Life in the freezer: The role of dimethylsulfoniopropionate (DMSP) in the physiological and biochemical adaptations of Antarctic microalgae

Cristin Sheehan

July 2019

Supervisors: Dr Katherina Petrou and Professor Peter Ralph

Climate Change Cluster

School of Life Sciences, University of Technology Sydney

This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Science

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Cristin Sheehan declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy in Science, in the School of Life Sciences at the University of Technology Sydney. This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research is supported by an Australian Government Research Training Program Scholarship.

Production Note:
Signature removed prior to publication.

Cristin Sheehan

July 2019

ACKNOWLEDGEMENTS

The support of many people has made this work possible. First and foremost, I need to thank my supervisors, Dr Katherina Petrou and Prof. Peter Ralph. Katherina whose passion not only inspired me to start a PhD but continues to inspire me by proving that with hard work, dedication and some smarts you can achieve so much and still have fun along the way! Peter for providing me with the opportunity to undertake a PhD as part of his incredible team at C3 and ongoing advice.

I would like to acknowledge the financial and technical support I received from UTS, the Australian Antarctic Division and the Australian Synchrotron. I'd like to express my gratitude to the UTS technical staff Gemma, Kun, and in particular, Paul Brooks for not running at the sight of me, instead finding a solution to the many and varied problems I'd come to him with. To UTS and my lab friends (especially James O'Brien and Steph Gardner) for sharing their skills and knowledge and a helping hand when needed.

Thanks to my amazing friends, Georgia, Janani, Steph and Jess for their continuous encouragement, understanding and the occasional motivation words or gifs when needed. My uni buddy, Caitlin Austin, who I convinced it would be fun to do a PhD together. It has been fun for the most part but for the times it wasn't, thank you for all the pep talks, providing the chocolate and baked goods, and reminding me I'm not alone in all this.

Lastly and by no means least, I cannot thank my family enough for their love, support and encouragement not only over the last four years but all the ones leading up to it. My parents and brother, Brendan for listening to me ramble on even when they didn't understand what I was talking about. Without their practical, emotional, financial and moral support none of this would have even been conceivable. Thank you.

ABSTRACT

Marine microalgae are the fuel of the Antarctic ecosystem and changes in primary production can impact the entire food web, as well as the nutritional value at the base of the food web which is dependant not only on biomass but also the macromolecular content of the individual species. Primary production by Antarctic microalgae is also of key importance in the biogeochemical cycling of carbon and sulfur. Antarctica has a unique and dynamic environment where microalgae are evolutionarily adapted to live in freezing temperatures under extreme and oscillating environmental gradients exposing them to solar, osmotic, oxidative and nutrient stress. This thesis investigated the physiological and biochemical adaptations of Antarctic microalgae, focusing on the role dimethylsulfoniopropionate (DMSP) plays in surviving in the harsh Antarctic environment. This thesis provides new knowledge into who are the DMSP producers in Antarctica, the spatial dynamics and role of DMSP in natural Antarctic microbial communities.

In a screening study, 16 species of Antarctic microalgae were characterised by their growth rates, physiological health, carbon content, DMSP production and DMSP lyase activity. We found that DMSP production and rates of lyase activity were species-specific, varying within taxa, and that diatom species can produce significant levels of DMSP, in the same magnitude as known DMSP producing haptophytes, *Phaeocystis spp.*.

In a descriptive study, we take a geographical look at the DMSP content and lyase activity, macromolecular profiles and productivity of three different Antarctic microalgal communities from three unique Antarctic environments; the open ocean to the sea ice and a hypersaline lake. We reveal that species diversity is reduced with more challenging environmental conditions and the species with the greatest phenotypic plasticity dominate in harsher settings. This thesis found that macromolecular content of microalgae changes based on environment, whereby sea-ice microalgae were higher in caloric value due to heavy investment in lipids compared to pelagic species.

Using manipulative laboratory studies, we delivered new insight into the response of DMSP to environmental stress and future climate change scenarios as well as macromolecular responses at the species and community levels. Exposure to hypersaline conditions did not induce increased DMSP production, potentially due to the salinity shift being too rapid. In addition, there was no significant change in DMSP or macromolecular concentrations in response to ocean acidification at the species level, however there was a difference at the community level due to a shift in community composition.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1. INTRODUCTION

GLOBAL IMPORTANCE OF MARINE PHYTOPLANKTON

Phytoplankton are unicellular phototrophic organisms key to the health and productivity of marine ecosystems. As primary producers, phytoplankton form the base of the marine pelagic food web and are the major source of energy and nutrients to the upper trophic levels (Twining et al. 2008). The nutritional value of phytoplankton and how efficiently biomass can be transferred between trophic levels is reliant on taxonomic phenotypic characteristics such as morphology, biochemical composition and digestibility (Brown 2002; Irigoien, Flynn & Harris 2005; Thompson, Guo & Harrison 1992; Van Donk, Ianora & Vos 2011). Surrounding environmental conditions dictate the biochemical composition of phytoplankton, with changing conditions often resulting in alterations to the lipid, protein and carbohydrate content of cells (Strandberg et al. 2015). For example, when grown outside of their optimum temperature, phytoplankton experience a change in total lipid content (Converti et al. 2009). Specifically, at lower temperatures the fluidity in the cell membrane is often reduced resulting in an increase in the production of unsaturated fatty acids in order to re-establish membrane fluidity (Juneja, Ceballos & Murthy 2013; Renaud et al. 2002). These unsaturated fatty acids are essential in the diet of zooplankton and fish, providing a supply of polyunsaturated fatty acids which they themselves cannot synthesis *de novo* (Brett & Müller-Navarra 1997; Tocher 2003). However, the biochemical composition of phytoplankton affects not only their nutritional value and thus trophic energy transfer, but also impacts upon nutrient cycling.

Marine phytoplankton are the biological link between oceanic and atmospheric processes, fundamental in the biogeochemical cycling of carbon and other major macronutrients – such as the nitrogen, phosphorus, iron, silica and sulfur cycles (Andreae 1990; Boyd & Ellwood 2010; Falkowski et al. 2000; Tréguer & De La Rocha 2013; Tyrrell 1999). Phytoplankton are significant carbon sinks, removing CO_2

from near surface waters and sequestering it to the deeper waters (known as the 'biological pump'), and while they account for only 0.2% of total global primary producer biomass they are responsible for almost half of total global carbon fixation (Field et al. 1998; Sabine et al. 2004), transforming inorganic forms of carbon into organic molecules through photosynthesis (Falkowski, Barber and Smetacek, 1998). All phytoplankton produce proteins and photosynthetic pigments that contribute to high amounts of nitrogen (Finkel et al. 2016; Geider & La Roche 2002), and lipids, nucleic acids and polyphosphates that contribute to the phosphorus cycle (Moreno & Martiny 2018; Sterner & Elser 2002). However, contributions to the cycling of some nutrients (eg. silica and sulfur) are limited to certain taxa and/or species. One particular taxa of phytoplankton, diatoms, produce an estimated 6 Tmol Si $year^{-1}$, and as the main organism responsible for the production of biogenic silica in the open ocean and coastal zones they are integral in the biogeochemical cycling of silica (DeMaster, Leynaert & Queguiner 1995; Tréguer & De La Rocha 2013). The contribution of phytoplankton to marine sulfur cycling is restricted to phytoplankton that produce the ubiquitous organic sulfur compound, dimethylsulfoniopropionate (Keller 1989a).

THE CHEMICAL ECOLOGY OF DIMETHYLSULFONIOPROPIONATE (DMSP)

Dimethylsulfoniopropionate (DMSP) is an abundant organosulfur compound in the marine environment. It is produced by phytoplankton living in the surface waters of the ocean and plays a key role in marine sulfur cycling (Malin 1996; Malin 2006), trophic interactions (Savoca & Nevitt 2014; Steinke, Malin & Liss 2002) and climate regulation (Bates, Charlson & Gammon 1987; Charlson et al. 1987). The role of DMSP in regional climate regulation is indirect; DMSP forms the main biogenic precursor to dimethylsulfide (DMS), a climatically important volatile sulfur compound that readily fluxes out of the surface waters into the atmosphere (Bates, Charlson & Gammon 1987). DMS has been described as a climate influencing gas because when oxidised to form sulfur dioxide (SO_2) and methanesulfonic acid (CH_3SO_3H) in the atmosphere, the acidic sulfate aerosols act as a source of cloud condensation nuclei (CCN), causing water molecules to amalgamate, form clouds and increase albedo (Malin 1996). It has been proposed that elevated temperature and/or increased irradiance encourages growth of DMSP-producing phytoplankton and dimethysulfide production, leading to an increase in the atmospheric concentration of DMS and therefore the formation of cloud cover (Figure 1.1). Enhanced cloud cover would result in more solar radiation being absorbed and reflected, reducing solar irradiance reaching the surface. This would alter the global radiation budget and theoretically decrease the temperature of the upper ocean, subsequently slowing the growth of the marine community that originally generated the DMSP (Charlson et al. 1987; Kiene, Linn & Bruton 2000; Simó 2001). This biologically driven negative feedback loop has been dubbed the CLAW hypothesis (Charlson et al. 1987) named after the initials of the four authors of the paper. Despite decades of research into DMSP-mediated ocean-atmosphere links, direct evidence of this process is still absent, and along with increasing significance of non-DMS sources of CCN, researchers now question the relevance of CLAW (Quinn & Bates 2011). However, this does not eliminate a link between ocean-derived CCN and climate; Instead, there may be multiple sources of these nuclei at the atmosphere-water boundary and it is clear

that the formation of clouds in response to these aerosols is more complex than first thought (Quinn & Bates 2011).

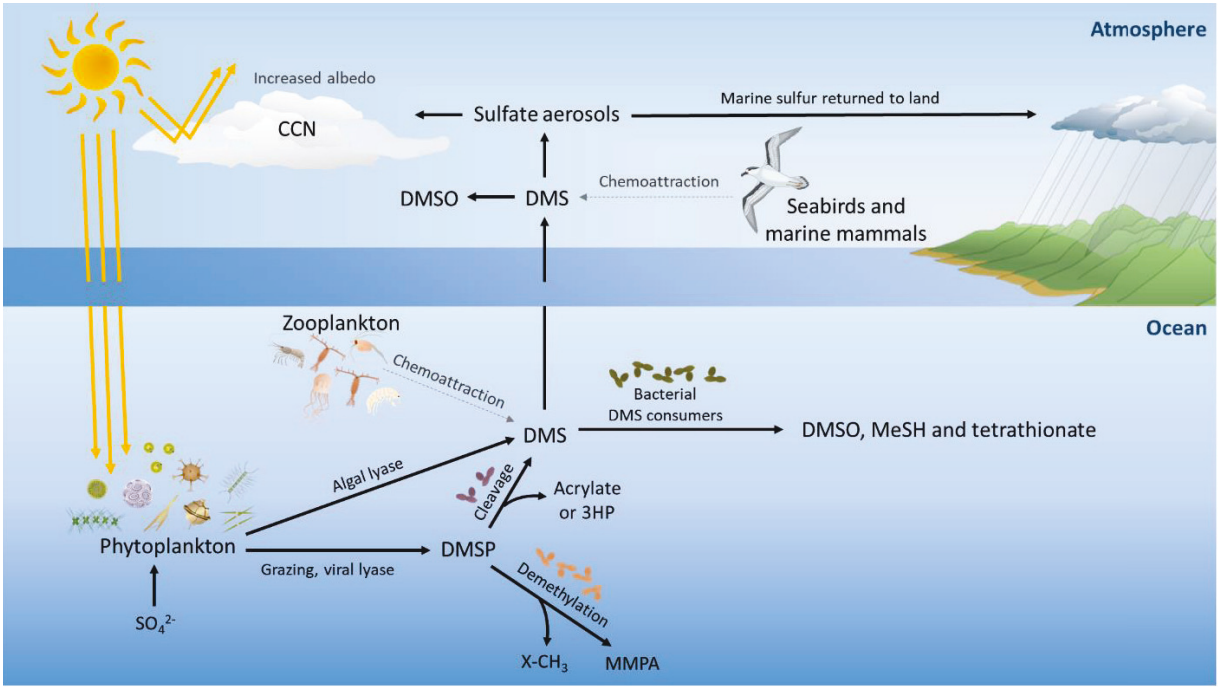


Figure 1.1 Conceptual model of the biogeochemical cycling of DMSP and DMS in seawater and the atmosphere. DMSP is released from phytoplankton through exudation, cell lysis and grazing. A fraction of that DMSP is assimilated by grazers and the rest is either cleaved to DMS by algal and bacterial lyases, or used by bacteria through other pathways. DMS is consumed by bacteria or lost through photooxidation. A small fraction of DMS is ventilated into the atmosphere where it is a precursor for sulphur containing aerosols that can influence the local climate through cloud formation and direct backscatter of solar radiation. Sulfur is also returned to the land via rain and snow, linking sea and land in the sulfur cycle. DMS has been shown to act as a foraging cue for zooplankton, seabirds and marine mammals (grey dotted line). *3HP – 3-hydroxypropionate, CCN – cloud condensation nuclei, DMS – dimethysulfide, DMSP – dimethylsulfoniopropionate, DMSO - dimethylsulfoxide, MeSH – methanethiol, MMPA – methylmercaptopropionate, X - tetrahydrofolate.* Adapted from Curson et al. (2011) and Alcolombri et al. (2015). Images from the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/)

In contrast to CLAW, another relationship between DMS and solar radiation has been described: the "anti-CLAW" hypothesis, defined by a positive feedback loop. In the anti-CLAW hypothesis, an increase in global temperatures would cause thermal stratification of ocean surface waters and limit the flux of nutrients from depth, thereby limiting phytoplankton growth and the production of DMSP, resulting in reduced DMS emissions and therefore a reduction in cloud albedo (Lovelock 2006). Like the CLAW hypothesis, the anti-CLAW hypothesis also faces wide ranging resistance, but modelling of future climate scenarios has shown a decrease in primary production under increased ocean stratification (Steinacher et al. 2010), suggesting some scope for a positive feedback loop in the sulfur cycle to exist. Given that DMS is the major biogenic source of sulfur to our atmosphere—accounting for 50-60% of all naturally occurring sulfur fluxes of which there is 10 million metric tonnes annually (Johnston 2015; Simó 2001), and that oceanic production accounts for up to 95% of this total biogenic emission (Yoch 2002)—the ability of DMSP and its breakdown products to influence atmospheric chemistry and climate highlights the central importance of this molecule in marine biogeochemical cycling and emphasises the need to understand it's role in the physiological and ecological processes that occur in the surface waters of the oceans.

The production of DMSP by phytoplankton accounts for the majority of organic sulfur fluxes from primary to secondary producers in marine microbial food webs (Malin 1996; Simó 2001; Vila-Costa et al. 2006). DMSP is a zwitterion, meaning it can only be released from cells with damaged membranes or through active transport (Simó et al. 1998). This means that for transfer and transport through the food web it has to be released into the surrounding waters through exudation, grazing or cell lysis, where it can then be either assimilated by grazers or metabolised by bacteria (Figure 1.1). There is another way that DMSP can leave an algal cell, this is through its conversion to the membrane permeable DMS or dimethylsulfoxide (DMSO). In marine microalgae, cleavage of DMSP is possible via enzymes known as DMSP lyases. In the cosmopolitan coccolithophore *Emiliana huxleyi*, the first

enzyme and corresponding gene (Alma1) to mediate DMSP lyase activity in microalgae was recently identified, cleaving DMSP into DMS and acrylate (Figure 1.2; (Alcolombri et al. 2015). To date, this is the only known microalgal DMS-releasing enzyme. However, other microalgae may contain different enzymes that lyase DMSP, with further research needed to identify potential lyases.

Once DMSP is released into the water column, there are several pathways for its degradation: one that results in its conversion to DMS, the other it's assimilation into the food web (Figure 1.1). The most predominant degradation pathway in the marine environment is demethylation, accounting for roughly 34-100% of DMSP turnover depending on the season (Curson et al. 2008; Kiene, Linn & Bruton 2000). This pathway involves the breakdown of dissolved DMSP into methanethiol and subsequently contributes to the carbon and sulfur nutritional pools in the cells (Curson et al. 2008). Demethylation occurs via the demethylase gene *dmdA*, which leads to the production of methylmercaptopropionate (MMPA) without the production of DMS (Howard et al. 2006). Around 60% of bacterioplankton in marine surface waters possess this gene, indicating that there is an ecological benefit for bacterioplankton to demethylate DMSP (Howard et al. 2008; Moran et al. 2011). Demethylation of DMSP by bacteria is used as a source of carbon and sulfur for metabolism, playing an important role in biogeochemical cycling, redirecting carbon and sulfur back into the microbial food web (Moran et al. 2011).

The other DMSP transformation pathway, mediated by bacteria, cleaves DMSP into DMS (Figure 1.1). This pathway accounts for 10% of the total dissolved DMSP pool, of which only 2-10% of DMS produced ventilates into the atmosphere, with the potential to influence cloud formation (Howard et al. 2008; Moran et al. 2011; Reisch, Moran & Whitman 2011). There are three bacterial cleavage pathways possible, each of which is mediated by separate enzymes, and while all three result in the formation of

DMS, they have different co-products and bear no resemblance to Alma1 (Curson et al. 2008; Johnston 2015; Kiene, Linn & Bruton 2000; Reisch, Moran & Whitman 2011; Sun et al. 2016). Cleavage by Ddd enzymes results in DMS and either 3-hydroxypropionate (3HP) or acrylate; *DddD* proteins generate 3HP rather than acrylate, while the enzymes *DddK, DddL, DddP, DddQ, DddW* and *DddY* convert DMSP into acrylate, releasing DMS and H+ (Curson et al. 2011; Reisch, Moran & Whitman 2011; Sun et al. 2016).

Almost all DMSP-catabolising bacteria belong to the Proteobacteria phylum, specifically, the marine gammaproteobacteria and alphaproteobacteria, which make up some of the most abundant chemo-organotrophic bacteria in the oceans (Curson et al. 2011; Sun et al. 2016). So while only a few types of bacteria process DMSP, the significant abundance of these bacteria means they are responsible for a large percentage of total bacterial activity (Simó 2001). The ability of marine bacteria to degrade DMSP with both DMS and non-DMS producing pathways means bacteria can control the flux of DMS. In some cases, bacteria can utilise both pathways, thus choosing the fate of dissolved DMSP, a process dubbed the 'bacterial switch' (Howard et al. 2006; Simó 2001). However, understanding what drives this switch is still in its infancy. In most cases, it is the strength of each pathway (demethylation and cleavage), the environmental conditions and the bacterial community present, that ultimately determines the amount of DMS released into the water column and thus ventilated into the atmosphere, influencing the dynamics of the region's climate and the marine chemical landscape.

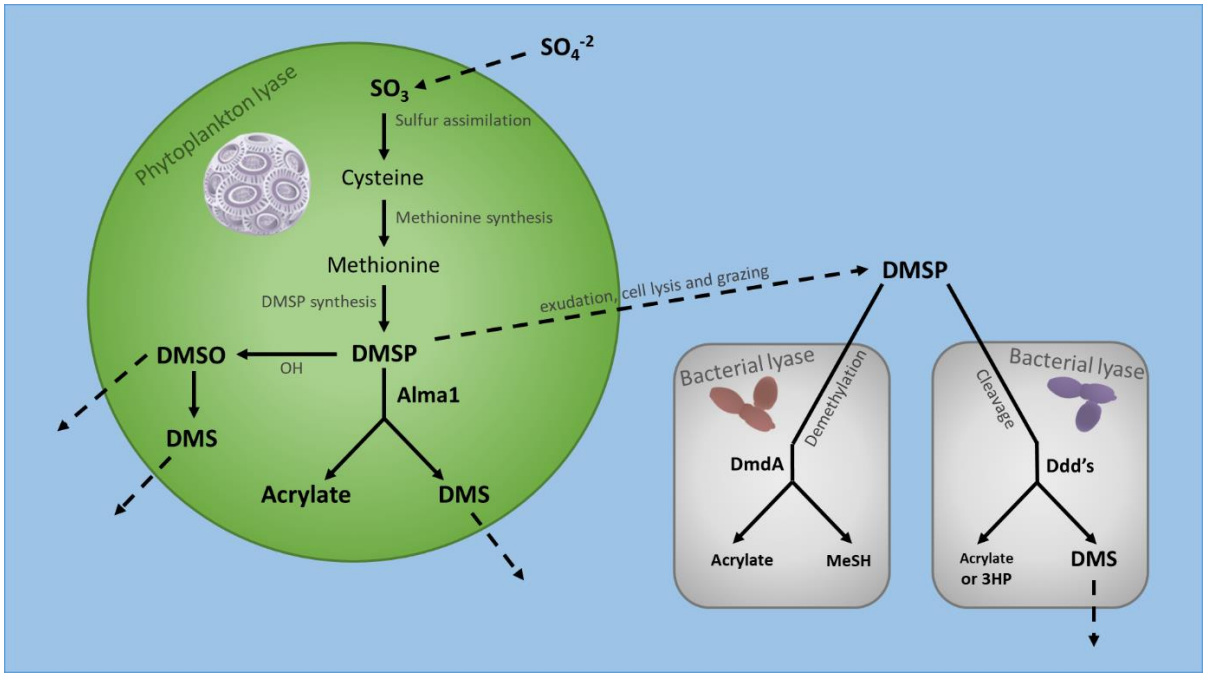


Figure 1.2. DMSP synthesis and the pathways for DMSP catabolism in the ocean including both microalgal and bacterial DMSP lyases. DMSP is synthesised by algae using sulfur which is abundant in seawater, via active transport across the cell membrane. Once inside the cell it undergoes multiple steps of assimilation and synthesis to produce DMSP. DMSP can be directly lysed within the cell by enzymes (Alma1 in *E. huxleyi*), resulting in acrylate and DMS, which is then released into the water column. Alternatively, DMSP can be released into the water (due to grazing or viral lysis) and catabolised by marine bacteria either via demethylation (DmdA) or cleavage enzymes (Ddd's). The demethylation pathway results in acrylate or methanethiol (MeSH) without the production of DMS, while the cleavagepathway results in either acrylate or 3-hydroxypropionate (3HP) and DMS. Adapted from Alcolombri et al. (2015) and Kettles, Kopriva & Malin (2014).

PRODUCTION AND FUNCTIONAL ROLES OF DMSP

DMSP is a secondary metabolite, it is produced in the cell following the uptake of sulfur from seawater via membrane transporters. Once transported into the cell, the sulfate is activated by ATP sulfurylase (ATPS) to adenosine 5 -phosphosulfate (APS), which is reduced to sulfite, then further reduced to sulfide by sulfite reductase (SiR) (Koprivova & Kopriva 2014). The sulfide is then incorporated into the amino acid skeleton of O-acetylserine (OAS) to make cysteine (Koprivova & Kopriva 2014), whichis then converted to methionine and finally to DMSP (Figure 1.2). DMSP has been found to accumulate in vacuoles, cytoplasm and chloroplasts – identified as possible storage sites (Raina et al. 2017). It was thought that eukaryotes were the only significant producer of DMSP until recently when the first DMSP biosynthesis gene in any organism, *dsyB*, was identified in a heterotrophic marine alphaproteobacteria (Curson et al. 2017). In a study of the model DMSP-producing bacteria *Labrenzia aggregate*, it was found that DMSP synthesis and the transcription of *dsyB* was upregulated when cells were exposed to increased salinity, nitrogen limitation and reduced temperatures (Curson et al. 2017). This lead to the discovery of methylthiohydroxybutryate methyltransferase enzyme (*DSYB*), a *dsyB* homologue, in the chloroplast and mitochondria of the haptophyte, *Prymnesium parvum*, and the identification of the site of DMSP synthesis in phytoplankton (Curson et al. 2018).

The production and regulation of cellular DMSP is highly dynamic, depending on the physiological conditions of the microalgal cell and the conditions of the surrounding environment. While it is only produced by some species, it can account for up to 10% of their cellular carbon (Stefels et al. 2007). The proposed cellular roles of DMSP are many, including an osmolyte or cryoprotectant (Karsten, Kirst & Wiencke 1992; Kirst et al. 1991; Lee & De Mora 1999; Motard-Côté & Kiene 2015), as well as an antioxidant (Sunda et al. 2002) and as a signalling molecule in the marine environment (Seymour et al.

2010). However, with no single function holding true in all cases, it is difficult to assign to it a primary function, which may differ over the lifespan of a cell, as well as between species.

Evidence for its role as an organic osmolyte comes from studies that have shown DMSP production to be upregulated under increased salinity conditions (Kirst et al. 1991). Furthermore, in bacterial cells exposed to acute osmotic stress, DMSP uptake and accumulation is enhanced, with a concomitant down-regulation in demethylation, suggesting that cells maintain dissolved DMSP untransformed in their cells for use as an osmoprotectant under hypersaline conditions (Motard-Côté & Kiene 2015). In conjunction with being an osmolyte, DMSP has also been suggested to act as a cryoprotectant, preventing bacteria and microalgae from freezing in polar waters (Karsten, Kirst & Wiencke 1992; Kirst et al. 1991; Lee & De Mora 1999; Nishiguchi & Somero 1992). In particular, concentrations of cellular DMSP were found to be higher in sea ice microalgal communities compared to those found in other polar environments and temperate regions (Lee et al. 2001).

DMSP has also been described as a general stress response molecule, where increases in DMSP concentrations have been observed under harmful environmental conditions, including; high light (Karsten, Wiencke & Kirst 1990; Stefels & van Leeuwe 1998), high UV (Archer et al. 2010; Slezak & Herndl 2003), as well as in other cases that result in high oxidative stress (Sunda et al. 2002). In microalgae, general stress responses will often result in changes to the photosystem. For example, excess light and UV can negatively impact photosystem II via damage to the key antenna protein D1, which is responsible for initial stages of photosynthetic electron transport and oxygen evolution (Aro, Virgin & Andersson 1993; Cirulis, Scott & Ross 2013; Niyogi 1999). This damage often results in a build-up of reactive oxygen species (ROS) in the cell (Bouchard et al. 2005; Edelman & Mattoo 2008; Krieger-Liszkay & Trebst 2006; Nishiyama, Allakhverdiev & Murata 2006). A general response to increased

cellular ROS, would be for cells to increase their production of antioxidants, such as superoxide dismutase, glutathione and catalase, which can work together to quench ROS and prevent cellular damage caused by ROS (Cirulis, Scott & Ross 2013; Niyogi 1999). With the evidence that DMSP, its breakdown products and the oxidation product (DMSO) can readily scavenge harmful hydroxyl radicals (Sunda et al 2002), it could be suggested that these sulfur compounds may function as an additional effective antioxidant system in phytoplankton cells experiencing oxidative stress (Harada et al. 2004; Keller et al. 1999; Sunda et al. 2002; Sunda et al. 2007).

Ecologically, the role of DMSP is equally complex. Despite relatively low concentrations compared to other biologically active molecules, it has been described as a 'molecule of keystone significance' for its ability to initiate trophic interactions across multiple levels, connecting microbial decomposers right up to apex predators (Ferrer & Zimmer 2012). Acting as an information-conveying chemical (infochemical), DMSP has been shown to act as a non-toxic chemical deterrent against some protist grazers via the production of acrylate (which is toxic to zooplankton) during cleavage (Strom et al. 2003; Wolfe, Steinke & Kirst 1997). Further to being a chemical deterrent, one study revealed that DMS along with acrylic acid had an antiviral effect suggesting the DMSP system in algae could function as a chemical defence against viral infection (Evans et al. 2006). In contrast to this, atmospheric emissions of DMS have been used as a chemosensory foraging cue attracting a variety of organisms over a vast range of spatial and temporal scales (Steinke, Malin & Liss 2002). In the marine environment, DMSP acts as a chemical attractant for some grazers and bacteria, stimulating foraging behaviour (Seymour et al. 2010; Steinke, Stefels & Stamhuis 2006; Zimmer-Faust, de Souza & Yoch 1996). Chemoattraction has been observed right up to apex predators such as procellariiform seabirds (Savoca & Nevitt 2014). In this study, the authors showed that procellariiform seabird species used DMS as a cue allowing them to selectively forage for phytoplankton grazers, and notes that their faecal matter delivered a valuable iron source that could enhance algal growth, suggesting that there is a tritrophic mutualist relationship

(Savoca & Nevitt 2014). The increasing interest in the physiological function of DMSP and its potential as an information-conveying chemical, or signal molecule, has meant that in the last few decades the diverse roles of DMS and its precursor DMSP have been investigated extensively, gathering many lines of evidence to confirm the importance of DMSP in shaping food webs and influencing climate.

BIOGEOGRAPHY OF DMSP PRODUCTION AND THE SPECIES RESPONSIBLE

Despite the ubiquity of DMSP in the marine environment and its many proposed physiological and ecological roles, only certain microalgal species are capable of DMSP biosynthesis, with its rates of production being species specific. Intracellular concentrations measured in cultured strains have been shown to range between 0-14700 *f*mol cm^{-3} per cell (Table 1.1; (Caruana & Malin 2014)). Yet its production is generally constrained to a few specific microalgal classes (Stefels 2000). Generally, small haptophytes and dinoflagellates are the greatest producers of DMSP, while diatoms, green algae and cyanobacteria are considered low or non-producers (Vila-Costa et al. 2006). As such, there have been comprehensive investigations into the production of DMSP and DMSP lyase activity in a broad range of dinoflagellates (Caruana et al. 2012; Caruana & Malin 2014) and also in the haptophytes *Phaeocystis* (Liss et al. 1994; Stefels & Van Boekel 1993) and *Emiliania huxleyi* (Steinke, Wolfe & Kirst 1998) (Table 1.1). However, DMSP studies involving diatoms are limited with many species found to be low or non-producers (Kasamatsu et al. 2004).

Table 1.1 Comparison of intracellular DMSP concentrations and DMSP lyase activity in microalgae from published data. 'Y' indicates lyase activity detected, 'N' indicates no lyase activity or below detection limit, '–' indicates not measured.

Species	**Strain**	**DMSP:CV (mM)**	**DMSP:cell (fmol cell^{-1})**	**DMSP:chla (mol mol^{-1})**	**Lyase (Y/N)**	**Paper**
Dinoflagellates						
(38 spp.)	107 strains	0.003–7590	0.1-14700	-	Y	Caruana & Malin (2014)
Chrysophytes						
Ochromonas sp.	IC	84.6	-	-	-	Keller (1989a)
Pelagococcus subviridus	PelaCl	29.9	-	-	-	Keller (1989a)
Green algae						
Dunaliella tertiolecta	DUN	0	0	0	-	Matrai & Keller (1994)
Blue-green algae						
Prochlorococcus marinus pastoris	CCMP-2389	-	-	-	N	Harada & Kiene (2011)
Synechococcus bacillaris	CCMP-1333	-	-	-	N	Harada & Kiene (2011)
Synechococcus enlongatus	CCMP-1630	-	-	-	N	Harada & Kiene (2011)
Synechococcus sp.	CCMP-1334	-	-	-	N	Harada & Kiene (2011)
Haptophytes						
Prymnesiophyceae (6 spp.)	28 strains	157	-	-	-	Keller (1989b)
Coccolithophores (11 spp.)	*17 strains*	174-715	-	-	Y	Franklin et al. (2010)
Emiliania huxleyi	CCMP-373	304	9.7	-	Y	Harada & Kiene (2011)
	CCMP-374	274	10.9	-	N	Harada & Kiene (2011)
	CCMP-374	198-393	-	63-101	-	Sunda et al. (2002)

	CCMP-1516	120	-	-	-	Franklin et al. (2012)
	8613C	124.4	-	-	-	Keller (1989a)
	6 strains	50-242	3.6-18.9	-	-	Steinke, Wolfe & Kirst (1998)
	NIOZ-L	197-233	5.03-8.86	-	-	Van Rijssel & Gieskes (2002)
	2 strains	107-113	3.58-7.59	-	Y	Wolfe & Steinke (1996)
Phaeocystis	677-3	261.1	-	-	-	Keller (1989a)
	K	71-150	-	-	Y	Stefels & Van Boekel (1993)
	-	-	2	-	-	Liss et al. (1994)
Isochrysis galbana	CCMP-1324	-	4	-	N	Niki, Kunugi & Otsuki (2000)
Gephyrocapsa oceanica	NIES-353	-	7	-	N	Niki, Kunugi & Otsuki (2000)
Diatoms						
Centric and pennate	22 strains	19	-	-	-	Keller (1989b)
Chaetoceros	B23-p2	0	0	0	-	Kasamatsu et al. (2004)
Cylindrotheca closterium	-	0.03-0.04	6-7	-	-	Van Bergeijk, Van der Zee & Stal (2003)
Navicula	P1-3	0.0016	-	-	-	Kasamatsu et al. (2004)
Nitzschia	P1-5	0.0034	-	-	-	Kasamatsu et al. (2004)
Skeletonema costatum	CCMP-1332	-	-	-	N	
	SKEL	0.035	-	-	-	Matrai & Keller (1994)
Thalassiosira oceanica	CCMP-1005	-	-	-	N	Harada & Kiene (2011)
	CCMP-1005	0.9	-	-	-	Vila-Costa et al. (2006)
Thalassiosira pseudonana	CCMP-1335	-	-	-	N	Harada & Kiene (2011)
	CCMP-1335	35	-	-	-	Franklin et al. (2012)

	CCMP-1335	0-8.9	-	-	-	Kettles, Kopriva & Malin (2014)
	CCMP-1335	0.85-1.46	-	-	-	Sunda et al. (2002)
	CCMP-1335	1.3	-	-	-	Vila-Costa et al. (2006)

In the last few decades, studies into the biogeographical distribution and abundance of DMSP and DMS have shown that the highest concentrations of DMS are found in areas of coastal upwelling (Galí & Simó 2010), such as the equatorial Pacific Ocean or off Guinea and Angola, and high latitudes or the polar oceans, in particular the Southern Ocean (Aumont, Belviso & Monfray 2002; Kettle et al. 1999; Lana et al. 2012). Equatorial and subtropical zones are low in total DMSP (5-25 nmol L^{-1}) year round, however like many areas of the world, DMSP production is seasonal, with more seasonal shifts seen in subtropical gyres compared to equatorial regions and latitudes above 35° where DMSP concentrations can exceed 25 nmol L^{-1} in the productive months (Galí et al. 2015). Most DMSP is produced during large blooms of producers (Lizotte et al. 2017), such as *E. huxleyi* (Levasseur et al. 1996; Matrai & Keller 1993) and *Phaeocystis* (Schoemann et al. 2005); This is especially seen in the waters around the North Atlantic, east of Patagonian waters, the North Pacific and New Zealand extending down to the Polar Seas in the summer (Galí et al. 2015).

Antarctica has been identified as a hot spot for DMSP production and DMS emission. In the Southern Ocean, DMS levels are highest in the mixed layer with lower concentrations in the deeper ocean (Curran, Jones & Burton 1998). High sulfur fluxes are closely associated with *Phaeocystis* blooms which often dominate in polar regions (DiTullio et al. 2000; Galí & Simó 2010). These dense blooms are highly seasonal and linked to sea ice formation and melting patterns (Trevena & Jones 2006). During the early summer when sea ice melts, elevated levels of DMS (21–37 nM) in surface waters have been recorded despite relatively low chlorophyll *a* concentrations, indicating that DMS and DMSP is released by the sea ice rather than an ice edge algal bloom (Trevena & Jones 2006). Interestingly, in the absence of large *Phaeocystis* blooms, Antarctic waters and sea ice are dominated by diatoms, yet despite this shift, DMS and DMSP concentrations can still be high. In lower latitudes, diatoms are generally considered non-producers, but background levels of DMSP and DMS in Antarctic waters would suggest

that diatoms from high latitudes are major contributors. While we have substantial species-specific data on dinoflagellates and haptophytes from lower latitudes, we have minimal data on polar species, with the exception of a few key species such as *Fragilariopsis cylindrus* and *Phaeocystis* (Table 1.2), as such detailed information on species-specific production of DMSP from Antarctic strains is lacking.

Table 1.2 Comparison of intracellular DMSP concentrations and DMSP lyase activity in Antarctic microalgae from published data. 'Y' indicates lyase activity detected, 'N' indicates no lyase activity or below detection limit, '–' indicates not measured.

Species	Strain	DMSP:CV (mM)	DMSP:cell (fmol cell^{-1})	DMSP:chla (nmol μg^{-1})	Lyase (Y/N)	Paper
Dinoflagellates						
Polarella glacialis	CCMP1138	94	8900	66	N	Caruana et al. (2012)
Polarella sp.	-	-	-	-	Y	Harada & Kiene (2011)
Haptophytes						
Phaeocystis sp.	-	-	87	-	-	Gibson et al. (1990)
Phaeocystis sp.	-	-	-	41	-	McTaggart & Burton (1992)
Phaeocystis sp.	-	-	-	11	-	Kirst et al. (1993)
Phaeocystis sp.	-	-	10	-	-	
P. antarctica		-	372.4	-	-	
P. antarctica	111103A2	-	-	-	Y	Harada & Kiene (2011)
Diatoms						
Ice diatoms	various	0.0002	-	9	-	Kirst et al. (1991)
Pelagic diatoms	various	-	-	3		Kirst et al. (1993)
Pelagic diatoms	various	-	-	35-52		Meyerdierks et al. (1994)
Fragilariopsis cylindrus	CCMP-1102	16-18	-	1.2-2.6	-	Lyon et al. (2016)
Surface pack ice	various	344	-	275.2	-	Trevena & Jones (2012)
Surface fast ice	various	100	-	215	-	Trevena & Jones (2012)

ANTARCTIC PHYTOPLANKTON ARE MAJOR CONTRIBUTORS TO GLOBAL DMSP

Globally marine phytoplankton produce 1 Gigaton of DMSP each year, releasing 10^6 metric tonnes of DMS into the atmosphere annually (Johnston 2015), with sunlight as an important modulator of DMS production (Galí et al. 2011). Antarctic algal species have been shown to contain higher concentrations of DMSP compared with their counterparts in temperate regions (Karsten, Kirst & Wiencke 1992). In the austral summer, the euphotic zone has constant exposure to sunlight, ideal for algal growth, resulting in more time for DMSP production. In contrast, for the other half of the year nearly 20 million km^2 of sea ice surrounds the Antarctic continent, making both sea ice and pelagic microalgal communities a significant source of DMSP in the Antarctic (Lee et al. 2001). Indeed, 'hot spots' of DMS in Antarctic waters have been linked to the melting of sea ice and release of sea ice algal DMS and DMSP (Trevena & Jones 2006). It has been estimated that the whole Antarctic sea ice zone may contain up to 9 Mmol sulfur in the form of DMSP (Trevena et al. 2003), with measurements ranging between 0-20 nM recorded in Antarctic coastal waters (Asher et al. 2017) and concentrations of over 200 nM measured within the sea ice brine (Asher et al. 2011). Taken together, the combined influence of the long daylight hours and massive sea ice extent makes the Antarctic environment a significant contributor to the global sulfur cycle.

Antarctic coastal DMSP concentrations show considerable seasonal variation ranging from 8-160 nM (Asher et al. 2017), and show no correlation with chlorophyll *a* measurements, indicating taxon dependency (Simó & Vila-Costa 2006). In the open pelagic waters of coastal Antarctica, two phytoplankton taxa dominate; the diatoms and a haptophyte, *Phaeocystis antarctica* (Figure 1.3). In coastal regions of the West Antarctic peninsula, diatoms and *Phaeocystis* blooms dominate the community in certain locations and at distinct times (Arrigo et al. 1999; Smith Jr et al. 2010; Smith &

Asper 2001). In spring, haptophytes normally dominate in the early season in the deeply mixed waters of the Ross Sea polynya; this is followed by a surge in diatom abundance in the shallow mixed layer, peaking around the western continental shelf and ice edge (Arrigo et al. 1999; Smith Jr et al. 2010; Smith & Asper 2001). The diatoms, particularly from the genera *Thalassiosira* spp. and *Fragilariopsis* spp., that dominate algal assemblages in the spring, are then replaced by flagellates or cryptophytes in the summer (Ducklow et al. 2007; Garibotti, Vernet, Ferrario, et al. 2003; Petrou et al. 2016). This transition has been correlated to glacial melt-water runoff and reduced surface water salinity causing a well stratified water column (Moline et al. 2004), as well as sedimentation, advection and grazing (Ducklow et al. 2007). Sea ice microalgal communities tend to have a similar composition to the pelagic communities (Ducklow et al. 2007), signifying the close-knit relationship between sea ice and water column. This is most likely due to the seeding of the water column during the spring ice melt and particle incorporation during new ice formation or infiltration of seawater in void spaces (Arrigo 2014).

In sea ice, pennate diatoms form the dominant taxa (Thomas & Dieckmann 2002) and although diatoms are generally not considered DMSP producing taxa, sea ice diatoms are known to produce significant amounts of DMS reaching up to 2,910 nM in a single ice core (Trevena & Jones 2006). Other studies have also found that sea ice microalgal assemblages are on average high DMSP producers where the assemblages were dominated by diatoms, making up more than 80% of the sampled community (Trevena et al. 2003). Overall, *Pseudonitzschia* spp., *Fragilariopsis* spp., and *Chaetoceros* spp. are the dominant diatom species found in the Southern Ocean, with *Fragilaropsis* spp. dominating the seasonal ice zone –with recorded DMSP concentrations of up to 1012 nM (Lyon et al. 2016)—and *Pseudonitzschia* spp. found to outnumber other diatoms in the southern Antarctic zone (Kopczyńska et al. 2007). Congruously DMS production is species specific, the diatom, *Chaetoceros socialis* produced more DMS (around 7 DMS:C fg^{-1} pg^{-1}), than other diatoms under the same conditions (*Nitzschia curta* at ~0.9

DMS:C fg^{-1} pg^{-1} and *Thalassiosira tumida* at ~1.8 DMS:C fg^{-1} pg^{-1}), only to be exceeded by the haptophyte, *Phaeocystis antarctica,* producing 26.6 DMS:C fg^{-1} pg^{-1} (Baumann, Brandini & Staubes 1994).

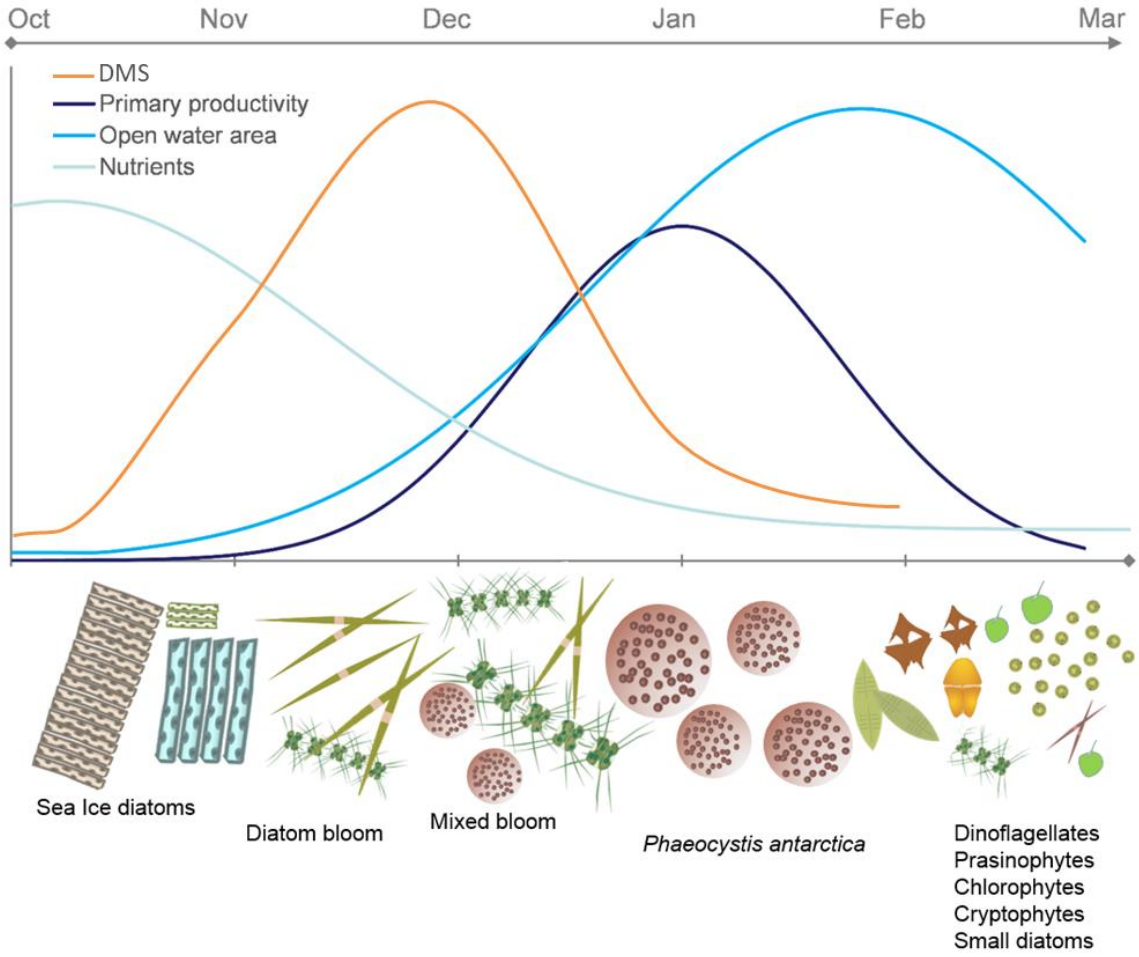


Figure 1.3 Antarctic Spring/Summer conditions and seasonal shift in phytoplankton community. Seasonal changes in DMS flux, phytoplankton primary productivity, area of open water and nutrient level in the seasonal sea ice zone from October to March. The melting out of the sea ice increases the pelagic zone and rapid nutrient drawdown driving an increase in primary production and the observed succession of Antarctic phytoplankton communities. Adapted from Petrou et al. (2016) and DMS flux taken from Trevena & Jones (2012).

Phaeocystis antarctica is able to survive long periods of freezing and darkness, then resume growth and photosynthesis after thawing, advantageous in the changing seasons of the Antarctic (Smith & Asper 2001; Tang et al. 2009). *P. antarctica* is a common bloom forming microalgal species in seasonal ice zones and Antarctic coastal waters, such as the Ross Sea (DiTullio et al. 2000; Smith & Asper 2001). One Antarctic study collected natural marine microbial communities from beneath the sea ice then exposed them to an abrupt increase in irradiance, a light regime that mimicked the rapid light changes during the break out of sea ice (Vance et al. 2013). They found that DMSP within the water rapidly increased from 16.6 nmol L^{-1} to 192.7 nmol L^{-1} over 2 days, which was attributed to the significant increase in abundance of *P. antarctica* (Vance et al. 2013). Another study found that in the coastal waters off Palmer Station the seasonal cycle of DMSP could also be linked to the abundance of *P. antarctica* (Asher et al. 2017). Intracellular DMSP concentrations measured in *Phaeocystis* blooms exceed global averages by more than an order of magnitude, ranging from 100 to 1650 nM (Kettle et al. 1999; Kirst et al. 1991; Van Duyl et al. 1998; Vance et al. 2013). Interestingly, studies have measured similar concentrations in sea-ice diatoms (Ditullio, Garrison & Mathot 1998; Trevena et al. 2003). While the high concentrations of DMSP and DMS produced by these algal blooms are generally and frequently seen during the spring and summer (Figure 1.3) with DMS flux peaking at 31 nM in December (Trevena & Jones 2012), studies conducted during the winter have found DMS(P) production in sea ice to also be significant during the winter months (Uhlig et al. 2014).

While Antarctica is characterised as a major hot spot for DMSP and DMS, there is still a lot of research needed to understand sulfur cycling in the Antarctic, particularly who the cyclers are and what drives their high production. Studies show that Antarctic coastal locations are usually characterised by the dominance of either *P. antarctica* or diatoms and it is unusual to find a location where significant concentrations of both groups do not co-occur (Garibotti, Vernet, Kozlowski, et al. 2003; Rodríguez et

al. 2002; Smith & Asper 2001). However, despite the considerable work on *P. antarctica*, little is known about the contributions of individual microalgal species, especially diatoms, to DMSP production in the Antarctic environment.

ANTARCTIC PHYTOPLANKTON PHYSIOLOGY AND ADAPTING TO A CHANGING CLIMATE

The Southern Ocean is a highly dynamic environment and as such, Antarctic phytoplankton are exposed to extreme and highly seasonal environmental conditions, from deeply mixed waters in summer to frozen ice sheets in winter (Gleitz & Thomas 1992). Phytoplankton in the winter are plunged into darkness, with most microalgae being restricted to the freezing temperatures within the sea ice, where brine channels can reach extreme salinity (Arrigo 2014). In spring, as the ice melts out, the sea ice algae are released into a lens of slightly fresher water (salinity above 25 (Untersteiner 1968)) that forms, reducing the mixed layer depth and increasing daily irradiance exposure (Figure 1.4). Summer is dominated by almost 24 h of sunlight and a stable salinity around 34, while deep mixing results in a variable light climate and high nutrient availability (Constable, Nicol & Strutton 2003). This seasonal variability has made many species highly plastic, enabling them to deal with rapid changes in temperature, salinity, pH and light, i.e. the seasonal variability experienced in the Antarctic (Arrigo 2014; Mock & Hoch 2005; Petrou et al. 2010). In helping to survive these environmental shifts, the plasticity of these microalgae can be seen via their adjustment of their photosystems (Lavaud, Strzepek & Kroth 2007; Petrou, Doblin & Ralph 2011a; Wagner, Jakob & Wilhelm 2006) and macromolecular composition (de Castro Araújo & Garcia 2005; Sackett et al. 2013). Furthermore, DMSP has been proposed to have multiple functions within the Antarctic microalgal cell, implicating a possible role for this compound in phytoplankton phenotypic plasticity.

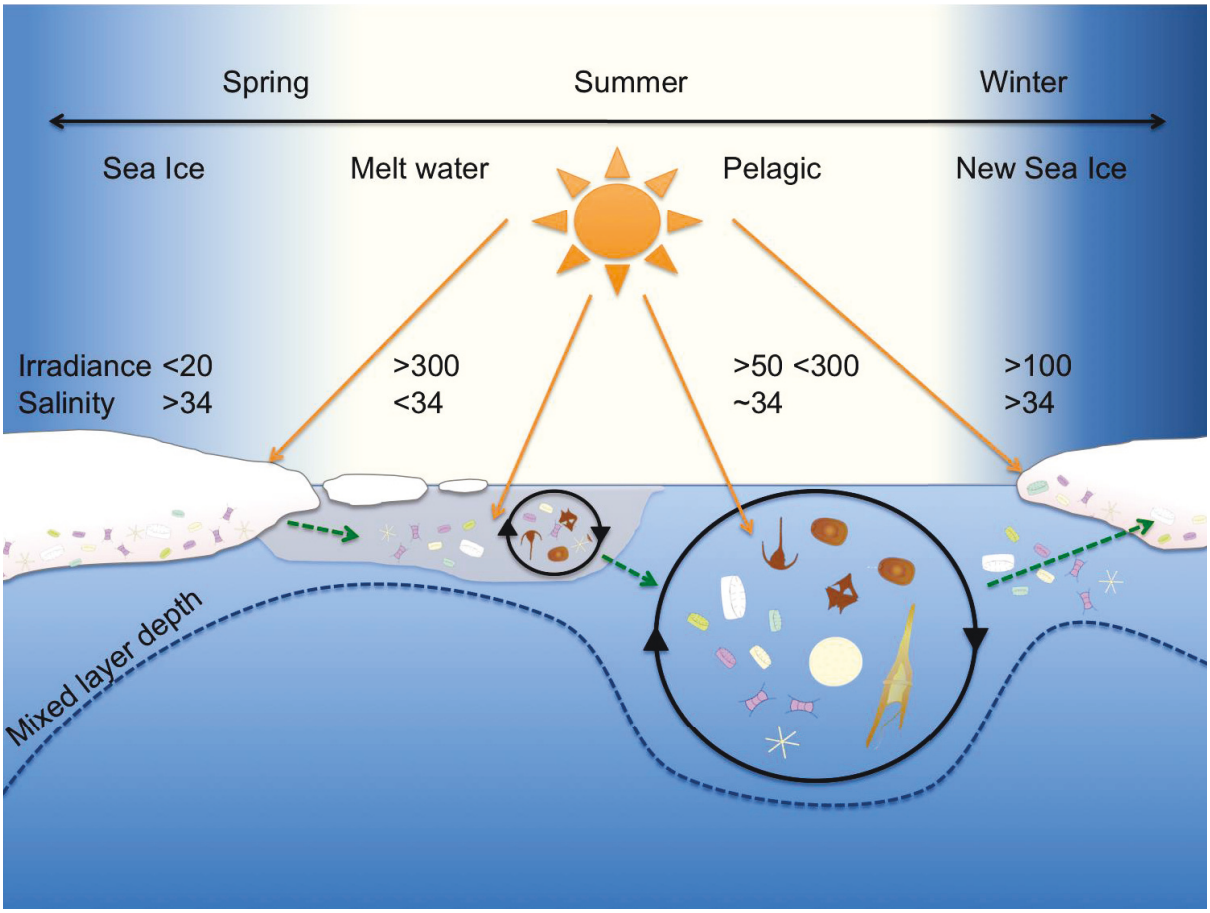


Figure 1.4 Spatial and temporal shift in the Antarctic coastal ecosystem. The seasonal pathway of phytoplankton from the winter sea ice to the melt water and pelagic environment is depicted (green arrows). Mixed layer depth (navy blue line) and light attenuation (orange line) are shown along with salinity and irradiance (in µmol photon m^{-2} s^{-1}) for each environment. Adapted from Petrou, Doblin & Ralph (2011b).

Sea ice diatoms have a variety of well-studied adaptations to living in the cold and salty brine channels of the sea ice matrix (Morgan-Kiss et al. 2006). Microalgae have been seen to alter their macromolecules, expressing higher cellular concentrations of lipids under sea-ice conditions compared to meltwater conditions in order to maintain cell membrane fluidity under freezing conditions (Brett & Müller-Navarra 1997; Fahl & Kattner 1993; Sackett et al. 2013). Alternatively they can modify their environment by producing antifreeze proteins that they secrete into brine channels. These antifreeze proteins or cryoprotectants, prevent the cells from freezing in sub-zero temperatures (Arrigo 2014).

DMSP is thought to function as a cryoprotectant within the cell (Lee & De Mora 1999), with a study conducted by Nishiguchi & Somero (1992) showing that DMSP was capable of stabilising enzyme proteins from low temperature induced denaturation. In a number of temperature studies, DMSP has been found to increase with a decrease in temperature. In the prasinophyte culture, *Tetraselmis subcordiformis,* when grown over a range of temperatures from 5 to 23 °C, intracellular DMSP content was 8 times greater at lower temperatures (Sheets & Rhodes 1996). In the case of diatoms, it was found that *N. curta, T. tumida, C. socialis* and the haptophyte, *P. antarctica,* grown at 1 °C and -1.6 °C experienced an increase in DMS emissions at the lower temperature (Baumann, Brandini & Staubes 1994). Suggesting that the production of DMSP in sea ice algae evolved due to its advantageous role as an effective cryoprotectant.

When exposed to sea-ice conditions, irrespective of temperature, microalgae produce less proteins at the highest salinities (Sackett et al. 2015). Surviving the extreme salinities within the sea ice and protecting these proteins requires many microalgae to produce osmolytes, organic solutes that are used to adjust the internal osmotic pressure in response to the external salinity (Hellebust 1985). Proline, mannitol and glycerol are well known secondary osmolytes and have been shown to shift with salinity (Chen, Jiang & Wu 2009; Hellebust 1976, 1985; Szabados & Savoure 2010). There have been numerous studies that suggest DMSP acts as an osmolyte (Kirst et al. 1991; Lyon et al. 2016). It has been suggested that although DMSP plays a role in osmotic adjustment, changes to intracellular concentration occur slowly and instead the already high concentration of DMSP within the cell acts as a buffer during the initial period of stress after hyperosmotic shock (Kirst 1996). DMSP concentrations measured in natural Antarctic communities revealed that DMSP content in sea ice algae was 25-56 times greater than the DMSP measured in the open water and over 100 times greater than that found in the under-ice water (Kirst et al. 1991). In *F. cylindrus* cultures, DMSP concentrations increased under hypersaline conditions

and experienced only partial impairment of growth rates, photosynthetic efficiency of PSII and reactive oxygen species, providing some indication that DMSP accumulation in algal cells may act as an effective osmolyte and antioxidant when faced with salinity stress (Lyon et al. 2016).

Antarctic microalgae are well adapted to the seasonal changes in current environmental conditions. However, changes induced by increasing greenhouse gas emissions have the potential to alter ecosystem processes and affect microalgal physiology and functioning. The solubility of CO_2 increases at cooler temperatures, therefore more CO_2 is absorbed in the polar regions than in temperate and equatorial waters. In fact, the Southern Ocean is the world's biggest CO_2 sink, absorbing 30% of anthropogenic CO_2 annually (Sabine et al. 2004). It is also the largest upwelling region in the world, bringing deep CO_2 rich waters to the surface (Orr et al. 2005; Smetacek, Assmy & Henjes 2004). The seasonal changes in pH, upwelling and cold temperatures make Antarctic waters highly susceptible to ocean acidification. Ocean acidification is the drop in ocean pH over a prolonged period, several decades or more, due primarily to increased absorption of CO_2 from the atmosphere (Gattuso & Hansson 2011). Once dissolved in seawater, CO_2 becomes a weak acid and can alter the carbonate chemistry of the water, increasing bicarbonate ions and dissolved inorganic carbon and decreasing pH, carbonate ions and the saturation state of carbonate ions (Gattuso & Hansson 2011).

Numerous studies have looked into the effects of increasing CO_2 on algal physiology, and while there is a negative impact upon calcifying phytoplankton whereby the rate of calcification of their plates is reduced (and most sensitive in cold-water) (Müller, Trull & Hallegraeff 2015), the non-calcifying phytoplankton have seen an increase in growth (Boyd et al. 2016) and productivity (Hopkinson et al. 2011; Trimborn et al. 2013). However, the effects of increasing anthropogenic CO_2 and the resulting

ocean acidification on DMSP production in the oceans are still poorly understood (Arnold, Kerrison & Steinke 2013). As DMS possesses the ability to increase albedo and result in cooling of the local climate, the consequences of anthropogenic climate change on DMS production should be of critical interest and importance. A mesocosm study on a natural Arctic community responses to increased pCO_2 found that DMSP concentrations increased at elevated pCO_2, doubling at 1420 µatm as the community shifted to be dominated by dinoflagellates (Archer et al. 2013). Another recent study using minicosms (600 L) to expose an early spring coastal microbial community to ocean acidification scenarios, found a critical threshold in tolerance to CO_2 levels at around 953 to 1140 µatm (Deppeler et al. 2018; Hancock et al. 2018). Increasing CO_2 had a negative impact on photosynthetic performance and primary production, reducing carbon fixation and chlorophyll *a* accumulation (Deppeler et al. 2018). This experiment also caused in a shift in microalgae community composition, from a starting community dominated by large diatoms and *Phaeocystis antarctica,* which increased abundance with a moderate increase in CO_2, to both populations declining dramatically above 953 µatm, resulting in small diatoms dominating the community (Hancock et al. 2018). These sorts of significant changes in community composition would likely result in major changes in the overall nutritional value and biomolecular composition of the community, as well as the Antarctic DMSP budget and in turn the sulfur cycle, however macromolecular changes, DMSP production and emissions of DMS under ocean acidification scenarios in the Antarctic are yet to be investigated.

THESIS OUTLINE

This thesis investigates the physiological and biochemical adaptations of Antarctic microalgae, focusing on the role of DMSP in the Antarctic environment. The following chapters are organised as follows:

Chapter 2 provides detailed materials and methods, for analyses that are repeated throughout the experimental chapters of this thesis and will be referred to in each chapter where applicable or amended with the minor changes from those described in Chapter 2. In the first experimental chapter, **Chapter 3**, 16 species of Antarctic microalgae are characterised in a screening study where their growth rates, physiological health, carbon content, DMSP production and DMSP lyase activity were measured to find out who are the producers in Antarctic waters. This work is used to inform the following chapters, which look at DMSP production in mixed communities. **Chapter 4** is a descriptive study that takes a geographical look at the DMSP content and lyase activity, macromolecular profiles and productivity of three different Antarctic microalgal communities from three unique Antarctic environments; the open ocean, the sea ice and a hypersaline lake. In **Chapter 5** the link between salinity stress and DMSP production in sea ice microalgal communities is examined, with the aim to link DMSP production with osmoregulation or antioxidant function. The final experimental chapter, **Chapter 6,** explores the effects of rising CO_2 on DMSP production and lyase activity, macromolecular profiles and productivity in a coastal microbial community.

To conclude, **Chapter 7** provides a general discussion, summarising the key findings and highlighting the contributions this thesis has made to the overall understanding of the role of DMSP in microalgae from the Antarctic environment. In addition, it provides new information that may be useful for the modelling of DMSP fluxes in Antarctic communities and discusses directions for future research.

CHAPTER 2. GENERAL METHODS

INTRODUCTION

Throughout this thesis, many of the methods used are common across several experimental chapters. These are presented below and are referred to throughout the thesis in the relevant sections. Specific methods to individual studies are provided within their respective chapters. Information on the statistical analyses employed has been omitted from this section, as methods differed depending on the specific dataset. Refer to the methods section of those particular chapters for further details.

PULSE AMPLITUDE MODULATED FLUOROMETRY

In all experiments, microalgal photosynthetic efficiency was measured using Pulse amplitude modulated (PAM) Fluorometry (Water PAM; Walz GmbH, Effeltrich, Germany). Aliquots (3 mL) of culture or seawater were pipetted into a quartz cuvette and dark-adapted for a minimum of 10 min whilst being continuously stirred. After base fluorescence (F_O) signal stabilised, a saturating pulse (Intensity 12, Width 0.8 s) was applied to measure maximum fluorescence (F_M) and from these two parameters, the maximum quantum yield (F_V/F_M) of photosystem II (PSII) was calculated using the formula $(F_M-F_O)/F_M$ (Baker 2008; Genty, Briantais & Baker 1989).

CELL COUNTS AND GROWTH RATE DETERMINATION FOR ALGAE AND BACTERIA

In all experiments, cell counts were determined using a Neubauer hemocytometer (Swastik Scientific, Mumbai, India) counting chamber (0.5 x 0.5 x 1 mm^3). In laboratory experiments (Chapter 3), cultures were subsampled a (1 mL) every 2 days and fixed in 1% glutaraldehyde for manual counting. Cell

density was estimated according to Guillard (1973) and specific growth rates (μ) calculated according to Wood, Everroad & Wingard (2005). For bacterial enumeration, a 2 mL aliquot was subsampled and fixed in 1% glutaraldehyde, snap frozen in liquid N_2 and stored at -80 °C. Bacterial cell counts were performed using flow cytometry (CytoFLEX S; Beckman Coulter, Inc., USA) according to Marie et al. (1999). Briefly, the aliquot was rapidly defrosted in hot water and 1 mL was run unstained, while the remaining 1 mL was stained with SYBR Green I Nucleic Acid Gel Stain (1:10,000) (Invitrogen, ThermoFisher Scientific, USA) for 15 min before running. The total stained cell count was then subtracted from the unstained cell count to determine total bacterial density.

CHLOROPHYLL *A* CONTENT

Samples for chlorophyll *a* concentration were filtered (6-15 mL of culture, or 50 mL of mixed community sample) onto GF/C filters which were then snap frozen in liquid N_2 and stored at -80 °C until analysis. Pigments were extracted in 90% acetone and incubated at 4 °C in the dark for 24 hours. Chlorophyll content was determined using a spectrophotometer (Cary50: Varian, Santa Clara, CA, USA) and calculated using the equations of Jeffery et al. (1999), modified by Ritchie (2006).

PRIMARY PRODUCTIVITY AND RESPIRATION

Using custom-built oxygen chambers connected to a four-channel fiber-optic oxygen meter (Pyroscience FireSting O2, Germany), net production and respiration were measured on whole communities (Chapters 4, 5 and 6). The custom-built system consisted of four 5.1 mL glass vials each containing an oxygen sensor spots (Pyroscience, Germany) located on the inside of the vial, which was attached using non-toxic silicon glue. To obtain dark respiration and net photosynthesis rates, each vial

was filled to the top and then sealed. Care was taken to avoid any bubbles being enclosed within the system to ensure that oxygen present in the system was due to the cells and not diffusion of gases from trapped bubbles. All vials were stirred continuously using cuvette stirrers to allow homogenous mixing of gases within the system. While in the dark, respiration rates were recorded until a linear rate could be obtained and then samples were exposed to light (a growth irradiance of 144 μmol photons m^{-2} s^{-1} in Chapters 4 and 6; or a light curve of increasing irradiance in Chapter 5) for several minutes until a steady net photosynthetic rate was obtained. Respiration and photosynthesis rates were determined from the slope of the change of oxygen concentration in the vials and gross productivity calculated by summing of respiration and net production rates. All measurements were conducted at 0.1 °C. At the end of measurements, a 1 mL aliquot from each vial was fixed with glutaraldehyde (1% v/v final concentration) for later determination of the cell density for normalisation.

QUANTIFICATION OF SULFUR COMPOUNDS

SAMPLE PRESERVATION AND EXTRACTION

For culture samples (Chapter 3), intracellular DMSP (DMSPp) was obtained by measuring total DMSP (DMSPt = DMSPp + dissolved DMSP + DMS) and subtracting the dissolved DMSP (DMSPd) fraction and DMS. To measure DMS, 2 mL of sample was filtered and placed into an amber vial along with 2 mL of MilliQ water, which was capped, crimped and analysed immediately via gas chromatography. To measure DMSPt, 1 mL of culture was placed in a 20 mL amber vial containing 1 mL of 0.75 M NaOH (used to convert DMSP into DMS), sealed and left to react at room temperature for at least 12 h before being used for quantification of total DMS. To measure dissolved DMSP (DMSPd), cultures were gravity filtered through a 2 μm filter and a 1 mL aliquot pipetted into an amber vial containing 1 mL of 0.75 M NaOH, and the vial immediately capped, crimped and stored at room temperature in the dark for at

least 24 h before analysis. For field samples (Chapters 4, 5 and 6), DMSPp was used as a conservative estimate of intracellular DMSP. Known volumes of seawater were gently centrifuged for 10 mins at 425 g. The supernatant was then removed and the algal pellet resuspended in 10 mL of 100% methanol, sonicated for 30 mins on ice and stored at 3 °C until analysis. The extract was dried in a rotary evaporator and the dried extract resuspended in 3 mL of milliQ water and vortexed for 10 seconds (Tapiolas et al. 2013). A 1 mL aliquot of the resuspended extract was then added to a 20 mL vial containing 1 mL of 0.75 M NaOH, sealed and left to react at room temperature for at least 12 h before being used for quantification of total DMS using gas chromatography.

GAS CHROMATOGRAPHY

DMS concentrations were quantified using a series of DMS standards that ranged between 1 and 100 pmol for purge and trap samples and 100 and 10,000 pmol for higher concentration samples measured via direct injection. DMS standards were generated by converting DMSP standards to DMS through known additions in 20 ml vials, in the same manner as the samples. They were then hydrolysed with NaOH, capped, crimped and left to react for a minimum of 12 h at room temperature before use. All sulfur compound analyses were performed on a Gas Chromatograph (GC) (GC-2010 Plus, Shimadzu) coupled with a flame photometric detector (FPD) set at 160°C with a hydrogen and instrument grade air flow rate set at 40 mL min^{-1} and 60 mL min^{-1}, respectively. DMS was eluted on a capillary column (30 m x 0.32 mm x 5 µm) set at 120°C using high purity Helium as the carrier gas at a constant flow rate of 5 mL min^{-1} and a split ratio of five. For low concentrations, samples were analysed using a purge and trap system attached to the GC. Samples were treated with gas stripping and purged with an inert gas (UP He), extracting all the volatile gas (including DMS) from the sample. The volatiles were then retained in the cryotrap in a sorbent bed i.e. a Teflon loop in liquid N_2, while the purge gas passes through a vent. After 4 min, the sample was released from the cryotrap by heating the trap and

allowing the volatiles to desorb. The trap was then back flushed with carrier gas and sample injected into the gas chromatograph, where the volatiles are separated and DMS detected. For high DMS concentrations (i.e *Phaeocystis* spp.) the purge and trap system was bypassed and direct injection was used to determine sulfur concentrations. A 500 µL of sample of headspace gas was taken from the vial using a gas tight syringe and directly injected into the gas chromatograph injection port through a septum, directly onto the capillary column and DMS detected.

DMSP Lyase Activity (DLA)

Phytoplankton DMSP lyase activity

In all chapters, an estimate of DLA in samples was measured as described by Harada et al. (2004). Algal cells from known volume of culture were gently filtered onto a 2.0 µm polycarbonate filter, snap frozen and stored at -80 °C until later analysis. After thawing on ice, the filter was transferred facedown into a gas-tight vial in 1 mL of pH 8.2 TRIS buffer, capped with a rubber stopper and vortexed for 10 s. After 20 min incubation in iced water, 20 µL of DMSP-HCl (Sigma Aldrich, USA) was added to a final concentration of 5 mM, and the vial sealed and crimped. The vial was vortexed vigorously for 10 s, put back in the ice water, the timer started and 100 µL of headspace immediately extracted using a gas tight syringe, which was then injected directly onto the gas chromatograph for quantification of DMS. DMS production was monitored over time with 4-5 sequential measurements and the exact time of headspace removal recorded. DMS production was linear over time and corrected for the abiotic cleavage activity found in buffer controls. DLA was then normalised to volume of water filtered and algal associated parameters (Chl a, C:N, cell density and volume).

BACTERIAL CLEAVAGE ENZYME ACTIVITY

In chapter 3, bacterial cleavage enzyme activity was measured by taking a known volume of 2.0 µm filtered culture and gently filtering onto a 0.2 µm polycarbonate filter (SterliTech, USA), the filter snap frozen and stored at -80 °C till later analysis. Enzyme activity was then measured as described above for phytoplankton lyase enzyme activity.

MACROMOLECULAR COMPOSITION

Fourier transform infrared (FTIR) spectroscopy is a powerful tool as it is a fast, inexpensive, quantitative, sensitive method providing multivariate data and most notably, when combined with a high intensity light beam and microscope, can be performed on single cells (Heraud et al. 2005). The technique involves irradiating a sample with specific wavelengths of light then measuring the absorbance by the sample. The resulting spectra consist of absorbance bands that are associated with different macromolecules, revealing quantitative variations in macromolecular composition (Heraud et al. 2007).

In Chapters 4 and 6, spectral data were collected on the Infrared Microspectroscopy Beamline (2BMIB) at the Australian Synchrotron, Melbourne, Australia in November 2015. Spectra were acquired over the measurement range 4000-800 cm-1 with a Vertex 80v FTIR spectrometer (Bruker Optics, Ettlingen, Germany) in conjunction with an IR microscope (Hyperion 2000, Bruker) fitted with a mercury cadmium telluride detector that was cooled with liquid nitrogen. To determine the macromolecular composition of cells, single cell analyses were conducted on formalin (2%) fixed cells. A patterned calcium fluoride (CaF_2) window (0.3 mm thick) with fabricated spacers was placed within a compression chamber (ThermoFisher, Waltham, MA, USA) and cells were analysed in hydrated form by pipetting ~2 µL of fixed sample directly onto the centre of the window. A second unpatterned CaF_2

window was placed on top and the lid to the compression chamber closed pushing the window down on the spacer and preventing evaporation before and during measurements (Tobin et al. 2010). The chamber was then placed under the IR microscope which was connected to a computer-controlled microscope stage contained within a specially designed box purged with dehumidified air. Cells identified as belonging to dominate species within the community were selected for spectral acquisition. Measurements were made in transmission mode and an aperture size of 5 μm x 5 μm. Spectral acquisition and instrument control were performed using Opus 6.5 software (Bruker).

Collected spectra were exported from OPUS and imported into multivariate statistical software program for spectroscopy data, The Unscrambler X (Camo Inc., Oslo, Norway). The regions of 3050-2800, 1770-1100 cm-1, which contain the major biological bands (Table 2.1), were selected for analysis. Data was smoothed (5 pts either side) and second derivative transformed (3^{rd} order polynomial) using Savitzky-Golay and then normalised using the SNV normalisation before running a Principal Component Analysis (PCA). Comparisons in macromolecular concentration between communities and species were done using the second derivative spectra by calculating the area under the peak corresponding to each macromolecule band (Table 2.1), peak areas for macromolecules of interest were quantified to determine the relative concentration of each macromolecule per species. This could be achieved because the macromolecule concentration is proportional to the area under the peak in the infrared spectrum corresponding to each macromolecule, previously demonstrated on microalgal cells (Giordano et al. 2001).

Table 2.1 Infrared band assignments of IR spectra.

Wavenumber (cm^{-1})	Assignment	Reference
~3010	*v*(C–H) of *cis*-alkene –HCCH–, from unsaturated fatty acid	(Vongsvivut et al. 2012)
~2960	v_{as}(C–H) from methyl (–CH_3), from saturated fatty acids	(Vongsvivut et al. 2012)
~2925	v_{as}(C–H) from methylene (–CH_2), from saturated fatty acids	(Vongsvivut et al. 2012)
~2855	v_s(C–H) from methylene (–CH_2), from saturated fatty acids	(Vongsvivut et al. 2012)
~1745	*v*(C=O) of ester functional groups, from membrane lipids and fatty acids	
~1720	v(C=O) of ester functional groups, carboxylic group of esters	(Murdock & Wetzel 2009)
~1540	δ(N–H) associated with proteins (amide II band)	(Giordano et al. 2001)
~1460	$\delta_{as}(CH_3)$ and $\delta_{as}(CH_2)$ of proteins (carboxylic group)	(Giordano et al. 2001; Murdock & Wetzel 2009)
~1400	$\delta_s(CH_3)$ and $\delta_s(CH_2)$ of proteins, and v_s(C-O) of COO^- groups (carboxylic group)	(Giordano et al. 2001)
~1200	*v*(C-O-C) of polysaccharides (carbohydrates)	(Giordano et al. 2001)

Note: *v*as = asymmetrical stretch; *v*s = symmetrical stretch; δ_{as} = asymmetrical deformation (bend); δ_s = symmetrical deformation (bend).

CHAPTER 3. CONCENTRATIONS OF DIMETHYLSULFONIOPROPIONATE AND ACTIVITIES OF DIMETHYLSULFIDE-PRODUCING ENZYMES IN BATCH CULTURES OF 15 ANTARCTIC PHOTOTROPHIC PHYTOPLANKTON SPECIES AND 1 PHAGOTROPHIC FLAGELLATE

INTRODUCTION

Dimethylsulfide (DMS) is an important volatile organosulfur compound that readily fluxes out of surface waters into the atmosphere and is responsible for over half of total global natural sulfur fluxes (Simó 2001). DMS has the ability to form cloud condensing nuclei, leading to the hypothesis that DMS is a climate influencing gas (Charlson et al. 1987). Since this hypothesis, research on DMS and its inclusion in environmental models has increased (Aumont, Belviso & Monfray 2002; Bopp et al. 2004; Le Clainche et al. 2010). The world's oceans contribute up to 95% of sulfur flux to the atmosphere, however the exchange of DMS in the oceans is regionally specific, as it is highly dependent on latitude and season (Galí et al. 2015; Lana et al. 2012; Yoch 2002).

The majority of the DMS pool in the ocean is resultant of the conversion of dimethylsulfoniopropionate (DMSP) to DMS by algal and bacterial enzymes (Alcolombri et al. 2015; Curson et al. 2011). While not all marine microalgae produce DMSP, extensive research on temperate microalgal species has shown dinoflagellates to be the most prolific producers of DMSP amongst marine microalgae, although highly variable in intracellular concentrations, ranging from 0.003 mM to 7590 mM (Caruana & Malin 2014). The other major group of DMSP producers are the prymnesiophytes, and as such, there has been substantial research into DMSP production in two prymnesiophytes, *Emiliana huxleyi* (Levasseur et al.

1996; Matrai & Keller 1993; Steinke, Wolfe & Kirst 1998) and *Phaeocystis* spp. (Liss et al. 1994; Mohapatra et al. 2013; Stefels & Van Boekel 1993), which represent common bloom-forming species and both of which have been shown to possess DMSP lyase capabilities (Franklin et al. 2010; Harada & Kiene 2011).

Antarctica is a recognised hot spot for DMS emissions, where in the austral summer, sea surface DMS emissions can reach 10 nM (Kettle et al. 1999). DMSP production and thus DMS flux, in summer is often attributed to the vast blooms of *Phaeocystis* that occur in coastal waters (DiTullio et al. 2000). However, high levels of DMSP have also been recorded in the sea ice meltwater, where diatoms dominate the community (Trevena & Jones 2006). In addition to *Phaeocystis* spp. other Southern Ocean phytoplankton have been shown to produce considerable amounts of DMSP (Baumann, Brandini & Staubes 1994; Stefels & Van Boekel 1993) and while temperate diatoms are not generally considered substantial DMSP producers, high levels of DMS have been recorded in Antarctic areas dominated by diatoms (Trevena et al. 2003).

In order to accurately predict the potential impact of phytoplankton communities on DMS emissions, we need to understand the variability and individual contributions of each species, in particular those that form algal blooms. A current challenge in the modelling of DMS production and its effect on the climate has been limited knowledge, particularly in the Antarctic, on which taxa synthesise DMSP, what are their intracellular concentrations, do they have the capacity to lyase DMSP and how does this vary with environmental conditions (Steiner et al. 2012). In this chapter, we present the DMSP concentrations and DMS-producing enzyme activity (DLA) for 15 species of Antarctic phototrophic phytoplankton (14 microalgae species and one cyanobacterium) and a phagotrophic flagellate (*Telonema* sp.), as well as their associated bacteria, along with corresponding C, N and Chl *a* measurements. Our overall aim was to increase the database available for describing and modelling the contribution of Southern Ocean phytoplankton to DMSP and DMS production.

METHODS

CELL CULTURING, MAINTENANCE AND SUBSAMPLING FOR EXPERIMENTATION

Cultures (13 species) were isolated from Prydz Bay, Davis Station, Antarctica (66°S, 77°E) during the Austral Spring (November 2014) and maintained in 0.2µm filtered seawater (salinity 35 psu) enriched with an adjusted L1 stock medium (Table 3.1). Three additional cultures (*Dunaliella*, *Phaeocystis cf. pouchetti*, and *Synechococcus*) were purchased from the CSIRO Australian National Algae Culture Collection (Table 3.1). Cultures were acclimated to an irradiance of 50 µmol photons m^{-2} s^{-1} (14:10 h light: dark cycle) and maintained at 3 ± 1 °C. Cultures were grown in quadruplicate for each species and growth curves obtained from cell counts (as per **General Methods**) to ascertain time of exponential growth. In a subsequent growth curve, each replicate was subsampled for DMSP and Chl *a* (**General Methods, Chapter 2**), as well as C:N analyses in mid-exponential phase. Sampling was undertaken during exponential growth phase to allow cells to be captured in balanced growth and to avoid nutrient limitation. All sampling was performed mid-way through the photoperiod (12 pm) to reduce physiological variation due to diel activity.

C:N ANALYSIS

A 5-20 mL aliquot of culture was filtered onto a GF/F filter (pre-combusted at 450°C for 4 h) and snap frozen in liquid nitrogen until later analysis. Prior to analysis, the sample and blank filters were dried at 35 °C for 48 h before being wrapped in tin foil and placed in ceramic boats with nickel boat liners (LECO Corporation, USA). Analysis was run on a Leco TruMac Carbon Nitrogen Analyser (LECO Corporation, USA). Carbon and nitrogen were combusted at 1300 °C and the infrared detector measures carbon as CO_2, while the thermal conductor detects nitrogen as N2. Concentrations were quantified using a series of soil reference material standards (LECO Corporation, USA) with calibration limits of 0.1 – 6 mg N and 1.2 – 223 mg C. C and N concentrations were corrected against blank

measurements (filters that have had L1 media run through them) and normalised to filtered volume and cell density.

Table 3.1 A summary table of the Antarctic cultures investigated.

Species	Strain code	Collection site	Medium	Grouping
Chaetoceros castracanei	PZB010	Prydz Bay	L1	Diatom
Chaetoceros sp.	-	Prydz Bay	L1	Diatom
Chrysophyte sp.	PZB025	Prydz Bay	L1	Other
Dunaliella sp.	CS-635	Organic Lake	L1	Other
Fragilariopsis sp.	PZB009	Prydz Bay	L1	Diatom
Fragilariopsis sp.	PZB060	Prydz Bay	L1	Diatom
Nitzschia sp.	PZB001	Prydz Bay	L1	Diatom
Phaeocystis cf. pouchetti	CS-243	Antarctica	L1	Haptophyte
Phaeocystis sp.	PZB016	Prydz Bay	L1	Haptophyte
Pseudonitzschia sp.	PZB063	Prydz Bay	L1	Diatom
Pyramimonas sp.	PZB033	Prydz Bay	L1	Other
Stellarima sp.	AAD015	Antarctica	L1	Diatom
Synechococcus sp.	CS-601	Ace Lake	L1	Other
Telonema sp.	PZB013	Prydz Bay	L1	Other
Thalassiosira sp.	PZB048	Prydz Bay	L1	Diatom
Thalassiosira sp.	PZB062	Prydz Bay	L1	Diatom

Medium was prepared as described in the Materials and methods section.

DATA ANALYSIS

IBM SPSS statistics software (version 20) was used to perform two sample Kolmogorov-Smirnov tests to compare distributions between taxa for carbon, nitrogen and Chl *a* content. SigmaPlot (version 11.0) was used to generate box and whisker plots for intracellular concentrations of C, N, Chl *a*, cell volume, DMSP and DMSP lyase data.

RESULTS

Exponential growth rates ranged from 0.12 to 0.49 day^{-1} across all species with the average growth rate of 0.22 day^{-1} (Table 2). The green algae *Dunaliella* and centric diatom *Stellarima* were the slowest growing species, while one of the species of *Chaetoceros* (PZB010) and the cyanobacterium *Synechoccocus* were the fastest growing cultures (Figure 3.1; Table 2).

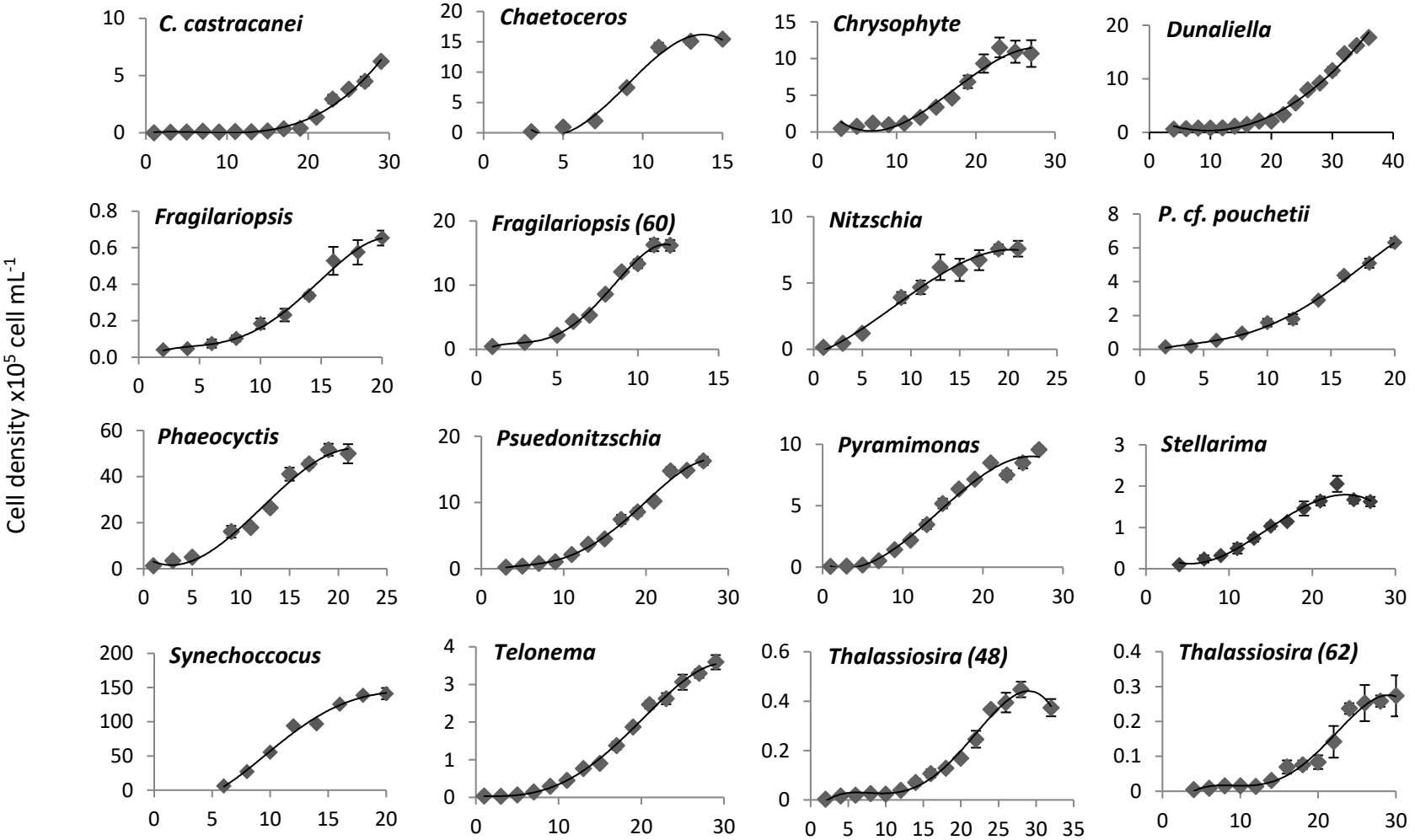


Figure 3.1 Growth curves for 16 Antarctic phytoplankton species. Cell density is presented in cells mL^{-1} (x10^5) ± SE (n=4). Sampling was taken during exponential phase.

Mean cell volume spanned several orders of magnitude between all 16 species (Table 3.2). The cyanobacterium *Synechococcus* was the smallest species (3 μm^3), followed by the green microalga *Dunaliella* (15 μm^3). There was a 100-fold difference in cell volume between the smallest diatom species (*Psuedonitzschia*, 97 μm^3) and the largest diatom species (*Stellarima*, 10,086 μm^3), which was also the largest species in this study. The two haptophytes, both from the genus *Phaeocystis,* ranged from 31-50 μm^3, while the cryptomonad-haptophtye species, *Telonema* was 238 μm^3 (Table 3.2).

As with cell volume, particulate organic carbon and nitrogen, chl *a*, and DMSP per cell ranged three orders of magnitude across all species (Figure 3.2A). However, when expressed per cell volume (CV), reduced variation was seen in C, N and chl *a*, whereas DMSP per CV remained highly variable ranging four orders of magnitude across all 16 species (Figure 3.2B).

Species contained on average 179 ± 92 pg C $cell^{-1}$ and 32 ± 18 pg N $cell^{-1}$, with an average C:N of 5.1 (Table 3.2). In general, diatoms had higher average C and N content than the other species, but being larger cells, this pattern was reversed when expressed per cell volume (Table 3.2; Figure 3.2B). Chlorophyll *a* content ranged from 0.01 pg $cell^{-1}$ in *Phaeocystis* to 31.75 pg $cell^{-1}$ in *Stellarima,* with an average of 3.67 ± 1.997 pg $cell^{-1}$ for all species, while diatoms had a higher average of 6.11 ± 3.396 pg $cell^{-1}$ ($F_{1,63}$= 8.905, P=0.004; Table 3.2). When normalised to cell volume, average chl *a* concentration was 6.4 ± 1.75 fg μm^3 for all species, with a lower average in diatoms 3.1 ± 0.82 fg μm^3. Chlorophyll *a* fluorescence varied between species, with maximum quantum yield of PSII (F_VF_M) ranging from 0.37 ± 0.008 in *Fragilariopsis* (60) to 0.68 ± 0.005 in the chrysophyte. The chrysophyte also had the highest effective quantum yield (EQY) at 0.63 ± 0.005, while the lowest EQY was observed in *Synechoccocus* at 0.29 ± 0.006 (Table 3.2).

Table 3.2 Specific growth rate (μ), cell volume, carbon, nitrogen and Chl *a* concentration and chlorophyll *a* fluorescence measured in 15Antarctic phototrophs and one flagellate. Displayed as means (n=4) ± standard error. F_V:F_M = maximum quantum yield of PSII and EQY = effective quantum yield of PSII.

Species	Specific growth rate		Volume		C				N				C:N ratio		Chl *a*				F_V:F_M		EQY	
	(μ day^{-1})		(μm^3)		pg cell^{-1}		pg μm^{-3}		pg cell^{-1}		pg μm^{-3}				pg cell^{-1}		fg μm^{-3}					
C. castracanei	0.20	(0.02)	334	(14)	78	(11.5)	0.23	(0.03)	24	(2.2)	0.07	(0.007)	3.4	(0.5)	0.52	(0.059)	1.6	(0.18)	0.41	(0.007)	0.37	(0.007)
Chaetoceros	0.49	(0.02)	17	(2)	1	(0.4)	0.04	(0.02)	-	-	-	-	-	-	0.16	(0.006)	9.2	(0.34)	0.45	(0.003)	0.42	(0.005)
Chrysophyte	0.15	(0.02)	22	(3)	5	(3.3)	0.24	(0.15)	10	(0.7)	0.48	(0.034)	0.5	(0.3)	0.26	(0.014)	11.9	(0.66)	0.68	(0.005)	0.63	(0.005)
Dunaliella	0.12	(0.00)	15	(1)	10	(0.8	0.67	(0.06)	5	(0.4)	0.31	(0.029)	2.2	(0.3)	0.32	(0.005)	20.8	(0.30)	0.57	(0.006)	0.49	(0.012)
Fragilariopsis (09)	0.21	(0.05)	3392	(242)	650	(58.1)	0.19	(0.02)	49	(5.4)	0.01	(0.002)	13.5	(1.1)	8.16	(0.329)	2.4	(0.10)	0.43	(0.010)	0.39	(0.008)
Fragilariopsis (60)	0.36	(0.01)	250	(15)	72	(14.7)	0.29	(0.06)	19	(0.9)	0.07	(0.004)	3.8	(0.6)	0.48	(0.028)	1.9	(0.11)	0.37	(0.008)	0.48	(0.004)
Nitzschia	0.23	(0.03)	221	(16)	18	(1.8)	0.08	(0.01)	7	(0.5)	0.03	(0.002)	2.8	(0.3)	0.33	(0.019)	1.5	(0.09)	0.55	(0.008)	0.42	(0.005)
P. cf. pouchetii	0.16	(0.03)	50	(4)	38	(5.7)	0.76	(0.11)	4	(1.1)	0.08	(0.015)	12.7	(1.2)	0.08	(0.016)	1.5	(0.32)	0.47	(0.003)	0.30	(0.003)
Phaeocystis	0.23	(0.01)	31	(2)	3	(1.7)	0.10	(0.06)	-	-	-	-	-	-	0.01	(0.002)	0.4	(0.05)	0.40	(0.010)	0.32	(0.012)
Pseudonitzschia	0.15	(0.01)	97	(11)	6	(2.5)	0.06	(0.03)	6	(0.9)	0.06	(0.009)	0.9	(0.3)	0.17	(0.004)	1.7	(0.05)	0.48	(0.010)	0.42	(0.005)
Pyramimonas	0.14	(0.02)	75	(8)	26	(1.2)	0.35	(0.02)	4	(0.4)	0.06	(0.006)	6.4	(0.6)	0.49	(0.019)	6.5	(0.25)	0.58	(0.001)	0.58	(0.002)
Stellarima	0.12	(0.01)	10086	(930)	1426	(230)	0.14	(0.02)	240	(13)	0.02	(0.001)	5.9	(0.9)	31.75	(1.806)	3.1	(0.18)	0.54	(0.010)	0.38	(0.009)
Synechococcus	0.45	(0.00)	3	(0.6)	3	(0.1)	0.89	(0.03)	1	(0.0)	0.16	(0.008)	5.5	(0.2)	0.07	(0.002)	23.2	(0.79)	0.41	(0.008)	0.29	(0.006)
Telonema	0.14	(0.02)	238	(67)	139	(48.0)	0.58	(0.20)	31	(8.3)	0.13	(0.035)	3.6	(1.2)	2.48	(0.125)	10.4	(0.52)	0.42	(0.009)	0.30	(0.006)
Thalassiosira (48)	0.14	(0.02)	2302	(225)	155	(67.4)	0.07	(0.03)	13	(13)	0.01	(0.006)	-	-	6.06	(0.876)	2.6	(0.38)	0.58	(0.016)	0.36	(0.003)
Thalassiosira (62)	0.19	(0.03)	1765	(391)	239	(147)	0.14	(0.08)	-	-	-	-	-	-	7.36	(1.412)	4.2	(0.80)	0.55	(0.005)	0.44	(0.005)
Averages																						
Diatoms	0.23	(0.04)	2051	(1080)	294	(157)	0.14	(0.03)	51	(32)	0.04	(0.011)	5.0	(1.8)	6.11	(3.396)	3.1	(0.82)	0.49	(0.025)	0.41	(0.012)
All species	0.22	(0.03)	1181	(644)	179	(92.6)	0.30	(0.07)	32	(18)	0.12	(0.038)	5.1	(1.2)	3.67	(1.997)	6.4	(1.75)	0.49	(0.022)	0.41	(0.024)

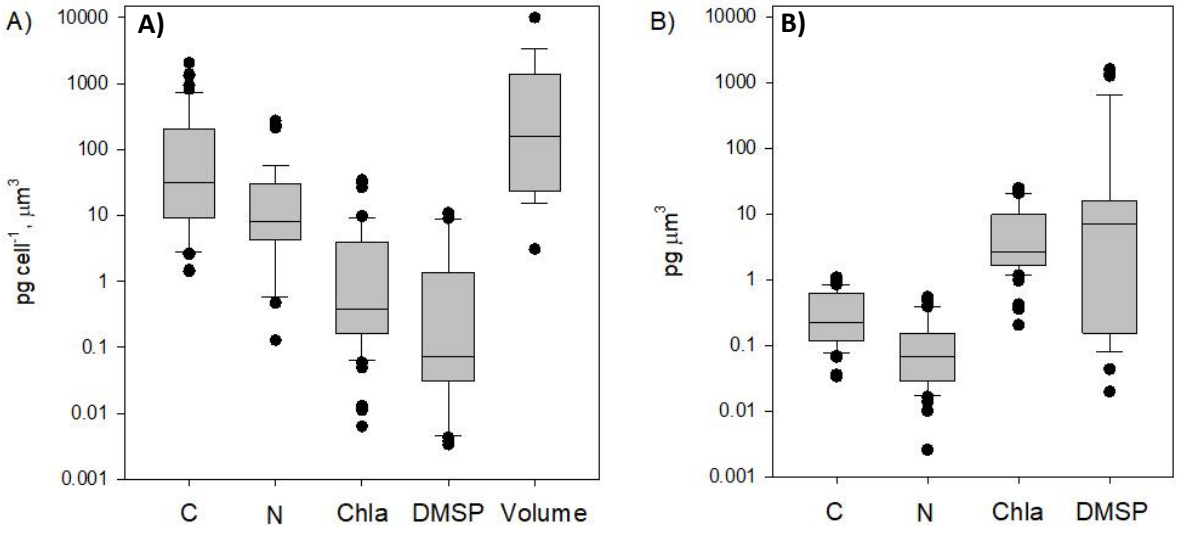


Figure 3.2 Antarctic DMSP producers A) intracellular concentrations of C, N, Chl a and DMSP in pg cell^{-1} as well as range of cell volume (CV) in µm^3 for 16 Antarctic phototrophic species and **B)** concentrations of C, N, Chl a, DMSP expressed per cell volume in pg µm^3.

Eleven out of the 16 Antarctic species produced DMSP, both cultures of *Chaetoceros*, the chrysophyte, *Dunaliella* and *Telonema* had no detectable levels of DMSP (Table 3.3). Of the species that did produce DMSP, concentrations per cell ranged from 0.004 pg cell^{-1} in *Synechoccocus* to 9.82 pg cell^{-1} in *Phaeocystis cf. pouchetii* (Table 3.3). DMSP content showed similar patterns whether normalised per CV, C, N or chl *a* (Table 3.3). DMSP per CV was elevated in *Phaeocystis*, but only in one strain (*P. cf. pouchetii*). The large pennate diatoms *Fragilariopsis* and *Nitzschia* had relatively high DMSP concentrations, while the majority of the other diatoms had low concentrations (Table 3.3).

Table 3.3 DMSP data for 16 Antarctic marine plankton species. DMSP particulate is expressed per total cell volume (CV), per cell, per carbon, per nitrogen and per chlorophyll *a*. C-DMSP:C indicates the DMSP-carbon to C ratio. For each species, the mean value for all replicates (n=4) is shown ± standard error in parentheses.

Species	DMSP:CV		DMSP:cell				DMSP:C		DMSP:N		DSMP:Chla		C-DMSP:C	
	mM		pg cell^{-1}		fmol cell^{-1}		mmol mol^{-1}		mmol mol^{-1}		mmol g^{-1}		%	
C. castracanei	BDL		BDL		BDL		BDL		BDL		BDL		BDL	
Chaetocerous	BDL		BDL		BDL		BDL		BDL		BDL		BDL	
Chrysophyte	BDL		BDL		BDL		BDL		BDL		BDL		BDL	
Dunaliella	BDL		BDL		BDL		BDL		BDL		BDL		BDL	
Fragilariopsis (09)	17.63	(0.96)	8.03	(0.438)	59.80	(0.003)	1.12	(0.086)	17.76	(2.17)	7.37	(0.58)	0.56	(0.04)
Fragilariopsis (60)	5.76	(0.46)	0.19	(0.015)	1.44	(0.000)	0.26	(0.031)	1.07	(0.04)	3.01	(0.08)	0.13	(0.02)
Nitzschia	46.64	(1.34)	1.38	(0.040)	10.31	(0.000)	7.04	(0.852)	22.07	(2.04)	31.81	(1.50)	3.52	(0.43)
P. cf. pouchetii	1460.12	(84.08)	9.82	(0.565)	73.17	(0.004)	23.89	(2.276)	302.14	(58.35)	1091.48	(215.93)	11.95	(1.14)
Phaeocystis	10.10	(0.96)	0.04	(0.004)	0.31	(0.000)	-		-		30.72	(5.73)	-	
Pseudonitzschia	1.85	(0.04)	0.02	(0.001)	0.18	(0.000)	-		0.48	(0.08)	1.07	(0.00)	-	
Pyramimonas	7.10	(0.24)	0.07	(0.002)	0.53	(0.000)	0.24	(0.004)	1.81	(0.16)	1.10	(0.05)	0.12	(0.00)
Stellarima	0.11	(0.02)	0.15	(0.029)	1.08	(0.000)	0.01	(0.003)	0.06	(0.01)	0.03	(0.01)	0.01	(0.00)
Synechococcus	9.45	(0.58)	0.004	(0.000)	0.03	(0.000)	0.13	(0.007)	0.82	(0.07)	0.41	(0.03)	0.06	(0.00)
Telonema	BDL		BDL		BDL		BDL		BDL		BDL		BDL	
Thalassiosira (48)	0.15	(0.02)	0.05	(0.006)	0.35	(0.000)	-		-		0.06	(0.00)	-	
Thalassiosira (62)	0.04	(0.03)	0.01	(0.007)	0.06	(0.000)	-		-		0.01	(0.01)	-	
Averages														
Diatoms	10.31	(6.51)	1.40	(1.119)	10.46	(8.34)	2.11	(1.662)	8.29	(4.80)	6.19	(4.39)	1.05	(0.74)
All species	141.72	(131.90)	1.80	(1.076)	13.39	(8.02)	4.67	(3.342)	305.97	(298.73)	106.10	(98.60)	2.34	(1.67)

BDL = Below Detection Limit

DMSP content (per cell) showed *Phaeocystis cf. pouchetii* was the highest producer of DMSP, diatoms were moderate and then *Synechococcus* produced the least (Figure 3.3A). Interestingly, the second strain of *Phaeocystis* amongst the lowest producers, demonstrating almost as much variation between genus as seen within class (Figure 3.3B). When grouped, DMSP content revealed diatoms to have a higher degree of variation compared to the haptophytes (Figure 3.3B), despite the difference in the two *Phaeocystis* strains, but overall concentrations overlapped in range. Less DMSP was found in the *Pyramimonas* and cyanobacteria species, which made up the 'Other' category.

Due to the difference in cell size, when DMSP was expressed per cell volume, the ranking order changed (Figure 3.4A) and *Thalassiosira* (48) had the lowest content per cell volume (0.04 mM), while *P cf. pouchetii* remained the species with the highest DMSP content (1460 mM), with intracellular concentrations more than order of magnitude higher than any other species (Figure 3.4A). Importantly, the second strain of *Phaeocystis* ranked higher and *Synechoccocus* became a mid-range producer. Under this normalisation, the centric diatoms were ranked lowest (Figure 3.4B). When visualised as a group, the median value for diatoms (~2 nM) was lower than the other two groups (haptophytes with a median of ~1000 nM and 'other' a median of ~10 nM). However, even though the 'Other' group median was higher than the diatom, due to the high variation in diatoms, the ranges overlapped (Figure 3.4B).

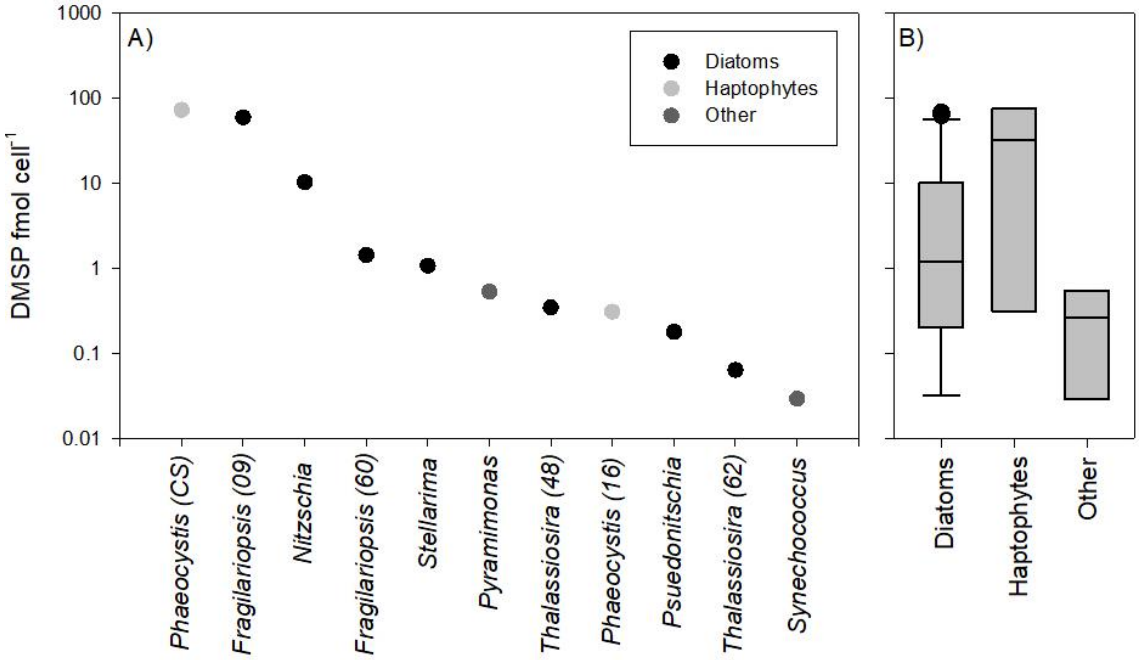


Figure 3.3 DMSP content in fmol per cell in the 11 Antarctic species that produce DMSP. A) Data arranged in decreasing order. Arranged by functional group. Diatoms – black, Haptophytes – light grey, Other – dark grey. **B)** Box and whisker plot showing the distribution for grouped data.

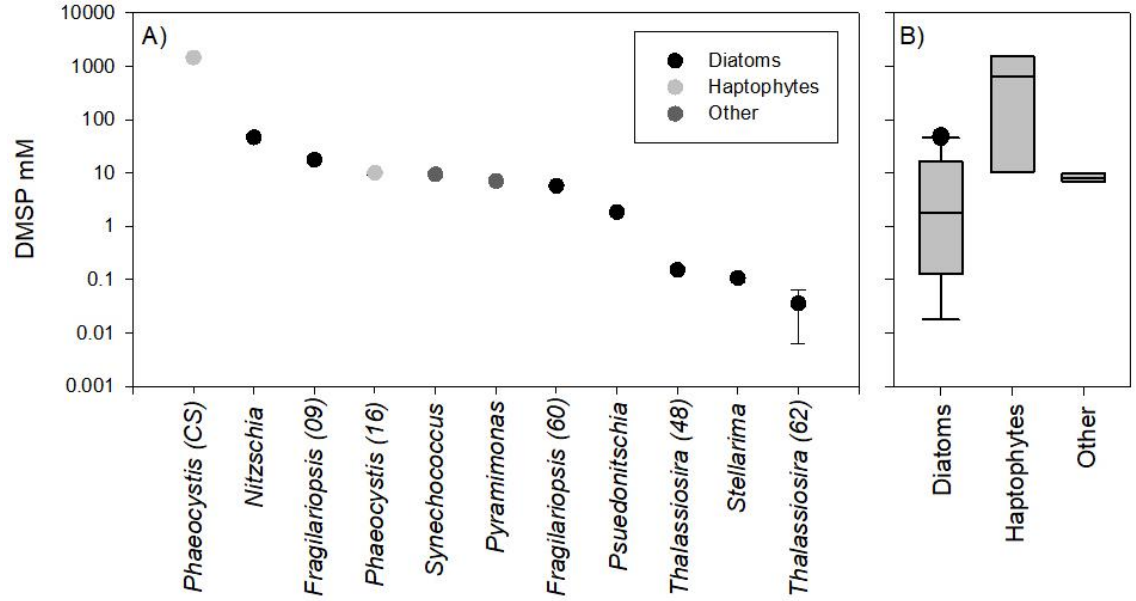


Figure 3.4 Intracellular DMSP concentrations (mM) in the 11 Antarctic species that produced DMSP. A) Data arranged in descending order based on concentration. Arranged by functional group. Diatoms – black, Haptophytes – light grey, Other – dark grey. **B)** Box and whisker plot showing the distribution for grouped data.

When considering all DMSP producing species, the overall variability in DMSP content per cell was the same as DMSP per cell volume (CV), C, N and chl *a* (Figure 3.5A). Variability of the interquartile range, was lowest when DMSP was normalised to C (Figure 3.5A). DMSP normalised to chl *a* showed the greatest amount of variability, except for in Haptophytes, where the least variable parameters were DMSP expressed per chl *a* or N (Figure 3.5C).

From the 16 Antarctic species that were screened, nine were found to have lyase activity, including both *Phaeocystis* cultures, both chlorophytes (*Dunaliella* and *Pyramimonas*) and several diatom species (Table 3.4). DLA per cell ranged from 0.02 fmol $cell^{-1}$ h^{-1} in *Dunaliella* and *Phaeocystis* to 1.98 fmol $cell^{-1}$ h^{-1} in *Pyramimonas,* whereas per cell volume, *Chaetoceros* had the highest rate of activity (19,366 fmol L^{-1} h^{-1}). There was a similar spread of data for all species capable of DMSP lyase, when expressed either per cell or per CV (Figure 3.5A). However, when looking at diatoms there was over an order of magnitude of difference in DLA per cell volume compared to per cell (Figure 3.5B).

Of the nine microalgal species that had detectable lyase activity, four also had DMSP lyase activity detected in their associated bacterial community (Table 3.4). Three cultures had relatively low levels of bacterial DLA ranging from 4.3 – 6.3 nmol L^{-1} h^{-1}, however in the *P. cf. pouchetii* culture, total bacterial community DLA was 100 times greater (456.2 nmol L^{-1} h^{-1}). Interestingly, in *P. cf. pouchetii* cultures the rate of DLA was greater in the bacterial fraction than the algal fraction (Table3.4). However, it cannot be ruled out that small *P. pouchetti* flagellates did not pass through the 2 µm filter, contributing to the bacterial lyase detected. The lowest intracellular rate of DLA was in bacteria associated with *Nitzschia* 0.2 fmol $cell^{-1}$ h^{-1} which was five times lower than the algal rate of DLA in *Nitzschia* at 0.04 fmol $cell^{-1}$ h^{-1} (Table 3.4).

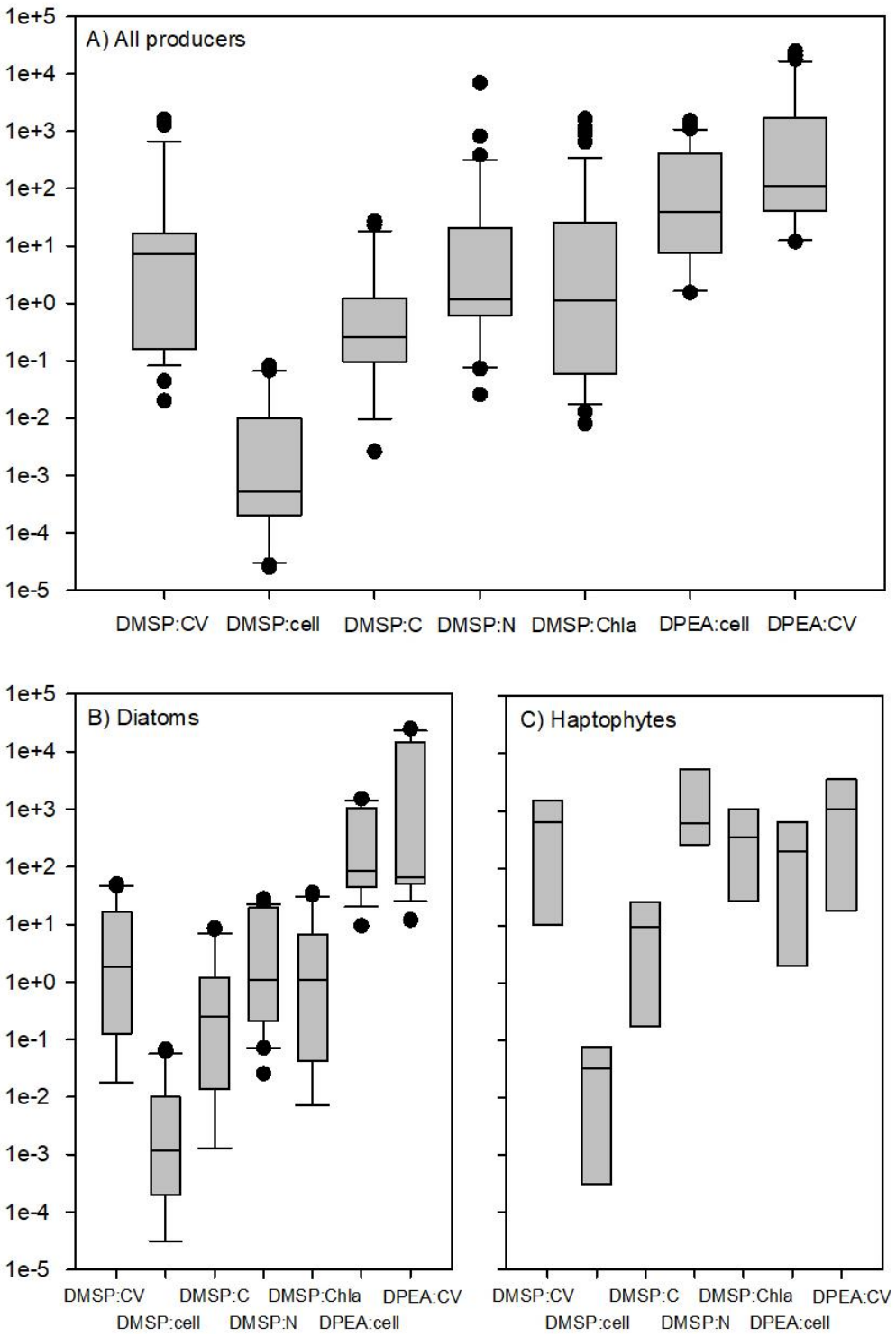


Figure 3.5 The DMSP data for species that produce DMSP and DMSP lyase activity (DLA) of species with lyase capability for groupings A) all species, **B)** diatoms only, **C)** haptophytes only. Y-axis for each parameter = DMSP:CV – DMSP per cell volume (CV) in mM, DMSP:cell – DMSP per cell in pmol cell-1, DMSP:C – DMSP per carbon in mmol mol^{-1}, DMSP:N – DMSP per nitrogen in mmol mol^{-1}, DMSP:Chla - DMSP per chlorophyll *a* in mmol g^{-1}, DLA:cell – Algal DLA per cell in fmol $cell^{-1}$ h^{-1}, DLA:CV – Algal DLA per cell volume in fmol L^{-1} h^{-1}.

Table 3.4 DMS content and *in vivo* DLA data for 16 Antarctic species and their associated bacterial consortia. For each species, the mean value for all replicates (n=4) is shown ± standard error.

Species	DMS	DLA_{algae}	DLA_{algae}:cell	DLA_{algae}:CV	DLA_{bact}	DLA_{bact}:cell
	pmol mL^{-1}	nmol L^{-1} h^{-1}	fmol $cell^{-1}$ h^{-1}	fmol L^{-1} h^{-1}	nmol L^{-1} h^{-1}	fmol $cell^{-1}$ h^{-1}
C. castracanei	BDL	BDL	- -	- -	BDL	- -
Chaetoceros	7 (0.37)	1493 (154)	1.19 (0.156)	19366.59 (2550.23)	BDL	- -
Chrysophyte	BDL	BDL	- -	- -	BDL	- -
Dunaliella	9 (0.21)	26 (6)	0.02 (0.005)	194.82 56.59	BDL	- -
Fragilariopsis (09)	BDL	BDL	- -	- -	BDL	- -
Fragilariopsis (60)	6 (0.12)	22 (6)	0.07 (0.015)	72.56 16.38	BDL	- -
Nitzschia	26 (0.28)	27 (3)	0.04 (0.006)	41.03 10.83	4.3 (0.3)	0.2 (0.01)
P. cf. pouchetii	1527 (57.27)	147 (24)	0.56 (0.095)	3112.92 527.58	456.2 (74.7)	271.9 (51.66)
Phaeocystis (16)	12 (0.49)	7 (2)	0.02 (0.005)	20.85 3.71	5.3 (2.6)	1.8 (0.87)
Pseudonitzschia	7 (0.25)	3 (1)	- -	- -	ND	- -
Pyramimonas	11 (2.51)	23 (2)	1.98 (0.174)	129.03 9.04	6.3 (0.9)	3.3 (0.58)
Stellarima	BDL	BDL	- -	- -	BDL	- -
Synechococcus	BDL	BDL	- -	- -	BDL	- -
Telonema	BDL	BDL	- -	- -	BDL	- -
Thalassiosira (48)	BDL	BDL	- -	- -	BDL	- -
Thalassiosira (62)	9 (0.41)	18 (4)	1.67 (0.521)	54.04 37.73	BDL	- -
Averages						
All producers	103 (94.98)	196 (163)	0.69 (0.270)	2873.98 2249.31	112.0 (87.5)	72.2 (52.4)

BDL = Below detection limit, ND = No data

DISCUSSION

The 16 Antarctic cultures covered a range of phytoplankton functional groups, including diatoms, haptophytes, green algae, a chrysophytes, a cryptomonad-haptophyte and a cyanobacteria. The growth rates calculated in this study are comparable to those observed in microalgae isolated from the Antarctic previously, 0.08-0.34 day^{-1} (Teoh et al. 2004). Chlorophyll *a* content however, was lower for *Phaeocystis* spp. and *Synechococcus* sp. than previous studies (Baumann et al. 1994; Buma et al. 1991; Rueter & Unsworth 1991), but this could be due to differences in growth conditions and strains (Powell et al. 2005). In this study, cultures were kept at 3 °C compared with previous work on a variety of polar *Phaeocystis* spp. where temperature ranged from -2 to 22 °C (Baumann et al. 1994). Reduced chl *a* could also be responsible for the unexpectedly lower maximum photochemical efficiency of *Phaeocystis* spp. and *Fragilariopsis* spp. compared to previous Antarctic photophysiology studies (Kropuenske et al. 2009; Lyon et al. 2016). The carbon content of *P. cf. pouchetii* and *Synechococcus* was in the same range as previous studies (Baumann et al. 1994; Biddanda & Benner 1997) and *P. cf. pouchetii* had the C:N ratio reflective of a nitrogen replete culture (Schoemann et al. 2005). The large variation seen in the carbon content of diatoms in this study has also been observed before in Antarctic diatoms, where small chain-forming cells of *Chaetoceros socialis* were much lower in carbon (8 pg $cell^{-1}$) than the large *Thalassiosira tumida* cells (1056 pg $cell^{-1}$), while *Fragilariopsis* sp. and *Nitzschia* sp. fell within that range (Baumann, Brandini & Staubes 1994; Young et al. 2015).

Not all the Antarctic species in this study produced DMSP, and of those that did, there was considerable variation between each species, with some possible grouping based on cell type. For example, within the diatoms, the pennate diatoms such as the *Fragilariopsis* spp., *Nitzschia* sp. and *Pseudonitzschia* sp. were greater producers of DMSP per cell volume when compared to the centric diatoms *Stellarima* sp. and *Thalassiosira* spp, which were some of the lowest producers amongst the diatom species. In a previous study, two pennate diatoms, *Nitzschia* sp. and *Navicula* sp. isolated from a seasonal sea-ice

zone in Saroma-Ko Lagoon, Hokkaido, Japan, were found to be DMSP producers, however, the DSMP concentrations measured were much lower, 0.0034 and 0.0016 mM, respectively (Kasamatsu et al. 2004). The pennate diatom, *Nitzschia* sp. in this study possessed the highest intracellular concentration (46 mM) for any of the diatom species, generating significantly higher amounts of DMSP than those previously recorded in *Nitzschia* spp (Kasamatsu et al. 2004).

The DMSP content of the centric diatoms measured in this study, *Stellarima* sp. and *Thalassiosira* spp., ranged from 0.04 – 0.11 mM, suggesting relatively low production by these cells. While seemingly low, these values are within the same range as temperate centric diatoms, *Thalassiosira oceanica* at 0.9 mM (Vila-Costa et al. 2006) and *T. pseudonana* at 0.85 - 1.46 mM (Sunda et al. 2002; Vila-Costa et al. 2006). Of note however, is that these data were collected on strains cultured under relatively standard growth conditions. Earlier work on *T. pseudonana* found that DMSP concentrations were below the detectable limit in cells before a shift in salinity, light and nitrogen starvation, which caused increases in intracellular DMSP concentration (up to 8.9 mM) (Kettles, Kopriva & Malin 2014). Similarly, another study showed that iron limitation caused an increase in cellular DMSP in *T. oceanica* (Bucciarelli et al. 2013). Therefore, it's possible that these Antarctic strains, while low producers under growth conditions, may in fact increase their DMSP production if exposed to environmental shifts such as increased salinity, light or nutrient limitation.

Two green algae were included in this screening study, *Dunaliella* sp., which was below detection in this study, but has been shown to produce trace levels of DMSP previously (Matrai & Keller 1994); and *Pyramimonas* sp., which was a moderate producer (7.1 mM). *Pyramimonas* has been seen to dominate sea ice communities alongside *Phaeocystis* (Vance et al. 2013) and a previous study found a temperate *Pyramimonas* sp. to be a low level DMSP producer at 0.02 fmol cell^{-1} (Keller 1989a). This is an order of magnitude less than the 0.53 fmol cell^{-1} detected in the *Pyramimonas* sp. of this study, validating that Antarctic isolates may produce more DMSP than their temperate counterparts. The *Pyramimonas* sp. in

this study also had substantial lyase activity (1.98 fmol $cell^{-1}$ h^{-1}), taken together, these data suggest that this species may contribute to sulphur production in the sea ice. However, in contrast to our results, a previous study on an Antarctic isolate of *Pyramimonas* sp. noted no detectable DMSP lyase activity (Harada & Kiene 2011), indicating it may have been a different strain to what was presently tested.

The cyanobacterium, *Synechococcus,* is a well-known consumer of DMSP (Malmstrom et al. 2005) and a possible significant producer (Wilson, Turner & Mann 1998). In this study, *Synechococcus* had intracellular concentrations of ~9.45 mM, making it a moderate producer across all species measured, however, DLA was below detection. This finding is supported by Malmstrom et al. (2005) who found axenic cultures of phycoerythrin containing *Synechococcus* were capable of DMSP transport, but did not produce any DMS.

In addition to species and functional group comparisons, we are also able to make some intra-genus comparisons, as the study included two species from the genera *Fragilariopsis*, *Phaeocystis, Chaetoceros* and *Thalassiosira*. Interestingly, DMSP content varied substantially within each genus, where *Fragilariopsis* strain PZB009 was a relatively moderate producer (59.80 fmol $cell^{-1}$), whereas PZB060 was a low producer (1.44 fmol $cell^{-1}$). One recent study on DSMP production in an Antarctic diatom, exposed *Fragilariopsis cylindrus* to salinity shifts representative of sea ice formation and melting, where DMSP ranged from 0-38 mM, acting as a solute across large salinity gradients (Lyon et al. 2016). At a salinity of 35, the DMSP content of *F. cylindrus* was between 16-18 mM, comparable to the 17.63 mM found in *Fragilariopsis* sp. (PZB009), this contrasts with the lower concentrations of DMSP (5.76 mM) recorded for *Fragilariopsis* sp. (PZB060) which was morphologically different and therefore likely to be different species, suggesting considerable strain variability amongst Antarctic taxa.

Due to the widespread distribution and prominence of *Phaeocystis* as a bloom forming species, as well as its link to DMS hot spots, particularly in the Southern Ocean, DMSP production in *Phaeocystis* has been well studied (Baumann, Brandini & Staubes 1994; DiTullio et al. 2000; Hefu & Kirst 1997; Liss et al. 1994; Stefels & Dijkhuizen 1996; Van Duyl et al. 1998). The two *Phaeocystis* strains in this study varied greatly, where *P. cf. pouchetii* strain was the highest producer (73.17 fmol cell^{-1}) compared to *Phaeocystis* PZB016 (0.31 fmol cell^{-1}). *P. cf. pouchetii* reached intracellular concentrations (1460 mM) far in excess of those seen in temperate species at 261.1 mM (Keller 1989a) or even North Sea isolates (grown at 10 °C) which ranged from 71 - 150 mM (Stefels & Van Boekel 1993). Higher concentrations of DMSP have been observed before in Antarctic *Phaeocystis* compared to temperate strains, such as in the six sites reviewed by Liss et al. (1994). DMSP concentrations reached 87 fmol cell^{-1} in *Phaeocystis* cells from Davis Station Antarctica (Gibson et al. 1990), comparable to the 73 fmol cell^{-1} of *P. cf. pouchettii* in this study, whereas the other *Phaeocystis* strain (PZB016) had cellular concentrations (0.31 fmol cell^{-1}) even smaller than *Phaeocystis* from lower latitude regions, which ranged from 2-13 fmol cell^{-1} (Liss et al. 1994). This variability means that certain strains of *Phaeocystis* are responsible for DMS hot spots.

Neither *Chaetoceros* spp. in this study produced DMSP, which is congruous with the negligible amounts also reported in a psychrophilic strain isolated from sea ice off the coast of Japan (Kasamatsu et al. 2004). There was however, a difference in their ability to lyase DMSP, with *C. castracanei* showing no lyase capability, whereas the other *Chaetoceros* sp. had significant amounts of lyase activity. It stands to reason that while a species may not be producing DMSP, there is a possibility for uptake of DMSP and subsequent lyase to occur (Vila-Costa et al. 2006). Both *Thalassiosira* cultures were relatively low producers, and similar to the *Chaetoceros*, *Thalassiosira* PZB048 did not show any lyase activity, whereas *Thalassiosira* PZB062 showed moderate levels of DLA. These species-specific results demonstrate the challenges in generalising based on genus and the complexity of modelling DMS flux

in Antarctica. It is important to remember that as the dominate species shift seasonally, community composition needs to be incorporated into estimates.

Our results highlight that DMSP concentration and lyase activity varies not only across species but also among strains from the same genus. However, rates of activity can be influenced by light and nutrients (Caruana & Malin 2014; Harada & Kiene 2011; Keller & Korjeff-Bellows 1996), and in this study, growth conditions were kept constant throughout, thereby not testing absolute capability for production, but rather generalising across species exposed to standardised (non-stressful) conditions. It is also known that pH can affect DLA, for example dinoflagellates maximise lyase activity at higher pH whereas prymnesiophytes have higher lyase activity at a lower pH (Harada & Kiene 2011). In this study we did not optimise the pH for each species in order to ascertain maximum lyase activity, instead a pH of 8.2 (close to seawater) was used as recommended in Caruana et al. (2012), reflecting activity that could be expected in a natural ocean environment.

In order to maximise the comparability of the data presented in this study and provide useful information for relevant climate models, we have expressed DMSP not only per cell and cell volume, but also per C, N and chl *a* content (Caruana et al. 2012; Stefels et al. 2007). This is because when measuring DMSP in the field, chl *a* is often used for normalisation and as a proxy for phytoplankton biomass, and while it can be hard to determine the exact input of specific species (particularly via satellite observations), it is an easy and readily available parameter, making it especially attractive to modellers (Caruana et al. 2012; Huot et al. 2007). Similarly, optical measurements of phytoplankton biomass can be expressed as carbon biomass (Behrenfeld & Boss 2006), with a lot of global climate models presented in units of carbon, and ecosystem models expressed as pools of carbon or nitrogen (Bucciarelli et al. 2013; Caruana et al. 2012; Stefels et al. 2007).

Some models based on remote sensing data (chl *a*, solar radiation and MLD) have successfully predicted DMS concentrations and replicated DMS seasonality (Simó & Dachs 2002; Vallina & Simó 2007), however these models appear limited in simulating DMS concentrations in shelf seas, equatorial and Antarctic regions (Halloran, Bell & Totterdell 2010). In the case of Antarctica, studies have shown that there is no correlation between chl *a* and DMS emissions, instead linking emissions to the presence of *Phaeocyctis* blooms (Kwint & Kramer 1996; Stefels et al. 2018). This study demonstrated that chlorophyll *a* may be an acceptable indicator of DMSP if the community is dominated by haptophytes with relatively small variation in DMSP:Chl *a* compared to other indices. However, unless you can determine the taxa contribution to chlorophyll *a* content, it may not be the best indicator for DMSP in mixed communities, as this parameter was the most variable and instead, DMSP expressed per unit C may be a better parameter, as in this study this parameter was less variable and there have been strong correlations detected between particulate organic carbon and DMSP (Stefels et al. 2018).

This study has provided the first comprehensive analysis of DMSP production and DLA in a suite phototrophic and phagotrophic species isolated from Antarctica. It has revealed 11 of the 16 species to have DMSP producing capabilities and nine species to possess DLA. The study highlights the variability in DMSP concentrations across multiple strains and that this variability is also present within genera. It was found that DMSP:C might provide a less variable parameter for measuring DMSP, particularly when dealing with mixed communities. Based on these findings, future studies, if presented with the opportunity, should consider expressing DMSP data not only by chl *a* but also per carbon, in order for modellers to parameterise DMSP pathways in the best way.

CHAPTER 4. MACROMOLECULAR COMPOSITION, PRODUCTIVITY, DIMETHYLSULPHONIOPROPIONATE (DMSP) CONTENT AND LYASE ACTIVITY IN ANTARCTIC MICROALGAL COMMUNITIES

INTRODUCTION

Marine microorganisms are influenced by the chemistry of the waters surrounding them and can in turn modify it (Thomas & Dieckmann 2009). For example, marine microalgae make use of available macro- and micronutrients for growth and productivity, yet they also play a vital role in recycling those nutrients via their growth, consumption, exudation of compounds and cell lysis (Buesseler 1998; Garibotti, Vernet, Ferrario, et al. 2003; Garibotti, Vernet, Kozlowski, et al. 2003). Microalgae are a diverse and adaptable group of organisms, with many species found inhabiting a variety of diverse ecological niches, however, only a few species will generally dominate a community (Fuhrman 2009). When a community is dominated by one taxa, the influence on nutrient cycling or biogeochemistry will vary, as each taxa has a different role in biogeochemistry i.e diatoms, which are closely linked to the silica cycle (Tréguer & De La Rocha 2013), coccolithophores, who produce $CaCO_3$ impacting the carbon cycle (Marsh 2003), or the haptophytes and dinoflagellates who are important contributors to the marine sulfur cycle (Keller 1989a).

In Antarctica, environmental conditions vary greatly from the open ocean to the frozen coastline and consequently, this environmental variability supports different assemblages of microorganisms. Marine microalgae, which form the base of the Antarctic food web, are the main source of nutrients and energy for higher trophic levels, and being a highly dynamic environment, Antarctic microalgae are well adapted to seasonally driven environmental conditions over an annual cycle (Gleitz & Thomas 1992).

Changes in surrounding environmental conditions can influence phenotypic traits in microalgae such as, photophysiology and macromolecular composition, consequently impacting on processes such as nutrient cycling, grazability and the transfer of energy to higher trophic levels. For example, diatoms, which dominate sea-ice communities, have a remarkable ability to rapidly acclimate to changed conditions, expressing high levels of phenotypic plasticity in traits such as photophysiology, morphology and macromolecular composition (Arrigo 2014; Mock & Thomas 2005; Morgan-Kiss et al. 2006; Petrou, Doblin & Ralph 2011b; Sackett et al. 2013; Villareal & Fryxell 1983). Under conditions equating to the sea-ice environment, *Fragilariopsis cylindrus* showed high levels photosynthetic plasticity, effectively adapting it's photosystem to cope with changes in salinity and temperature, while *Psuedonitzschia subcurvata* utilised a strategy to reduce antenna size limiting absorption of photons in order to protect the photosystems from damage (Petrou, Doblin & Ralph 2011b). Morphologically, it has been shown that the polar diatoms *Thalassiosira antarctica* and *Porosira glacialis* displayed a change in valve structure and reduced external tubes when grown at lower temperatures (Villareal & Fryxell 1983). Biochemically, Antarctic microalgal cultures exposed to a variety of temperature and salinity regimes showed macromolecular shifts occurred during seasonal ice formation and decay (Sackett et al, 2013). Using Fourier transform infrared spectroscopy (FTIR), the study revealed species-specific responses to changes in macromolecular content, whereby widespread polar species, such as *F. cylindrus* and *Chaetoceros simplex*, displayed greater phenotypic plasticity and elevated lipid accumulation in the low temperature, high salinity treatments (representative of sea-ice), while the pelagic species *P. subcurvata* had a lower degree of plasticity under the same conditions, showing minimal variation in macromolecules between treatments (Sackett et al. 2013). Changes in temperature, salinity and light, like those experienced during a sea-ice break out, have also been seen to induce a rapid increase and subsequent drop in DMSP production from a semi-natural Antarctic community, where it has been suggest DMSP may be used as antioxidant within the cell allowing them

to quickly adapt to stress (Vance et al. 2013). Together, these studies demonstrate how environmental conditions influence species phenotype and highlights how seasonal environmental shifts may alter food quality and biogeochemistry.

Unique aquatic environments are also found inland, as Antarctica has many isolated lakes. These lakes have formed over thousands of years through seawater trapped in basins, and following millennia of evaporation have become hypersaline, wherein only a few extreme halophiles can survive (Yau et al. 2013). Antarctic lakes experience their own seasonal changes, as solar radiation levels change and snow and ice cover vary throughout the year. The seasonal light-dark cycle further influences the microbial diversity and function annually (Burton 1981, Vick-Majors et al 2014). Microbes able to tolerate and thrive under these extreme hypersaline conditions include a range of archaea, the cyanobacteria *Synechococcus* and the green algae *Dunaliella* (Cavicchioli 2015). While most species of green algae are moderate halophiles, there are a few extreme halophilic species such as *Dunaliella salina* and *Asteromonas gracilis* (DasSarma & Arora 2002). Halophiles generally prevent water loss to the external medium through the accumulation of high solute concentrations in the cytoplasm and osmolytes, such as amino acids and polyols, for example glycerol is synthesized by *D. salina* in response to osmotic stress (DasSarma & Arora 2002). The sulphur compound dimethylsulphoniopropionate (DMSP) has been identified as an osmolyte (Motard-Côté & Kiene 2015), as well as cryoprotectant (Kirst et al. 1991) making it a solute of interest in understanding acclimation in these habitats. Furthermore, high concentrations of DMSP have been recorded in the hypersaline lakes of Antarctica where halophiles such as *D. salina* have been found (Franzmann et al. 1987).

This study characterises the phenotypic traits of Antarctic microalgae from three unique aquatic habitats in the Antarctic environment. In particular, it characterises the community composition, physiology, macromolecular composition, DMSP content and DMSP lyase activity of three Antarctic

microalgal communities from aquatic environments surrounding Davis Station, Antarctica, namely the sea-ice, coastal ocean and a hypersaline lake.

METHODS

SAMPLE COLLECTION

Natural microalgal communities were collected from three Antarctic environments, the sea-ice, the coastal open ocean and a shallow, meromitic, hypersaline lake. Coastal water samples were taken from open water in Prydz Bay (66°S, 77°E), lake samples were collected from Organic Lake (68°S, 78°E) in the Vestfold Hills, while samples of sea-ice algae were obtained from ice cores (66°S, 77°E) at Prydz Bay, Davis Station. All samples were collected in November 2014. Pelagic surface water samples were collected by helicopter using a Bambi Bucket and stored for 2 days in a 650 L temperature and light controlled tank prior to sampling. Lake samples were collected from a 5 m depth via a pump system and transported back to Davis Station where they were kept in the dark at 0 °C for 24 h until sampling. Sea-ice samples were collected by shaving off the bottom 10 cm of 220 mm diameter ice cores and the ice shavings melted in the dark at 0 °C with regular additions of filtered seawater (0.22 µm) to prevent the salinity of the water dropping too low and potentially damaging the cells (Petrou et al. 2010).

PHYSIOLOGICAL MEASUREMENTS

The photophysiological condition of the microalgal community from each site was determined using variable chlorophyll *a* fluorescence measured with a Pulse Amplitude Modulated fluorometer (Water PAM, Walz GmbH, Germany). Briefly, a 3 mL aliquot was dark adapted for 30 min before a steady state light curve was conducted from which the photophysiological parameters, F_V/F_M (maximum quantum yield of PSII), $\Delta F/F_M'$ (effective quantum yield), NPQ (non-photochemical quenching) and rETR

(relative electron transport rate) were obtained. Net oxygen production and respiration were measured and rates normalised to chlorophyll *a* concentration. DMSP, DMSP lyase activity (DLA) and macromolecular content were analysed from 50 ml samples collected from each site. DMSP content and lyase activity were normalised to cell density. For detailed methods on chlorophyll *a* content, primary productivity, DMSP content and lyase activity and macromolecular composition please refer to the **General Methods** (**Chapter 2**).

Steady-State Light Curve

A 5-step steady-state light curve (SSLC) was conducted for each site (n = 3). Samples from the open ocean were exposed to light levels of 130, 307, 600, 973, 1450 µmol photons m^{-2} s^{-1}, and samples from the sea ice and Organic Lake light levels of 40, 92, 206, 435, 1450 µmol photons m^{-2} s^{-1} which were applied for 5 min before recording the light-adapted minimum (Ft) and maximum fluorescence (Fm') values. There was a 30 s 'recovery' period between each stepped increase in light. Fluorescence data were fitted according to a double exponential function (Ralph & Gademann 2005) and light utilisation efficiency (α), minimum saturating irradiances (E_K), and maximum electron transport rate (ETRmax) obtained from the curve following Ralph & Gademann (2005).

Data analysis

IMB SPSS statistics (version 20) software was used to perform one-way analysis of variance (ANOVA) to determine statistically significant difference in chlorophyll *a* fluorescence, gross O_2 productivity, DMSP, DLA and macromolecular content. Levene's test was used to test the assumption of homogeneity of variance required for the one-way ANOVA. Student Newman Kewell (SNK, p=0.05) and Tukey's post hoc tests were used to compare difference amongst groups (ie. communities or species).

RESULTS

HABITAT CHARACTERISTICS

The physical characteristics varied between habitats, where open ocean was characterised by a salinity of 30 and water temperature of 1.1 °C (Table 4.2), while the bottom sea-ice community temperature was -1.4 °C and the salinity ranged between 16 and 29 upon the ice melting. For Organic Lake, salinity was measured at over 200 with a temperature of -11 °C (Table 4.2).

Table 4.1 Physical parameters, including temperature (°C) and salinity recorded at each site; and the depth (m) at which the water sample was taken.

	Temperature (°C)	Salinity	Depth (m)
Open Ocean	1.1	30	1-2 (surface waters)
Sea-ice	-1.4	16-29	1.5 (sea-ice/water interface)
Organic Lake	-11	200	5

COMMUNITY COMPOSITION

Total cell abundance for the three communities were 1,446,429 cells L^{-1} for the open ocean 2,065,800 cells L^{-1} for sea-ice and 2,352,900 cells L^{-1} for Organic Lake. The open ocean had the most diverse community. Although dominated by haptophytes, in particular, *Phaeocystis* spp. which accounted for more than half the open ocean community (66 %). Diatoms were the most diverse group, with 17 taxa, while their abundance accounted for ~21% of the community. Dinoflagellates and cryptophytes were also present in lower numbers, contributing only 5 % and 12 %, respectively (Figure 4.1). The sea-ice community was comprised of a variety of diatoms, in particular *Odontella* spp. accounting for 47 % of the population, *Entomoneis* spp. (32 %), *Fragilariopsis* spp. (16 %), *Psuedonitzschia* spp. (3 %) and *Nitzschia* spp. (0.2 %). For Organic Lake, the community was dominated by the one species of green algae *Dunaliella* sp. (99.9 %), however, a single cell of unknown species from the *Chaetoceros* genus was also found.

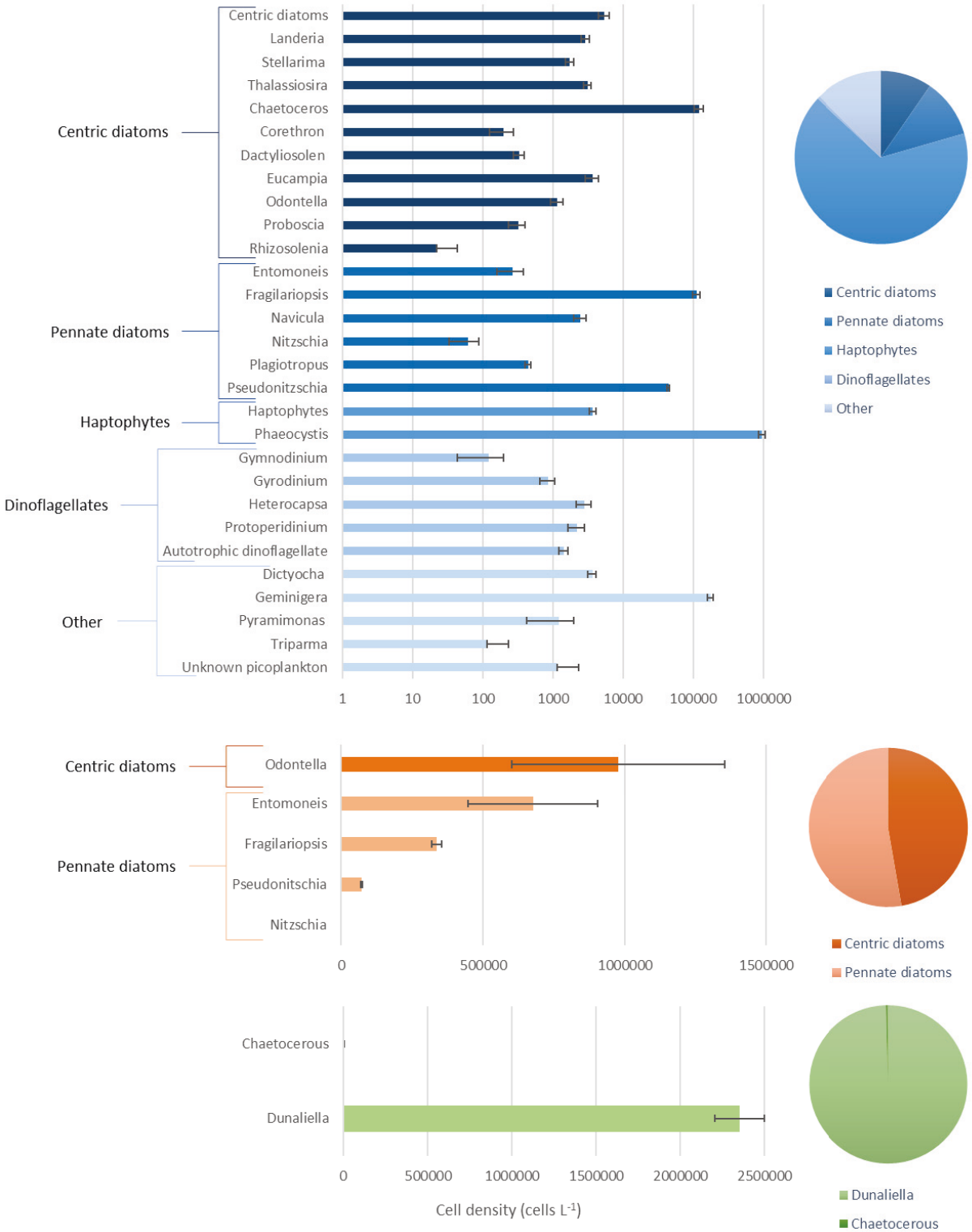


Figure 4.1 Community composition and cell density (cells L^{-1}) from three natural Antarctic microalgal communities (n=3). Blue - open ocean, Orange - sea-ice, Green - organic lake. Pie charts illustrate proportional contribution of each taxonomic group. Note log scale on x-axis for open ocean.

The photophysiological response differed between the three communities with the highest effective quantum yield (Fig 4.2A) and non-photochemical quenching (NPQ) (Fig 4.2C) measured in the open ocean community. In all communities, the effective quantum yield declined at irradiances above 975 µmol photons m^2 s^{-1} with non-photochemical quenching increasing reciprocally. While there was no significant difference in chlorophyll *a* fluorescence, the sea-ice community did show an increase in NPQ at the highest irradiance which was similar to the open ocean community, while the community from Organic Lake had a comparatively lower NPQ at the highest irradiance (Fig 4.2C). Relative electron transport rates (rETR) were highest for the open ocean community, with a maximum ETR rate (rETRmax) over 200 for the open ocean and 115 the sea-ice, compared to the *Dunaliella* dominated Organic Lake community, where rETRmax was significantly lower at 29.9 (ANOVA, $F_{2,8}$= 15.442, P = 0.004; Table 4.3). There was no significant difference between light utilisation efficiency (α) and habitat, sea-ice and Organic Lake had α values of 0.37 and 0.30, respectively, approx. half that of the open ocean (0.63) (Table 4.3). There was no significant difference in minimum saturating irradiance (E_k) despite an E_k of over 350 µmol photons m^{-2} s^{-1} in both the open ocean and sea-ice, compared to Organic Lake, which had an E_k of 100 µmol photons m^{-2} s^{-1} (Table 4.3).

Gross primary productivity corresponded with the photophysiological data, where the open ocean community was significantly more productive (ANOVA, $F_{1,6}$= 75.449, P <0.001) than the sea-ice community at 0.44 and 0.12 µmol s^{-1} mg chl *a*, respectively (Fig 4.2D). Unfortunately, due to the interference of organic matter for the optical oxygen sensor, no productivity data was able to be collected for Organic Lake.

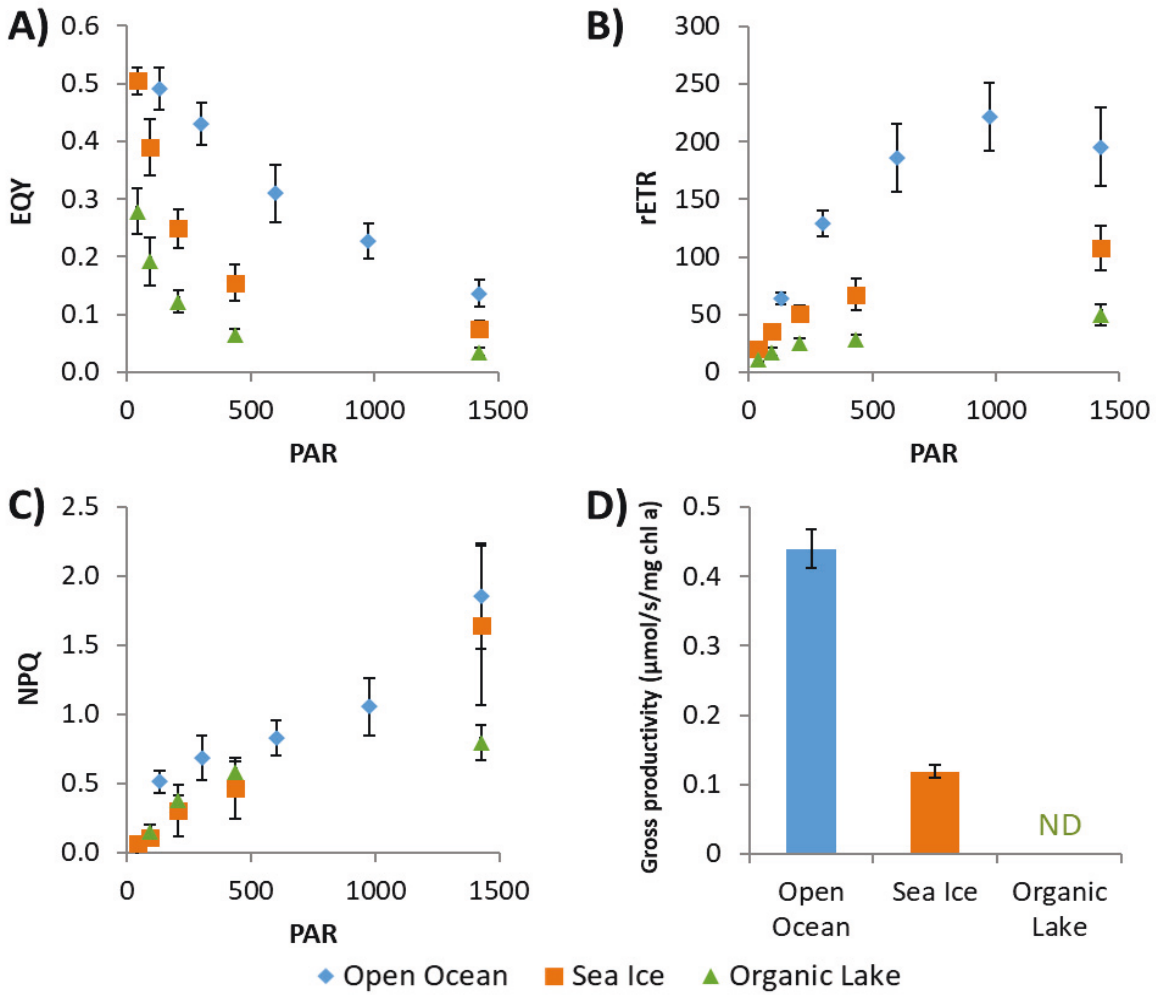


Figure 4.2 Photophysiology and gross productivity for Antarctic microalgal communities from three unique environments A) Effective quantum yield (EQY) of PSII (n=3), **B)** Relative electron transport rate (rETR, n=3), **C)** Non-photochemical quenching (NPQ, n=3), **D)** Gross primary productivity (n=4). Data represent mean ± SE. Open Ocean – Blue, Sea-ice – Orange, Organic Lake – Green. ND - No Data

Table 4.2 Photosynthetic parameters ± SD Light utilisation effieciency - α, relative maximum eletcron transport rate - $rETR_{max}$, minimum saturating irradiance - I_k.

	OPEN OCEAN	SEA-ICE	ORGANIC LAKE
α	0.63 ± 0.17	0.37 ± 0.17	0.30 ± 0.03
ETR_{MAX}*	211 ± 17	115 ± 65	29.9 ± 17
E_K	353 ± 100	366 ± 226	100 ± 51

DIMETHYLSUFIONOPROPIONATE CONTENT AND LYASE ACTIVITY

DMSP content varied between communities (ANOVA, $F_{2,20}$ =15.213, P<0.001). It was highest in the open ocean community at concentrations of 44.3 fmol DMSP per cell, three times the concentrations found in the sea-ice algal communities (14.9 fmol DMSP per cell), while microalgae from Organic Lake had the lowest DMSP content at only 0.8 fmol DMSP per cell (Fig 4.3A). Similarly, DMSP lyase activity (DLA) was greatest in the open ocean (ANOVA, $F_{2,7}$ = 1972.459, P<0.001) producing 27.2 fmol DMS $cell^{-1}$ h^{-1} (Fig 4.3B). These rates were much higher than the DLA values of 2.8 and 3.1 fmol $cell^{-1}$ h^{-1}, which were measured in the sea-ice and Organic Lake communities, respectively (Fig 4.3B).

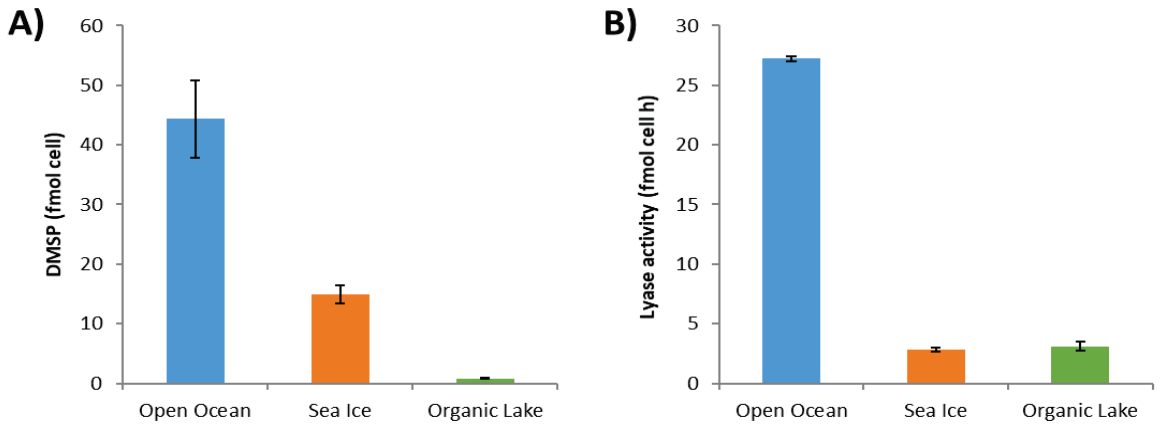


Figure 4.3 A) DMSP content (n=3) and **B)** DMSP lyase activity (n=3) within Antarctic microalgal communities from three unique habitats. Data represent the mean ± SE.

MACROMOLECULAR CONTENT

Normalised infrared absorbance spectra showed typical bands associated with microalgal samples, with clear distinction of community profiles based on species composition. The 2nd derivative spectra of the selected biologically relevant regions revealed the specific absorbance bands representing key macromolecules of interest, from which a total of 10 bands with unequivocal band assignments were selected for comparison across environments (Table 2). Whole community Principal Component Analysis (PCA) of the biologically relevant spectra revealed the main variation in environmental profiles

was driven by habitat, which displayed a tight grouping along the PC-1 axis (with the exception of one species in the sea-ice) explaining 26% of variation, while PC-2 explained 11% variation (Fig 4.4B). The biomolecules of greatest influence, represented as changes along PC-1, were saturated fatty acids (bands 2960, 2925 and 2855 cm^{-1}) and amides (band 1540 and 1400 cm^{-1}). Saturated fatty acids were highest in sea-ice (ANOVA, $F_{2,326}$ = 45.638, P<0.001) as were unsaturated fatty acids (ANOVA, $F_{2,326}$ = 124.090, P<0.001), while open ocean and sea-ice had higher concentrations of both amides II and III compared to Organic Lake (Fig 4.4C). The second most profound response, represented by deviations along PC-2, was again in amides, but also carbohydrates (band ~1200 cm^{-1}) (Fig 4.4B). The Organic Lake community had almost double the carbohydrate content of the open ocean and sea-ice communities (ANOVA, $F_{2,326}$ = 90.129, P<0.001) and no unsaturated fatty acids content calculated due to an absence of peak for unsaturated fatty acids (Fig 4.4C).

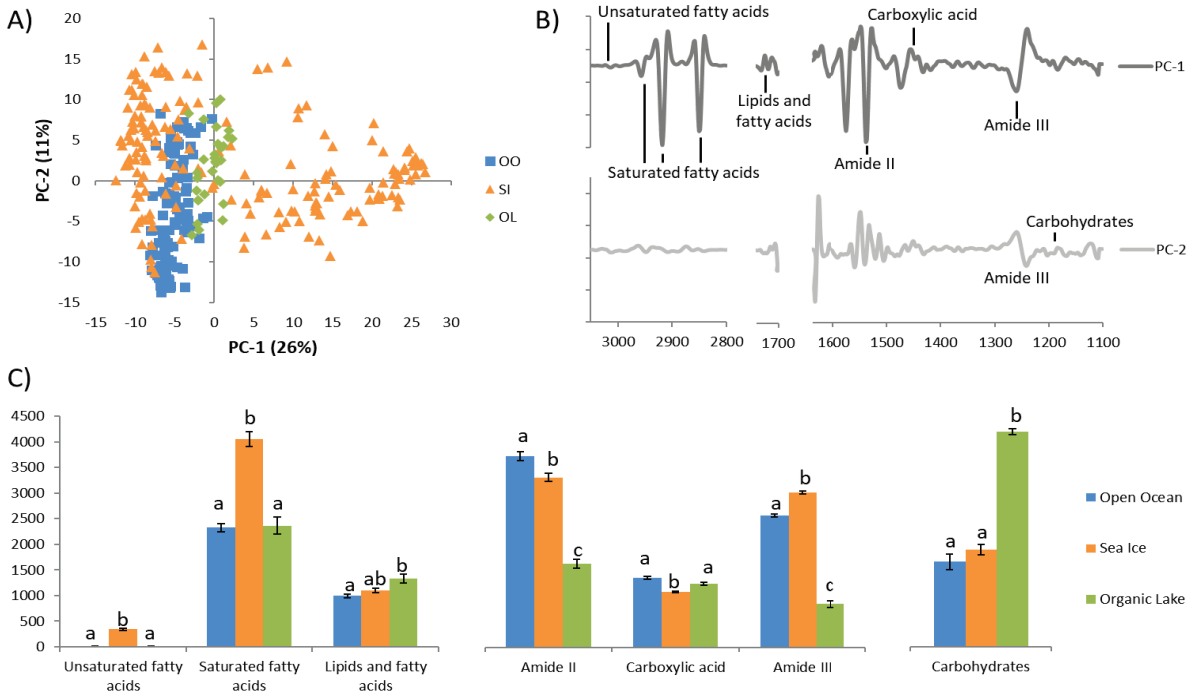


Figure 4.4 Macromolecular composition of Antarctic microalgalcommunities, from the open ocean, sea-ice and hypersaline lake. A) Scores plot based on infrared spectra of species across all habitats; **B)** PC-1 and PC-2 loadings plots; **C)** Mean relative cellular concentrations of macromolecule ± SE. The concentration is proportional to the area under the peak in the infrared spectrum corresponding to each macromolecule. Open ocean – Blue, Sea-ice – Orange, Organic Lake – Green. Superscript letters denote significant differences at p<0.05.

To obtain a better understanding of species-specific differences underpinning the community response, macromolecular profiles for representative taxa within habitats were analysed. In the open ocean this consisted of four diatom species, in the sea ice three diatom species and in Organic Lake a single species of green algae. Species-specific macromolecular profiles within the open ocean community indicated the major influence on species profiles was due to proteins, explaining 18% of variation, while PC-2 was driven by carbohydrates, explaining 13% variation (Fig 4.5B). The biggest influence, represented as changes along PC-1, was found in amide II (band 1540 cm^{-1}) where *Stellarima* and *Thalassiosira* had a higher content than *Fragilariopsis* and *Chaetoceros*, the latter having the least (ANOVA, $F_{3,112}$ =41.930, P<0.001). The second most profound response, represented by PC-2, was again driven by changes in the amides, but also carbohydrates (band ~1200 cm^{-1}), lipids and fatty acids (band 1745 cm^{-1}) (Fig 4.5B). The pennate diatom, *Fragilariopsis,* had the highest carbohydrate, lipid and fatty acid content of all four open ocean species analysed (Fig 4.5). In contrast, the large centric diatoms, *Stellarima* and *Thalassiosira,* had lower carbohydrate content compared to both *Fragilariopsis* and *Chaetoceros* (ANOVA, $F_{3,112}$ =50.425, P<0.001).

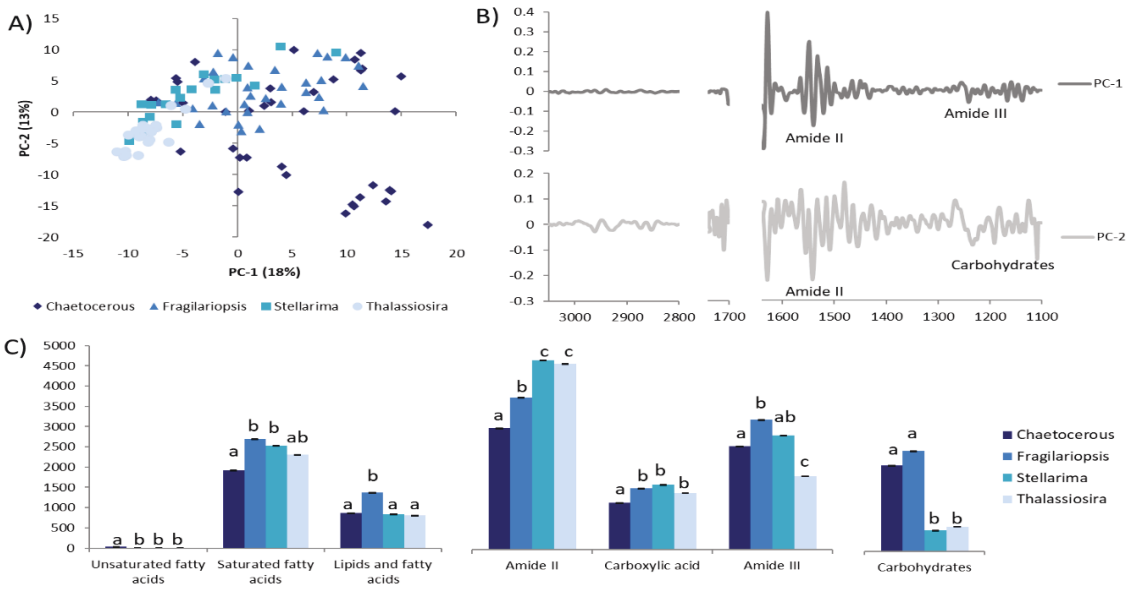


Figure 4.5 Macromolecular composition of species found within the Open ocean environment, namely *Chaetoceros, Fragilariopsis, Stellarima* and *Thalassiosira* A) Scores plot based on infrared spectra of open ocean species; **B)** PC-1 and PC-2 loadings plots; **C)** Mean cellular concentrations of macromolecules ± SE. Superscript letters denote significant differences at p<0.05.

The species-specific data for the sea-ice community revealed a large population spread, with thegrouping of *Odontella* and *Fragilariopsis* along PC1, compared to a large spread in *Entomoneis* (Fig 4.6A). The main effects on sea-ice species profiles were proteins (band 1540 and 1400 cm^{-1}), lipids and fatty acids (bands 2960, 2925, 2855 and 1745 cm^{-1}), explaining 48% of variation, while PC-2 was driven by fatty acids, including unsaturated fatty acids (band 3010 cm^{-1}), explaining 13% variation (Fig 4.6B). *Entomoneis* had the highest saturated fatty acid (ANOVA, $F_{2,182}$ =119.035, P<0.001), amide II (ANOVA, $F_{2,182}$ =25.551, P<0.001) and carboxylic acid (band 1460 cm^{-1}) content (ANOVA, $F_{2,182}$ =23.166, P<0.001) of any species, while *Odontella* had the highest unsaturated fatty acids (ANOVA, $F_{2,182}$ =17.309, P<0.001), lipids and fatty acids (ANOVA, $F_{2,182}$ =36.884, P<0.001) and amide III (ANOVA, $F_{2,182}$ =123.490, P<0.001) content of all three sea-ice species measured (Fig 4.6C).

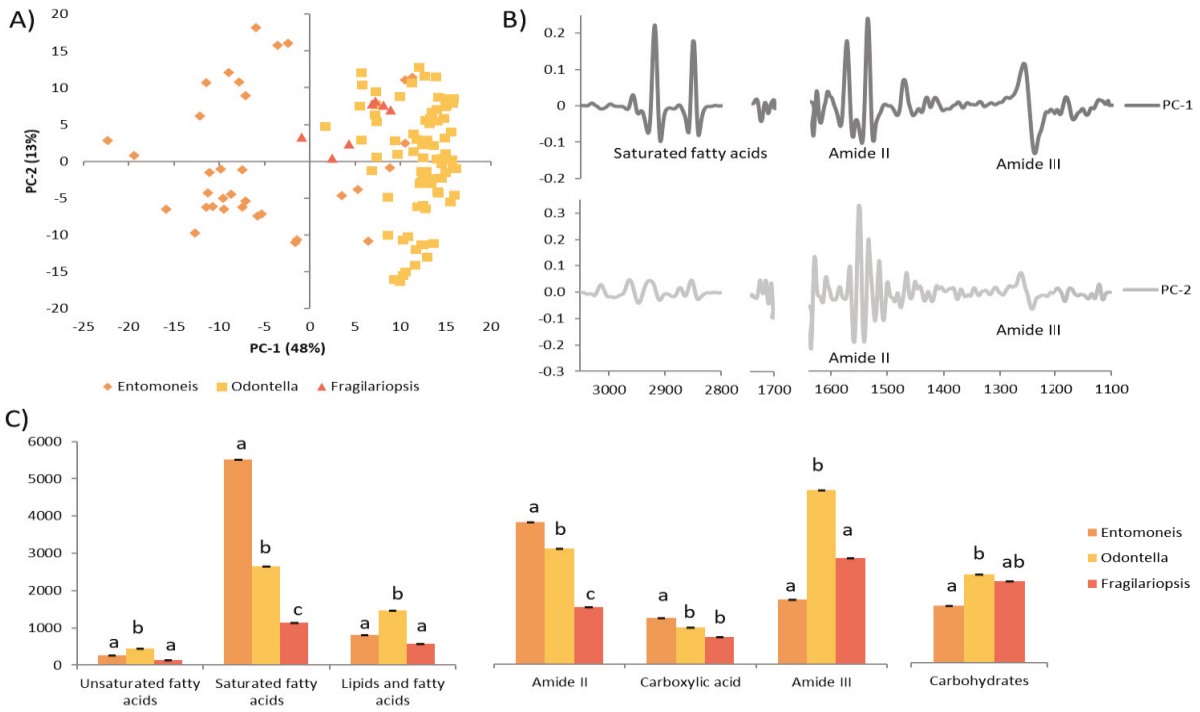


Figure 4.6 Macromolecular composition of three dominant diatoms found within the sea-ice environment, namely *Entomoneis, Odontella* and *Fragilariopsis*. A) Scores plot based on infrared spectra of three sea-ice species; **B)** PC-1 and PC-2 loadings plots; **C)** Mean cellular concentrations of macromolecules ± SE. Superscript letters denote significant differences at p<0.05.

Dunaliella, the dominant species in Organic Lake, had equal relative ratios of lipids:protein:carbohydrates (32:32:36). However, when comparing all species across habitats, clear shifts in relative proportions of macromolecules were evident (Figure 4.7). *Dunaliella* showed a relatively stronger investment in carbohydrates (36 %) than either the sea-ice or open ocean species, which only had an average relative proportion of carbohydrate of 16 % and 11 %, respectively (Figure 4.7). Comparing within the same taxa, *Fragilariopsis* in the sea-ice had a higher proportion of carbohydrates (24 %) than *Fragilariopsis* from the open ocean (17 %). In general, the sea-ice diatoms had more lipids compared to the open ocean species, in particular, *Entomoneis* had very high contribution into lipids (45 %). In contrast, proteins made up 56 - 69% in diatoms from the open ocean compared to only 45 – 56 % in sea-ice diatoms (Figure 4.7).

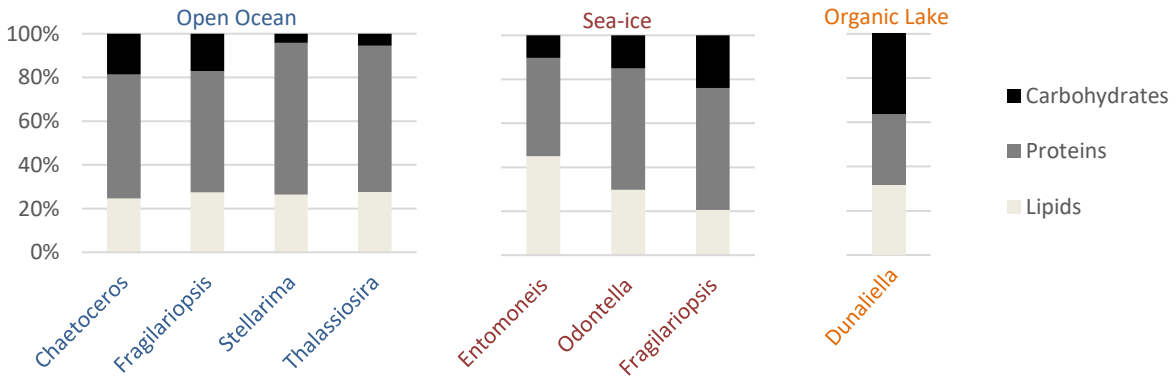


Figure 4.7 Ratio of lipids, protein and carbohydrate content for species from three Antarctic communities: the open ocean, sea-ice and Organic Lake. Lipids – light grey, Protein – dark grey, carbohydrates – black

DISCUSSION

This study characterised the community composition, primary productivity, photophysiology, DMSP content and macromolecular composition in microalgae from three natural Antarctic environments. We found strong variations in the measured parameters and these differences were likely attributed to

their niche environment characteristics, such as salinity, light and temperature. This pattern was also reflected in the differences in the community composition data, where locations with more challenging environmental conditions, microalgal diversity decreased, such a trend has been determined previously for microbial communities (Barton et al. 2010; Pedrós-Alió et al. 2000; Salazar & Sunagawa 2017).

In this study, the open ocean community had the greatest microalgal diversity, consisting of at least 29 taxa made up of haptophytes, diatoms, dinoflagellates, silicoflagellates, cryptophytes and others. *Phaeocystis* and diatoms (in particular *Fragilariopsis* spp.) are considered the main species dominating Antarctic pelagic environments, alongside other phytoflagellates, *Cryptomonas* spp., *Pyramimonas* and dinoflagellates, and there is a correlation between biomass and water column physical characteristics (Arrigo et al. 1999; Garibotti, Vernet, Ferrario, et al. 2003). We found that this open ocean pelagic community, in the short term, was the most resilient to photoinhibition under high irradiance and had the highest productivity rates of all three communities. This high diversity and potential productivity are likely to stem from the relatively favourable physical characteristics found in the open ocean environment, which is generally well mixed, with high nutrient availability, a variable light climate and moderate, stable salinity (Constable, Nicol & Strutton 2003). Fast ice algal communities have also been shown to be resilient under high irradiance with the ability to rapidly adapt to changing light, some adapting within hours (McMinn, Ryan & Gademann 2003) and in other cases taking days (Mock & Hoch 2005). Bottom sea-ice algae are extremely shade-adapted, growing at irradiances as low as 0.1 µmol photon m^{-2} s^{-1} (McMinn, Ryan & Gademann 2003) and shade-adapted communities experience photoinhibition at lower irradiances. As our measurements were conducted within hours and not days, the reduced photophysiology results of this sea-ice community, in comparison to the pelagic community, could indicate that the sea-ice algae did not fully acclimate to a change in light and could display results just as resilient in the long term.

Within the open ocean community, the small chain forming diatoms, *Chaetoceros* and *Fragilariopsis* had similar macromolecular profiles, while the larger centric diatoms *Stellarima* and *Thalassiosira* shared a similar macromolecular signature, with lower carbohydrate content in the latter two. Due to the unique morphology of diatoms and the restriction of cytoplasm to a thin parietal layer inside the frustule, a negative relationship between cellular concentrations such as carbohydrate vs cell volume has been previously recorded (Hitchcock 1982), which would support the lower relative carbohydrate content found in the two larger diatom species. The predominance of these diatoms and *Phaeocystis* is likely due to their photosynthetic plasticity, high capacity for photoprotection and ability to bloom during austral spring (Lavaud, Strzepek & Kroth 2007; Tang et al. 2009).

There was a high concentration of DMSP in the open ocean samples, which we attribute to the high abundance of *Phaeocystis* (>50%). While dinoflagellates are commonly accepted as the most prolific producers of DMSP (Caruana & Malin 2014), and the small population found in the open ocean would contribute to the high DMSP concentrations found in open ocean community; the abundance of *Phaeocystis* colonies, also prolific DMSP producers, make them the most likely contributors to our results. In addition, diatoms, such as *Fragilariopsis* and *Nitzschia* were shown to be considerable DMSP producers in Chapter 3. Given their abundance, their contribution to the open ocean community DMSP concentration should not be overlooked. *Phaeocystis* is said to contain DMSP lyases (Harada & Kiene 2011) which cleave DMSP to produce dimethylsulfide (DMS), resulting in some of the highest concentrations of DMS recorded during blooms (Vance et al. 2013). Indeed, in Chapter 3, both strains of *Phaeocystis* possessed DLA, although to differing degrees. This lyase capacity was also likely responsible for the significantly higher DLA values measured in the open ocean community.

The sea-ice community had lower diversity, with a community comprised of a small number of diatom species, including several large species of pennate diatoms (*Entomoneis*, *Fragilaropsis* and *Nitzschia*), a couple of smaller species of pennate diatoms (*Psuedonitzschia* and *Fragilariopsis*) and a single species

of centric diatom (*Odontella*) which made up nearly half the biomass (47%). Because the microalgal species that live in the sea-ice need to be able to tolerate conditions such as a freezing substrate, a low light climate and high salinity, a lower diversity was expected (Pedrós-Alió et al. 2000). However, the absence of non-diatom species can be explained by the fact that diatoms are highly efficient at converting light into energy under fluctuating light regimes and can effectively regulate photosynthesis over a wide range of polar temperatures, rapidly acclimating in as little as three days (Mock & Hoch 2005; Petrou, Doblin & Ralph 2011b; Wagner, Jakob & Wilhelm 2005), making them well-equipped to deal with the extreme and changing conditions associated with freeze thaw cycles of the Antarctic sea ice. *Odontella*, *Entomoneis* and *Fragilariopsis* were the most abundant diatoms found in the sea-ice samples and are also common bloom forming species in coastal areas of Antarctica (Garibotti, Vernet, Ferrario, et al. 2003). Overall, these three taxa showed relatively high proportions of lipids and proteins compared to their investment in carbohydrates, which is common among Antarctic microalgal species (Teoh et al. 2004). In particular, the bottom ice algae living in extreme shaded conditions have high levels of protein and low storage polymers like carbohydrates (Morris 1981; Smith, Clement & Head 1989). Lipids and proteins are disproportionately necessary for rapid growth (Wikfors, Ferris & Smith 1992), compared with the need for carbohydrate stores. Lipids are important in a range of cellular functions including, energy storage, metabolism, photosynthesis, integrity of the membrane and its structure, as well as cell signalling (Guschina & Harwood 2009; Harwood 1998; Murata & Siegenthaler 1998). Low temperature and low light conditions result in greater lipid production in polar algae (Smith & Morris 1980; Smith, Clement & Head 1989). The sea-ice community was higher in lipids than the other communities in agreeance with the results of Cota & Smith (1991), however this is in contrast to Duerksen et al. (2014)who found pelagic diatoms had significantly higher levels of polyunsaturated fatty acids compared to ice-associated producers. Furthermore, when comparing the same species across both sea ice and open ocean habitats this was not the case. The environmental conditions such

as temperature and salinity affect macromolecular content as well as nutrient availability (Lizotte & Sullivan 1992; Smith, Gosselin & Taguchi 1997). In this study, *Fragilariopsis,* one of the most abundant genera in the Southern Ocean, had higher lipid and protein content in the open ocean samples compared to the *Fragilariopsis* found in the sea-ice. This would suggest the *Fragilariopsis* in the open ocean, was exposed to nutrient replete conditions and therefore a higher capacity for rapid growth and thus greater ability to form large blooms. In a previous study, *Fragilariopsis* was shown to adapt effectively to sea-ice conditions, but its productivity and photophysiology was more plastic under environmental conditions representative of open ocean and meltwater environments (Petrou, Doblin & Ralph 2011b).

In order to survive the freezing conditions and osmotic stress experienced within the sea-ice, organisms such as diatoms are required to adjust their cellular concentrations of osmolytes (such as proline and glycine betaine) and production of antifreeze proteins, which act as a form of cryoprotection (Thomas & Dieckmann 2002). Despite the high concentrations of DMSP often measured in sea ice and the important role of DMSP in cryoprotection and osmoregulation (Lyon et al. 2016; Thomas & Dieckmann 2002), our study found DMSP concentrations at a per cell basis to be relatively low in the sea ice community compared to that of the open ocean. This discrepancy was likely the result of community composition, whereby the open ocean *Phaeocystis* dominated community meant that DMSP concentrations were well above those of the diatom dominated sea ice community. Despite recent research that has shown that some diatoms can be significant DMSP producers (Chapter 3), which is supported by the concentration recorded in the sea-ice community, DMSP production was not high enough to match the production of the haptophyte, supporting the findings of Chapter 3. These results are also consistent with the work of Stefels et al. (2012) who determined that the high levels on DMSP in sea ice were the result of an extreme abundance of diatoms that have modest amounts of DMSP, rather than themselves being significant producers of DMSP. However, it cannot be ruled out that the

change in conditions during melt out of the microalgae from the ice, may have resulted in alterations to DMSP production and retention by the cells. Therefore, future work should consider measuring DMSP content directly from sea ice samples, without prior manipulation of the environmental conditions.

Lipids, carbohydrates and proteins are important macromolecules in microalgal productivity. This study has highlighted environmental factors, such as salinity and temperature, as key drivers for community differences in macromolecular composition, where microalgae from the open ocean showed elevated levels of proteins compared to the sea-ice and lake communities. This has been shown before, where the pelagic diatom, *Pseudonitzschia subcurvata* expressed the highest concentration of proteins under treatment conditions representative of the pelagic environment and lowest protein content when subjected to conditions representative of the sea-ice (Sackett et al. 2013).

Antarctic sea-ice microalgal communities are often found to be high in lipid content (Fahl & Kattner 1993; Mock & Kroon 2002). In this study, we found the lipid content of the sea-ice community was relatively high in both saturated and unsaturated fatty acids, particularly for *Entomoneis*, which contrasted with the other two communities. These results were expected, as environmental conditions of sea-ice are known to induce high concentrations of polyunsaturated fatty acids, especially during winter (Asher et al. 2011). This is because lipids are a primary form of energy storage and used in the maintenance of membrane structure and function (Mock and Kroon, 2002). They are also high in caloric value, therefore any shifts in community composition could affect the number of calories available to those higher trophic levels feeding on microalgae. While lipids are calorie heavy, it is important to look at their composition; saturated fatty acids are mainly used for energy storage, whereas unsaturated fatty acids are important in physiological processes and membrane fluidity (Brett & Müller-Navarra 1997). This study found that sea-ice microalgae were higher in relative unsaturated fatty acid content compared with the open ocean, suggesting that sea-ice microalgae preferentially invest in unsaturated fatty acids, possibly to ensure membrane fluidity is maintained during freezing.

Ecologically, a high unsaturated fatty acid content in microalgae is important in the maintenance of high growth, survival and reproductive rates in zooplankton grazers (Brett & Müller-Navarra 1997; Demott & Müller-Navarra 1997; Søreide et al. 2010). Given that sea-ice algae are the main source of food for zooplankton during austral winter, these differences in macromolecular content likely underpin the food quality that is transferred through the trophic web during winter, which in turn, may be key to the productivity and survival of the Antarctic marine ecosystem.

When macromolecular profiles were teased apart based on species, there were clear species-specific signatures. These species-specific signatures highlight the variation in cellular levels of major classes of macromolecules (lipids, proteins and carbohydrates) in response to environmental conditions. Within the sea-ice community, there was a distinct difference between the lipid and protein content of *Entomoneis*, *Odontella* and *Fragilariopsis* spp. where *Entomoneis* and *Odontella* had greater relative amounts of lipids (predominantly saturated fatty acids) than *Fragilariopsis* spp.. Sackett et al. (2013) previously found variation in macromolecular composition to be species-specific, with all species responding differentially to changed environmental conditions. These results indicate that when presented with the same situation, different species may employ different strategies for acclimation.

The hypersaline conditions of Organic Lake make it inhospitable to many organisms, with salinity reaching in excess of 200, meaning only extreme halophiles are able to survive. The extreme salinity and low temperatures of Organic Lake also form barriers to biological productivity, limiting the number of interactions with higher trophic levels (Perriss & Laybourn-Parry 1997). While Organic Lake has been found to have one of the highest concentrations of DMS of any natural water body (Franzmann et al. 1987), in this study, only trace concentrations of DMSP where detected. Furthermore, its dominant species, *Dunaliella,* showed only moderate levels of DMSP lyase activity. Therefore, it is likely that most of the DMS recorded in the lake is a result of bacterial DMSP production and cleavage (Curson et al. 2017; Yau et al. 2013). The low DMSP content we detected in *Dunaliella* combined with the high

carbohydrate signature from the FTIR, may indicate that *Dunalliela* uses glycerol as an osmolyte instead of DMSP. Indeed, *Dunaliella* have previously been shown to maintain osmotic balance by metabolising intracellular glycerol in response to salinity stress (Chen, Jiang & Wu 2009; Goyal 2007). In order to synthesise glycerol the cell breaks down starch reserves (Goyal 2007) and previous studies have shown that even algal species tolerant of variations in salinity, macromolecular composition can be affected, specifically with an increase in carbohydrates at the highest salinities (de Castro Araújo & Garcia 2005). When considering the extremely high salinity of Organic Lake, the high carbohydrate content found within *Dunaliella* in this study, could be linked to high glycerol production.

This study characterised the productivity, DMSP content and macromolecular composition of Antarctic microalgae from three unique ecological habitats that encompassed a range of temperature, salinity and light conditions. Here, the open ocean community was characterised by high diversity and productivity, high DMSP content and DMSP lyase activity, as well as elevated levels of proteins. These characteristics combined, demonstrate the communities high level of plasticity and highlights their importance for supporting the marine food web. The key characteristics for sea ice microalgae were a community dominated by diatoms, elevated unsaturated and saturated fatty acids which are high in caloric value and provide a major food source for the Antarctic ecosystem. The presence of DMSP and DMSP lyase activity supports the use of DMSP as an osmolyte and cryoprotectant in sea-ice diatoms, however, how these data were measured needs to be considered in future studies. Organic Lake was characterised by low microalgal diversity, harbouring only one species. *Dunaliella*, showed low levels of productivity and high carbohydrate content consistent with its hypersaline environment. Despite only very low levels of DMSP, *Dunaliella* did have DMSP lyase activity, equivalent to that of sea-ice community. This would indicate, that despite not being a major producer, it could still contribute to DMSP cleavage and that the high concentrations of DMSP previously recorded in Organic Lake, are likely a result of bacterial production.

CHAPTER 5. SHORT-TERM HYPERSALINITY STRESS IN SEA-ICE ALGAE AND THE ROLE OF DIMETHYLSULFONIOPROPIONATE

INTRODUCTION

Marine phytoplankton produce one Gigaton of dimethylsulfoniopropionate (DMSP) each year, releasing 10^6 metric tonnes of dimethylsulfide (DMS) into the atmosphere annually (Johnston 2015). The production of DMSP by algae and its subsequent conversion to DMS is known to play a significant role in the marine sulfur cycle and form a source of cloud condensing nuclei, biologically controlling the Earth's albedo (Charlson et al. 1987). The significance of this algal derived DMS in climate control has recently been supported by a new study that shows marine DMS is responsible for increasing low-level cloud formation (Fiddes et al. 2018).

The Southern Ocean has been identified as a significant source of DMS emissions particularly in summer (Curran & Jones 2000; Kettle et al. 1999), as it constitutes 5 % of the world's oceans but contributes 20 % of sulfur flux, contributing 5.9 Tg S annually (Kettle et al. 1999) and a summer flux estimated at 3.4 Tg S (Jarníková & Tortell 2016), while the global average is estimated at 24-27 Tg S annually (Boucher et al. 2003). These high concentrations are linked with large blooms of *Phaeocystis*, which is known to dominate the Antarctic waters in late spring (Schoemann et al. 2005). *Phaeocystis* are one of the most prolific DMSP producing algae with intracellular concentrations of 5 to 50 nM (Liss et al. 1994), and blooms resulting in ocean concentrations as high as 1650 nM (Van Duyl et al. 1998). The sea-ice is considered another source of DMS and DMSP in the Antarctic, with concentrations of DMS and DMSP up to 500 times higher in the ice than the underlying water (Turner et al. 1995), and DMSP concentrations of over 200 nM recorded in sea-ice brine channels and bottom ice algal

assemblages (Asher et al. 2011; Trevena et al. 2000). Given that the sea-ice covers 2 million km² of ocean during the austral summer and 19 million km² in the winter (Comiso 2010), and that the primary production of sea-ice algae has been associated with DMSP production (Levasseur, Gosselin & Michaud 1994), with seasonal variation (Asher et al. 2017), it is likely that the contribution from the sea-ice microalgal community is significant.

Sea-ice microalgae are faced with a variety of tough conditions living within the sea-ice, such as light and nutrient limitation, freezing temperatures and steep salinity gradients (Arrigo 2014). Salinity varies within the sea-ice as salts are excreted during its formation. The ice-atmosphere interface has a low salinity (can reach low as 1) and salinity rapidly increases from the surface down until reaching the underlying seawater (~35), while brine channels within the sea-ice can reach over 150 (Arrigo 2014). Salinity stress can cause a number of pathophysiological responses including photosynthetic damage, increased respiration and oxidative stress (Sudhir & Murthy 2004). In particular, hypersaline conditions can cause oxidative stress within microalgal cells (Jahnke & White 2003), causing additional stress on the antioxidant system to quench any build-up of ROS that could lead to photosystem damage.

DMSP is a zwitterion, with a similar structure to other solutes like proline and glycine betaine; and is thought to act as an antioxidant, as well as an osmolyte (Sunda et al. 2002). Changes in DMSP concentrations from below detection to 8.9 mM were observed in the diatom *Thalassiosira psuedonana*, when salinity was increased from 10 psu to 35 psu (Kettles, Kopriva & Malin 2014). Similarly, the sea-ice diatom *Fragilariopsis cylindrus* when exposed to gradual salinity shifts increased intracellular DMSP concentrations at elevated salinity, and decreased DMSP concentrations at low-salinity, thereby determining DMSP to be a compatible solute in the acclimation to large salinity gradients in that species (Lyon et al. 2016).

This study investigated the role of DMSP in sea-ice microalgal cell function during a rapid osmotic shift that might be expected during seasonal sea-ice formation. The aim of this experiment was to determine whether DMSP production in a natural sea-ice community is enhanced as a result of increased salinity (i.e. testing it's osmoregulatory role) and determine how that increase related to photophysiological stress and cellular oxidative stress (i.e. testing its antioxidant role). By measuring salinity stress responses, we aimed to gain deeper insight into how sea-ice microalgae respond or adjust to changes in the abiotic environment, and in turn provide new information on marine biogeochemical cycling.

Materials and Methods

Species collection and experimental set up

A natural sea-ice microalgal community was obtained from an ice core (66 °S, 77 °E) taken 26th November 2014 at Prydz Bay, Davis Station. The bottom 1 cm of the ice core was melted in the dark for 48 h, with regular additions of 0.22 µm filtered seawater to prevent the salinity of the water dropping too low and damaging the cells during melt out. The community was then homogenised and split into two treatments where the salinity was then adjusted (to 35 and 70 psu) over 4 h with hourly additions of sodium chloride (NaCl) salt (Sigma) and confirmed with a salinity probe. The treatments were incubated for 24 h in a temperature-controlled incubator set to -1 °C under moderate light i.e. 112 µmol photons m^{-2} s^{-1} measured in air using a 2Pi light meter (LI-250A, LI-COR, USA).

Measurements and analysis

The microalgae community was subsampled after 24 h for species identification to determine community composition (n=3), cell density (n=3) and cell volume (n=10) using light microscopy

(Nikon Eclipse Ni-E, Nikon Instruments, USA, 100x magnification). The photophysiological condition of the algal community was measured for each treatment using Pulse Amplitude Modulated fluorometer (Water PAM, Walz GmbH, Germany) as detailed in the **General Methods**. Methanol preserved community samples (stored at 3 °C until analysis) were collected for DMSP measurements, along with community samples collected on 0.2 µm filters (GF/F, Whatman), snap frozen and stored at -80 °C until analysis) for DMSP lyase activity, determined using gas chromatography (GC) as described in the **General Methods**.

Alkalinity and DIC

Practical alkalinity was calculated from DIC and pH_T measurements. DIC was measured using an AS-C3 analyzer (Apollo SciTech, USA) equipped with a LICOR7000 CO_2/H_2O Gas Analyzer (LI-COR, USA). pH_T was measured according to the Dickson et al. (2007) spectrophotometric approach, using the pH indicator m-cresol purple on an UV-Vis 916 spectrophotometer (GBC Scientific Equipment, USA) in a 10 cm thermostated cuvette.

Gross productivity

Gross productivity was measured for each treatment using oxygen microsensors (as per **General Methods**) and a light curve performed. During the light curve, 4 light levels were applied (88, 241, 321 and 482 µmol photons m^{-2} s^{-1}) for 5 min each and net productivity measured, with respiration measured in the dark before each light step. Gross productivity was calculated at each light level by summing of respiration and net production rates. For normalisation of O_2 data, 1 mL of sample was collected to determine cell density. Primary productivity was normalised to cell density, plotted against PAR and fitted according to a double exponential function (Ralph & Gademann 2005). Light utilisation

efficiency (α), minimum saturating irradiances (E_K), and maximum electron transport rate (ETRmax) obtained from the curve following Ralph & Gademann (2005).

PIGMENT ANALYSIS

Pigment concentrations were determined using high-performance liquid chromatography (HPLC), where a 50 mL subsamples were filtered onto 25 mm GF/F filters (Whatman), snap-frozen and stored at -80 °C. Pigments were extracted according to van Huekelem and Thomas (2001) and run on a HPLC system that consisted of a pump, temperature-controlled auto-injector (Waters Australia Pty Ltd, Woolloongabba, QLD, Australia), C8 column (150 9 4.6 mm; Eclipse XDB) and photodiode array detector (Waters Australia Pty Ltd, Woolloongabba, QLD, Australia). Calibration standards (DHI, Horsholm, Denmark) were used to identify each pigment by retention time and spectra. Empower Pro software (Waters Australia Pty Ltd, Woolloongabba, QLD, Australia) was used to integrate peak area and manually checked for baseline accuracy and comparison to standards.

Chlorophyll *a* was taken for normalisation of DMSP and DMSP lyase activity. A 15 mL subsample was filtered onto GF/C filters which were then snap frozen in liquid N2 and stored at -80 °C until analysis. Chlorophyll pigments were extracted in 90% acetone and incubated at 4 °C in the dark for 24 h, determined spectrophotometrically (Cary50: Varian, Santa Clara, CA, USA) and calculated using the equations of Jeffery et al. (1999), modified by Ritchie (2006).

ANTIOXIDANT ACTIVITY

The antioxidant, superoxide dismutase (SOD), was selected for measurement in each treatment as a representative of the antioxidant response within the cells. A 15 mL subsample of each treatment was centrifuged at 3600 g for 10 mins, the supernatant removed, and the algal pellet snap frozen and stored

at -80 °C. The algal pellets were resuspended in 2 mL filtered sea water and sonicated on ice for 3 rounds of 10 seconds using a sonicator probe (Vibra-Cell VC 50T, Sonics & Materials Inc., USA) to rupture the cells. The suspension was then centrifuged at approximately 3600 g for 10 min at 4°C to pellet the cell wall fragments, and the supernatant analysed for enzyme activity and the detection of total protein. Total SOD (including Cu/Zn and Mn) was measured using a SOD activity determination kit (SOD-560, Applied Bioanalytical Labs) as described by the manufacturer. Total protein analysis was conducted using the Pierce™ BCA protein assay kit (Thermo Scientific, USA) and used to normalise SOD enzyme activity.

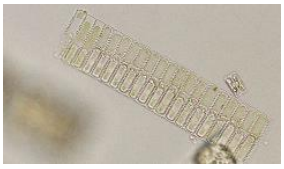
Data analysis

Tests were performed using IBM SPSS Statistics (version 20) to determine statistically significant differences. One-way analysis of variance (ANOVA) for chlorophyll *a* fluorescence, photosynthetic and photoprotective pigments, SOD activity, DMSP content and lyase activity data. Test for normality and Levene's test for homogeneity of variance were conducted *apriori*, before the one-way ANOVA.

Results

The bottom sea-ice community was comprised of diatoms (Table 5.1), with a final cell concentration of 1,678.05 cells mL^{-1} used for the incubations. The most dominant species were *Fragilariopsis* spp. (45 %) and *Melosira* (38 %). While *Entomoneis* (8 %), *Odontella* (5 %), *Psuedonitzschia* (4 %) and *Thalassiosira* sp. (<1 %) were also present, but in lower abundances. Cell volume varied four orders of magnitude with *Psuedonitzschia* being the smallest cells at 505 μm^3 and *Odontella* the largest at 121,951 μm^3.

Table 5.1 Community composition of sea-ice, including species, volume (µm³) and abundance (cells L⁻¹). Displayed as means ± SE. Scale bar = 20 µm³.

Species	Photograph	Volume (µm³)	Abundance (cells L⁻¹)
Entomoneis sp.		20,000 ± 808	138,600 ± 49,500
Fragilariopsis spp.		1,106 ± 47	762,300 ± 29,700
Melosira sp.		34,559 ± 962	628,650 ± 84,150
Odontella spp.		121,951 ± 21,822	84,150 ± 44,550
Psuedonitzschia sp.		505 ± 85	59,400 ± 19,800
Thalassiosira sp.		22,574 ± 1,665	4,950 ± 4,950
Total			**1,678,050 ± 123,750**

Under the salinity treatment of 35 psu, representative of normal seawater, pH was recorded as 8.2, with DIC and total alkalinity of 1785.7 µmol/kg and 2164.7 µmol/kg respectively. At 70 psu, DIC was 1823.3 µmol/kg and total alkalinity was 2055.3 µmol/kg (Table 5.2). For the 70 psu, pH was not able to be determined because of a malfunction using an old electrode.

Table 5.2 Water carbonate chemistry data. ND = no data.

Treatment Salinity	DIC µmol/kg	Total alkanity µmol/kg	pH (at 25 °C)
35	1785.7	2164.7	8.242
70	1823.3	2055.3	ND

Increased salinity lead to a decline in gross photosynthesis (Figure 5.1), where there was a lower light utilisation efficiency (α) under high salinity ($F_{(1,7)}$ = 8.0, P= 0.030). However, no difference in the maximum electron transport rate and minimum saturating irradiance with increased salinity was detected (Table 5.3).

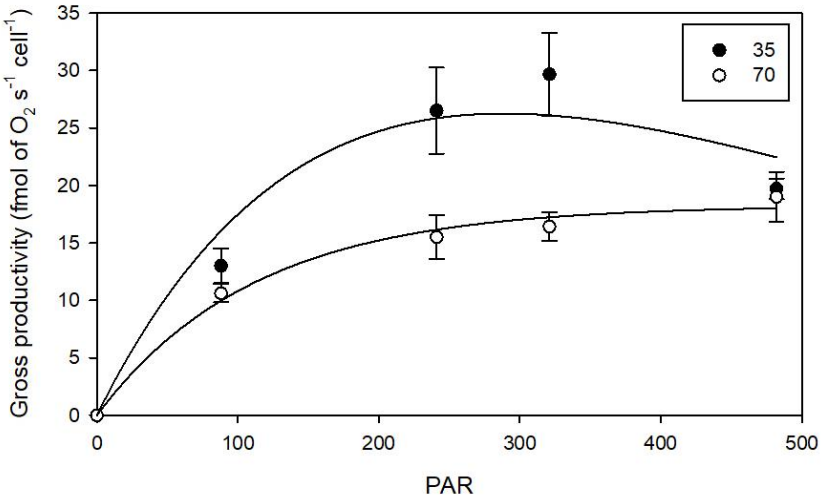


Figure 5.1 Gross primary productivity of a natural sea-ice community under control and hypersaline conditions (expressed per cell in fmol of O_2 per sec). Data represent mean ± SE (n=4).

Table 5.3 Photosynthetic parameters: Light utilisation effieciency - α, maximum eletcron transport rate - ETR_{max}, minimum saturating irradiance - E_k. Data represent mean ± SE (n=4). Red asterisk * indicates significant difference in treatments.

	35	70
α*	0.26 ± 0.07	0.19 ± 0.06
ETR_{max}	26.23 ± 4.65	18.84 ± 4.82
E_k	104.15 ± 25.34	117.06 ± 62.88

Congruent with the productivity data, there was a significant decline in photosynthetic health of the microalgal community when exposed to hypersaline conditions (Figure 5.2). Maximum quantum yield of PSII (F_V/F_M) was reduced by 52 % ($F_{1,7}$= 240.771, P<0.001), and light-adapted effective quantum yield of PSII (EQY) by 43 % ($F_{1,7}$= 43.548, P=0.001) under hypersaline conditions (Fig. 5.1 A and B). The relative electron transport rate (rETR) was also negatively affected, showing a significant reduction of 43 % when salinity doubled ($F_{1,7}$= 42.697, P=0.001; Fig. 5.1 C). There was a clear counter response in the non-photochemical quenching (NPQ), which more than doubled under high salinity ($F_{1,7}$= 14.650, P=0.009; Fig. 5.1 D).

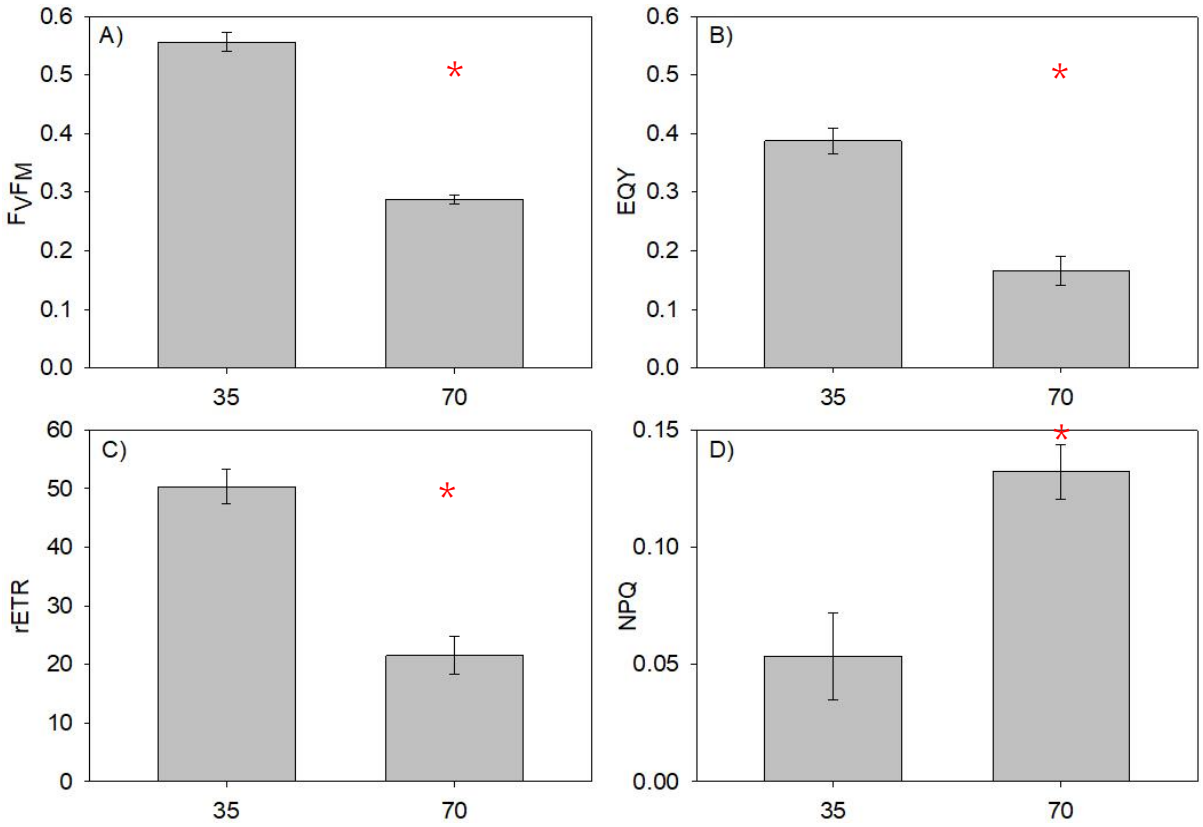


Figure 5.2 Photophysiology for a natural Antarctic sea-ice community under control and hypersaline conditions. A) Maximum quantum yield of PSII (F_V/F_M) **B)** Effective quantum yield of PSII (EQY) **C)** Relative electron transport rate (rETR) **D)** Non-photochemical quenching (NPQ). Data represent means ± SE (n=4). Red asterisk * indicates significant difference in treatments.

Pigment analysis revealed cells exposed to hypersaline conditions had significantly lower concentrations of fucoxanthin ($F_{1,3}$ = 246.4, P= 0.004), diadinoxanthin ($F_{1,3}$=428.9, P=0.002), diatoxanthin ($F_{1,3}$= 78.6, P=0.012) and chlorophyll *a* ($F_{1,3}$=26.05, P=0.036), though there was no significant difference in the chlorophyll c2 and zeaxanthin content between salinity treatments (Table 5.4). When pigments were pooled based on function (photosynthetic vs photoprotective), there was a decrease in both total photosynthetic pigments ($F_{1,3}$=38.5, P=0.025) and total photoprotective pigments ($F_{1,3}$=222, P=0.004) with increased salinity (Table 5.4). The ratio of photoprotective pigments to total pigments (PP:Total) was significantly higher at 35 psu than 70 psu ($F_{1,3}$=283378, P<0.001). There were no differences in ratios of chl *c2*: *a*, chl a:fucoxanthin or the de-epoxidation ratio with treatment (Table 5.4).

Table 5.4 Pooled and ratiometric pigment concentrations in µg/L and pg/cell of sea-ice communities. Data represent the mean (n=2). Chlorophyll – Chl, Photosynthetic pigments (chlorophyll *a* and c_2) – PSP, Photoprotective pigments (Diadinoxanthin and diatoxanthin) – PP, Chlorophyll – Chl, Fucoxanthin – Fuc. Red asterisk * indicates difference in treatments.

	35		**70**	
	µg/L	**pg/cell**	**µg/L**	**pg/cell**
Chl *a**	45.43	25.21	9.48	5.26
Chl c_2	17.14	9.51	4.77	2.65
Fucoxanthin*	31.87	17.69	8.25	4.58
Diadinoxanthin*	6.27	3.48	0.24	0.13
Diatoxanthin*	0.87	0.49	0.02	0.01
Zeaxanthin	2.07	1.15	0.27	0.15
Total PSP*	62.57	34.73	14.25	7.91
Total PP*	5.46	1.68	0.01	<0.01
PP:Total*	0.08	0.05	<0.01	<0.01
Chl c_2:*a*	0.39	0.39	0.52	0.52
Chl *a*:Fuc	1.42	1.42	1.15	1.15
De-epoxidation ratio	0.12	0.12	0.09	0.09

There was no significant difference in superoxide dismutase (SOD) content, between salinity treatments (Figure 5.3A). DMSP content also did not vary between salinity treatments ranging between 0.65 – 0.66 µmol / µg chl *a* (Figure 5.3B), and while there was an apparent reduction in DMSP lyase activity under hypersaline conditions, this difference was not significant (Figure 5.3C).

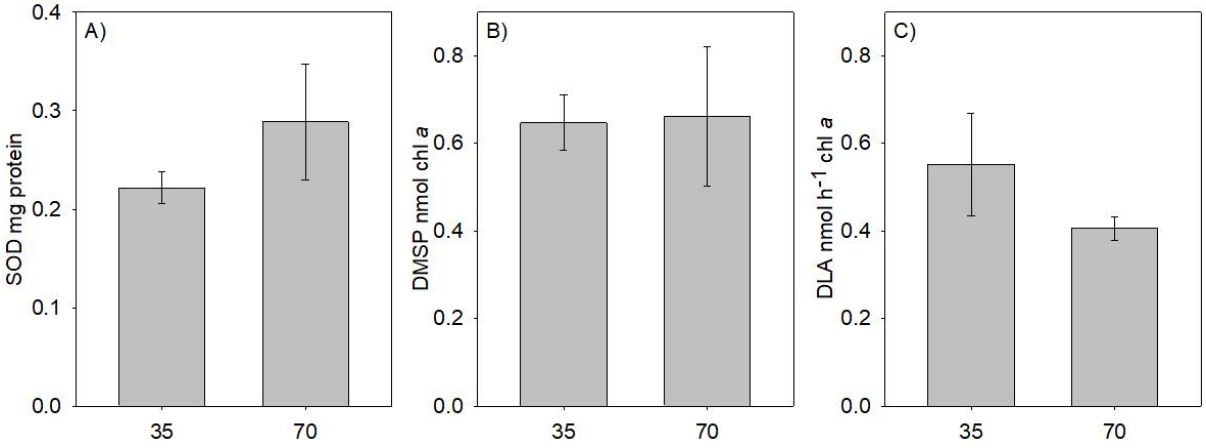


Figure 5.3 A) Superoxide dismutase (SOD) content per unit of protein (n=2) **B)** DMSP content in nmol per µg chl *a* (n=3) and **C)** DMSP lyase activity in nmol per hour per µg chl *a* (n=3) in sea-ice communities exposed to two salinity treatments, that of ambient seawater (35 psu) and hypersaline conditions (70 psu). Data represent the mean ± SE.

DISCUSSION

The highest concentrations of dimethylsulfide (DMS) have been found in polar regions, particularly the Southern Ocean (Curran & Jones 2000; Kettle et al. 1999), where high sulfur fluxes have been closely linked to the presence of blooms of the haptophyte, *Phaeocystis* (Brussaard et al. 1996; DiTullio et al. 2000). However, in the absence of *Phaeocystis* blooms, DMS and DMSP concentrations in surface waters can still be high, indicating that rather than being a product of an ice edge bloom, DMS and DMSP is released into the water column from the sea-ice itself (Trevena & Jones 2006). This study collected the sea-ice community from the bottom of the sea-ice core, where 90% of the sea-ice algae are found and concentrations of DMSP are among the highest (Trevena et al. 2000). In this study, diatoms

dominated the community, with a high prevalence of the pennate diatom *Fragilariopsis* sp., which is typical of land fast ice (Andreoli et al. 2000).

Salinity has been labelled the most dominant abiotic stress on sea-ice diatoms (Krell 2006). Despite this, sea-ice diatoms survive a broad range of salinities and salinity shifts during natural sea-ice freeze-thaw cycles. For cells trapped within the sea-ice matrix, the environment consists of brine channels where salinity can reach up to three times the salinity of seawater (Arrigo 2014). Then in the spring/summer as the sea-ice thaws, cells are released back into seawater or into melt ponds that can form from snowmelt on the surface of the sea-ice where salinity within the ponds is around that of freshwater (Arrigo 2014). Microalgae have developed physiological mechanisms to deal with this dynamic environment and the fast-changing conditions inherent to the seasonal sea-ice environment, being more tolerant to decreases in salinity than increases in salinity (Kirst & Wiencke 1995). Some species adapted better than others to a sea-ice environment, showing a high photosynthetic plasticity and are able to acclimate across a range of environmental conditions (Arrigo & Sullivan 1992; Petrou, Doblin & Ralph 2011a; Ryan, Ralph & McMinn 2004), with tolerance to high salinities even increasing at lower temperatures (Aletsee & Jahnke 1992; Gleitz & Thomas 1992). In sea-ice diatoms, the xanthophyll cycle and D1-protein repair are key to photoprotection in cells under stress, such as those caused by changes in salinity and temperature (Galindo et al. 2017; Kropuenske et al. 2009; Petrou, Doblin & Ralph 2011a). The de-epoxidation reaction of the xanthophyll cycle plays an important role in protecting against photooxidative damage, by converting diadinoxanthin to diatoxanthin, which is used to dissipate excess light as heat (Goss & Jakob 2010; Lavaud, Rousseau & Etienne 2002). While this response was reflected in the increase in non-photochemical quenching (NPQ), suggesting cells were experiencing photosynthetic stress under high salinity, the pigment data showed no difference in the de-epoxidation ratio. In addition to this, the lack of SOD activity would suggest that the xanthophyll cycle wasn't activated at all due to the incubation irradiance, being relatively low and close to the

minimum saturating irradiance of both treatments, it was insufficient to cause oxygen radical formation. Instead, there was an overall decline in photoprotective pigments, conflicting with previous studies where photoprotective pigments increase under increased salinity and low temperature (Petrou, Doblin & Ralph 2011a). Cellular pigment concentrations are regulated by changes in rates of synthesis and/or loss (Kana, Geider & Critchley 1997).The overall decline in pigments would indicate possible pigment bleaching or loss as a result of the high salinity, whereby the photosystem was maintaining electron transport, possibly at the expense of cell damage. If pigments were insufficient to protect the photosystem, photoinhibition would likely be the result, however this is only speculative as photoinhibition was not measured in this study.

In support of cells struggling under the high salinity treatment, we saw lowered photosynthetic activity (including F_V/F_M, EQY, gross primary productivity and light utilisation effieciency) and a strong reduction in light harvesting pigments at a salinity of 70. The loss in chlorophyll *a* content (80 %) was greater than that previously observed in marine diatoms, *Cylindrotheca closterium* (22 %) exposed to the same salinity shift, but over 24 h rather than 4h as was done in this study. This would suggest that time could play an important role in successful osmotic adjustment (Rijstenbil 2003). However, there was consistency between both studies, where the ratios of chlorophyll c2 to chlorophyll *a* and fucoxanthin to chlorophyll *a*, showed no change with salinity (Rijstenbil 2003). The loss of pigments observed in this study is of concern and it is likely that the community in this study was more adversely affected than intended and cells were experiencing damage to or breakdown of protein pigments under the rapid (4 h) salinity doubling.

Sea-ice algae, if not provided the time to gradually acclimate to changes in salinity, are susceptible to osmotic shock (Ryan, Ralph & McMinn 2004). DMSP has been described as a compatible solute allowing for osmoregulation of cells. In a study of two dinoflagellate species, there was an increase in DMS per cell with increased salinity, indicating that DMSP is upregulated when cells are exposed to

osmotic stress (Zhuang et al. 2011), with similar findings in diatom species (Kettles, Kopriva & Malin 2014; Lyon et al. 2016). In contrast, our study found that there was no significant difference in the intracellular DMSP of a sea-ice community when experiencing salinity stress. This could be due to the protective properties of DMSP, as a comparative study of various organic solutes confirmed that not all solutes provide equivalent protective outputs, in particular, DMSP while it does have a role in osmotic adjustment, it is a less effective solute than betaine, proline or glycerol, due to it slow response to osmotic demands (Kirst 1996). In addition to no measurable increase in DMSP content with increased salinity, DMSP concentration was extremely low at 0.65 nmol chl *a*, compared to concentrations previously recorded for diatoms 4 mmol : g chl *a* (Stefels et al. 2007), and particularly in Antarctic sea ice 215- 275.2 nmol : µg chl a (Trevena & Jones 2012) and while DMSP production is slow compared to other solutes, DMSP is usually already present in high concentrations acting as a buffer during the initial stress of salinity shock (Kirst 1996). Given the low DMSP detected in the samples and the apparent severe physiological stress that the microalgae in this study were under, it is possible that the cells simply did not have sufficient energy to upregulate their production of DMSP and that any DMSP that was present was lost before analysis.

Environmental factors such as extreme salinity can result in the excess production of reactive oxygen species (ROS) in marine microalgae, altering or halting certain biochemical activity and leading to oxidative stress (Mallick & Mohn 2000). Microalgal cells have developed antioxidant systems in which to combat the toxic effects of ROS. One of the first lines of defence is the antioxidant, superoxide dismutase (SOD), which has been seen to increase (from ~100 to ~200 U mg protein^{-1}) in the marine diatom, *C. closterium* when salinity is increased and more than double (~360 U mg protein^{-1}) under the combined effects of salinity and UV-B stress (Rijstenbil 2003). While the photosynthetic data and pigment concentrations indicated that the sea-ice community was experiencing stress under high salinity, we detected no difference in the antioxidant activity of SOD. This may indicate that the cells

were using another antioxidant to combat their oxidative stress. Indeed, a study on the chlorophyte *Dunaliella tertiolecta* showed that while cells displayed significant changes in various antioxidant enzymes and substrates under oxidative stress from hypo and hyper-saline conditions, there detected no change in SOD activity (Jahnke & White 2003). DMSP and its breakdown products (DMS, dimethylsulphoxide (DMSO), methane sulphinic acid and acrylate) have been shown to act as a possible antioxidant system in marine microalgae, scavenging hydroxyl radicals and other reactive oxygen species, mediated by the rate of DMSP lyase within the cell (Spiese et al. 2017; Sunda et al. 2002). In this study, we saw no difference in DMSP concentration or DMSP lyase activity with salinity treatment, indicating DMSP was not regulated within the cell under the salinity conditions that we applied and therefore, may not in this instance have been utilised as an ROS quencher. One could speculate that that while no detectable increase in DMSP occurred with osmotic shift, any upregulation of DMSP may have been masked by an increase in its utilisation by the cell to cope with additional ROS. However, in the absence of direct measurements of ROS, and no measurements of DMSO production, it is impracticable to draw any definitive conclusions.

This study set out to investigate the osmoregulatory and antioxidant roles of DMSP in sea-ice microalgae during a rapid osmotic shift that might be expected during seasonal sea-ice formation. However, the results from this study were inconclusive. Unfortunately, the osmotic shift may have proved too rapid, preventing cellular adjustment to the environmental conditions of this study, instead causing damage to the cells, thus confounding any DMSP response, whether osmotic or antioxidant in nature. Despite this limitation, this study characterised the community structure, photophysiology and DMSP content of a bottom sea-ice microalgal community, providing good baseline data from which future studies can use and build upon.

CHAPTER 6. OCEAN ACIDIFICATION AND MARINE TROPHIC DYNAMICS: INVESTIGATING DIMETHYLSULFONIOPROPIONATE (DMSP) AND MACROMOLECULAR CONTENT IN ANTARCTIC PHYTOPLANKTON IN HIGH CO2 WATERS

INTRODUCTION

Over the last 150 years the composition of our atmosphere has changed at an alarming rate, with atmospheric concentrations of CO_2 in 2013 exceeding 400ppm, a first in over 3 million years (Bala 2013). The Antarctic marine ecosystem will be amongst the earliest to respond to a high CO_2 world, due to the high solubility of carbon dioxide and calcium carbonate in cold waters (McNeil & Matear 2008). Significant changes in ocean chemistry will influence marine productivity and ecosystem functioning through initiating shifts in phytoplankton community structure (Deppeler et al. 2018; Hancock et al. 2018) and prompting shifts in individual phytoplankton physiologies (Petrou et al. 2016). What is unknown is whether or not enhanced CO_2 will result in adjustments in the macromolecular composition of individual species. Indeed, changes in the relative proportions of proteins, lipids and carbohydrates have been measured in phytoplankton cells grown under altered environmental conditions, such as iron limitation or thermal stress (Stehfest et al. 2005; Sackett et al., 2013). However, little is known of the effect of increased CO_2 will have on phytoplankton nutritional value.

In Antarctic waters, phytoplankton account for the majority of biomass, therefore any shift in their community structure or individual physiologies will influence food web dynamics and biogeochemical cycling. Studies have shown that enhanced CO_2 can cause changes in the size spectrum of

phytoplankton impacting their metabolism, availability to grazers and the efficiency of carbon cycling (Davidson et al. 2016; Engel et al. 2007). There are varying reports on the effects of elevated pCO2, with several studies demonstrating an increase in the primary productivity of some diatom species (Trimborn et al. 2013; Wu, Gao & Riebesell 2010) while others were unaffected (Chen et al. 2015; Hoppe et al. 2015; Trimborn et al. 2013). In contrast, there are studies that observed, a decline in primary production and growth rates (Barcelos e Ramos et al. 2014; Deppeler et al. 2018; Hoppe et al. 2015; Shi, Xiahou & Wu 2017) and change in community structure at high pCO_2, with increased abundance of small species and fewer large diatoms (Hancock et al. 2018; Hare et al. 2007), indicating changes to food web structure and carbon sequestration. If you add to that the potential for a shift in the macromolecular composition of the community, affecting the overall nutritional value of the phytoplankton, such changes could have significant implications for grazers and higher trophic levels. In biogeochemical terms, shifts to a community of smaller cells will lead to a reduction in carbon export (bigger cells sink faster) and carbon dioxide uptake (lower production = less CO_2 drawn down), thereby reducing carbon cycling efficiency, however less is known about the impact of ocean acidification on the marine sulfur cycle. Mesocosm and incubation studies have resulted in conflicting reports where higher pCO2 leads to lower DMS and DMSP concentrations (Avgoustidi et al. 2012; Hopkins et al. 2010; Lee et al. 2009); or alternatively it has elevated levels of DMS and DMSP, most likely as a result of increased primary production (Wingenter et al. 2007) and increased grazing activity (Kim et al. 2010).

This study aims to determine the potential impact of increased CO_2 levels on a natural Antarctic microbial community, focusing on the effects on DMSP production and DMSP lyase activity and macromolecular composition of a coastal Antarctic microbial community.

METHODS

EXPERIMENTAL SET UP

An un-replicated experiment, using six 650 L polyurethane tanks (minicosms) housed within a temperature-controlled shipping container, was conducted on natural microbial communities exposed to a range of CO_2 levels (343, 506, 634, 953, 1140 and 1641 µatm) see Deppeler et al. (2018) and Davidson et al. (2016). Before use, the minicosms were acid rinsed with 10% vol:vol AR grade HCl, followed by MilliQ water and finally filled and drained with seawater from the sampling site.

Seawater was collected from an open area among broken fast ice approximately 1 km from shore, in Prydz Bay, Davis Station, Antarctica (68° 35' S, 77° 58' E) on the 19th November, 2014. Seawater was transferred, via helicopter and a clean 720 L Bambi Bucket, to a 7000 L reservoir tank before being gravity fed into the minicosms via a Teflon lined hose with an in-line 200 µm Arkal filter to remove metazooplankton, allowing all tanks to be filled simultaneously, ensuring a homogenous starting community for each tank.

Each tank was sealed with an acrylic lid and contained a shielded high-density polyethylene auger that provided gentle mixing (15 rpm). The water temperature was kept at 0.0 °C ± 0.5 °C and regulated through a balance of room cooling and two 300W aquarium heaters (Fluval) which were connected to temperature controllers (Carel) running a temperature control program. Each tank was exposed to a 19:5 h light : dark cycle using two 150WHQI-TS/NDL (Osram) metal halide lamps (transmission spectra; Deppeler, Davidson & Schulz (2017)) with a light-scattering filter (LSF) and a one-quarter colour temperature (CT) blue filter (Arri) attached in order to convert the tungsten light to a natural daylight spectral distribution.

The fugacity of carbon dioxide (fCO_2) was adjusted over a five-day acclimation period during which phytoplankton growth was attenuated by reducing light intensity to 0.9±0.2 µmol photons m^{-2} s^{-1} by

attaching two 90% neutral density (ND) filters (Arri). After the five-day acclimation period the two 90% ND filters were replaced with one 60% ND filter for 24 h before the final light intensity was achieved on day 7 with the LSF and CT blue filter (Arri).

Sampling took place on day 14 of the experiment during exponential growth. For the FTIR data, samples were collected at the end of the experiment (Day 18).

MEASUREMENTS AND ANALYSIS

Samples were collected for analysis of community composition, chlorophyll *a* content, gross O2 productivity, chlorophyll *a* fluorescence and processed according to the methods outlined in the **General Methods** chapter.

A 5-step steady-state light curve was conducted on each minicosm (n = 3). Samples from each tank were exposed to 130, 307, 600, 973, 1450 µmol photons m^{-2} s^{-1}, applied for 5 min before recording the light-adapted minimum (Ft) and maximum fluorescence (Fm') values. There was a 30 s 'recovery' period between each stepped increase in light. Fluorescence data were fitted according to a double exponential function (Ralph & Gademann 2005) and light utilisation efficiency (α), minimum saturating irradiances (E_K), and maximum electron transport rate (ETRmax) obtained from the curve following Ralph & Gademann (2005).

Species identification was conducted using an automated upright fluorescence microscope (Nikon Eclipse Ni-E, Nikon Instruments, USA, 100x magnification). The most common species in each community were selected for FTIR analysis. Methanol preserved samples were collected and analysed for DMSPp. Microalgal samples were filtered onto 0.2 µm GF/F filter papers, snap-frozen and analysed

for DMSP lyase activity using gas chromatography and macromolecular content using FTIR analysis as detailed in the **General Methods** chapter.

DATA ANALYSIS

Statistical tests were performed using IBM SPSS Statistics (version 20). A one-way analysis of variance (ANOVA) was used to determine statistically significant differences in total cell density, chlorophyll *a* content, gross O_2 production, light curve parameters, SOD, DMSP and lyase activity. Levene's was used establish homogeneity of variance required for the one-way ANOVA and Tukey's post hoc tests were used to find where the differences lie. The six level fCO_2 gradient approach meant that some of our data could be analysed using a regression model, allowing us to identify a functional relationship between our CO_2 treatment and our response variable. Linear regression analyses were performed on the chlorophyll *a* and FTIR data to explore relationships between pigment or macromolecular content and fCO_2.

RESULTS

Carbonate chemistry on day 14 varied between minicosm tanks, with DIC and CO_2 increasing from 2186.40 and 328.66 in Tank 1, to 2378.90 DIC and 1684.17 CO_2 in Tank 6 (Table 6.1). Salinity was consistent across tanks at 34.53 psu however pH, temperature and alkalinity showed slight variation across tanks ranging from 7.71 – 8.11, -0.3 – 0.1 °C and 2310.76 – 2330.08, respectively. There was some variation in nutrients between tanks with the highest concentrations found in Tank 6. Nitrate and nitrite (NOx) ranged from 14.28 – 19.28 across tanks and soluble reactive phosphorus ranged from 0.87 – 1.26, while silicate remained constant at 124.62 across all Tanks except 6 which had a higher concentration of 128.18 (Table 6.1).

Table 6.1 Environmental conditions of minicosm tanks on day of subsampling (day 14).*

Tank	DIC	pH	Salinity	Temp	PA	fCO_2	NOx	SRP	Si
1	2186.40	8.11	34.53	-0.2	2328.87	328.66	14.28	0.97	124.62
2	2245.10	7.95	34.53	-0.3	2328.15	501.97	17.13	1.07	124.62
3	2267.40	7.87	34.53	0.1	2330.08	600.82	13.56	0.87	124.62
4	2309.00	7.71	34.53	0.1	2323.49	891.95	14.99	0.94	124.62
5	2334.50	7.62	34.53	-0.3	2321.88	1096.69	17.13	1.07	124.62
6	2378.90	7.44	34.53	-0.1	2310.76	1684.17	19.28	1.26	128.18

*Data provided by Deppeler, Davidson & Schulz (2017).

While there was no difference in chlorophyll *a* between tanks 1,2 3, at fCO_2 concentrations above 600, chlorophyll *a* decline steadily as CO_2 increased (Adj-R^2 = 0.909, P = 0.002). There was a significant difference in gross productivity ($F_{5,22}$= 101.612, P < 0.001) with fCO_2 minicosms, where Tank 6 had the highest gross productivity and the lowest chlorophyll *a* content (Figure 6.1A, 6.1B) and Tank 2 had elevated gross productivity compared to Tanks 1, 3, 4 and 5. The relative electron transport rate (rETR) was reduced at higher irradiance in the highest fCO_2 treatments (Figure 6.1C), with a significant difference in the maximum electron transport rate ($F_{5,17}$= 3.131, P= 0.049) between tanks 2 and 6 (Table 6.2). There was no difference in light utilisation efficiency or minimum saturating irradiance with fCO_2.

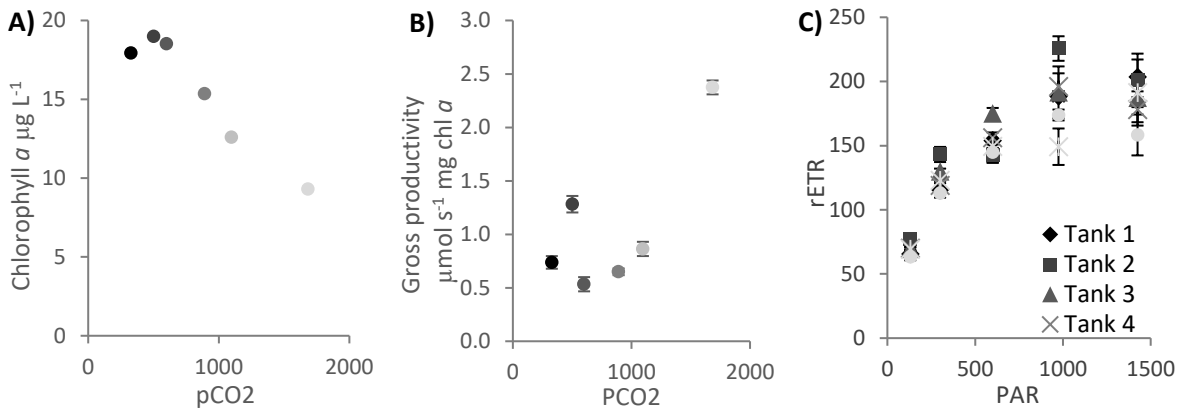


Figure 6.1 A) Chlorophyll *a* content (n=1), **B)** Gross productivity (n=4), **C)** relative electron transport rate (n=3)

Table 6.2 Photosynthetic parameters: Light utilisation effieciency - α, maximum eletcron transport rate - ETR_{max}, minimum saturating irradiance - E_k. Data represent the means (n=3) ± SE. Red asterisk * indicates significant difference in treatments

	1		2		3		4		5		6	
α	0.51	± 0.02	0.60	± 0.04	0.66	± 0.05	0.56	± 0.02	0.63	± 0.04	0.56	± 0.04
$rETR_{max}$*	202	± 16	218	± 12	194	± 11	194	± 6	174	± 2	168	± 8
E_k	395	± 21	368	± 40	302	± 42	346	± 25	279	± 21	306	± 21

The total phytoplankton cell density varied between tanks ($F_{5,23}$ = 3.388, p= 0.025), with greatest cell numbers in Tank 5 (2581 cell mL^{-1}) after which cell density more than halved in Tank 6 (1094 cells mL^{-1}). The phytoplankton communities of all six minicosms were dominated by diatoms, in particular, a small, chain forming *Chaetoceros* spp., which made ~ 51-67 % of the community. There were also a few large individual *Chaetoceros* spp. present in all minicosms (Figure 6.2A). There were large populations of centric diatoms, *Melosira* sp., *Thalassiosira* sp and *Proboscia* sp., as well as smaller populations of *Stellarima microtrias* and pennate diatoms, *Pseudonitzschia* sp. and *Fragilariopsis* spp. in most tanks

(Figure 6.2A). In all tanks, there was an even contribution from the colony forming haptophyte *Phaeocystis*, with colonies constituting 3-7 % of the community in all tanks. Dinoflagellates and the silicoflagellate, *Dictyocha speculum*, were present in all six tanks, but only made up a tiny fraction of the community (<3 %). Under the highest fCO_2 (Tank 6), there was a notable absence of the large diatoms from the genera *Eucampia* sp., *Odontella* spp. and *Nitzschia* sp. (Figure 6.2).

When concentrating on the DMSP producing portion of phytoplankton within each community, the abundance of diatom species that were determined to be significant DMSP producers (ascertained in Chapter 3) made up 6-24% of the community and differed significantly between tanks ($F_{5,23} = 7.341$, $P = 0.001$), with the greatest density of known DMSP producers found in Tank 3 ($fCO_2 = 601$) at a concentration of 638,550 cells L^{-1} and the smallest populations found in Tanks 4 ($fCO_2 = 892$) and 6 ($fCO_2 = 1684$), at concentrations of 277,200 and 252,450 cells L^{-1}, respectively (Figure 6.2B). In contrast, the DMSP producing haptophytes, *Phaeocystis* spp. showed no difference in the number of colonies between tanks ranging from 56,925 to 103,950 L-1 (Figure 6.2C) and dinoflagellates constituted less than 3% of the community in each minicosm (Figure 6.2A).

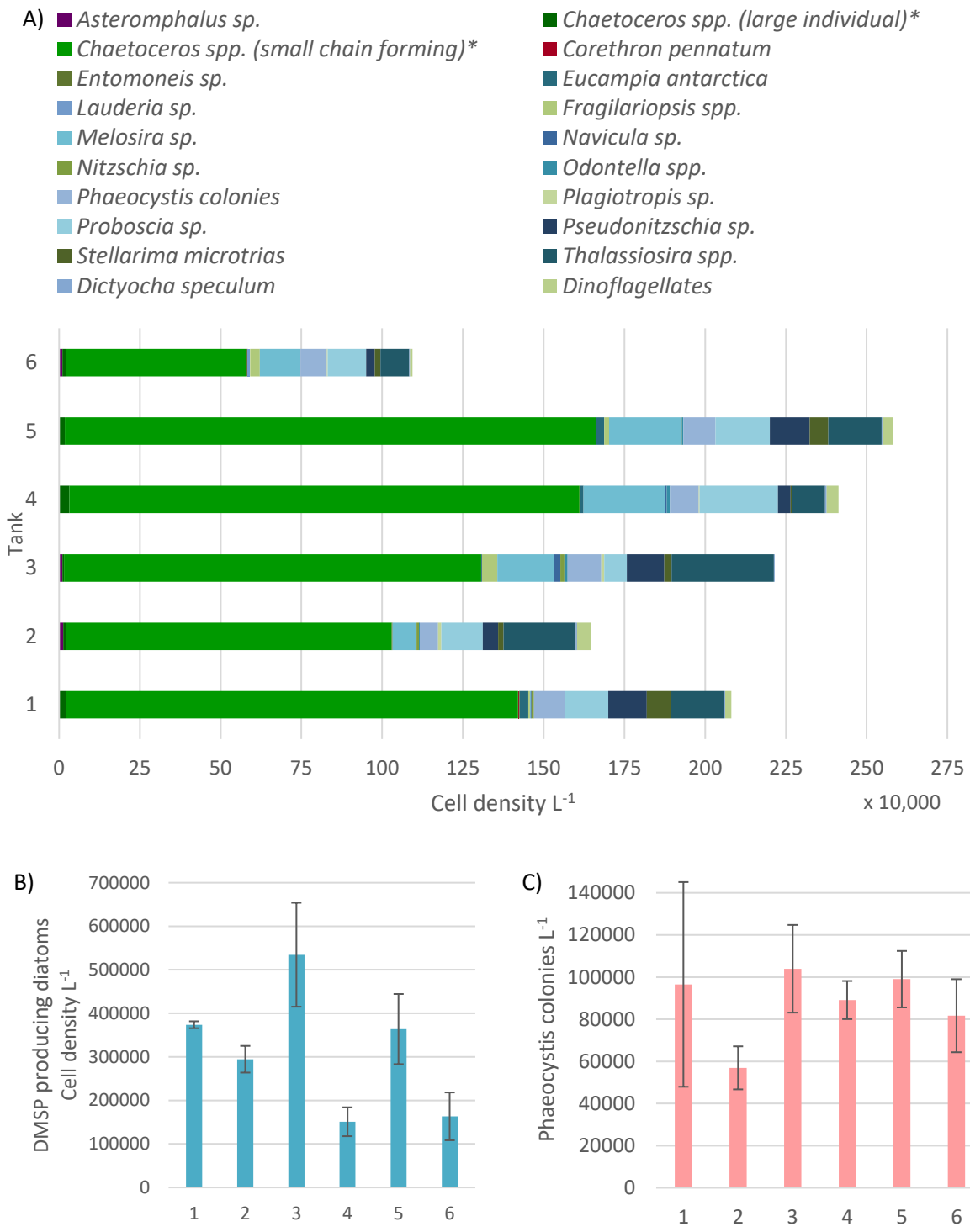


Figure 6.2 A) Community composition. Data represent the mean abundance in cells L^{-1}. DMSP producers are in bold and species that do not produce DMSP are marked with an * (as determined in Chapter 3). **B) cell density of DMSP producing diatoms** (as determined in Chapter 3) and **C) average abundance of colonies of *Phaeocystis* sp.** Data represent the mean ± SE (n=4).

Particulate DMSP concentration varied between minicosm tanks ($F_{5,17}$= 142.803, P < 0.001), where DMSP was highest in Tank 3 (Figure 6.3A). Similarly, when DMSP was normalised to chl *a* it was highest in Tank 3 ($F_{5,17}$= 116.524, P < 0.001), but was also elevated in Tank 5 (fCO_2 = 1079) (Figure 6.3A, 6.3B). DMSP lyase activity showed a similar pattern to intracellular DMSP in which DMSP lyase was highest at 600 fCO_2 in Tank 3, both per cell and per chl *a* (Figure 6.3C, 6.3D).

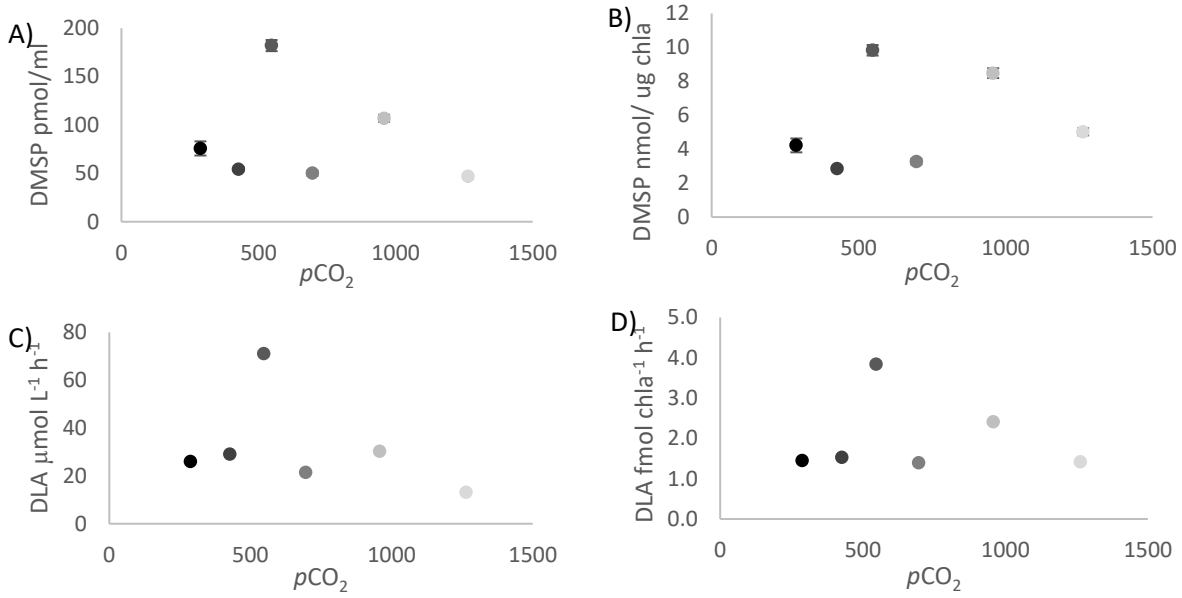


Figure 6.3 DMSP content where data represent the means of three technical replicates A) per mL and **B)** per ug chl a (please note that SE error bars are too small to be seen); **and DMSP lyase activity (n=1) C)** per L and **D)** per fmol chl a.

Spectral data revealed the macromolecular content of some of the dominant diatom species showed a relationship between cellular macromolecules and fCO_2 (Figure 6.4A). Analysis (PCA) of the biologically relevant spectra revealed the main variation in macromolecular profiles of each tank was driven by amides and fatty acids, which displayed a tight grouping along the PC-1 axis explaining 32% of variation while PC-2 explained 7% (Figure 6.4B). The biggest biomolecular influences were amide II (band 1540 cm^{-1}) and saturated fatty acids (bands 2960 and 2875 cm^{-1}), both represented as positive changes along PC-1, while tyrosine (band 1515 cm-1) and lipids (band 1745 cm-1) were represented as negative changes along PC-1 (Figure 6.4C). The second biggest influences were represented as positive

changes in saturated fatty acids (band 2930 cm^{-1}) and carboxylates (band 1450 cm^{-1}) and negative changes in amide II (band 1540 cm^{-1}) along the PC-2 axis.

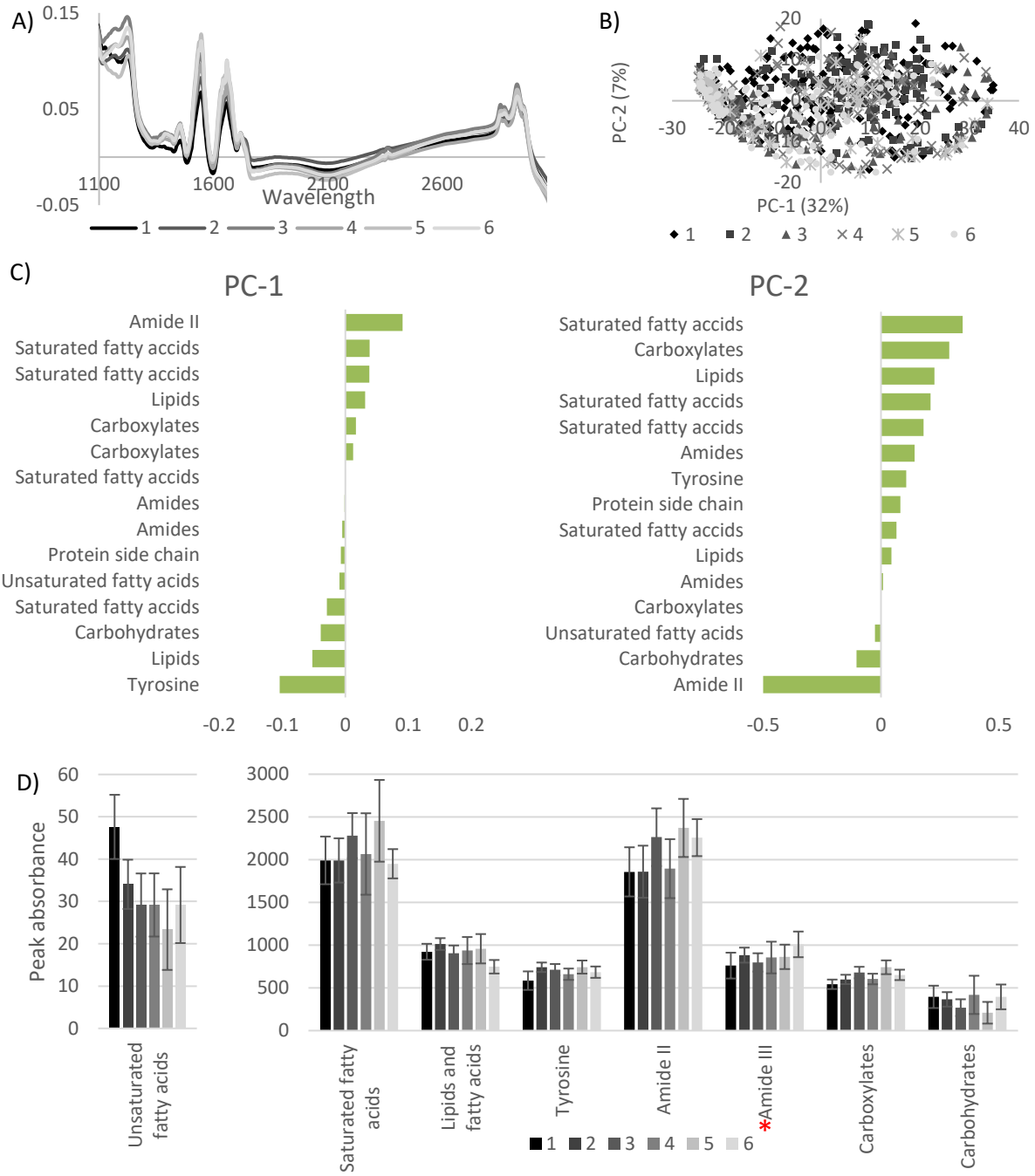


Figure 6.4 Macromolecular composition of selected phytoplankton from minicosms exposed to increasing levels of fCO_2 A) average IR spectra **B)** Scores plot based on infrared spectra of species across all minicosms; **C)** PC-1 and PC-2 loadings plots. **D)** Mean content of key macromolecules of dominant species in each minicosm. The concentration is proportional to the area under the peak in the infrared spectrum corresponding to each macromolecule. Bars represent mean ± SE (n= 91 – 177). Red asterisk * indicates significant difference in treatments.

The macromolecular composition of dominant species within the community showed all minicosms were high in saturated fatty acids and Amide II compared to other macromolecules (Figure 6.4D). While there was no correlation between lipid content and CO_2, there was a negative correlation between the ratio of unsaturated:saturated fatty acids, which decreased with increased CO_2 (Table 6.3; 6.4). Linear regression of community macromolecule content revealed there was a positive correlation between Amide III content and increasing CO_2 (Table 6.4). As the dominant species in each minicosm comprised of diatoms, carbohydrate information was limited due to the silicate peak, which overlapped with the bands for carbohydrates, however in the available carbohydrate data there was no clear trend in carbohydrate content and CO_2 (Figure 6.4D).

Table 6.3 Ratio of unsaturated fatty acids to saturated fatty acids (Unsat:Sat). Data represent mean ± SE (n= 91 – 177)

Tank	Unsat:Sat	SE
1	0.05	0.003
2	0.04	0.003
3	0.03	0.003
4	0.02	0.003
5	0.01	0.002
6	0.02	0.002

Table 6.4 Regression analysis of the whole community macromolecular content in relation to fCO_2. Ratio of unsaturated to saturated fatty acids – Unsat:Sat. Bold indicates a significant relationship at $p<0.05$.

	R Square	$F_{1,5}$	P-value
Unsaturated fatty acids	0.387	2.524	0.187
Saturated fatty acids	0.001	0.002	0.964
Lipids and fatty acids	0.549	4.873	0.092
Tyrosine	0.378	0.287	0.620
Amide II	0.067	2.427	0.194
Amide III	**0.765**	**12.996**	**0.023**
Carboxylates	0.267	1.457	0.294
Carbohydrates	0.000	0.001	0.975
Unsat:Sat fatty acids	**0.636**	**9.719**	**0.036**

Looking at species-specific macromolecular profiles, all species experienced an overall downward trend in unsaturated fatty acids with increased CO_2 except for *Fragilariopsis* sp., *Proboscia* sp. and to a lesser extent *Thalassiosira* spp. which started to increase above 1097 µatm (Figure 6.5A). There was an initial increase then decline in saturated fatty acids and lipids and fatty acids in *Proboscia* sp., while there was an overall decrease in the large centric diatoms, *Melosira* sp. and *Thalassiosira* spp. (Figure 6.5A). Linear regression revealed a negative correlation between lipids and fatty acids and increased Co_2 in *Melosira* (Table 6.8) and *Thalassiosira* (Table 6.10).

In *Asteromphalus* sp., *Fragilariopsis* spp., *Melosira* sp. and *Thalassiosira* spp. there was a peak in the amino acid, tyrosine at 502 µatm (Tank 2), whereas *Chaetoceros* spp. and *Proboscia* spp. dipped before an increase in tyrosine at the highest CO_2 levels (Figure 6.5B). Linear regression revealed a positive correlation between Amide II and CO_2 in *Proboscia* sp. (Table 6.9). While there was no significant relationship between Amide III and CO_2 for any species, *Chaetoceros* sp., *Proboscia* sp. and *Thalassiosira* sp. experienced an initial decrease in Amide III before reaching 892 µatm in Tank 4 then increased again (Figure 6.5B).

Carboxylates increased with increased CO_2 before reaching an asymptote in *Asteromphalus* sp. (Figure 6.5C), while *Proboscia* sp. experienced an increase with increased CO_2 (Table 6.9). Linear regression showed that there was no correlation between carbohydrate content and CO_2 in any species, except for *Melosira* sp. which decreased with decreased CO_2 (Table 6.8). However, the large centric diatoms, *Asteromphalus* sp. and *Thalassiosira* sp. displayed an initial decline, while *Chaetoceros* spp. and *Proboscia* sp. also displayed an initial decrease before increased in carbohydrate content in *Chaetoceros* spp. above 601 µatm and *Proboscia* sp. above 892 µatm (Figure 6.5C). The carbohydrate content of *Fragilariopsis* spp. fluctuated across CO_2 treatments but remained unchanged overall (Figure 6.5C).

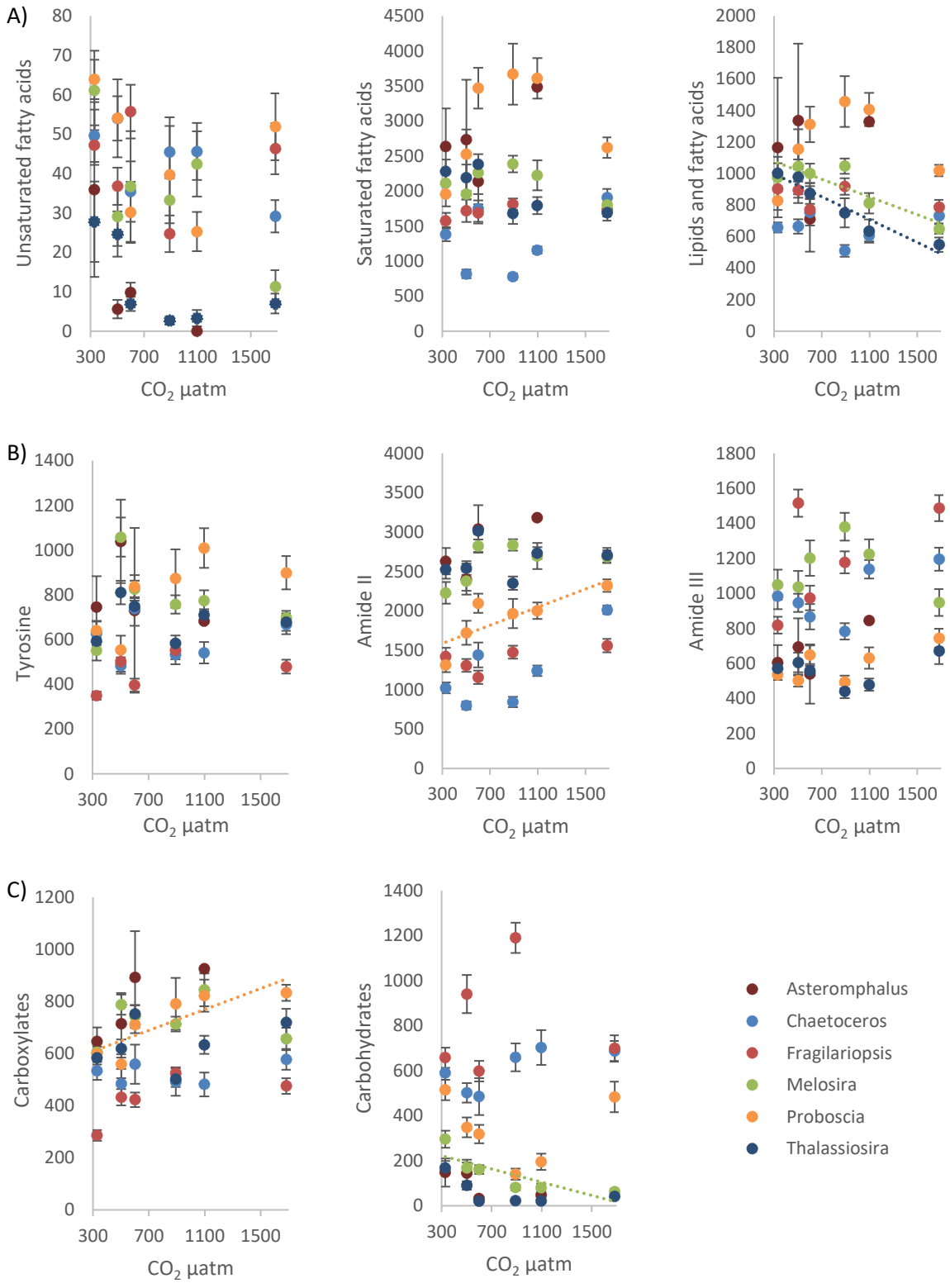


Figure 6.5 Key macromolecules of dominate species in each minicosm. The concentration is proportional to the area under the peak in the infrared spectrum corresponding to each macromolecule. Trendlines are included where a significant regression was detected.

Table 6.5 Regression analysis of *Asteromphalus* macromolecular content in relation to fCO_2. Bold indicates a significant relationship at p<0.05.

Asteromphalus	**R Square**	**$F_{1,3}$**	**P-value**
Unsaturated fatty acids	0.575	2.711	0.241
Saturated fatty acids	0.520	2.169	0.278
Lipids and fatty acids	0.056	0.118	0.763
Tyrosine	0.173	0.419	0.584
Amide II	0.610	2.859	0.233
Amide III	0.410	3.088	0.221
Carboxylates	0.716	5.035	0.154
Carbohydrates	0.480	1.852	0.307

Table 6.6 Regression analysis of *Chaetoceros* macromolecular content in relation to fCO_2. Bold indicates a significant relationship at p<0.05.

Chaetoceros	**R Square**	**$F_{1,5}$**	**P-value**
Unsaturated fatty acids	0.506	4.103	0.113
Saturated fatty acids	0.161	0.765	0.431
Lipids and fatty acids	0.006	0.022	0.889
Tyrosine	0.016	0.065	0.811
Amide II	0.568	5.264	0.083
Amide III	0.409	2.765	0.172
Carboxylates	0.104	0.466	0.532
Carbohydrates	0.527	4.449	0.103

Table 6.7 Regression analysis of *Fragilariopsis* macromolecular content in relation to fCO_2. Bold indicates a significant relationship at p<0.05.

Fragilariopsis	**R Square**	**$F_{1,4}$**	**P-value**
Unsaturated fatty acids	0.003	0.010	0.928
Saturated fatty acids	0.287	1.206	0.352
Lipids and fatty acids	0.237	0.932	0.405
Tyrosine	0.197	0.736	0.454
Amide II	0.411	2.089	0.244
Amide III	0.338	1.534	0.304
Carboxylates	0.380	1.834	0.268
Carbohydrates	0.000	0.001	0.972

Table 6.8 Regression analysis of *Melosira* macromolecular content in relation to fCO_2. Bold indicates a significant relationship at p<0.05.

Melosira	**R Square**	**$F_{1,5}$**	**P-value**
Unsaturated fatty acids	0.555	4.979	0.604
Saturated fatty acids	0.146	0.685	0.454
Lipids and fatty acids	**0.754**	**12.235**	**0.025**
Tyrosine	0.022	0.091	0.778
Amide II	0.274	1.508	0.287
Amide III	0.025	0.104	0.763
Carboxylates	0.001	0.006	0.944
Carbohydrates	**0.678**	**8.440**	**0.044**

Table 6.9 Regression analysis of *Proboscia* macromolecular content in relation to fCO_2. Bold indicates a significant relationship at p<0.05.

Proboscia	**R Square**	**$F_{1,5}$**	**P-value**
Unsaturated fatty acids	0.055	0.232	0.655
Saturated fatty acids	0.068	0.293	0.617
Lipids and fatty acids	0.024	0.099	0.769
Tyrosine	0.497	3.945	0.118
Amide II	**0.662**	**7.832**	**0.049**
Amide III	0.540	4.690	0.096
Carboxylates	**0.712**	**9.912**	**0.035**
Carbohydrates	0.001	0.005	0.948

Table 6.10 Regression analysis of *Thalassiosira* macromolecular content in relation to fCO_2. Bold indicates a significant relationship at p<0.05.

Thalassiosira	**R Square**	**$F_{1,5}$**	**P-value**
Unsaturated fatty acids	0.432	3.036	0.156
Saturated fatty acids	0.653	7.520	0.052
Lipids and fatty acids	**0.926**	**50.18**	**0.002**
Tyrosine	0.005	0.021	0.892
Amide II	0.014	0.056	0.825
Amide III	0.051	0.215	0.667
Carboxylates	0.090	0.398	0.563
Carbohydrates	0.327	1.944	0.236

DISCUSSION

Recent studies of the effect of ocean acidification on key Antarctic phytoplankton have revealed mixed responses. One study found *Phaeocystis* abundance to decrease under an ocean acidification scenario after a period of exponential growth (Hancock et al. 2018), while another revealed *Phaeocystis* abundance increased (Trimborn et al. 2017). In contrast, our study found ocean acidification had no impact on the number of *Phaeocystis* colonies during exponential growth, which is in agreeance with earlier studies (Tortell et al. 2008; Trimborn et al. 2013). The reduced cell density at highest CO_2, in addition to an absence of large diatoms and smaller species being dominant has been seen in previous studies (Davidson et al. 2016; Hoppe et al. 2013). This change in community composition could significantly impact the food chain, as krill, the keystone species of the Antarctic marine ecosystem, find it difficult to eat small cells, feeding preferentially on larger diatoms (Walsh et al. 2001), effecting availability of phytoplankton to grazers and flow on effects up the food web. The decline in the abundance of large diatoms, as well as the chain forming *Chaetoceros* spp. indicates ocean acidification could have a significant impact on biogeochemical cycling, as these bloom forming species have a high capacity for carbon export via sedimentation (Stickley et al. 2005).

Phytoplankton play a key role in biosphere-atmosphere interactions, producing volatile organic compounds, such as DMS and isoprene, which photo-oxidise in the atmosphere to form cloud condensing nuclei and have the ability to influence the global climate (Carpenter, Archer & Beale 2012; Liss & Johnson 2014). Phytoplankton are thought to produce either DMS or isoprene, with emissions governed by various physical and biological factors (Dani & Loreto 2017). DMS is favoured in polar waters while isoprene is more common in warmer waters, suggesting a change in global water temperatures could lead to a shift in the geographic distribution of both with an overall increase in isoprene and decrease in DMS (Dani & Loreto 2017). Early ocean acidification models predicted there would be a decrease in DMS emissions, but only at the equator, while the opposite was forecast for

higher latitudes, with an increase of over 150% in DMS fluxes in the Southern Ocean alone (Cameron-Smith et al. 2011). However, subsequent studies on ocean acidification in polar regions have found DMS emissions to decrease (Archer et al. 2013; Arnold, Kerrison & Steinke 2013). An ocean acidification mesocosm study based in the Arctic found that while DMS decreased in the high CO_2 waters, there was an increase in DMSP, due to an increase in dinoflagellate biomass (Archer et al. 2013). This decrease in DMS production under increased CO_2 was also observed in the coccolithophore, *Emiliania huxleyi*, a species particularly susceptible to ocean acidification because of its calcium carbonate shell, however, under combined high temperature and elevated CO_2 there was an increase in DMS production and intracellular DMSP (Arnold, Kerrison & Steinke 2013). In our study, we found no correlation between intracellular DMSP and CO_2, but we did find intracellular DMSP concentrations matched the pattern seen in the abundance of known DMSP producing diatoms, as determined in Chapter 3.

A key influence on DMS emissions is the biomass of DMSP producing species, indicating that the changes in phytoplankton biomass and species composition of the community under ocean acidification will have a flow on effect, impacting on the global marine sulfur cycle. *Phaeocystis* is a significant producer of DMSP in the Antarctic (Stefels 2000), with significant lyase capabilities (also shown in Chapter 3), and acidification has been seen to cause an increase in the conversion of DMSP to DMS in *Phaeocystis* cultures (Kinsey & Kieber 2016). However, in this study, we found no correlation between DMSP or DMSP lyase activity and the number of *Phaeocystis* colonies present in each tank. Similarly, while many pennate diatoms such as *Fragilariopsis*, *Nitzschia* and *Pseudonitzschia* possess DMSP lyase capabilities, albeit at much lower rates than *Phaeocystis* (Chapter 3), when all species with the potential for DMSP lyase were grouped we found no correlation between DMSP lyase activity and species abundance across the tanks. This could be due to the variability of lyase activity within taxonomic groups, whereby many of the species present in this study, while from the same genus, were not the same as those measured in the laboratory study (Chapter 3).

The nutritional value of phytoplankton is determined by cell size, shape, digestibility and macromolecular composition (lipids, proteins and carbohydrates), as well as the requirements of the grazer (El-Hady et al. 2016). Lipids, proteins and carbohydrates are important macromolecules in algal productivity, with sufficient quantities of both lipids and proteins needed for rapid growth (Wikfors, Ferris & Smith 1992). Lipids are high in caloric value and shifts in not only species content, but the species that make up the community, affect the number of calories available to higher trophic levels. In phytoplankton, lipids are used as a reservoir of carbon for overnight protein biosynthesis (Terry, Hirata & Laws 1983). Saturated fatty acids in particular, are a primary form of long term energy storage and used in the maintenance of membrane structure and function (Mock & Kroon 2002), while unsaturated fatty acids are important in many physiological processes and regulating membrane fluidity (Brett & Müller-Navarra 1997). Long-chain polyunsaturated fatty acids (LC-PUFA) are of particular importance, as most vertebrates have a limited ability to synthesise LC-PUFA and therefore must consume them (Cook & McMaster 2002; Parrish 2009). Macromolecular composition of phytoplankton is affected by the surrounding environment conditions and will therefore be susceptible to future changes in climate. A multi-trophic study demonstrated that increased CO_2 caused a decline in total fatty acids and a decrease in the ratio of LC-PUFA to saturated fatty acids of diatom *Thalassiosira psuedonana*, resulting in a decrease in copepod somatic growth and egg production (Rossoll et al. 2012). A review of six major groups of marine and freshwater phytoplankton found similar results in fatty acid profiles with increased temperature; When grouped together there was a decrease in the proportion of unsaturated fatty acids and an increase in saturated fatty acids (Hixson & Arts 2016). However, work on the synergistic effects of pCO_2 and temperature on sea-ice diatom, *Navicula lecointei*, revealed that while there was an overall decrease in fatty acids at high CO_2 at - 1.8 °C, there were no changes at 2.5 °C (Torstensson et al. 2013). Initially the saturated fatty acid content of the community increased with increased CO_2, however there was a decrease at the highest CO_2 levels. The inverse relationship was

observed in the unsaturated fatty acids of the combined community data, as seen by the decrease in ratio of unsaturated to saturated fatty acids with increased CO_2, which was driven by the decline in the large centric diatoms, *Asteromphalus* sp., *Melosira* sp. and *Thalassiosira* sp.. There was a decline in overall cell density, but particularly large diatoms at the highest CO_2 treatment, which has potential implications for krill abundance, as they can have difficulty grazing on smaller cells (McClatchie & Boyd 1983). This absence of a large diatom species, high in unsaturated fatty acid, along with a shift from unsaturated to saturated fatty acids in high CO_2 waters has potentially negative implications for the zooplankton that graze upon these phytoplankton to maintain their own high growth, survival and reproductive rates (Brett & Müller-Navarra 1997; Demott & Müller-Navarra 1997) and vertebrates relying on phytoplankton as their source of LC-PUFAs, constricting trophic transfer.

Phytoplankton cells can invest in proteins, however, not only do proteins have half the caloric value of lipids, but an increase in protein synthesis usually results in a downturn in the lipid fraction (Morris, Glover & Yentsch 1974; Whyte 1987). Antarctic phytoplankton have been found to invest up to 60% of cellular carbon into protein synthesis, with a high availability of macronutrients indicated as the driver for enhanced protein synthesis (Song et al. 2016). All five of the most dominant diatom species showed elevated levels of at least one amide peak under high CO_2 and increases in both the Amide II and Amide III at the community level suggest that protein content could increase under ocean acidification. Phytoplankton high in protein are generally indicative of healthy cells with high growth rates, and no nitrogen limitation (Springer & McRoy 1993), but at the highest CO_2 the decline in biomass and drop in relative electron transport rate, could suggest larger cells were under stress.

Carbohydrates are an important form of energy storage in phytoplankton, especially in the Antarctic due to its variable light climate. Carbohydrates are used as a source of energy and carbon skeletons for protein synthesis during periods of limited light (Granum & Myklestad 2002; Van Oijen et al. 2005). Investigations of the carbohydrate band is limited due to the overlap with the silica band, a common

problem when studying diatoms. However, the negative relationship in both saturated fatty acids and carbohydrate content and increase in protein synthesis with acidification suggests that *Melosira* sp. and *Thalassiosira* spp. are not storing as much of their energy under high CO_2 conditions, while a decrease in chlorophyll *a* could indicate that there is less energy being captured. As diatoms are a major food source of krill in Antarctica, preferred over prymnesiophytes and cryptophytes (Haberman, Ross & Quetin 2003; Head & Harris 1994), this has implications for the nutritional quality supplied to the base of the food web.

Due to the unreplicated gradient design of this mesocosm study, conclusions from this study must be interpreted conservatively. However, the observed changes in phytoplankton community composition, biomass and macromolecular composition due to elevated CO_2 all occurred above an *f*CO2 of 890 µatm, suggesting a possible CO_2 threshold above which significant changes can be expected. This threshold is projected to be reached by the end of the century. It is clear from the shifts in phytoplankton physiology and biomolecular composition seen in this study and others, that these changes have the potential to alter biogeochemical cycling, and marine food web dynamics of the future.

CHAPTER 7. GENERAL DISCUSSION AND THESIS SUMMARY

The studies presented in this thesis investigated the physiological and biochemical adaptations of Antarctic microalgae and the role of dimethylsulfoniopropionate (DMSP) in relation to these adaptations. The results of these studies have provided new knowledge into who are the DMSP producers in Antarctica, the spatial dynamics and role of DMSP in natural Antarctic microbial communities. Furthermore, the use of manipulative laboratory studies has delivered new insight into the response microalgae have to environmental stress and future climate change scenarios at the cellular, species and community levels. In this chapter, the key findings and implications of this new information is discussed, along with future research directions.

PRIMARY PRODUCTION IN ANTARCTICA

Antarctic marine microalgae are key to primary production and carbon export in the Southern Ocean and inhabit a variety of environmental niches. In winter almost half the Southern Ocean is covered in sea-ice and not only does the sea-ice provide a habitat for microbes but the sea-ice makes a significant contribution to primary production during the winter period (Arrigo et al. 1998; Lizotte 2001). However, primary productivity is at its highest in summer when the ice edge retreats and conditions are ideal for microalgal blooms (Arrigo et al. 1998). In this thesis, we demonstrated that species diversity is reduced with more challenging environmental conditions and the species with the greatest phenotypic plasticity tend to dominate these more challenging environments (Chapter 4 and 6). As a result of the changing community composition, primary productivity varies between environmental niches (Chapter 4), whereby the community is less productive under increased environmental stress such as salinity, pH and temperature (Chapter 4, 5 and 6). Similar findings have also been observed

over longer timescales where net primary productivity decreased with increased temperature and number of open water days (Schine, van Dijken & Arrigo 2016).

Differences in primary production can have impacts up the food web, where there are changes in nutritional value at the base of the food web not only due to biomass, but also the macromolecular content of the individual species. With each species having a unique macromolecular profile, taxonomic shifts over the spring/summer season can result in changes to the elemental stoichiometry of the community. In this thesis, we found that macromolecular content of microalgae differed depending on environment, whereby sea-ice microalgae were higher in caloric value, due to heavy investment in lipids compared to their pelagic counterparts (Chapter 4), making their nutritional value ideal for winter grazers when food supply is reduced. The change in community composition under ocean acidification resulted in a change to community macromolecular content and available nutritional pools, specifically, we saw a decrease in protein and shift in the ratio of unsaturated and saturated fatty acids, where the ratio unsaturated:saturated fatty acids decreased with increased CO_2. There were also shifts in species-specific macromolecular content with increased *f*CO2, with a decrease in lipids and fatty acids for the centric diatoms, *Melosira* and *Thalassiosira*, and an increase in carbohydrates for *Melosira* and proteins for *Proboscia*. These changes to community structure and macromolecular content can impact grazability and transfer of nutrients between trophic levels. Based on the results of this thesis and current literature, there is the potential for this to have a negative impact on krill populations as they have trouble grazing on smaller cells and the microalgae would be of lesser nutritional quality. In addition, the constriction of essential fatty acids could have flow on effects at higher trophic levels. Therefore, this work highlights that the collection of taxon-specific macromolecular data is important in ecosystem structure and function and essential for understanding the relationship between primary productivity, food web interactions and potential fisheries yields.

CONTRIBUTION TO THE ANTARCTIC SULFUR CYCLE

Microalgae are the biological link between oceanic and atmospheric processes and Antarctic microalgae play a vital part in the sulfur cycle. While microalgae are known producers of the sulfur compound DMSP, not all taxa produce the same amount or even produce it at all. Until now, little was known about which Antarctic species produced DMSP and what significance their individual or collective contribution might be. The work contained in this thesis confirms that Antarctic diatoms, compared to their temperate counterparts, are significant producers of DMSP and major contributors to high sulfur emissions in the Antarctic (Chapter 3). We were able to confirm that Antarctic diatoms can produce DMSP per cell in the same range as the prolific DMSP producer *Phaeocystis*.

The new information on species-specific DMSP production and lyase activity (Chapter 3) provides support that links patterns in DMS emission with phytoplankton succession. Every year there is a seasonal shift in community composition; at the start of spring (October) before the sea-ice has melted, the microalgal community is confined to the sea-ice and is dominated by sea-ice diatoms. At this time, DMS flux measurements are at their lowest. As the sea-ice melts, the sea-ice community is released into the open water, causing diatoms to bloom and DMS flux increases (Trevena & Jones 2012). Our measurements on diatom DMSP production provide support for the potential significance of their contribution to DMS flux in the Antarctic (Chapter 3). As the season progresses a mixed community forms dominated by *Phaeocystis* and diatoms in December, this corresponds to a peak in DMS (Trevena & Jones 2006). Our data presented in Chapter 4 which showed highest concentrations of DMSP in our mixed open ocean community dominated by *Phaeocystis* (66 %) and diatoms (21 %), supports this peak production in December. In January, when *Phaeocystis* blooms take over, DMSP tends to drop, probably indicative of acclimation to higher light and therefore reduced production (Van Duyl et al. 1998). This bloom gives way in late summer, leaving a diverse community of various phytoplankton taxa from numerous classes, in which DMS emissions are low again (Petrou et al. 2016; Trevena &

Jones 2006). We found that in general, pennate diatoms had higher intracellular DMSP concentrations than centric diatoms (Chapter 3). Given that pennate diatoms dominate the sea-ice, this may help to explain the high DMS emissions measured as the sea-ice melts out in summer. Although we found that the concentrations of DMSP in open ocean were greater than sea-ice, this was likely caused by the substantial contribution of the prolific DMSP producing haptophyte *Phaeocystis* which made up more than 50% of the community (Chapter 4).

Anthropogenic induced climate change has the potential to impact significantly on the Antarctic sulfur cycle. Our ocean acidification experiment revealed a change in community structure (Chapter 6) and when considering that DMSP production and lyase activity are species-specific (Chapter 3), any shifts in community composition has the potential to impact DMS flux within the region. In addition to ocean acidification, it has been predicted that the warming surface waters will increase stratification reducing the mixed layer depth and increasing the total daily irradiance impacting on DMS emissions (Vallina & Simó 2007), which has been shown to increase DMS production in phytoplankton and decrease bacterial DMS consumption (Levine et al. 2012; Slezak, Brugger & Herndl 2001; Toole et al. 2006). Therefore, DMS emissions could change under future ocean conditions, possibly increasing, due to shifts in the species that make up the community and environmental condition.

ROLE OF DMSP IN ANTARCTIC MICROALGAE

DMSP varied between communities and taxa, and even within taxa (Chapter 3, 4 and 6). This could be indicative of the multiple possible roles of DMSP as a cryoprotectant, osmolyte and antioxidant. The pelagic community had the highest DMSP content and DMSP lyase activity of all three environments tested (Chapters 4 and 6). Microalgae in the Southern Ocean are exposed to high UV radiation in the summer and it is possible the potential antioxidant function of DMSP was being activated here. DMSP

was not as high in the sea-ice community, showing minimal support for DMSP being used as a principle osmolyte or cryoprotectant. Despite the potential osmotic and temperature challenges, the sea-ice community, dominated by diatoms, had less than half the DMSP per cell and lower lyase activity than those measured in the open ocean community. Although Organic Lake, which consisted of the one species of green alga, was low in DMSP, it did have high DMSP lyase activity, indicative of living in an environment high in DMSP. Given the lack of DMSP production however, it was concluded that this species likely employs an alternative osmoregulatory method and that production of DMSP in this system is most likely driven by bacterial processes. While, our study on the osmoregulatory role of DMSP in the Antarctic sea-ice community was inconclusive due to the salinity shift being too rapid, DMSP has previously been demonstrated to act as an osmolyte under gradual salinity shifts in the sea-ice diatom, *Fragilariopsis cylindrus* (Lyon et al. 2016). In addition to changes in temperature, salinity and irradiance this thesis also explored the effects of ocean acidification and found that CO_2 did not affect DMSP production in the individual species of the open ocean community, but rather the changes in the biomass of known diatom DMSP producing species (Chapter 3) had the potential to drive changes in the community contribution to DMSP (Chapter 6).

FUTURE DIRECTIONS

The chapters within this thesis provide new information into who are some of the DMSP producers in Antarctica and how DMSP is used by these species. Chapter 3 measured DMSP concentration and DMSP lyase activity in 16 Antarctic species with large variation within and between taxa, highlighting that Antarctic diatoms can be significant DMSP producers. To increase the database of DMSP producers and those capable of DMSP lyase, future research should focus on conducting studies with an increased variety of Antarctic species and investigating these processes under different environmental conditions.

By sampling more of the species that make up the community and under varying conditions, we can provide more accurate data for global estimations and predictions of sulfur fluxes originating from the Southern Ocean.

In Chapters 5 and 6, we exposed natural Antarctic microbial communities, identified to genus, to environmental stress and sampled for DMSP content and lyase activity at the community level. As species composition of the community changed in response to environmental stressors, any subsequent change in DMSP content was attributed to changes in biomass of known producers. Building on these results, future studies should include investigating responses at the cellular level, determining the extent of sensitivity of individual species and whether the intracellular concentration and rate of DMSP lyase changes over environmental gradients. Furthermore, the possibility that species that do not produce DMSP, but rather have the ability to take it up from the environment is still to be explored, with research needed to determine which species take up, the rate at which they remove DMSP from the pool, and if this changes with environmental condition. This will improve our understanding of production and cycling in oceanic systems.

Finally, Antarctica is highly seasonal and there is an imbalance of studies conducted between the winter and summer months with most research conducted during the biologically active spring and summer months. However, in order to form a complete picture of the contribution of Antarctic microbial communities to the sulfur cycle more research during the winter needs to be conducted.

CONCLUSION

Marine microalgae are important, particularly in the Antarctic, where they not only fuel the trophic exchange in the ecosystem, but are of key importance in the biogeochemical cycling of carbon and sulfur. Unfortunately, due to the logistical difficulties of working in an isolated location under such

extreme conditions and the sheer expense of Antarctic research, knowledge is limited. Most initial studies focused on ecology and the taxonomic diversity in Antarctica but over time with the establishment of research stations and advancement in scientific tools, particularly over the last decade, there have been studies done down to the molecular level (Lyon & Mock 2014; Mock & Thomas 2005). Physiology studies have shown Antarctic microalgae are evolutionarily adapted to live in freezing temperatures under extreme and oscillating environmental gradients exposing them to solar, osmotic, oxidative and nutrient stress (Lyon & Mock 2014; Morgan-Kiss et al. 2006). With the growing evidence that the Earth's climate is changing, it is clear that polar environments are susceptible to these shifts in environmental condition and as such, will be undergoing change (Bala 2013; McNeil & Matear 2008). Understanding how composition and distribution of Antarctic microalgal communities respond to environmental change will allow for more accurate predictions of how the Antarctic ecosystem will function in the future and of potential impacts to biogeochemical cycles.

The work done in this thesis highlighted the multiple possible functions DMSP could have within the cell. While our assessment of the osmoregulatory role of DMSP was inconclusive, the higher production of DMSP in pelagic waters compared to sea-ice might indicate that the primary role of DMSP in the Antarctic is that of an antioxidant rather than its role as an osmo-cryoprotectant, as a result of the different stressor being present. However, more work is needed to separate these responses from species shifts. Indeed, our findings show that in addition to the physical and chemical conditions of the environment, community composition has a strong influence on DMSP production, degradation and subsequent DMS flux, highlighting its significant influence on sulfur cycling. Throughout the thesis we also investigated shifts in macromolecular composition in relation to both changes in phytoplankton assemblages and environmental condition. Taken together, the new information in this thesis will help towards improving numerical models for carbon and sulfur cycling in the Antarctic and allow for more accurate future predictions of food web dynamics and DMSP production in the Antarctic.

REFERENCES

Alcolombri, U., Ben-Dor, S., Feldmesser, E., Levin, Y., Tawfik, D.S. & Vardi, A. 2015, 'Identification of the algal dimethyl sulfide–releasing enzyme: a missing link in the marine sulfur cycle', *Science*, vol. 348, no. 6242, pp. 1466-9.

Aletsee, L. & Jahnke, J. 1992, 'Growth and productivity of the psychrophilic marine diatoms Thalassiosira antarctica Comber and Nitzschia frigida Grunow in batch cultures at temperatures below the freezing point of sea water', *Polar biology*, vol. 11, no. 8, pp. 643-7.

Andreae, M.O. 1990, 'Ocean-atmosphere interactions in the global biogeochemical sulfur cycle', *Marine Chemistry*, vol. 30, pp. 1-29.

Andreoli, C., Moro, I., La Rocca, N., Valle, L.D., Masiero, L., Rascio, N. & Vecchia, F.D. 2000, 'Ecological, physiological, and biomolecular surveys on microalgae from Ross Sea (Antarctica)', *Italian Journal of Zoology*, vol. 67, no. S1, pp. 147-56.

Archer, S., Kimmance, S., Stephens, J., Hopkins, F., Bellerby, R., Schulz, K.G., Piontek, J. & Engel, A. 2013, 'Contrasting responses of DMS and DMSP to ocean acidification in Arctic waters', *Biogeosciences*, vol. 10, no. 3, pp. 1893-908.

Archer, S.D., Ragni, M., Webster, R., Airs, R.L. & Geider, R.J. 2010, 'Dimethyl sulfoniopropionate and dimethyl sulfide production in response to photoinhibition in Emiliania huxleyi', *Limnology and Oceanography*, vol. 55, no. 4, pp. 1579-89.

Arnold, H.E., Kerrison, P. & Steinke, M. 2013, 'Interacting effects of ocean acidification and warming on growth and DMS-production in the haptophyte coccolithophore *Emiliania huxleyi*', *Global change biology*, vol. 19, no. 4, pp. 1007-16.

Aro, E.-M., Virgin, I. & Andersson, B. 1993, 'Photoinhibition of photosystem II. Inactivation, protein damage and turnover', *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, vol. 1143, no. 2, pp. 113-34.

Arrigo, K.R. 2014, 'Sea ice ecosystems', *Annual review of marine science*, vol. 6, pp. 439-67.

Arrigo, K.R., Robinson, D.H., Worthen, D.L., Dunbar, R.B., DiTullio, G.R., VanWoert, M. & Lizotte, M.P. 1999, 'Phytoplankton community structure and the drawdown of nutrients and CO2 in the Southern Ocean', *Science*, vol. 283, no. 5400, pp. 365-7.

Arrigo, K.R. & Sullivan, C.W. 1992, 'THE INFLUENCE OF SALINITY AND TEMPERATURE COVARIATION ON THE PHOTOPHYSIOLOGICAL CHARACTERISTICS OF ANTARCTIC SEA ICE MICROALGAE 1', *Journal of Phycology*, vol. 28, no. 6, pp. 746-56.

Arrigo, K.R., Worthen, D., Schnell, A. & Lizotte, M.P. 1998, 'Primary production in Southern Ocean waters', *Journal of Geophysical Research: Oceans*, vol. 103, no. C8, pp. 15587-600.

Asher, E., Dacey, J., Stukel, M., Long, M. & Tortell, P. 2017, 'Processes driving seasonal variability in DMS, DMSP, and DMSO concentrations and turnover in coastal Antarctic waters', *Limnology and Oceanography*, vol. 62, no. 1, pp. 104-24.

Asher, E.C., Dacey, J.W., Mills, M.M., Arrigo, K.R. & Tortell, P.D. 2011, 'High concentrations and turnover rates of DMS, DMSP and DMSO in Antarctic sea ice', *Geophysical Research Letters*, vol. 38, no. 23.

Aumont, O., Belviso, S. & Monfray, P. 2002, 'Dimethylsulfoniopropionate (DMSP) and dimethylsulfide (DMS) sea surface distributions simulated from a global three-dimensional ocean carbon cycle model', *Journal of Geophysical Research: Oceans (1978–2012)*, vol. 107, no. C4, pp. 4-1-4-19.

Avgoustidi, V., Nightingale, P.D., Joint, I., Steinke, M., Turner, S.M., Hopkins, F.E. & Liss, P.S. 2012, 'Decreased marine dimethyl sulfide production under elevated CO2 levels in mesocosm and in vitro studies', *Environmental Chemistry*, vol. 9, no. 4, pp. 399-404.

Baker, N.R. 2008, 'Chlorophyll fluorescence: a probe of photosynthesis in vivo', *Annu. Rev. Plant Biol.*, vol. 59, pp. 89-113.

Bala, G. 2013, 'Digesting 400 ppm for global mean CO2 concentration', *Current science*, vol. 104, no. 11, pp. 1471-2.

Barcelos e Ramos, J., Schulz, K.G., Brownlee, C., Sett, S. & Azevedo, E.B. 2014, 'Effects of increasing seawater carbon dioxide concentrations on chain formation of the diatom Asterionellopsis glacialis', *PloS one*, vol. 9, no. 3, p. e90749.

Barton, A.D., Dutkiewicz, S., Flierl, G., Bragg, J. & Follows, M.J. 2010, 'Patterns of diversity in marine phytoplankton', *Science*, vol. 327, no. 5972, pp. 1509-11.

Bates, T.S., Charlson, R.J. & Gammon, R.H. 1987, 'Evidence for the climatic role of marine biogenic sulphur', *Nature*, vol. 329, no. 6137, pp. 319-21.

Baumann, M., Lancelot, C., Brandini, F., Sakshaug, E. & John, D. 1994, 'The taxonomic identity of the cosmopolitan prymnesiophyte *Phaeocystis*: a morphological and ecophysiological approach', *Journal of Marine Systems*, vol. 5, no. 1, pp. 5-22.

Baumann, M.E., Brandini, F.P. & Staubes, R. 1994, 'The influence of light and temperature on carbon-specific DMS release by cultures of *Phaeocystis antarctica* and three antarctic diatoms', *Marine Chemistry*, vol. 45, no. 1-2, pp. 129-36.

Behrenfeld, M.J. & Boss, E. 2006, 'Beam attenuation and chlorophyll concentration as alternative optical indices of phytoplankton biomass', *Journal of Marine Research*, vol. 64, no. 3, pp. 431-51.

Biddanda, B. & Benner, R. 1997, 'Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton', *Limnology and Oceanography*, vol. 42, no. 3, pp. 506-18.

Bopp, L., Boucher, O., Aumont, O., Belviso, S., Dufresne, J.-L., Pham, M. & Monfray, P. 2004, 'Will marine dimethylsulfide emissions amplify or alleviate global warming? A model study', *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 61, no. 5, pp. 826-35.

Bouchard, J.N., Roy, S., Ferreyra, G., Campbell, D.A. & Curtosi, A. 2005, 'Ultraviolet-B effects on photosystem II efficiency of natural phytoplankton communities from Antarctica', *Polar Biology*, vol. 28, no. 8, pp. 607-18.

Boucher, O., Moulin, C., Belviso, S., Aumont, O., Bopp, L., Cosme, E., Kuhlmann, R.v., Lawrence, M., Pham, M. & Reddy, M. 2003, 'DMS atmospheric concentrations and sulphate aerosol indirect radiative forcing: a sensitivity study to the DMS source representation and oxidation', *Atmospheric Chemistry and Physics*, vol. 3, no. 1, pp. 49-65.

Boyd, P., Dillingham, P., McGraw, C., Armstrong, E., Cornwall, C., Feng, Y.-y., Hurd, C., Gault-Ringold, M., Roleda, M. & Timmins-Schiffman, E. 2016, 'Physiological responses of a Southern Ocean diatom to complex future ocean conditions', *Nature Climate Change*, vol. 6, no. 2, p. 207.

Boyd, P. & Ellwood, M. 2010, 'The biogeochemical cycle of iron in the ocean', *Nature Geoscience*, vol. 3, no. 10, p. 675.

Brett, M. & Müller-Navarra, D. 1997, 'The role of highly unsaturated fatty acids in aquatic foodweb processes', *Freshwater Biology*, vol. 38, no. 3, pp. 483-99.

Brown, M.R. 2002, 'Nutritional value and use of microalgae in aquaculture', *Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola*, vol. 3, pp. 281-92.

Brussaard, C., Gast, G., Van Duyl, F. & Riegman, R. 1996, 'Impact of phytoplankton bloom magnitude on a pelagic microbial food web', *Marine ecology progress series*, pp. 211-21.

Bucciarelli, E., Ridame, C., Sunda, W.G., Dimier-Hugueney, C., Cheize, M. & Belviso, S. 2013, 'Increased intracellular concentrations of DMSP and DMSO in iron-limited oceanic phytoplankton *Thalassiosira oceanica* and *Trichodesmium erythraeum*', *Limnology and Oceanography*, vol. 58, no. 5, pp. 1667-79.

Buesseler, K.O. 1998, 'The decoupling of production and particulate export in the surface ocean', *Global Biogeochemical Cycles*, vol. 12, no. 2, pp. 297-310.

Buma, A., Bano, N., Veldhuis, M. & Kraay, G. 1991, 'Comparison of the pigmentation of two strains of the prymnesiophyte Phaeocystis sp', *Netherlands Journal of Sea Research*, vol. 27, no. 2, pp. 173-82.

Cameron-Smith, P., Elliott, S., Maltrud, M., Erickson, D. & Wingenter, O. 2011, 'Changes in dimethyl sulfide oceanic distribution due to climate change', *Geophysical Research Letters*, vol. 38, no. 7.

Carpenter, L.J., Archer, S.D. & Beale, R. 2012, 'Ocean-atmosphere trace gas exchange', *Chemical Society Reviews*, vol. 41, no. 19, pp. 6473-506.

Caruana, A., Steinke, M., Turner, S. & Malin, G. 2012, 'Concentrations of dimethylsulphoniopropionate and activities of dimethylsulphide-producing enzymes in batch cultures of nine dinoflagellate species', *Biogeochemistry*, vol. 110, no. 1-3, pp. 87-107.

Caruana, A.M. & Malin, G. 2014, 'The variability in DMSP content and DMSP lyase activity in marine dinoflagellates', *Progress in Oceanography*, vol. 120, pp. 410-24.

Cavicchioli, R. 2015, 'Microbial ecology of Antarctic aquatic systems', *Nature Reviews Microbiology*, vol. 13, no. 11, p. 691.

Charlson, R.J., Lovelock, J.E., Andreae, M.O. & Warren, S.G. 1987, 'Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate', *Nature*, vol. 326, no. 6114, pp. 655-61.

Chen, H., Guan, W., Zeng, G., Li, P. & Chen, S. 2015, 'Alleviation of solar ultraviolet radiation (UVR)-induced photoinhibition in diatom Chaetoceros curvisetus by ocean acidification', *Journal of the Marine Biological Association of the United Kingdom*, vol. 95, no. 4, pp. 661-7.

Chen, H., Jiang, J.-G. & Wu, G.-H. 2009, 'Effects of salinity changes on the growth of *Dunaliella salina* and its isozyme activities of glycerol-3-phosphate dehydrogenase', *Journal of agricultural and food chemistry*, vol. 57, no. 14, pp. 6178-82.

Cirulis, J.T., Scott, J.A. & Ross, G.M. 2013, 'Management of oxidative stress by microalgae', *Canadian journal of physiology and pharmacology*, vol. 91, no. 1, pp. 15-21.

Comiso, J. 2010, 'Fundamental characteristics of the Polar Oceans and their sea ice cover', *Polar Oceans from Space*, Springer, pp. 19-71.

Constable, A.J., Nicol, S. & Strutton, P.G. 2003, 'Southern Ocean productivity in relation to spatial and temporal variation in the physical environment', *Journal of Geophysical Research: Oceans*, vol. 108, no. C4.

Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P. & Del Borghi, M. 2009, 'Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production', *Chemical Engineering and Processing: Process Intensification*, vol. 48, no. 6, pp. 1146-51.

Cook, H.W. & McMaster, C.R. 2002, 'Fatty acid desaturation and chain elongation in eukaryotes', *New comprehensive biochemistry*, vol. 36, Elsevier, pp. 181-204.

Cota, G.F. & Smith, R.E. 1991, 'Ecology of bottom ice algae: III. Comparative physiology', *Journal of Marine Systems*, vol. 2, no. 3-4, pp. 297-315.

Curran, M.A. & Jones, G.B. 2000, 'Dimethyl sulfide in the Southern Ocean: Seasonality and flux', *Journal of Geophysical Research: Atmospheres*, vol. 105, no. D16, pp. 20451-9.

Curran, M.A., Jones, G.B. & Burton, H. 1998, 'Spatial distribution of dimethylsulfide and dimethylsulfoniopropionate in the Australasian sector of the Southern Ocean', *Journal of Geophysical Research: Atmospheres (1984–2012)*, vol. 103, no. D13, pp. 16677-89.

Curson, A., Rogers, R., Todd, J., Brearley, C. & Johnston, A. 2008, 'Molecular genetic analysis of a dimethylsulfoniopropionate lyase that liberates the climate-changing gas dimethylsulfide in several marine α-proteobacteria and *Rhodobacter sphaeroides*', *Environmental Microbiology*, vol. 10, no. 3, pp. 757-67.

Curson, A.R., Liu, J., Martínez, A.B., Green, R.T., Chan, Y., Carrión, O., Williams, B.T., Zhang, S.-H., Yang, G.-P. & Page, P.C.B. 2017, 'Dimethylsulfoniopropionate biosynthesis in marine bacteria and identification of the key gene in this process', *Nature Microbiology*, vol. 2, p. 17009.

Curson, A.R., Todd, J.D., Sullivan, M.J. & Johnston, A.W. 2011, 'Catabolism of dimethylsulphoniopropionate: microorganisms, enzymes and genes', *Nature Reviews Microbiology*, vol. 9, no. 12, pp. 849-59.

Curson, A.R., Williams, B.T., Pinchbeck, B.J., Sims, L.P., Martínez, A.B., Rivera, P.P.L., Kumaresan, D., Mercadé, E., Spurgin, L.G. & Carrión, O. 2018, 'DSYB catalyses the key step of dimethylsulfoniopropionate biosynthesis in many phytoplankton', *Nature microbiology*, vol. 3, no. 4, p. 430.

Dani, K.S. & Loreto, F. 2017, 'Trade-off between dimethyl sulfide and isoprene emissions from marine phytoplankton', *Trends in plant science*, vol. 22, no. 5, pp. 361-72.

DasSarma, S. & Arora, P. 2002, 'Halophiles', *eLS*.

Davidson, A.T., McKinlay, J., Westwood, K., Thomson, P.G., van den Enden, R., de Salas, M., Wright, S., Johnson, R. & Berry, K. 2016, 'Enhanced CO2 concentrations change the structure of Antarctic marine microbial communities', *Marine Ecology Progress Series*, vol. 552, pp. 93-113.

de Castro Araújo, S. & Garcia, V.M.T. 2005, 'Growth and biochemical composition of the diatom *Chaetoceros cf. wighamii* brightwell under different temperature, salinity and carbon dioxide levels. I. Protein, carbohydrates and lipids', *Aquaculture*, vol. 246, no. 1, pp. 405-12.

DeMaster, D.J., Leynaert, A. & Queguiner, B. 1995, 'The silica balance in the world ocean: a reestimate', *Science*, vol. 268, p. 21.

Demott, W. & Müller-Navarra, D. 1997, 'The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with Daphnia, a cyanobacterium and lipid emulsions', *Freshwater Biology*, vol. 38, no. 3, pp. 649-64.

Deppeler, S., Davidson, A. & Schulz, K. 2017, 'Environmental data for Davis 14/15 ocean acidification minicosm experiment, Australian Antarctic Data Centre'.

Deppeler, S., Petrou, K., Schulz, K.G., Westwood, K., Pearce, I., McKinlay, J. & Davidson, A. 2018, 'Ocean acidification of a coastal Antarctic marine microbial community reveals a critical threshold for CO 2 tolerance in phytoplankton productivity', *Biogeosciences*, vol. 15, no. 1, p. 209.

DiTullio, G., Grebmeier, J., Arrigo, K., Lizotte, M., Robinson, D., Leventer, A., Barry, J., VanWoert, M. & Dunbar, R. 2000, 'Rapid and early export of *Phaeocystis antarctica* blooms in the Ross Sea, Antarctica', *Nature*, vol. 404, no. 6778, pp. 595-8.

Ditullio, G.R., Garrison, D.L. & Mathot, S.L. 1998, 'Dimethylsulfoniopropionate in sea ice algae from the Ross Sea polynya', *Antarctic Sea Ice: Biological Processes, Interactions and Variability*, pp. 139-46.

Ducklow, H.W., Baker, K., Martinson, D.G., Quetin, L.B., Ross, R.M., Smith, R.C., Stammerjohn, S.E., Vernet, M. & Fraser, W. 2007, 'Marine pelagic ecosystems: the west Antarctic Peninsula', *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 362, no. 1477, pp. 67-94.

Duerksen, S.W., Thiemann, G.W., Budge, S.M., Poulin, M., Niemi, A. & Michel, C. 2014, 'Large, omega-3 rich, pelagic diatoms under Arctic sea ice: sources and implications for food webs', *PloS one*, vol. 9, no. 12, p. e114070.

Edelman, M. & Mattoo, A.K. 2008, 'D1-protein dynamics in photosystem II: the lingering enigma', *Photosynthesis Research*, vol. 98, no. 1-3, pp. 609-20.

El-Hady, H.H.A., Fathey, S.A., Ali, G.H. & Gabr, Y.G. 2016, 'Biochemical profile of phytoplankton and its nutritional aspects in some khors of Lake Nasser, Egypt', *Egyptian Journal of Basic and Applied Sciences*, vol. 3, no. 2, pp. 187-93.

Engel, A., Schulz, K., Riebesell, U., Bellerby, R., Delille, B. & Schartau, M. 2007, 'Effects of CO 2 on particle size distribution and phytoplankton abundance during a mesocosm bloom experiment (PeECE II)', *Biogeosciences Discussions*, vol. 4, no. 6, pp. 4101-33.

Evans, C., Malin, G., Wilson, W.H. & Liss, P.S. 2006, 'Infectious titres of *Emiliania huxleyi* virus 86 are reduced by exposure to millimolar dimethyl sulfide and acrylic acid', *Limnology and oceanography*, vol. 51, no. 5, pp. 2468-71.

Fahl, K. & Kattner, G. 1993, 'Lipid content and fatty acid composition of algal communities in sea-ice and water from the Weddell Sea (Antarctica)', *Polar Biology*, vol. 13, no. 6, pp. 405-9.

Falkowski, P., Scholes, R., Boyle, E., Canadell, J., Canfield, D., Elser, J., Gruber, N., Hibbard, K., Högberg, P. & Linder, S. 2000, 'The global carbon cycle: a test of our knowledge of earth as a system', *science*, vol. 290, no. 5490, pp. 291-6.

Ferrer, R.P. & Zimmer, R.K. 2012, 'Community ecology and the evolution of molecules of keystone significance', *The Biological Bulletin*, vol. 223, no. 2, pp. 167-77.

Fiddes, S.L., Woodhouse, M.T., Nicholls, Z., Lane, T.P. & Schofield, R. 2018, 'Cloud, precipitation and radiation responses to large perturbations in global dimethyl sulfide', *Atmospheric Chemistry and Physics*, vol. 18, no. 14, pp. 10177-98.

Field, C.B., Behrenfeld, M.J., Randerson, J.T. & Falkowski, P. 1998, 'Primary production of the biosphere: integrating terrestrial and oceanic components', *Science*, vol. 281, no. 5374, pp. 237-40.

Finkel, Z.V., Follows, M.J., Liefer, J.D., Brown, C.M., Benner, I. & Irwin, A.J. 2016, 'Phylogenetic diversity in the macromolecular composition of microalgae', *PloS one*, vol. 11, no. 5, p. e0155977.

Franklin, D.J., Airs, R.L., Fernandes, M., Bell, T.G., Bongaerts, R.J., Berges, J.A. & Malin, G. 2012, 'Identification of senescence and death in Emiliania huxleyi and Thalassiosira pseudonana: cell staining, chlorophyll alterations, and dimethylsulfoniopropionate (DMSP) metabolism', *Limnology and Oceanography*, vol. 57, no. 1, pp. 305-17.

Franklin, D.J., Steinke, M., Young, J., Probert, I. & Malin, G. 2010, 'Dimethylsulphoniopropionate (DMSP), DMSP-lyase activity (DLA) and dimethylsulphide (DMS) in 10 species of coccolithophore', *Marine Ecology Progress Series*, vol. 410, pp. 13-23.

Franzmann, P., Deprez, P., Burton, H. & Van den Hoff, J. 1987, 'Limnology of Organic Lake, Antarctica, a meromictic lake that contains high concentrations of dimethyl sulfide', *Marine and Freshwater Research*, vol. 38, no. 3, pp. 409-17.

Fuhrman, J.A. 2009, 'Microbial community structure and its functional implications', *Nature*, vol. 459, no. 7244, pp. 193-9.

Galí, M., Devred, E., Levasseur, M., Royer, S.-J. & Babin, M. 2015, 'A remote sensing algorithm for planktonic dimethylsulfoniopropionate (DMSP) and an analysis of global patterns', *Remote Sensing of Environment*, vol. 171, pp. 171-84.

Galí, M., Saló, V., Almeda, R., Calbet, A. & Simó, R. 2011, 'Stimulation of gross dimethylsulfide (DMS) production by solar radiation', *Geophysical Research Letters*, vol. 38, no. 15.

Galí, M. & Simó, R. 2010, 'Occurrence and cycling of dimethylated sulfur compounds in the Arctic during summer receding of the ice edge', *Marine Chemistry*, vol. 122, no. 1-4, pp. 105-17.

Galindo, V., Gosselin, M., Lavaud, J., Mundy, C.J., Else, B., Ehn, J., Babin, M. & Rysgaard, S. 2017, 'Pigment composition and photoprotection of Arctic sea ice algae during spring', *Marine Ecology Progress Series*, vol. 585, pp. 49-69.

Garibotti, I.A., Vernet, M., Ferrario, M.E., Smith, R.C., Ross, R.M. & Quetin, L.B. 2003, 'Phytoplankton spatial distribution patterns along the western Antarctic Peninsula (Southern Ocean)', *Marine Ecology Progress Series*, vol. 261, pp. 21-39.

Garibotti, I.A., Vernet, M., Kozlowski, W.A. & Ferrario, M.E. 2003, 'Composition and biomass of phytoplankton assemblages in coastal Antarctic waters: a comparison of chemotaxonomic and microscopic analyses', *Marine Ecology Progress Series*, vol. 247, pp. 27-42.

Gattuso, J.-P. & Hansson, L. 2011, 'Ocean acidification: background and history', *Ocean acidification*, pp. 1-20.

Geider, R.J. & La Roche, J. 2002, 'Redfield revisited: variability of C [ratio] N [ratio] P in marine microalgae and its biochemical basis', *European Journal of Phycology*, vol. 37, no. 1, pp. 1-17.

Genty, B., Briantais, J.-M. & Baker, N.R. 1989, 'The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence', *Biochimica et Biophysica Acta (BBA)-General Subjects*, vol. 990, no. 1, pp. 87-92.

Gibson, J., Garrick, R., Burton, H. & McTaggart, A. 1990, 'Dimethylsulfide and the alga *Phaeocystis pouchetii* in antarctic coastal waters', *Marine Biology*, vol. 104, no. 2, pp. 339-46.

Giordano, M., Kansiz, M., Heraud, P., Beardall, J., Wood, B. & McNaughton, D. 2001, 'Fourier transform infrared spectroscopy as a novel tool to investigate changes in intracellular macromolecular pools in the marine microalga Chaetoceros muellerii (Bacillariophyceae)', *Journal of Phycology*, vol. 37, no. 2, pp. 271-9.

Gleitz, M. & Thomas, D.N. 1992, 'Physiological responses of a small Antarctic diatom (*Chaetoceros* sp.) to simulated environmental constraints associated with sea-ice formation', *Mar Ecol Prog Ser*, vol. 88, pp. 278-87.

Goss, R. & Jakob, T. 2010, 'Regulation and function of xanthophyll cycle-dependent photoprotection in algae', *Photosynthesis research*, vol. 106, no. 1-2, pp. 103-22.

Goyal, A. 2007, 'Osmoregulation in *Dunaliella*, Part II: Photosynthesis and starch contribute carbon for glycerol synthesis during a salt stress in *Dunaliella tertiolecta*', *Plant Physiology and Biochemistry*, vol. 45, no. 9, pp. 705-10.

Granum, E. & Myklestad, S.M. 2002, 'A simple combined method for determination of β-1, 3-glucan and cell wall polysaccharides in diatoms', *Hydrobiologia*, vol. 477, no. 1-3, pp. 155-61.

Guschina, I.A. & Harwood, J.L. 2009, 'Algal lipids and effect of the environment on their biochemistry', *Lipids in aquatic ecosystems*, Springer, pp. 1-24.

Haberman, K.L., Ross, R.M. & Quetin, L.B. 2003, 'Diet of the Antarctic krill (Euphausia superba Dana): II. Selective grazing in mixed phytoplankton assemblages', *Journal of Experimental Marine Biology and Ecology*, vol. 283, no. 1-2, pp. 97-113.

Halloran, P., Bell, T. & Totterdell, I. 2010, 'Can we trust empirical marine DMS parameterisations within projections of future climate?', *Biogeosciences*, vol. 7, no. 5, pp. 1645-56.

Hancock, A.M., Davidson, A.T., McKinlay, J., McMinn, A., Schulz, K. & van den Enden, R.L. 2018, 'Ocean acidification changes the structure of an Antarctic coastal protistan community'.

Harada, H. & Kiene, R.P. 2011, 'Assessment and characteristics of DMSP lyase activity in seawater and phytoplankton cultures'.

Harada, H., Rouse, M.-A., Sunda, W. & Kiene, R.P. 2004, 'Latitudinal and vertical distributions of particle-associated dimethylsulfoniopropionate (DMSP) lyase activity in the western North Atlantic Ocean', *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 61, no. 5, pp. 700-11.

Hare, C.E., Leblanc, K., DiTullio, G.R., Kudela, R.M., Zhang, Y., Lee, P.A., Riseman, S. & Hutchins, D.A. 2007, 'Consequences of increased temperature and CO2 for phytoplankton community structure in the Bering Sea', *Marine Ecology Progress Series*, vol. 352, pp. 9-16.

Harwood, J.L. 1998, 'Membrane lipids in algae', *Lipids in photosynthesis: structure, function and genetics*, Springer, pp. 53-64.

Head, E. & Harris, L. 1994, 'Feeding selectivity by copepods grazing on natural mixtures of phytoplankton determined by HPLC analysis of pigments', *Marine Ecology-Progress Series*, vol. 110, pp. 75-.

Hefu, Y. & Kirst, G. 1997, 'Effect of UV-radiation on DMSP content and DMS formation of Phaeocystis antarctica', *Polar Biology*, vol. 18, no. 6, pp. 402-9.

Hellebust, J.A. 1976, 'Effect of salinity on photosynthesis and mannitol synthesis in the green flagellate Platymonas suecica', *Canadian Journal of Botany*, vol. 54, no. 15, pp. 1735-41.

Hellebust, J.A. 1985, 'Mechanisms of response to salinity in halotolerant microalgae', *Biosalinity in Action: Bioproduction with Saline Water*, Springer, pp. 69-81.

Heraud, P., Wood, B.R., Beardall, J. & McNaughton, D. 2007, 'Probing the influence of the environment on microalgae using infrared and raman spectroscopy', *K KneippR ArocaH KneippE Wentrup-Byrne. New Approaches in Biomedical Spectroscopy. American Chemical Society, Washington, DC*, pp. 85-106.

Heraud, P., Wood, B.R., Tobin, M.J., Beardall, J. & McNaughton, D. 2005, 'Mapping of nutrient-induced biochemical changes in living algal cells using synchrotron infrared microspectroscopy', *FEMS Microbiology Letters*, vol. 249, no. 2, pp. 219-25.

Hitchcock, G.L. 1982, 'A comparative study of the size-dependent organic composition of marine diatoms and dinoflagellates', *Journal of plankton research*, vol. 4, no. 2, pp. 363-77.

Hixson, S.M. & Arts, M.T. 2016, 'Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton', *Global Change Biology*, vol. 22, no. 8, pp. 2744-55.

Hopkins, F.E., Turner, S.M., Nightingale, P.D., Steinke, M., Bakker, D. & Liss, P.S. 2010, 'Ocean acidification and marine trace gas emissions', *Proceedings of the National Academy of Sciences*, vol. 107, no. 2, pp. 760-5.

Hopkinson, B.M., Dupont, C.L., Allen, A.E. & Morel, F.M. 2011, 'Efficiency of the CO2-concentrating mechanism of diatoms', *Proceedings of the National Academy of Sciences*, vol. 108, no. 10, pp. 3830-7.

Hoppe, C.J., Hassler, C.S., Payne, C.D., Tortell, P.D., Rost, B. & Trimborn, S. 2013, 'Iron limitation modulates ocean acidification effects on Southern Ocean phytoplankton communities', *PLoS One*, vol. 8, no. 11, p. e79890.

Hoppe, C.J., Holtz, L.M., Trimborn, S. & Rost, B. 2015, 'Ocean acidification decreases the light-use efficiency in an A ntarctic diatom under dynamic but not constant light', *New Phytologist*, vol. 207, no. 1, pp. 159-71.

Howard, E.C., Henriksen, J.R., Buchan, A., Reisch, C.R., Bürgmann, H., Welsh, R., Ye, W., González, J.M., Mace, K. & Joye, S.B. 2006, 'Bacterial taxa that limit sulfur flux from the ocean', *Science*, vol. 314, no. 5799, pp. 649-52.

Howard, E.C., Sun, S., Biers, E.J. & Moran, M.A. 2008, 'Abundant and diverse bacteria involved in DMSP degradation in marine surface waters', *Environmental Microbiology*, vol. 10, no. 9, pp. 2397-410.

Huot, Y., Babin, M., Bruyant, F., Grob, C., Twardowski, M. & Claustre, H. 2007, 'Does chlorophyll a provide the best index of phytoplankton biomass for primary productivity studies?', *Biogeosciences discussions*, vol. 4, no. 2, pp. 707-45.

Irigoien, X., Flynn, K. & Harris, R. 2005, 'Phytoplankton blooms: a 'loophole'in microzooplankton grazing impact?', *Journal of Plankton Research*, vol. 27, no. 4, pp. 313-21.

Jahnke, L.S. & White, A.L. 2003, 'Long-term hyposaline and hypersaline stresses produce distinct antioxidant responses in the marine alga Dunaliella tertiolecta', *Journal of Plant Physiology*, vol. 160, no. 10, pp. 1193-202.

Jarníková, T. & Tortell, P.D. 2016, 'Towards a revised climatology of summertime dimethylsulfide concentrations and sea–air fluxes in the Southern Ocean', *Environmental Chemistry*, vol. 13, no. 2, pp. 364-78.

Johnston, A.W. 2015, 'Who can cleave DMSP?', *Science*, vol. 348, no. 6242, pp. 1430-1.

Juneja, A., Ceballos, R.M. & Murthy, G.S. 2013, 'Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: a review', *Energies*, vol. 6, no. 9, pp. 4607-38.

Kana, T.M., Geider, R.J. & Critchley, C. 1997, 'Regulation of photosynthetic pigments in micro-algae by multiple environmental factors: a dynamic balance hypothesis', *The New Phytologist*, vol. 137, no. 4, pp. 629-38.

Karsten, U., Kirst, G. & Wiencke, C. 1992, 'Dimethylsulphoniopropionate (DMSP) accumulation in green macroalgae from polar to temperate regions: interactive effects of light versus salinity and light versus temperature', *Polar biology*, vol. 12, no. 6-7, pp. 603-7.

Karsten, U., Wiencke, C. & Kirst, G. 1990, 'The effect of light intensity and daylength on the β-dimethylsulphoniopropionate (DMSP) content of marine green macroalgae from Antarctica', *Plant, Cell & Environment*, vol. 13, no. 9, pp. 989-93.

Kasamatsu, N., Hirano, T., Kudoh, S., Odate, T. & Fukuchi, M. 2004, 'Dimethylsulfoniopropionate production by psychrophilic diatom isolates', *Journal of phycology*, vol. 40, no. 5, pp. 874-8.

Keller, M., Kiene, R., Matrai, P. & Bellows, W. 1999, 'Production of glycine betaine and dimethylsulfoniopropionate in marine phytoplankton. I. Batch cultures', *Marine Biology*, vol. 135, no. 2, pp. 237-48.

Keller, M.D. 1989a, 'Dimethyl sulfide production and marine phytoplankton: the importance of species composition and cell size', *Biological Oceanography*, vol. 6, no. 5-6, pp. 375-82.

Keller, M.D. 1989b, 'Dimethyl sulfide production in marine phytoplankton', *Biogenic sulfur in the environment*.

Keller, M.D. & Korjeff-Bellows, W. 1996, 'Physiological aspects of the production of dimeyhtlsulfoniopropionate (DMSP) by marine phytoplankton', *Biological and environmental chemistry of DMSP and related sulfonium compounds*, Springer, pp. 131-42.

Kettle, A., Andreae, M., Amouroux, D., Andreae, T., Bates, T., Berresheim, H., Bingemer, H., Boniforti, R., Curran, M. & DiTullio, G. 1999, 'A global database of sea surface dimethylsulfide (DMS) measurements and a procedure to predict sea surface DMS as a function of latitude, longitude, and month', *Global Biogeochemical Cycles*, vol. 13, no. 2, pp. 399-444.

Kettles, N.L., Kopriva, S. & Malin, G. 2014, 'Insights into the regulation of DMSP synthesis in the diatom *Thalassiosira pseudonana* through APR activity, proteomics and gene expression analyses on cells acclimating to changes in salinity, light and nitrogen', *PloS one*, vol. 9, no. 4, p. e94795.

Kiene, R.P., Linn, L.J. & Bruton, J.A. 2000, 'New and important roles for DMSP in marine microbial communities', *Journal of Sea Research*, vol. 43, no. 3, pp. 209-24.

Kim, J.-M., Lee, K., Yang, E.J., Shin, K., Noh, J.H., Park, K.-t., Hyun, B., Jeong, H.-J., Kim, J.-H. & Kim, K.Y. 2010, 'Enhanced production of oceanic dimethylsulfide resulting from CO2-induced grazing activity in a high CO2 world', *Environmental science & technology*, vol. 44, no. 21, pp. 8140-3.

Kinsey, J.D. & Kieber, D.J. 2016, 'Microwave preservation method for DMSP, DMSO, and acrylate in unfiltered seawater and phytoplankton culture samples', *Limnology and Oceanography: Methods*, vol. 14, no. 3, pp. 196-209.

Kirst, G. 1996, 'Osmotic adjustment in phytoplankton and macroalgae', *Biological and environmental chemistry of DMSP and related sulfonium compounds*, Springer, pp. 121-9.

Kirst, G., Thiel, C., Wolff, H., Nothnagel, J., Wanzek, M. & Ulmke, R. 1991, 'Dimethylsulfoniopropionate (DMSP) in icealgae and its possible biological role', *Marine Chemistry*, vol. 35, no. 1, pp. 381-8.

Kirst, G., Wanzek, M., Haase, R., Rapsomanikis, S., De Mora, S., Schebeske, G. & Andreae, M. 1993, 'Ecophysiology of ice algae (Antarctica): Dimethylsulfoniopropionate content and release of dimethylsulfide during ice melt', *Dimethylsulfide: Oceans, Atmosphere and Climate*, pp. 23-6.

Kirst, G.O. & Wiencke, C. 1995, 'Ecophysiology of polar algae', *Journal of Phycology*, vol. 31, no. 2, pp. 181-99.

Kopczyńska, E.E., Savoye, N., Dehairs, F., Cardinal, D. & Elskens, M. 2007, 'Spring phytoplankton assemblages in the Southern Ocean between Australia and Antarctica', *Polar Biology*, vol. 31, no. 1, pp. 77-88.

Koprivova, A. & Kopriva, S. 2014, 'Molecular mechanisms of regulation of sulfate assimilation: first steps on a long road', *Frontiers in plant science*, vol. 5.

Krell, A. 2006, 'Salt stress tolerance in the psychrophilic diatom Fragilariopsis cylindrus', Universität Bremen.

Krieger-Liszkay, A. & Trebst, A. 2006, 'Tocopherol is the scavenger of singlet oxygen produced by the triplet states of chlorophyll in the PSII reaction centre', *Journal of experimental botany*, vol. 57, no. 8, pp. 1677-84.

Kropuenske, L.R., Mills, M.M., van Dijken, G.L., Bailey, S., Robinson, D.H., Welschmeyer, N.A. & Arrigoa, K.R. 2009, 'Photophysiology in two major Southern Ocean phytoplankton taxa: photoprotection in Phaeocystis antarctica and Fragilariopsis cylindrus', *Limnology and Oceanography*, vol. 54, no. 4, pp. 1176-96.

Kwint, R.L. & Kramer, K.J. 1996, 'Annual cycle of the production and fate of DMS and DMSP in a marine coastal system', *Marine Ecology Progress Series*, vol. 134, pp. 217-24.

Lana, A., Simó, R., Vallina, S.M. & Dachs, J. 2012, 'Re-examination of global emerging patterns of ocean DMS concentration', *Biogeochemistry*, vol. 110, no. 1-3, pp. 173-82.

Lavaud, J., Rousseau, B. & Etienne, A.-L. 2002, 'In diatoms, a transthylakoid proton gradient alone is not sufficient to induce a non-photochemical fluorescence quenching', *Febs Letters*, vol. 523, no. 1-3, pp. 163-6.

Lavaud, J., Strzepek, R.F. & Kroth, P.G. 2007, 'Photoprotection capacity differs among diatoms: possible consequences on the spatial distribution of diatoms related to fluctuations in the underwater light climate', *Limnology and Oceanography*, vol. 52, no. 3, pp. 1188-94.

Le Clainche, Y., Vézina, A., Levasseur, M., Cropp, R.A., Gunson, J.R., Vallina, S.M., Vogt, M., Lancelot, C., Allen, J. & Archer, S.D. 2010, 'A first appraisal of prognostic ocean DMS models and prospects for their use in climate models', *Global biogeochemical cycles*, vol. 24, no. 3.

Lee, P.A. & De Mora, S.J. 1999, 'Intracellular dimethylsulfoxide (DMSO) in unicellular marine algae: speculations on its origin and possible biological role', *Journal of Phycology*, vol. 35, no. 1, pp. 8-18.

Lee, P.A., De Mora, S.J., Gosselin, M., Levasseur, M., Bouillon, R.C., Nozais, C. & Michel, C. 2001, 'Particulate dimethylsulfoxide in Arctic sea-ice algal communities: The cryoprotectant hypothesis revisited', *Journal of Phycology*, vol. 37, no. 4, pp. 488-99.

Lee, P.A., Rudisill, J.R., Neeley, A.R., Maucher, J.M., Hutchins, D.A., Feng, Y., Hare, C.E., Leblanc, K., Rose, J.M. & Wilhelm, S.W. 2009, 'Effects of increased pCO2 and temperature on the North Atlantic spring bloom. III. Dimethylsulfoniopropionate', *Marine Ecology Progress Series*, vol. 388, pp. 41-9.

Levasseur, M., Gosselin, M. & Michaud, S. 1994, 'A new source of dimethylsulfide (DMS) for the arctic atmosphere: ice diatoms', *Marine Biology*, vol. 121, no. 2, pp. 381-7.

Levasseur, M., Michaud, S., Egge, J., Cantin, G., Nejstgaard, J., Sanders, R., Fernandez, E., Solberg, P., Heimdal, B. & Gosselin, M. 1996, 'Production of DMSP and DMS during a mesocosm study of an Emiliania huxleyi bloom: influence of bacteria and Calanus finmarchicus grazing', *Marine Biology*, vol. 126, no. 4, pp. 609-18.

Levine, N.M., Varaljay, V.A., Toole, D.A., Dacey, J.W., Doney, S.C. & Moran, M.A. 2012, 'Environmental, biochemical and genetic drivers of DMSP degradation and DMS production in the Sargasso Sea', *Environmental microbiology*, vol. 14, no. 5, pp. 1210-23.

Liss, P., Malin, G., Turner, S. & Holligan, P. 1994, 'Dimethyl sulphide and Phaeocystis: a review', *Journal of Marine Systems*, vol. 5, no. 1, pp. 41-53.

Liss, P.S. & Johnson, M.T. 2014, *Ocean-Atmosphere Interactions of Gases and Particles*, Springer.

Lizotte, M., Levasseur, M., Law, C.S., Walker, C.F., Safi, K.A., Marriner, A. & Kiene, R.P. 2017, 'Dimethylsulfoniopropionate (DMSP) and dimethyl sulfide (DMS) cycling across contrasting biological hotspots of the New Zealand subtropical front', *Ocean Science*, vol. 13, no. 6, p. 961.

Lizotte, M.P. 2001, 'The contributions of sea ice algae to Antarctic marine primary production', *American zoologist*, vol. 41, no. 1, pp. 57-73.

Lizotte, M.P. & Sullivan, C.W. 1992, 'Biochemical composition and photosynthate distribution in sea ice microalgae of McMurdo Sound, Antarctica: evidence for nutrient stress during the spring bloom', *Antarctic Science*, vol. 4, no. 1, pp. 23-30.

Lovelock, J. 2006, 'The Revenge of Gaia: Earth's Climate in Crisis and the Fate of Humanity New York City', USA: Basic Books.

Lyon, B.R., Bennett-Mintz, J.M., Lee, P.A., Janech, M.G. & DiTullio, G.R. 2016, 'Role of dimethylsulfoniopropionate as an osmoprotectant following gradual salinity shifts in the sea-ice diatom *Fragilariopsis cylindrus*', *Environmental Chemistry*, vol. 13, no. 2, pp. 181-94.

Lyon, B.R. & Mock, T. 2014, 'Polar microalgae: new approaches towards understanding adaptations to an extreme and changing environment', *Biology*, vol. 3, no. 1, pp. 56-80.

Malin, G. 1996, 'The role of DMSP and DMS in the global sulfur cycle and climate regulation', *Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds*, Springer, pp. 177-89.

Malin, G. 2006, 'New pieces for the marine sulfur cycle jigsaw', *Science*, vol. 314, no. 5799, pp. 607-8.

Mallick, N. & Mohn, F.H. 2000, 'Reactive oxygen species: response of algal cells', *Journal of Plant Physiology*, vol. 157, no. 2, pp. 183-93.

Malmstrom, R.R., Kiene, R.P., Vila, M. & Kirchman, D.L. 2005, 'Dimethylsulfoniopropionate (DMSP) assimilation by Synechococcus in the Gulf of Mexico and northwest Atlantic Ocean', *Limnology and oceanography*, vol. 50, no. 6, pp. 1924-31.

Marie, D., Partensky, F., Vaulot, D. & Brussaard, C. 1999, 'Enumeration of phytoplankton, bacteria, and viruses in marine samples', *Current protocols in cytometry*, vol. 10, no. 1, pp. 11.. 1-.. 5.

Marsh, M. 2003, 'Regulation of CaCO3 formation in coccolithophores', *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 136, no. 4, pp. 743-54.

Matrai, P. & Keller, M. 1994, 'Total organic sulfur and dimethylsulfoniopropionate in marine phytoplankton: intracellular variations', *Marine Biology*, vol. 119, no. 1, pp. 61-8.

Matrai, P.A. & Keller, M.D. 1993, 'Dimethylsulfide in a large-scale coccolithophore bloom in the Gulf of Maine', *Continental Shelf Research*, vol. 13, no. 8-9, pp. 831-43.

McClatchie, S. & Boyd, C.M. 1983, 'Morphological study of sieve efficiencies and mandibular surfaces in the Antarctic krill, Euphausia superba', *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 40, no. 7, pp. 955-67.

McMinn, A., Ryan, K. & Gademann, R. 2003, 'Diurnal changes in photosynthesis of Antarctic fast ice algal communities determined by pulse amplitude modulation fluorometry', *Marine Biology*, vol. 143, no. 2, pp. 359-67.

McNeil, B.I. & Matear, R.J. 2008, 'Southern Ocean acidification: A tipping point at 450-ppm atmospheric CO2', *Proceedings of the National Academy of Sciences*, vol. 105, no. 48, pp. 18860-4.

McTaggart, A.R. & Burton, H. 1992, 'Dimethyl sulfide concentrations in the surface waters of the Australasian Antarctic and Subantarctic oceans during an austral summer', *Journal of Geophysical Research: Oceans*, vol. 97, no. C9, pp. 14407-12.

Meyerdierks, D., Bolt, B., Kirst, G. & Scharek, R. 1994, 'Spatial and vertical distribution of algal dimenthylsulfoniumpropionate (DMSP between the retreating pack-ice zone and the Polar Front in the Southern Ocean during austral spring in 1992', *EOS (abstracts)*.

Mock, T. & Hoch, N. 2005, 'Long-term temperature acclimation of photosynthesis in steady-state cultures of the polar diatom *Fragilariopsis cylindrus*', *Photosynthesis research*, vol. 85, no. 3, pp. 307-17.

Mock, T. & Kroon, B.M. 2002, 'Photosynthetic energy conversion under extreme conditions—I: important role of lipids as structural modulators and energy sink under N-limited growth in Antarctic sea ice diatoms', *Phytochemistry*, vol. 61, no. 1, pp. 41-51.

Mock, T. & Thomas, D.N. 2005, 'Recent advances in sea-ice microbiology', *Environmental Microbiology*, vol. 7, no. 5, pp. 605-19.

Mohapatra, B.R., Rellinger, A.N., Kieber, D.J. & Kiene, R.P. 2013, 'Comparative functional characteristics of DMSP lyases extracted from polar and temperate Phaeocystis species', *Aquatic Biology*, vol. 18, no. 2, pp. 185-95.

Moline, M.A., Claustre, H., Frazer, T.K., Schofield, O. & Vernet, M. 2004, 'Alteration of the food web along the Antarctic Peninsula in response to a regional warming trend', *Global Change Biology*, vol. 10, no. 12, pp. 1973-80.

Moran, M.A., Reisch, C.R., Kiene, R.P. & Whitman, W.B. 2011, 'Genomic insights into bacterial DMSP transformations'.

Moreno, A.R. & Martiny, A.C. 2018, 'Ecological stoichiometry of ocean plankton', *Annual review of marine science*, vol. 10, pp. 43-69.

Morgan-Kiss, R.M., Priscu, J.C., Pocock, T., Gudynaite-Savitch, L. & Huner, N.P. 2006, 'Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments', *Microbiology and Molecular Biology Reviews*, vol. 70, no. 1, pp. 222-52.

Morris, I. 1981, 'Photosynthetic products, physiological state, and phytoplankton growth', *Canadian Bulletin of Fisheries and Aquatic Sciences*.

Morris, I., Glover, H. & Yentsch, C. 1974, 'Products of photosynthesis by marine phytoplankton: the effect of environmental factors on the relative rates of protein synthesis', *Marine Biology*, vol. 27, no. 1, pp. 1-9.

Motard-Côté, J. & Kiene, R.P. 2015, 'Osmoprotective role of dimethylsulfoniopropionate (DMSP) for estuarine bacterioplankton', *Aquatic Microbial Ecology*, vol. 76, no. 2, pp. 133-47.

Müller, M.N., Trull, T.W. & Hallegraeff, G.M. 2015, 'Differing responses of three Southern Ocean Emiliania huxleyi ecotypes to changing seawater carbonate chemistry', *Marine Ecology Progress Series*, vol. 531, pp. 81-90.

Murata, N. & Siegenthaler, P.-A. 1998, 'Lipids in photosynthesis: an overview', *Lipids in photosynthesis: structure, function and genetics*, Springer, pp. 1-20.

Murdock, J.N. & Wetzel, D.L. 2009, 'FT-IR microspectroscopy enhances biological and ecological analysis of algae', *Applied Spectroscopy Reviews*, vol. 44, no. 4, pp. 335-61.

Niki, T., Kunugi, M. & Otsuki, A. 2000, 'DMSP-lyase activity in five marine phytoplankton species: its potential importance in DMS production', *Marine Biology*, vol. 136, no. 5, pp. 759-64.

Nishiguchi, M.K. & Somero, G.N. 1992, 'Temperature-and concentration-dependence of compatibility of the organic osmolyte β-dimethylsulfoniopropionate', *Cryobiology*, vol. 29, no. 1, pp. 118-24.

Nishiyama, Y., Allakhverdiev, S.I. & Murata, N. 2006, 'A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II', *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, vol. 1757, no. 7, pp. 742-9.

Niyogi, K.K. 1999, 'Photoprotection revisited: genetic and molecular approaches', *Annual review of plant biology*, vol. 50, no. 1, pp. 333-59.

Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A., Gnanadesikan, A., Gruber, N., Ishida, A. & Joos, F. 2005, 'Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms', *Nature*, vol. 437, no. 7059, pp. 681-6.

Parrish, C.C. 2009, 'Essential fatty acids in aquatic food webs', *Lipids in aquatic ecosystems*, Springer, pp. 309-26.

Pedrós-Alió, C., Calderón-Paz, J.I., MacLean, M.H., Medina, G., Marrasé, C., Gasol, J.M. & Guixa-Boixereu, N. 2000, 'The microbial food web along salinity gradients', *FEMS Microbiology Ecology*, vol. 32, no. 2, pp. 143-55.

Perriss, S.J. & Laybourn-Parry, J. 1997, 'Microbial communities in saline lakes of the Vestfold Hills (eastern Antarctica)', *Polar Biology*, vol. 18, no. 2, pp. 135-44.

Petrou, K., Doblin, M. & Ralph, P. 2011a, 'Heterogeneity in the photoprotective capacity of three Antarctic diatoms during short-term changes in salinity and temperature', *Marine biology*, vol. 158, no. 5, pp. 1029-41.

Petrou, K., Doblin, M. & Ralph, P. 2011b, 'Photosynthesis and net primary productivity in three Antarctic diatoms: possible significance for their distribution in the Antarctic marine ecosystem'.

Petrou, K., Hill, R., Brown, C.M., Campbell, D.A., Doblin, M.A. & Ralph, P.J. 2010, 'Rapid photoprotection in sea-ice diatoms from the East Antarctic pack ice', *Limnology and Oceanography*, vol. 55, no. 3, pp. 1400-7.

Petrou, K., Kranz, S.A., Trimborn, S., Hassler, C.S., Ameijeiras, S.B., Sackett, O., Ralph, P.J. & Davidson, A.T. 2016, 'Southern Ocean phytoplankton physiology in a changing climate', *Journal of plant physiology*, vol. 203, pp. 135-50.

Powell, L., Bowman, J., Skerratt, J., Franzmann, P. & Burton, H. 2005, 'Ecology of a novel Synechococcus clade occurring in dense populations in saline Antarctic lakes', *Marine Ecology Progress Series*, vol. 291, pp. 65-80.

Quinn, P. & Bates, T. 2011, 'The case against climate regulation via oceanic phytoplankton sulphur emissions', *Nature*, vol. 480, no. 7375, pp. 51-6.

Raina, J.-B., Clode, P.L., Cheong, S., Bougoure, J., Kilburn, M.R., Reeder, A., Foret, S., Stat, M., Beltran, V. & Thomas-Hall, P. 2017, 'Subcellular tracking reveals the location of dimethylsulfoniopropionate in microalgae and visualises its uptake by marine bacteria', *Elife*, vol. 6, p. e23008.

Ralph, P.J. & Gademann, R. 2005, 'Rapid light curves: a powerful tool to assess photosynthetic activity', *Aquatic botany*, vol. 82, no. 3, pp. 222-37.

Reisch, C.R., Moran, M.A. & Whitman, W.B. 2011, 'Bacterial catabolism of dimethylsulfoniopropionate (DMSP)', *Frontiers in microbiology*, vol. 2.

Renaud, S.M., Thinh, L.-V., Lambrinidis, G. & Parry, D.L. 2002, 'Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures', *Aquaculture*, vol. 211, no. 1, pp. 195-214.

Rijstenbil, J. 2003, 'Effects of UVB radiation and salt stress on growth, pigments and antioxidative defence of the marine diatom Cylindrotheca closterium', *Marine Ecology Progress Series*, vol. 254, pp. 37-48.

Rodríguez, J., Jiménez-Gómez, F., Blanco, J.M. & Figueroa, F.L. 2002, 'Physical gradients and spatial variability of the size structure and composition of phytoplankton in the Gerlache Strait (Antarctica)', *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 49, no. 4-5, pp. 693-706.

Rossoll, D., Bermúdez, R., Hauss, H., Schulz, K.G., Riebesell, U., Sommer, U. & Winder, M. 2012, 'Ocean acidification-induced food quality deterioration constrains trophic transfer', *PLoS one*, vol. 7, no. 4, p. e34737.

Rueter, J.G. & Unsworth, N.L. 1991, 'RESPONSE OF MARINE SYNECHOCOCCUS (CYANOPHYCEAE) CULTURES TO IRON NUTRITION 1', *Journal of phycology*, vol. 27, no. 2, pp. 173-8.

Ryan, K., Ralph, P. & McMinn, A. 2004, 'Acclimation of Antarctic bottom-ice algal communities to lowered salinities during melting', *Polar Biology*, vol. 27, no. 11, pp. 679-86.

Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L., Wanninkhof, R., Wong, C., Wallace, D.W. & Tilbrook, B. 2004, 'The oceanic sink for anthropogenic CO2', *science*, vol. 305, no. 5682, pp. 367-71.

Sackett, O., Petrou, K., Reedy, B., De Grazia, A., Hill, R., Doblin, M., Beardall, J., Ralph, P. & Heraud, P. 2013, 'Phenotypic plasticity of southern ocean diatoms: key to success in the sea ice habitat?', *PloS one*, vol. 8, no. 11, p. e81185.

Sackett, O., Petrou, K., Reedy, B., Hill, R., Doblin, M., Beardall, J., Ralph, P. & Heraud, P. 2015, 'Snapshot prediction of carbon productivity, carbon and protein content in a Southern Ocean diatom using FTIR spectroscopy', *The ISME journal*.

Salazar, G. & Sunagawa, S. 2017, 'Marine microbial diversity', *Current Biology*, vol. 27, no. 11, pp. R489-R94.

Savoca, M.S. & Nevitt, G.A. 2014, 'Evidence that dimethyl sulfide facilitates a tritrophic mutualism between marine primary producers and top predators', *Proceedings of the National Academy of Sciences*, vol. 111, no. 11, pp. 4157-61.

Schine, C.M., van Dijken, G. & Arrigo, K.R. 2016, 'Spatial analysis of trends in primary production and relationship with large-scale climate variability in the Ross Sea, Antarctica (1997–2013)', *Journal of Geophysical Research: Oceans*, vol. 121, no. 1, pp. 368-86.

Schoemann, V., Becquevort, S., Stefels, J., Rousseau, V. & Lancelot, C. 2005, 'Phaeocystis blooms in the global ocean and their controlling mechanisms: a review', *Journal of Sea Research*, vol. 53, no. 1-2, pp. 43-66.

Seymour, J.R., Simó, R., Ahmed, T. & Stocker, R. 2010, 'Chemoattraction to dimethylsulfoniopropionate throughout the marine microbial food web', *Science*, vol. 329, no. 5989, pp. 342-5.

Sheets, E.B. & Rhodes, D. 1996, 'Determination of DMSP and other onium compounds in Tetraselmis subcordiformis by plasma desorption mass spectrometry', *Biological and environmental chemistry of DMSP and related sulfonium compounds*, Springer, pp. 55-63.

Shi, Q., Xiahou, W. & Wu, H. 2017, 'Photosynthetic responses of the marine diatom Thalassiosira pseudonana to CO 2-induced seawater acidification', *Hydrobiologia*, vol. 788, no. 1, pp. 361-9.

Simó, R. 2001, 'Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and evolutionary links', *Trends in Ecology & Evolution*, vol. 16, no. 6, pp. 287-94.

Simó, R. & Dachs, J. 2002, 'Global ocean emission of dimethylsulfide predicted from biogeophysical data', *Global Biogeochemical Cycles*, vol. 16, no. 4.

Simó, R., Hatton, A.D., Malin, G. & Liss, P.S. 1998, 'Particulate dimethyl sulphoxide in seawater: production by microplankton', *Marine Ecology Progress Series*, pp. 291-6.

Simó, R. & Vila-Costa, M. 2006, 'Ubiquity of algal dimethylsulfoxide in the surface ocean: Geographic and temporal distribution patterns', *Marine chemistry*, vol. 100, no. 1-2, pp. 136-46.

Slezak, D., Brugger, A. & Herndl, G.J. 2001, 'Impact of solar radiation on the biological removal of dimethylsulfoniopropionate and dimethylsulfide in marine surface waters', *Aquatic Microbial Ecology*, vol. 25, no. 1, pp. 87-97.

Slezak, D. & Herndl, G.J. 2003, 'Effects of ultraviolet and visible radiation on the cellular concentrations of dimethylsulfoniopropionate (DMSP) in Emiliania huxleyi (strain L)', *Marine Ecology Progress Series*, vol. 246, pp. 61-71.

Smetacek, V., Assmy, P. & Henjes, J. 2004, 'The role of grazing in structuring Southern Ocean pelagic ecosystems and biogeochemical cycles', *Antarctic Science*, vol. 16, no. 4, pp. 541-58.

Smith, A.E. & Morris, I. 1980, 'Synthesis of lipid during photosynthesis by phytoplankton of the Southern Ocean', *Science*, vol. 207, no. 4427, pp. 197-9.

Smith Jr, W.O., Dinniman, M.S., Tozzi, S., DiTullio, G.R., Mangoni, O., Modigh, M. & Saggiomo, V. 2010, 'Phytoplankton photosynthetic pigments in the Ross Sea: patterns and relationships among functional groups', *Journal of Marine Systems*, vol. 82, no. 3, pp. 177-85.

Smith, R., Gosselin, M. & Taguchi, S. 1997, 'The influence of major inorganic nutrients on the growth and physiology of high arctic ice algae', *Journal of Marine Systems*, vol. 11, no. 1-2, pp. 63-70.

Smith, R.E., Clement, P. & Head, E. 1989, 'Biosynthesis and photosynthate allocation patterns of arctic ice algae', *Limnology and Oceanography*, vol. 34, no. 3, pp. 591-605.

Smith, W.O. & Asper, V.L. 2001, 'The influence of phytoplankton assemblage composition on biogeochemical characteristics and cycles in the southern Ross Sea, Antarctica', *Deep Sea Research Part I: Oceanographic Research Papers*, vol. 48, no. 1, pp. 137-61.

Song, H.J., Kang, J.J., Kim, B.K., Joo, H., Yang, E.J., Park, J., Lee, S.H. & Lee, S.H. 2016, 'High protein production of phytoplankton in the Amundsen Sea', *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 123, pp. 50-7.

Søreide, J.E., Leu, E., Berge, J., Graeve, M. & Falk-Petersen, S. 2010, 'Timing of blooms, algal food quality and Calanus glacialis reproduction and growth in a changing Arctic', *Global change biology*, vol. 16, no. 11, pp. 3154-63.

Spiese, C.E., Sanford, J.L., Bowling, M.N., Tatarkov, E.A. & Pinkney, A.L. 2017, 'Methanesulfonate supports growth as the sole sulfur source for the marine diatom Thalassiosira pseudonana NCMA 1335', *Aquatic Microbial Ecology*, vol. 78, no. 3, pp. 177-85.

Springer, A.M. & McRoy, C.P. 1993, 'The paradox of pelagic food webs in the northern Bering Sea—III. Patterns of primary production', *Continental shelf research*, vol. 13, no. 5-6, pp. 575-99.

Stefels, J. 2000, 'Physiological aspects of the production and conversion of DMSP in marine algae and higher plants', *Journal of Sea Research*, vol. 43, no. 3, pp. 183-97.

Stefels, J., Carnat, G., Dacey, J.W., Goossens, T., Elzenga, J.T.M. & Tison, J.-L. 2012, 'The analysis of dimethylsulfide and dimethylsulfoniopropionate in sea ice: Dry-crushing and melting using stable isotope additions', *Marine chemistry*, vol. 128, pp. 34-43.

Stefels, J. & Dijkhuizen, L. 1996, 'Characteristics of DMSP-lyase in *Phaeocystis* sp.(Prymnesiophyceae)', *Marine Ecology Progress Series*, pp. 307-13.

Stefels, J., Steinke, M., Turner, S., Malin, G. & Belviso, S. 2007, 'Environmental constraints on the production and removal of the climatically active gas dimethylsulphide (DMS) and implications for ecosystem modelling', *Biogeochemistry*, vol. 83, no. 1-3, pp. 245-75.

Stefels, J. & Van Boekel, W. 1993, 'Production of DMS from dissolved DMSP in axenic cultures of the marine phytoplankton species Phaeocystis sp', *Marine Ecology Progress Series*, pp. 11-8.

Stefels, J. & van Leeuwe, M.A. 1998, 'Effects of iron and light stress on the biochemical composition of Antarctic *Phaeocystis* sp. (Prymnesiophyceae). I. Intracellular DMSP concentrations', *Journal of Phycology*, vol. 34, no. 3, pp. 486-95.

Stefels, J., van Leeuwe, M.A., Jones, E.M., Meredith, M.P., Venables, H.J., Webb, A.L. & Henley, S.F. 2018, 'Impact of sea-ice melt on dimethyl sulfide (sulfoniopropionate) inventories in surface waters of Marguerite Bay, West Antarctic Peninsula', *Phil. Trans. R. Soc. A*, vol. 376, no. 2122, p. 20170169.

Steinacher, M., Joos, F., Frolicher, T., Bopp, L., Cadule, P., Cocco, V., Doney, S.C., Gehlen, M., Lindsay, K. & Moore, J.K. 2010, 'Projected 21st century decrease in marine productivity: a multi-model analysis', *Biogeosciences*, vol. 7, no. 3.

Steiner, N.S., Robert, M., Arychuk, M., Levasseur, M.L., Merzouk, A., Peña, M.A., Richardson, W.A. & Tortell, P.D. 2012, 'Evaluating DMS measurements and model results in the Northeast subarctic Pacific from 1996–2010', *Biogeochemistry*, vol. 110, no. 1-3, pp. 269-85.

Steinke, M., Malin, G. & Liss, P.S. 2002, 'Trophic interactions in the sea: An ecological role for climate relevant volatiles?', *Journal of Phycology*, vol. 38, no. 4, pp. 630-8.

Steinke, M., Stefels, J. & Stamhuis, E. 2006, 'Dimethyl sulfide triggers search behavior in copepods', *Limnology and oceanography*, vol. 51, no. 4, pp. 1925-30.

Steinke, M., Wolfe, G.V. & Kirst, G.O. 1998, 'Partial characterisation of dimethylsulfoniopropionate (DMSP) lyase isozymes in 6 strains of *Emiliania huxleyi*', *Marine Ecology Progress Series*, pp. 215-25.

Sterner, R.W. & Elser, J.J. 2002, *Ecological stoichiometry: the biology of elements from molecules to the biosphere*, Princeton University Press.

Stickley, C., Pike, J., Leventer, A., Dunbar, R., Domack, E., Brachfeld, S., Manley, P. & McClennan, C. 2005, 'Deglacial ocean and climate seasonality in laminated diatom sediments, Mac. Robertson Shelf, Antarctica', *Palaeogeography, Palaeoclimatology, Palaeoecology*, vol. 227, no. 4, pp. 290-310.

Strandberg, U., Taipale, S., Hiltunen, M., Galloway, A., Brett, M. & Kankaala, P. 2015, 'Inferring phytoplankton community composition with a fatty acid mixing model', *Ecosphere*, vol. 6, no. 1, pp. 1-18.

Strom, S., Wolfe, G., Slajer, A., Lambert, S. & Clough, J. 2003, 'Chemical defense in the microplankton II: Inhibition of protist feeding by β-dimethylsulfoniopropionate (DMSP)', *Limnology and Oceanography*, vol. 48, no. 1, pp. 230-7.

Sudhir, P. & Murthy, S. 2004, 'Effects of salt stress on basic processes of photosynthesis', *Photosynthetica*, vol. 42, no. 2, pp. 481-6.

Sun, J., Todd, J.D., Thrash, J.C., Qian, Y., Qian, M.C., Temperton, B., Guo, J., Fowler, E.K., Aldrich, J.T. & Nicora, C.D. 2016, 'The abundant marine bacterium Pelagibacter simultaneously catabolizes dimethylsulfoniopropionate to the gases dimethyl sulfide and methanethiol', *Nature microbiology*, vol. 1, p. 16065.

Sunda, W., Kieber, D., Kiene, R. & Huntsman, S. 2002, 'An antioxidant function for DMSP and DMS in marine algae', *Nature*, vol. 418, no. 6895, pp. 317-20.

Sunda, W.G., Hardison, R., Kiene, R.P., Bucciarelli, E. & Harada, H. 2007, 'The effect of nitrogen limitation on cellular DMSP and DMS release in marine phytoplankton: climate feedback implications', *Aquatic Sciences-Research Across Boundaries*, vol. 69, no. 3, pp. 341-51.

Szabados, L. & Savoure, A. 2010, 'Proline: a multifunctional amino acid', *Trends in plant science*, vol. 15, no. 2, pp. 89-97.

Tang, K.W., Smith, W.O., Shields, A.R. & Elliott, D.T. 2009, *Survival and recovery of Phaeocystis antarctica (Prymnesiophyceae) from prolonged darkness and freezing*, Journal Article, vol. 276.

Tapiolas, D.M., Raina, J.-B., Lutz, A., Willis, B.L. & Motti, C.A. 2013, 'Direct measurement of dimethylsulfoniopropionate (DMSP) in reef-building corals using quantitative nuclear magnetic resonance (qNMR) spectroscopy', *Journal of Experimental Marine Biology and Ecology*, vol. 443, pp. 85-9.

Teoh, M.-L., Chu, W.-L., Marchant, H. & Phang, S.-M. 2004, 'Influence of culture temperature on the growth, biochemical composition and fatty acid profiles of six Antarctic microalgae', *Journal of Applied Phycology*, vol. 16, no. 6, pp. 421-30.

Terry, K.L., Hirata, J. & Laws, E.A. 1983, 'Light-limited growth of two strains of the marine diatom Phaeodactylum tricornutum Bohlin: chemical composition, carbon partitioning and the diel periodicity of physiological processes', *Journal of Experimental Marine Biology and Ecology*, vol. 68, no. 3, pp. 209-27.

Thomas, D. & Dieckmann, G. 2002, 'Antarctic sea ice - a habitat for extremophiles', *Science*, vol. 295, no. 5555, pp. 641-4.

Thomas, D.N. & Dieckmann, G.S. 2009, *Sea ice*, John Wiley & Sons.

Thompson, P.A., Guo, M.-x. & Harrison, P.J. 1992, 'Effects of variation in temperature. I. on the biochemical composition of eight species of marine phytoplankton', *Journal of Phycology*, vol. 28, no. 4, pp. 481-8.

Tobin, M.J., Puskar, L., Barber, R.L., Harvey, E.C., Heraud, P., Wood, B.R., Bambery, K.R., Dillon, C.T. & Munro, K.L. 2010, 'FTIR spectroscopy of single live cells in aqueous media by synchrotron IR microscopy using microfabricated sample holders', *Vibrational Spectroscopy*, vol. 53, no. 1, pp. 34-8.

Tocher, D.R. 2003, 'Metabolism and functions of lipids and fatty acids in teleost fish', *Reviews in fisheries science*, vol. 11, no. 2, pp. 107-84.

Toole, D., Slezak, D., Kiene, R., Kieber, D. & Siegel, D. 2006, 'Effects of solar radiation on dimethylsulfide cycling in the western Atlantic Ocean', *Deep Sea Research Part I: Oceanographic Research Papers*, vol. 53, no. 1, pp. 136-53.

Torstensson, A., Hedblom, M., Andersson, J., Andersson, M. & Wulff, A. 2013, 'Synergism between elevated pCO 2 and temperature on the Antarctic sea ice diatom Nitzschia lecointei', *Biogeosciences*, vol. 10, no. 10, pp. 6391-401.

Tortell, P.D., Payne, C.D., Li, Y., Trimborn, S., Rost, B., Smith, W.O., Riesselman, C., Dunbar, R.B., Sedwick, P. & DiTullio, G.R. 2008, 'CO2 sensitivity of Southern Ocean phytoplankton', *Geophysical Research Letters*, vol. 35, no. 4.

Tréguer, P.J. & De La Rocha, C.L. 2013, 'The world ocean silica cycle', *Annual review of marine science*, vol. 5, pp. 477-501.

Trevena, A. & Jones, G. 2012, 'DMS flux over the Antarctic sea ice zone', *Marine Chemistry*, vol. 134, pp. 47-58.

Trevena, A.J. & Jones, G.B. 2006, 'Dimethylsulphide and dimethylsulphoniopropionate in Antarctic sea ice and their release during sea ice melting', *Marine Chemistry*, vol. 98, no. 2, pp. 210-22.

Trevena, A.J., Jones, G.B., Wright, S.W. & Van den Enden, R.L. 2000, 'Profiles of DMSP, algal pigments, nutrients and salinity in pack ice from eastern Antarctica', *Journal of Sea Research*, vol. 43, no. 3-4, pp. 265-73.

Trevena, A.J., Jones, G.B., Wright, S.W. & Van den Enden, R.L. 2003, 'Profiles of dimethylsulphoniopropionate (DMSP), algal pigments, nutrients, and salinity in the fast ice of Prydz Bay, Antarctica', *Journal of Geophysical Research: Oceans (1978–2012)*, vol. 108, no. C5.

Trimborn, S., Brenneis, T., Sweet, E. & Rost, B. 2013, 'Sensitivity of Antarctic phytoplankton species to ocean acidification: Growth, carbon acquisition, and species interaction', *Limnology and Oceanography*, vol. 58, no. 3, pp. 997-1007.

Trimborn, S., Thoms, S., Brenneis, T., Heiden, J.P., Beszteri, S. & Bischof, K. 2017, 'Two Southern Ocean diatoms are more sensitive to ocean acidification and changes in irradiance than the prymnesiophyte Phaeocystis antarctica', *Physiologia plantarum*, vol. 160, no. 2, pp. 155-70.

Turner, S., Nightingale, P., Broadgate, W. & Liss, P. 1995, 'The distribution of dimethyl sulphide and dimethylsulphoniopropionate in Antarctic waters and sea ice', *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 42, no. 4-5, pp. 1059-80.

Twining, B.S., Baines, S.B., Vogt, S. & de JONGE, M.D. 2008, 'Exploring Ocean Biogeochemistry by Single-Cell Microprobe Analysis of Protist Elemental Composition 1', *Journal of eukaryotic microbiology*, vol. 55, no. 3, pp. 151-62.

Tyrrell, T. 1999, 'The relative influences of nitrogen and phosphorus on oceanic primary production', *Nature*, vol. 400, no. 6744, p. 525.

Uhlig, C., Rintala, J., Tison, J.-L., Carnat, G., Dieckmann, G., Delille, B. & Damm, E. 2014, 'Dimethyl sulfide and dimethylsulfoniopropionate profiles from winter sea ice-Influence of physical parameters and speciation'.

Untersteiner, N. 1968, 'Natural desalination and equilibrium salinity profile of perennial sea ice', *Journal of Geophysical Research*, vol. 73, no. 4, pp. 1251-7.

Vallina, S.M. & Simó, R. 2007, 'Strong relationship between DMS and the solar radiation dose over the global surface ocean', *Science*, vol. 315, no. 5811, pp. 506-8.

Van Bergeijk, S.A., Van der Zee, C. & Stal, L.J. 2003, 'Uptake and excretion of dimethylsulphoniopropionate is driven by salinity changes in the marine benthic diatom Cylindrotheca closterium', *European Journal of Phycology*, vol. 38, no. 4, pp. 341-9.

Van Donk, E., Ianora, A. & Vos, M. 2011, 'Induced defences in marine and freshwater phytoplankton: a review', *Hydrobiologia*, vol. 668, no. 1, pp. 3-19.

Van Duyl, F.C., Gieskes, W.W., Kop, A.J. & Lewis, W.E. 1998, 'Biological control of short-term variations in the concentration of DMSP and DMS during a Phaeocystis spring bloom', *Journal of Sea Research*, vol. 40, no. 3-4, pp. 221-31.

Van Oijen, T., Veldhuis, M., Gorbunov, M., Nishioka, J., Van Leeuwe, M. & De Baar, H. 2005, 'Enhanced carbohydrate production by Southern Ocean phytoplankton in response to in situ iron fertilization', *Marine chemistry*, vol. 93, no. 1, pp. 33-52.

Van Rijssel, M. & Gieskes, W.W. 2002, 'Temperature, light, and the dimethylsulfoniopropionate (DMSP) content of Emiliania huxleyi (Prymnesiophyceae)', *Journal of Sea Research*, vol. 48, no. 1, pp. 17-27.

Vance, T.R., Davidson, A.T., Thomson, P.G., Levasseur, M., Lizotte, M., Curran, M.A. & Jones, G.B. 2013, 'Rapid DMSP production by an Antarctic phytoplankton community exposed to natural surface irradiances in late spring', *Aquat. Microb. Ecol*, vol. 71, pp. 117-29.

Vila-Costa, M., Simó, R., Harada, H., Gasol, J.M., Slezak, D. & Kiene, R.P. 2006, 'Dimethylsulfoniopropionate uptake by marine phytoplankton', *Science*, vol. 314, no. 5799, pp. 652-4.

Villareal, T. & Fryxell, G. 1983, 'Temperature effects on the valve structure of the bipolar diatoms Thalassiosira antarctica and Porosira glacialis', *Polar Biology*, vol. 2, no. 3, pp. 163-9.

Vongsvivut, J., Heraud, P., Zhang, W., Kralovec, J.A., McNaughton, D. & Barrow, C.J. 2012, 'Quantitative determination of fatty acid compositions in micro-encapsulated fish-oil supplements using Fourier transform infrared (FTIR) spectroscopy', *Food chemistry*, vol. 135, no. 2, pp. 603-9.

Wagner, H., Jakob, T. & Wilhelm, C. 2005, 'Balancing the energy flow from captured light to biomass under fluctuating light conditions', *New Phytologist*, vol. 169, no. 1, pp. 95-108.

Wagner, H., Jakob, T. & Wilhelm, C. 2006, 'Balancing the energy flow from captured light to biomass under fluctuating light conditions', *New Phytologist*, vol. 169, no. 1, pp. 95-108.

Whyte, J.N. 1987, 'Biochemical composition and energy content of six species of phytoplankton used in mariculture of bivalves', *Aquaculture*, vol. 60, no. 3-4, pp. 231-41.

Wikfors, G.H., Ferris, G.E. & Smith, B.C. 1992, 'The relationship between gross biochemical composition of cultured algal foods and growth of', *Aquaculture*, vol. 108, pp. 135-54.

Wilson, W., Turner, S. & Mann, N. 1998, 'Population dynamics of phytoplankton and viruses in a phosphate-limited mesocosm and their effect on DMSP and DMS production', *Estuarine, Coastal and Shelf Science*, vol. 46, no. 2, pp. 49-59.

Wingenter, O.W., Haase, K.B., Zeigler, M., Blake, D.R., Rowland, F.S., Sive, B.C., Paulino, A., Thyrhaug, R., Larsen, A. & Schulz, K. 2007, 'Unexpected consequences of increasing CO2 and ocean acidity on marine production of DMS and CH2ClI: Potential climate impacts', *Geophysical Research Letters*, vol. 34, no. 5.

Wolfe, G.V. & Steinke, M. 1996, 'Grazing-activated production of dimethyl sulfide (DMS) by two clones of Emiliania huxleyi', *Limnology and Oceanography*, vol. 41, no. 6, pp. 1151-60.

Wolfe, G.V., Steinke, M. & Kirst, G.O. 1997, 'Grazing-activated chemical defence in a unicellular marine alga', *Nature*, vol. 387, no. 6636, pp. 894-7.

Wu, Y., Gao, K. & Riebesell, U. 2010, 'CO2-induced seawater acidification affects physiological performance of the marine diatom Phaeodactylum tricornutum', *Biogeosciences (BG)*, vol. 7, no. 9, pp. 2915-23.

Yau, S., Lauro, F.M., Williams, T.J., DeMaere, M.Z., Brown, M.V., Rich, J., Gibson, J.A. & Cavicchioli, R. 2013, 'Metagenomic insights into strategies of carbon conservation and unusual sulfur biogeochemistry in a hypersaline Antarctic lake', *The ISME journal*, vol. 7, no. 10, pp. 1944-61.

Yoch, D.C. 2002, 'Dimethylsulfoniopropionate: its sources, role in the marine food web, and biological degradation to dimethylsulfide', *Applied and Environmental Microbiology*, vol. 68, no. 12, pp. 5804-15.

Young, J.N., Goldman, J.A., Kranz, S.A., Tortell, P.D. & Morel, F.M. 2015, 'Slow carboxylation of Rubisco constrains the rate of carbon fixation during Antarctic phytoplankton blooms', *New Phytologist*, vol. 205, no. 1, pp. 172-81.

Zhuang, G., Yang, G., Yu, J. & Gao, Y. 2011, 'Production of DMS and DMSP in different physiological stages and salinity conditions in two marine algae', *Chinese Journal of Oceanology and Limnology*, vol. 29, no. 2, pp. 369-77.

Zimmer-Faust, R.K., de Souza, M.P. & Yoch, D.C. 1996, 'Bacterial chemotaxis and its potential role in marine dimethylsulfide production and biogeochemical sulfur cycling', *Limnology and oceanography*, vol. 41, no. 6, pp. 1330-4.