm GF/F) of a salinity between 33 and 34.5 PSU which coincided with surface salinity measurements at all sample sites. In situ environmental conditions, specifically salinity, can be used as a buffering method, to provide the most accurate biological results (Ryan et al., 2004). 400 g of rFS were then melted without the use of a buffer medium.

Two semi-consolidated and consolidated sea-ice cores were collected using a sea-ice corer from 56 80 178 °S, 0 30 262 °E (MIZ S) and 58 13 783°S, 0 00 442 °E (MIZ 2), respectively (**Figure 3. 1**). Sea-ice cores were transported in black plastic bags on board to avoid light exposure and prevent further photosynthetic responses. Sea-ice cores averaged approximately 60 cm in depth, and were cut with an electrical band saw into 10 cm depth sections. Three sections (top, interior, and bottom) from each core was used for analyses. One core from each sampling site was melted at 4 °C for approximately 8 hours in filtered seawater (FSW, 0.3 μm) using a dilution ratio of 1:8 for each 10 cm section. Sea-ice samples were stirred every hour to homogenise the sample and prevent rapid warming. One core from each sampling site was melted with no buffer medium (direct melting), at room temperature, for a period of approximately 12 hours, in the dark.

Sea-ice samples were then examined for chlorophyll a and sea-ice algae identification using light microscopy (refer to Chapter 2). Note that chlorophyll a was not measured for all sea-ice samples since sea-ice volume after direct melting was not sufficient for analysis.

Table 4. 1: Sea-ice procedure according to sea-ice type and sampling location.

Location	Ice type	Name	Buffered (dilution ratio)
S1 (-57° 00' 007" S; 00° 31' 300" E)	Frazil	FS	N
S1 (-57° 00' 007" S; 00° 31' 300" E)	Frazil	rFS	Y (2:1)
S2 (- 57° 34' 503" S; 00° 00' 28" E)	Frazil	FS	N
S2 (- 57° 34' 503" S; 00° 00' 28" E)	Frazil	rFS	Y (2:1)
S3 (-56° 80' 178" S; 00° 30' 262" E)	Pancake ice	Ice core (semi-consolidated)	N
S3 (-56° 80' 178" S; 00° 30' 262" E)	Pancake ice	Ice core (semi-consolidated)	Y (8:1)
S4 (-58 13783 °S; -0 00442°E)	Pancake ice	Ice core (consolidated)	N
S4 (-58 13783 °S; -0 00442°E)	Pancake ice	Ice core (consolidated)	Y (8:1)

4.2.2 Statistical analysis

Non-parametric statistics was performed using IBM SPSS 26 package. Sea-ice algae primary groups were summarised for quantitative measures (descriptive statistics) and a Wilcoxon signed-rank test was used to compare the two related samples (from corresponding sea-ice core/frazil sample), and to test the hypothesis that the sea-ice algae population mean ranks differ significantly ($p < 0.05$) between the two melting methods.

Multivariate statistical analysis was performed using the Primer 6, Permanova + package. A one way permutational multivariate analysis of variance (PERMANOVA) was employed to test if the ordination of the sea-ice algae community distance between centroids differ significant between sea-ice samples directly melted and melted with FSW (abundance and community structure) ($p < 0.05$). The similar percentage routine (SIMPER) using Bray Curtis distance matrix was used to identify species contributing the most towards dissimilarity between melting methods (shown as species contribution (%) and average abundance). Standard diversity indices was determined using the Shannon Wiener diversity index with respect to melting method (Hayek & Buzas, 2010). Refer to Chapter 2 for further clarification on statistical methods applied.

4.3 Results

Average abundance (cells/ml) of most functional protist groups were higher in sea-ice samples melted with FSW, compared to samples directly melted (**Figure 4. 1**, **Figure 4. 2**, **Table 4. 2**). Conversely, sillico flagellate cells showed higher average abundance in samples melted directly, and were absent from frazil ice samples melted with FWS. Centric diatoms also showed higher average abundance in sea-ice cores melted directly opposed to frazil ice melted directly. Dinoflagellate abundance was significantly ($p<0.05$) lower when samples were melted directly compared to those melted in FWS.

Average species richness (d), number of species (S), and species diversity (Shannon, H') was higher in samples directly melted (**Figure 4. 3**). Species were mostly evenly distributed (Pielou J') except for FS directly melted (J= 0.5).

A total dissimilarity of 57.6 % in sea-ice protist community structure was found between sea-ice samples directly melted and melted with FSW. However, according to the one-way permutational multivariate analysis of variance (PERMANOVA), no significant difference in species community ordination was found. Sea-ice algae contributing the most to dissimilarity between sea-ice samples directly melted and melted with FSW were identified as: *Fragilariopsis* spp., *Pseudo-nitzschia* spp., unknown pennate diatoms and diatoms, *Leptocylindrus* spp., unknown centric diatoms, *Cylindrotheca Closterium*, and *Guinardia* spp. (**Figure 4. 4**).
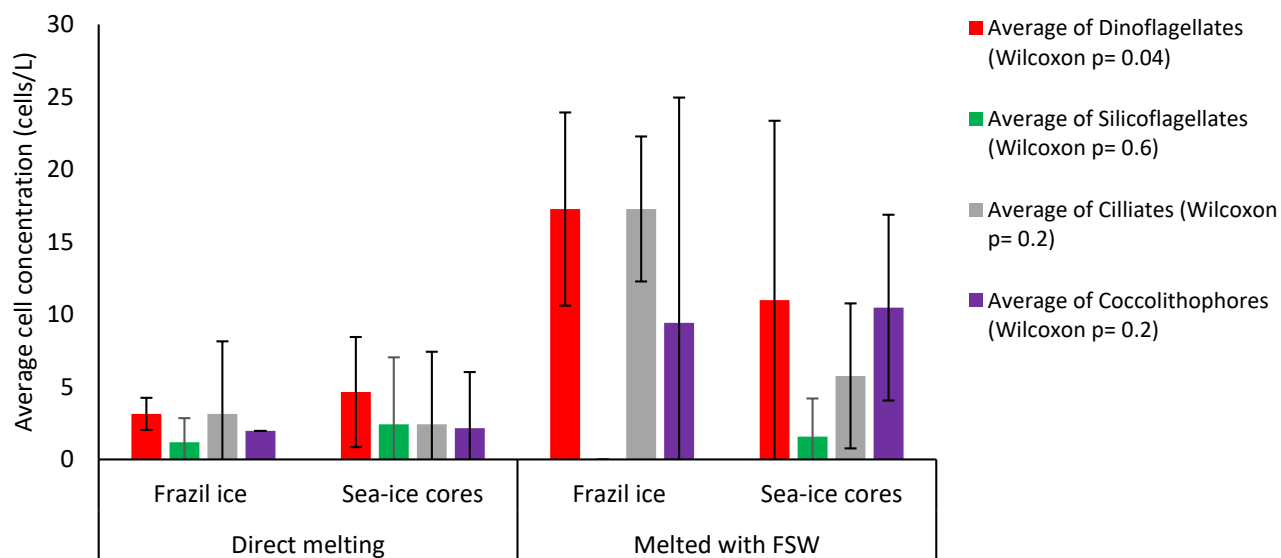

Figure 4. 1: Average cell concentration of primary protist sea-ice groups according to melting procedure Vertical bars denote standard deviation (+/-SD) of the mean.
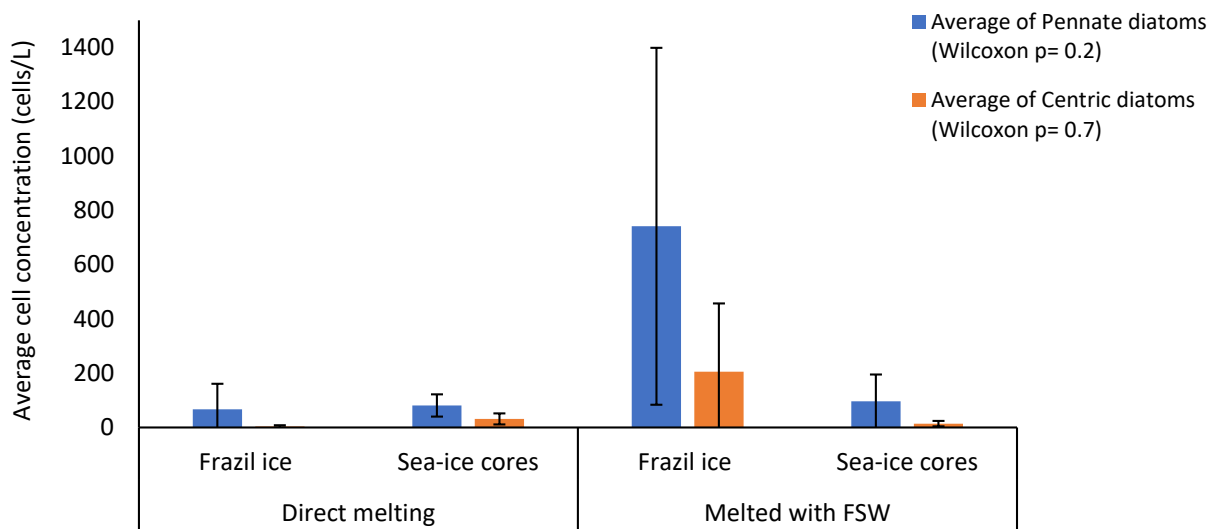
Figure 4. 2: Average cell concentration of diatoms according to melt procedure and sea-ice type. Vertical bars denote standard deviation (+/-SD) of the mean.

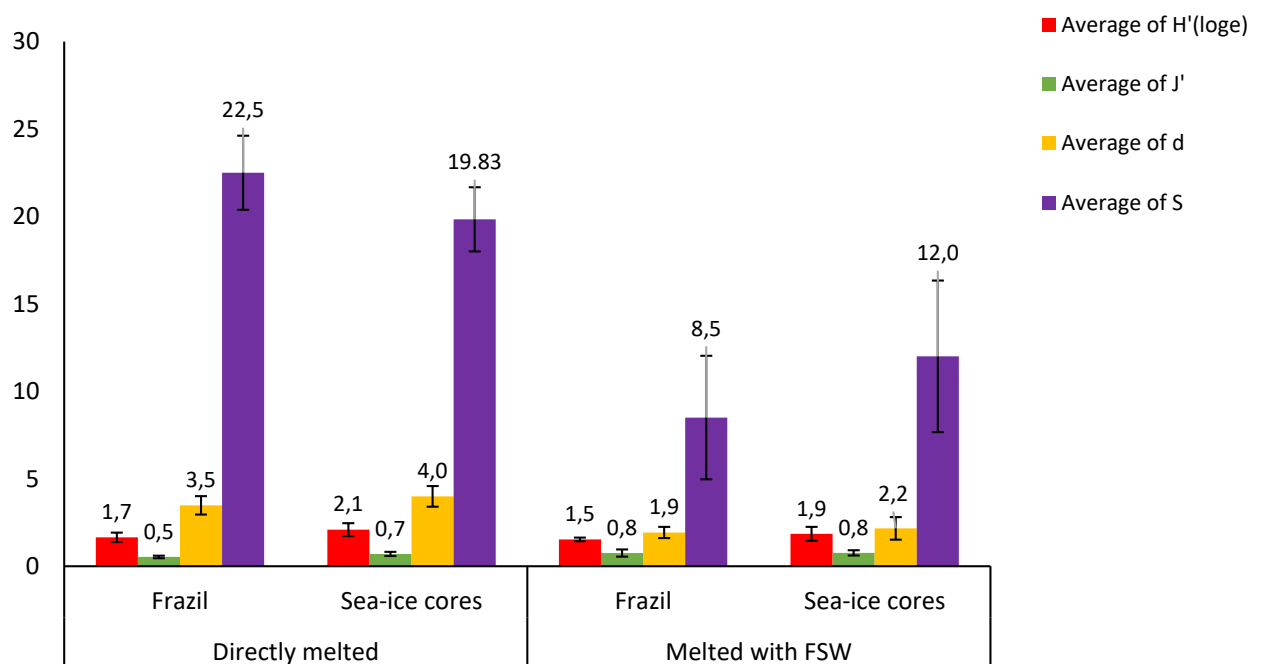**Figure 4. 3:** Average diversity indices according to melt procedure and sea-ice type. Vertical bars denote standard deviation (+/-SD) of the mean.
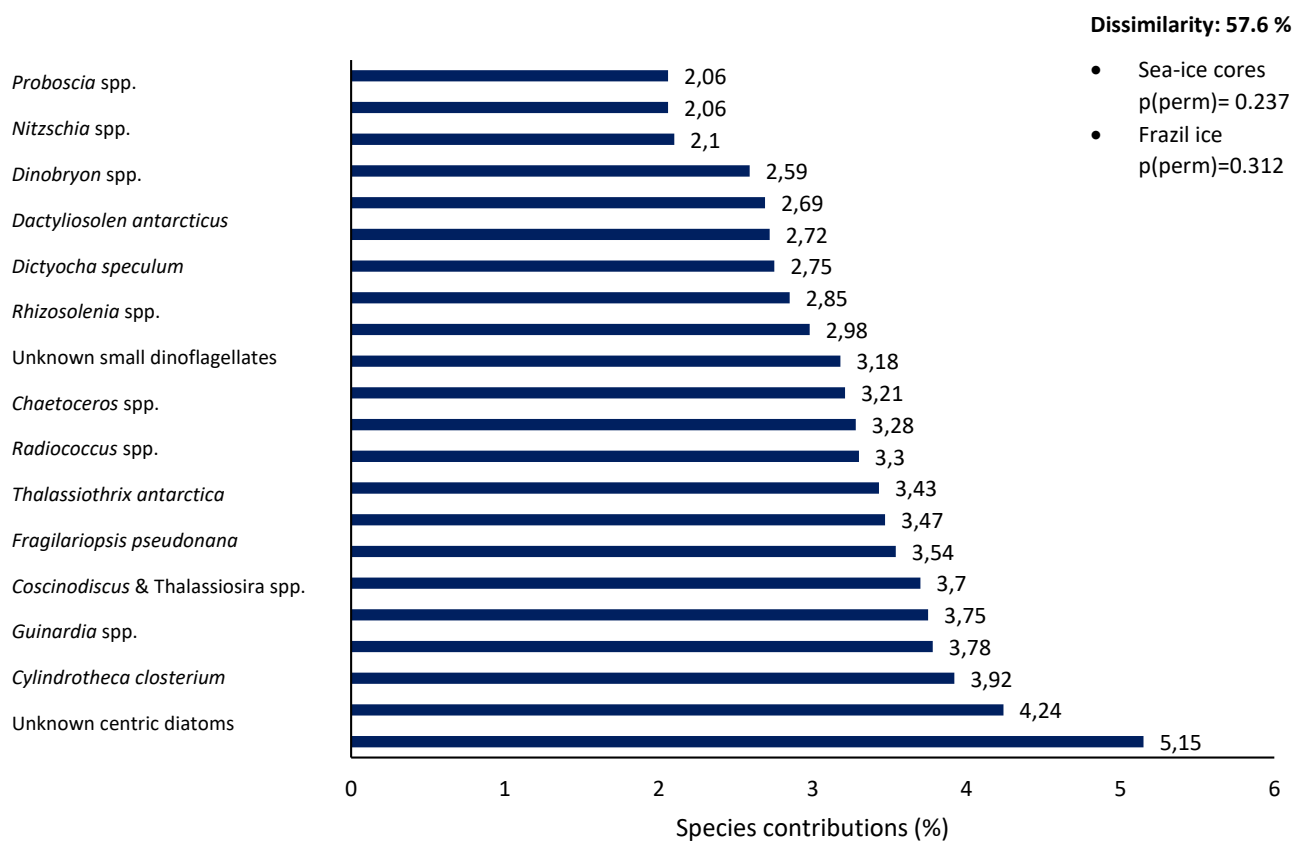
Figure 4. 4: Contribution of each species (%) to the dissimilarity of community structure (SIMPER) between sea-ice melting methods. Total dissimilarity (57.6 %) and significant difference between species community ordination according to sea ice melting method indica

Table 4. 2: Average abundance of protist genus across sea-ice samples according to melting method.

Species	Melted With FSW Ave. Abundance (Cells/ml)	Directly Melted Ave. Abundance (Cells/ml)
Fragilariopsis spp.	2.93	2.31
Pseudo-Nitzschia spp.	1.76	0.86
Unknown coccolithophores	0.96	0.72
Unknown pennate diatoms	2.02	1.76
Unknown ciliates	1.16	0.93
Unknown diatoms	1.38	0.85
Leptocylindrus danicus	1.04	1.06
Unknown centric diatoms	0.88	1.07
Cylindrotheca closterium	0.5	0.91
Guinardia spp.	0.33	0.95
Coscinodiscus spp.	0.93	0.79
Fragilariopsis p*seudonana*	0.67	1.01
Thalassiothrix antarcticus	0.82	0.44
Radiococcus spp.	0.46	0.53
Chaetoceros spp.	0.5	0.66
Unknown small dinoflagellates	1.56	1.17
Rhizosolenia spp.	0.78	0.22
Dictyocha speculum	0.36	0.69
Dactyliosolen antarcticus	0.42	0.69
Dinobryon spp.	0.47	0.14
Nitzschia spp.	0.6	0.12
Proboscia spp.	0.3	0.45

4.4 Discussion

Melting of solid sea-ice is usually conducted in the dark to avoid light stress in dark-adapted microorganisms often found in polar regions (Campbell et al., 2019). Conversely, factors such as melting time, temperature, buffer-medium type, and buffer-medium quantity is inconsistent in most melting procedures reported (Campbell et al., 2019), providing no fixed standard.

4.4.1 Species-specific response to melting process

The current study identified no significant ($p(perm)<0.05$) variation in species community-structure between samples directly melted or melted with FSW. However, species richness, number of species, and species diversity was lower in all sea-ice samples melted with FSW. Therefore, this indicates that sea-ice algae groups with low abundance (such as the silicoflagellates) might be missed in the FSW samples because of the dilution factor. It should also be noted that the small volume of sea-ice sample used for taxonomic identification could bias findings for samples "buffered" with FSW (refer to section 4.2). Sea-ice algae species containing a more rigid cell walls (e.g. *Stichococcus bacillaris*) are often able to tolerate and photosynthesise during hypo osmotic conditions better compared to high salinity environments (Kirst, 1990; Ryan et al., 2004). For that reason, species may dominate community structure due to species-specific osmoadaptation. However, this is not mutually exclusive from osmoacclimation (Kirst, 1990). Species such as *Nitzschia stellata* have dominated Antarctic sea-ice community structures during high salinities and vice versa for species of *Pleurosigma/Amphiprora/Pinnularia* (Ryan et al., 2004). Therefore, species that acclimate better in hypo osmotic conditions are more likely to be recorded in samples buffered with FSW.

Large dissimilarity (57.6 %) between groups indicated that pennate species of the genera *Cylindrotheca*, *Fragilariopsis* and *Pseudo-nitzschia* spp. contributed the most towards dissimilarity between sea-ice samples melted with FSW and directly melted, the latter likely due to higher average abundance in samples melted with FSW. Higher average abundance of *Pseudo-nitzschia* species could be due to osmotic adaptation, slow acclimation or species specific tolerances due to morphological disparities. Dominance of *F. curta* and *F. cylindrus* in both fast and pack ice, ice edge blooms, and open water are widely reported (von Quillfeldt, 2004). Widespread distribution is due to naturally tolerating a wide range of salinity each season, while having an strong affinity to colder regions (von Quillfeldt, 2004). Accumulation of the amino acid proline during sea-ice formation (conditions characterised by decreased temperature and increased salinity) could contribute to the initial survival of pennate diatom *F. cylindrus* as shown during experimental growth experiments (Krell et al., 2007). The importance of proline during cold and salt stress is also indicated where genes and proteins associated with the amino acid are elevated during synthetisation (Lyon & Mock, 2014).

Rigidity of cell structure might have played a role in the difference between measurements of the communities after the different melting methods . The weakly silicified cell wall of *Pseudo-nitzschia* spp. reduces their tolerance to osmotic stress, and likely explains their higher abundance in samples melted with FSW (Hasle, 1994). However, *Pseudo-nitzschia* spp. are known to tolerate salinities up to 45 PSU (Thessen et al., 2005). It is important to note however, that even within a genus, optimum growth within a specified salinity range can vary (Sugie et al., 2020; Thessen et al., 2005).

Dinoflagellate abundance was measured to be significantly different ($p<0.05$) between the two melting methods, with a higher average abundance in samples melted with FSW. Therefore, over-representation of a diatom dominated community was likely recorded in sea-ice samples melted relatively quickly, and with no buffer medium. (Roukaerts et al., 2018; Mikkelsen & Witkowski, 2010). Again, this is the likely impact of a rigid cell structure slowing down the process of cell lysis, which is thought to affect species in the flagellate group more negatively (Roukaerts et al., 2018). A significant difference between community structure and abundance of protist functional groups has been previously recorded when sea-ice is melted at 20 °C with no buffer medium, and when using a buffer medium (Mikkelson & Witkowski, 2010). In the Mikkelson & Witkowski (2010) study, the authors found that flagellates, chrysophytes, chlorophytes, and dinoflagellates were significantly different, while diatoms, cyst and cryptophyte functional groups were not (Mikkelson & Witkowski, 2010). As Mikkelson & Witkowski, (2010) predominantly focussed on microscopic identification of primary sea-ice algae functional groups, and not all protist groups, caution is encouraged when comparing findings directly with this study. Although this study is clearly dominated by diatoms in both melting methods, similar to findings of Mikkelson & Witkowski, (2010). The dominance of diatoms in sea-ice algae communities over other functional protist groups could also be due to preservation methods of sea-ice samples (Garrison & Buck, 1986). Garrison & Buck, (1986), recorded a loss of 65 % to 95 % of flagellate species when sea-ice algae species were counted and identified as a preserved sample rather than in situ counting using no preservation substance added directly after collection. The ciliate group was also found to be the most affected by preservation method. Similarly therefore, counts of hetero and autotrophic flagellate groups from Garrison & Buck, (1986) should be compared cautiously to those found in this study.

4.4.2 Temporal effect of melting procedures

Phytoplankton can adapt to a new temperature and light regime in just 35 hours while a new generation can be observed in <1.5 days (Rintala et al., 2014). Although in this study, sea-ice samples were melted over a short period (8 hours), biochemical processes have been found to occur over a much faster rate of approximately four hours (Campbell et al., 2019). This has been observed where the pigment diatoxanthin concentrations can change within minutes (Rintala et al., 2014). Rintala et al., (2014) recommend the avoidance of the use of artificial seawater as a buffer medium or leaving the sample in the dark for a

prolonged period. Melting sea-ice at colder temperatures prolongs the melting process. This in turn increases the likely continuation of biological processes, such as predation and bacterial degradation (Rintala et al., 2014). Other biological processes that could continue include growth, pigment alterations, and change in community structure (therefore resulting in erroneous assumptions regarding ecological function) (Rintala et al., 2014). Additionally, melting samples in the dark for a prolonged period could also induce light starvation and subsequent death of autotrophic species (Rintala et al., 2014). However, Mikkelson & Witkowski (2010) suggest that slow melting procedures at 4 °C and with a buffer medium of salinities between 10 and 20 could allow for historic community taxonomic comparison. Mikkelson & Witkowski (2010) also highlight that slow melting (measured in days) relieves osmotic stress on sea-ice algae cells, and species are able to adapt (osmotic acclimation) to varying salinities. According to Mikkelsen & Witkowski (2010), melting method could either be short or extended over a longer period of time and with or without a buffer medium. It has been shown that the longer the period of melting time (of approximately >1 day and < 1 week), the use of a buffer medium, and direct melting at 4 °C showed comparable taxonomic structure (Mikkelsen & Witkowski, 2010). It is therefore reasonable to assume that the microbial community had time to acclimate to salinity shifts using both a buffer medium and no medium at all. This result is comparable to this study, however even a short period of melting could be applied.

4.5 Conclusion

The use of a buffer medium when melting sea-ice sample has been well documented (Roukaerts et al., 2018; Garrison & Buck, 1986). A minimum dilution ratio of 1:2 with a salinity above 28 % is suggested to minimize osmotic stress (Ryan et al., 2004). The use of a FSW three to four times the volume of the respective sea-ice sample have also been recommend by multiple studies as a viable solution to alleviated stresses associated to osmotic shock (listed in Campbell et al., 2019).

Sea-ice algae species in this study did not respond to melting procedure on a species specific level, as no significant variation in community structure is observed; this is comparable to results shown in Mikkelsen & Witkowski, (2010). No clear conclusion can be drawn regarding higher abundance of any particular species, when ice is melted with a buffer medium (FSW) with the exception to the dinoflagellate functional groups. It is common for flagellate species to be more susceptible to osmotic stress due to their morphological inferior cellular structure compared to diatoms. This further elucidates the higher abundance of most protist groups in samples melted with FSW. However, biochemical parameters not measured and thus unaccounted for, such as EPS and other osmolytes could further motivate this study's findings. It is possible to postulate however that an acclimation time of 8 hours and a salinity similar to that of the surface water of sample site clearly favoured growth of some species (higher abundance), especially in frazil sea-ice samples. Therefore, serious consideration to melting procedure should be made with respect to sea-ice type that is being processed.

To deduce a viable melting process for Antarctic sea-ice algae and protists communities, it is necessary to differentiate between qualitative and quantitative accuracy. This study shows that sea-ice algae community structure can be accurately demonstrated with both direct melting at room temperature and with buffer medium (FSW) at 4 °C. However, some species might be absent when melting with FSW at a ratio of 1:8 for a duration of 8 hours. Therefore, this work highlights the importance of the melting process, similar to the study conducted by Rintala et al., (2014). Sea-ice samples should be melted over short period of time (12 hours) at room temperature, and gently shaken to maintain a homogeneous cool temperature. Accurate representation of all protists should also be considered, given the possible underestimation of dinoflagellate abundance found in this study.

4.6 References

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General conclusion

During winter field campaigns conducted in 2017 and 2019 in the MIZ of the SO, sea-ice and underlying seawater samples were collected for the analysis of phytoplankton abundance, biomass, species description and nutrient concentrations. It is hypothesised that variability in these parameters can explain any change in dominance of sea-ice algae functional groups. It may subsequently be possible to identify sea-ice-driven modifications of higher trophic food webs in the SO pelagic environment.

Comparison between the two winter campaigns revealed marked differences in sea-ice algae biomass and distribution. In 2017 sea-ice algae formed microhabitats throughout the sea-ice depth profile, proliferating within the sea-ice interior reaching chlorophyll a concentrations of 8 µg/L. This was in contrast to the absence of microhabitats observed in 2019, where sea-ice algae were concentrated in the bottom layers, reaching a concentration of 2.2 µg/L. These biological trends, and other biochemical sea-ice dynamics (algae-macronutrient relationships, stoichiometry ratios, and physical sea-ice properties) investigated, made it possible to conclude that the sea-ice algae community was well-established in 2017. However, this study cannot confidently corroborate if the sea-ice algae community in the 2019 winter campaign was a function of circumstantial conditions (temporarily residing as an assemblage), or a different successional stage community living in sea-ice, since species metabolism and nutrient stoichiometry could not be compared to surrounding pelagic phytoplankton community. Therefore, large heterogeneity in sea-ice algae community observations over the two-winter campaign motivates for increase sampling efforts during winter, particularly in the Southern-Atlantic and Indian MIZ.

Despite these regional differences, morphological traits revealed several similarities between the two-winter campaigns. For example; sea-ice algae taxa showed similar dominant species from the genera; *Fragilariopsis, Pseudo-nitzschia, Coscinodiscus* and *Chaetoceros* spp., while algae cells were mostly observed in the larger cell size class >2.7µm. Additionally, each winter campaign revealed a much greater species abundance (particularly pennate diatoms), and biomass in all sea-ice types when compared to the underlying water. For this reason, this study highlights the importance of sea-ice within the MIZ during winter as an ecologically significant feature impacting overwintering protist and other plankton communities such as krill. This finding has substantial implications for the greater SO food web due to the ever-increasing ice-free ocean environment predicted as a result of global climate change.

Overwintering sea-ice algae communities (in both consolidated and semi-consolidated pancake ice) generally were not correlated to underlying pelagic phytoplankton communities. Therefore, it is hypothesised that despite the dominant ice type (i.e. variation in biogeochemical and physical sea-ice properties) during winter, large sea-ice algae species can be found within the sea-ice, either growing or passively accumulated (spores).

Numerous parameters could have contributed to (dis)similarities observed in this study, such as synoptic-scale weather events, time of sea-ice formation, physical sea-ice properties, and availability of macronutrients and micronutrients.

As adverse anthropogenic effects continue to potentially cause anomalous variability in SO sea-ice dynamics (sea-ice concentration, type, and time of ice-advancement and melt), it will be important to determine biogeochemical drivers and productivity of the SO pelagic and sea-ice ecosystem over complete seasons. These biogeochemical parameters will enable remote sensing efforts to understand past, present, and future trends. Additionally, remote sensing methods are increasingly favoured for data acquisition as opposed to in-situ sampling methods due to greater spatial and temporal datasets, and cost- efficacy. However, in-situ data required for remote-sensing proxies is still lacking for austral winter in the MIZ, particularly within this study's sampling locations. For example; depth-integrated chlorophyll estimates during winter months are mostly inaccurate due to discrepancies in satellite signals concerning estimation in sea-ice concentration. Therefore, this study provides significant biochemical records that can be introduced in satellite measuring tools and climatic models calculating depth-integrated chlorophyll and other biogeochemical parameters.