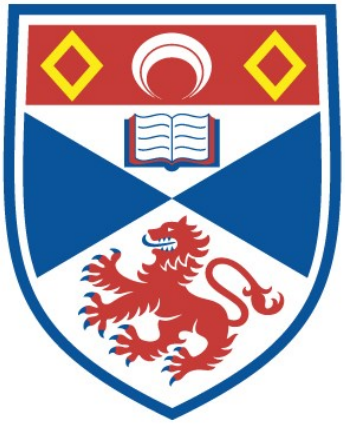
CLIMATE CHANGE EFFECTS ON DIMETHYLATED SULPHUR DYNAMICS IN TROPICAL CORAL REEF SYSTEMS

Tamara Kirsty Green

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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For mum, who helped me write my first word and all the others that followed

Julie Green

4 th September 1955 –3 rd October 2015

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Thank you all

Tammy
8th July 2018

List of Abbreviations

Abbreviation	Full meaning
ANOVA	Analysis of variance
AOC	Antioxidant capacity
BCA	Bicinchoninic acid assay
BSA	Bovine serum albumin
CCA	Crustose coralline algae
CCN	Cloud condensation nuclei
CLAW	Charlson, Lovelock, Andreae & Warren
CPC	Coral point count
CRM	Certified reference material
CV	Coral View
DI	Deionised water
DIC	Dissolved inorganic carbon
DMS	Dimethyl sulphide
DMSO	Dimethyl sulphoxide
DMSP	Dimethylsulphoniopropionate
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DOM	Dissolved organic matter
DSC	Dimethylated sulphur compounds
EDTA	Ethylenediaminetetraacetic acid
EMF	Electromotive force
FMN	Flavin mononucleotide
FPD	Flame photometric detector
GBR	Great Barrier Reef
GC	Gas chromatography
GP	Gross production
GPS	Global Positioning System
LB	Little Bight
LED	Light emitting diode
MCE	Mesophotic coral ecosystem
MMPA	Methylmercaptopropionate
MPA	Mercaptopropionate
MSA	Methanesulphonic acid
NIST	National Institute of Standards and Technology
OA	Ocean acidification
PAR	Photosynthetically active radiation
PTFE	Polytetrafluoroethylene
RC	Raggedy Cay
RCP	Representative concentration pathway
ROS	Reactive oxygen species
RP	Rocky Point
SCUBA	Self - contained underwater breathing apparatus

SE	Standard error
SPR	Sand, pavement, rubble
SST	Sea surface temperature
STE	Sodium Chloride - Tris - EDTA
TA	Total alkalinity
TMA	The Maze
UK	United Kingdom
USA	United States
UV	Ultraviolet
UVA	Ultraviolet - A radiation
UVB	Ultraviolet - B radiation

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Abstract:

Dimethylsulphoniopropionate (DMSP) and dimethylsulphoxide (DMSO) (collectively DMSP/O) are produced by marine algae, including symbiotic algae within corals. These sulphur compounds are important not only in sulphur cycle dynamics but also in potentially mediating atmospheric conditions, alleviating the effects of climate change and contributing to reef health.

Most research has focused on the production of DMSP and its major degradation product, the climatically active gas, dimethylsulphide (DMS) by *Acropora* corals in the Great Barrier Reef. However, mechanisms for the production and release of DMSP/O by different reef taxa is poorly understood. Recently the importance of mesophotic reefs as refugia for shallow water corals has been postulated, however their role in the marine sulphur cycle is unknown. This research aimed to improve our understanding of the contemporary and climate change induced seawater and tissue production of DMSP/O in a range of reef environments and taxa. This was achieved through a combination of laboratory and field - based studies, using modern and established techniques.

An effect of both elevated temperature and OA on increased tissue and seawater concentrations of DMSP/O production is reported in field and laboratory studies. Contrasting effects of benthic cover on tissue DMSP/O distributions and seawater DMSP are also noted. The importance of the physical and hydrodynamic environment on biogeochemical connectivity both within a reef and between neighbouring reefs is also focussed on. Crucially, however, the novel tissue and seawater data from mesophotic sites suggests that deeper reefs could affect the biogeochemistry of their shallow water counterparts. The key finding from this work is that climate change will result in increased seawater DMSP concentrations via two mechanisms; through the increase of cellular production of DMSP/O in all reef taxa, and by increasing the biomass of prolific DMSP producers as reefs transition to a fleshy/macroalgal assemblage. Whilst this could potentially mediate the effects of climate change, it will probably also worsen overall reef health, lead to a restructuring of reef communities from the microbial level upwards and will have possibly permanent and deleterious effects on overall ecosystem function.

Contents

1 Introduction

1.1 The global significance of coral reefs

Coral reefs are fascinating and complex ecosystems of which 20 % have already been seriously damaged and an additional 50 % are under constant human pressure and imminent threat of collapse (Burke et al. 2011). Although coral reefs represent a tiny fraction (~ 0.2 %) of the world's ocean area, they are of great environmental and financial importance (Harrison and Booth 2007; Hoegh-Guldberg et al. 2017). The reason for this attention lies partly in the diversity of life concentrated around reef habitats. The complex three - dimensional structure of coral reefs and their high productivity provide a habitat and an active source of sustenance food and nutrients for many thousands of species of invertebrates, fish, reptiles, birds and marine mammals, with coral reefs representing 25 % of the total marine biodiversity (Harrison and Booth, 2007). As a result, more than 500 million people draw food and resources from coral reef ecosystems, meaning the health of coral reefs will directly impact more than 25 % of the world's population (Hoegh-Guldberg 2011).

1.2 Spatial variability and ecology of reef systems

Coral reef zonation (Fig 1.1) from the landward margin includes a low wave energy back reef zone dominated by lagoons with sea grasses and patch reefs at depths from 2 to 10 m. The next zone is the reef flat leading to the reef crest, also known as the algal ridge, which is composed of consolidated calcareous material, corals and crustose coralline algae and is where high energy waves break and lose their energy before entering the back reef.

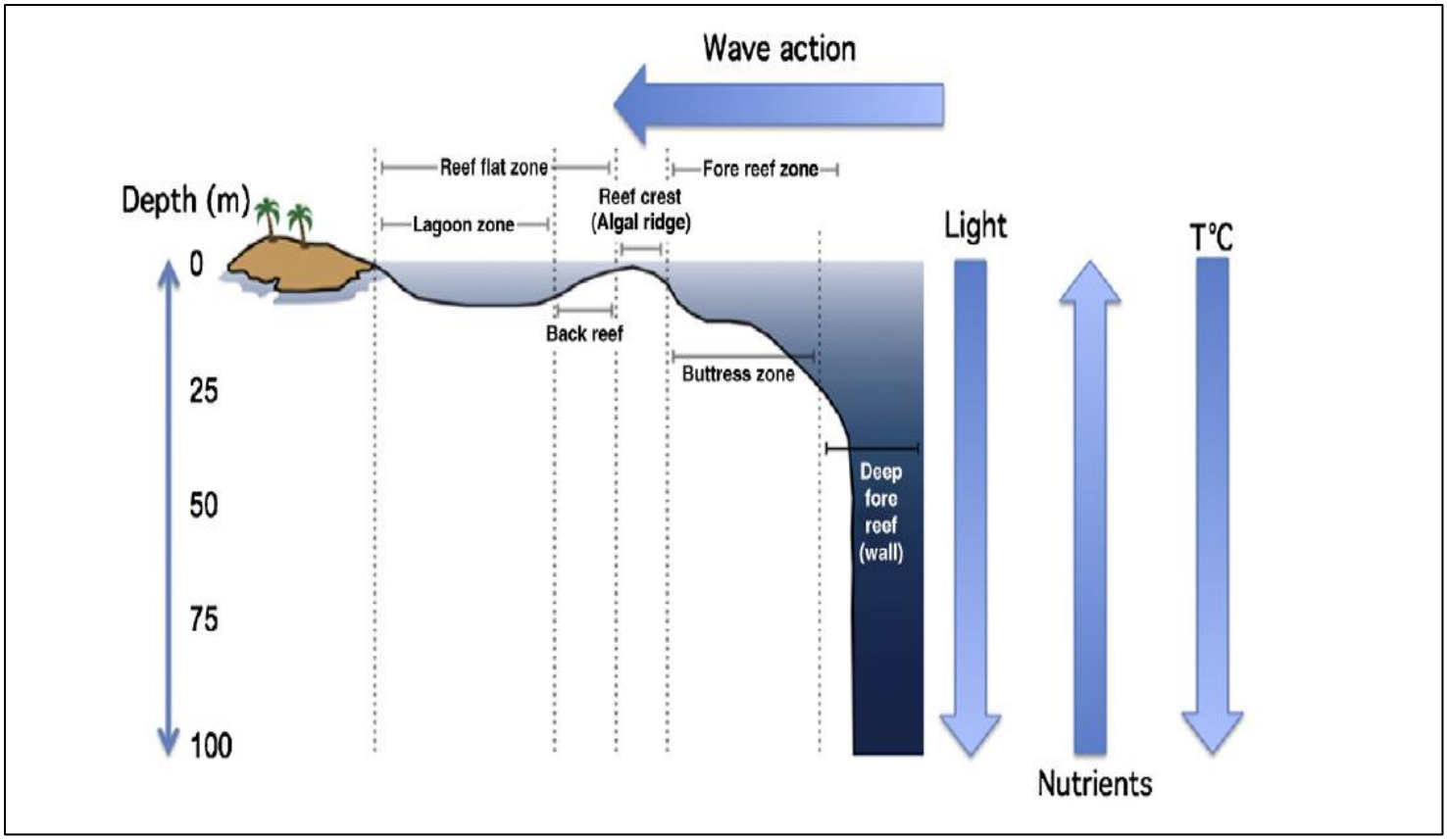


Figure 1.1 Generalised coral reef zonation, showing gradients of light, temperature and nutrients with changes in depth from shallow to mesophotic reefs. Image reproduced from Lesser et al. (2009).

The fore reef zone extends from 1 to ~ 30 m in depth and can include spur and groove formations, as well as deep buttress zones of well developed, coral dominated, communities. The fore - reef then slopes down to the low energy deep fore reef zone, where steep gradients of light (Fig 1.2) dominate the habitat and where upwelling or internal wave induced delivery of nutrients and particulate organic material to shallower depths occurs (Lesser et al. 2009a).

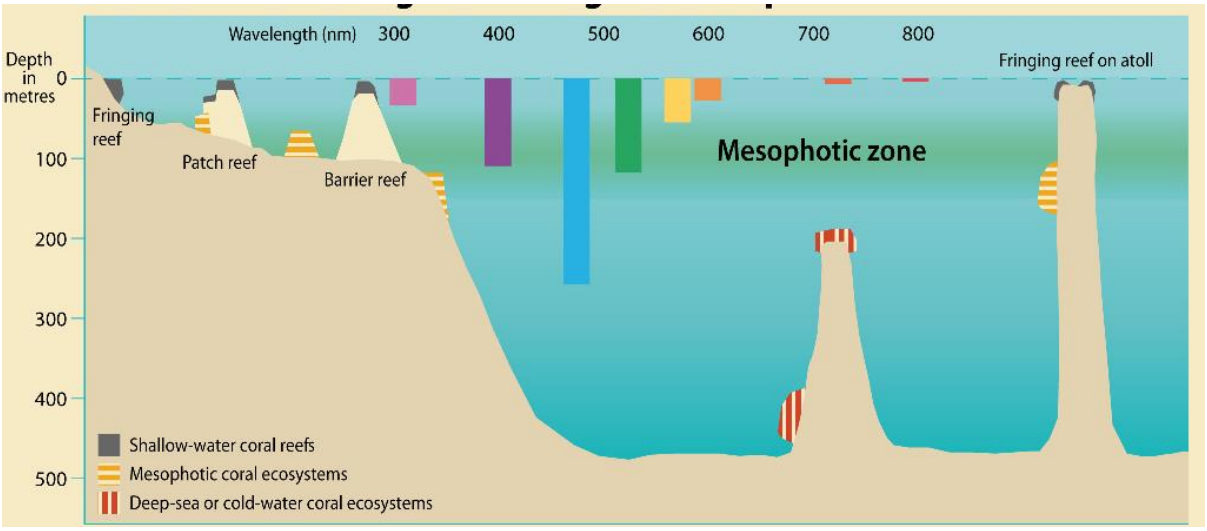


Figure 1.2 Conceptual model of light penetration in the ocean. Blue light dominates the photic zone below 30 m, but the actual depth of light penetration is site - specific and dependent on a variety of physical factors, such as suspended particulate matter (Baker et al. 2016)

Temperature also decreases from the surface to mesophotic depths, but is probably not a significant abiotic factor in structuring mesophotic communities (Slattery et al. 2011). It is generally suggested that mesophotic reef waters are 5 °C lower than surface waters, down to depths of ~ 100 m (Slattery et al. 2011).

1.3 Mesophotic reefs

Mesophotic coral ecosystems (MCEs) are deep fore reef communities that occur at intermediate depths (30–200 m) of the photic zone where light - dependent (zooxanthellate) corals are present, as well as many other shallow water coral reef taxa. MCEs are typically found along island and continental slopes as well as on top of seamounts yet are distinct from 'true' deep - water coral ecosystems that occur at greater depths and that are not light - dependent, therefore comprised of azooxanthellate corals. Recent work has demonstrated that MCEs are much more prevalent than previously thought; studies so far suggest that MCE areal coverage may equal or surpass that of shallow reefs in many of the world's coral reef systems, including the Great Barrier Reef (Harris et al. 2013) and the Caribbean (Locker et al. 2010). MCEs also exhibit a higher percentage of coral cover (40–60 % in MCEs) compared with ~ 20 % at shallower depths (Bak et al. 2005; Slattery et al. 2011),

suggesting that they may play a critical role in the connectivity/continuance of shallower coral reef environments under environmental stress. Whilst the physical environment of MCEs differs from shallower reefs, they are not ecologically distinct and many of the same coral species that occur in shallow reefs also occur in MCEs. This has led to the suggestion that MCEs might act as refugia for shallow water reefs, known as The Deep Reef Refugia Hypothesis, (Lesser et al. 2009a; Bongaerts et al. 2010). The low - light environment of MCEs drives variations in morphology in mesophotic corals, which form plate like structures rather than having boulder/branching morphologies. Studies to investigate the vertical or inter-reef spatial distribution of *Symbiodinium* species have been conducted with interesting results. Previous studies have reported similar community composition of symbionts in mesophotic reefs, with those observed in shallow reef systems (Bongaerts et al. 2011). Further studies have also shown that there does appear to be some degree of depth specialisation between hosts and symbionts (Pochon et al. 2015). Given the importance of *Symbiodinium* in production of dimethylated sulphur compounds (DSC), and the greater areal coverage of MCEs compared with shallow reefs, decreases in either coral or symbiont abundance is likely to result in decreases to DSC production, thereby impacting global marine sulphur biogeochemical cycling.

1.4 The global sulphur cycle

The global sulphur cycle involves the atmosphere, lithosphere, hydrosphere and biosphere, making it one of the most significant biogeochemical cycles on Earth. The oceans are especially important because they act as a source and sink of sulphur compounds, the most significant of which is dimethyl sulphide (DMS) and which accounts for the vast majority of oceanic sulphur emissions (Bates et al. 1992). DMS is an especially important compound in both marine and terrestrial environments; atmospheric oxidation products of DMS not only contribute to acid precipitation (Bates et al. 1992), but also represent important sources of carbon, reduced sulphur and energy for bacterioplankton (Kiene et al. 2000). Interest in DMS and its precursors was triggered in 1987, when it was suggested that atmospheric DMS oxidation products could act as cloud condensation nuclei (CCN), seeding the growth of new clouds or expanding the size of existing ones (Charlson et al. 1987). This CLAW hypothesis (Fig 1.3, named as an acronym of the surnames of the hypothesis proposers (Charlson,

Lovelock, Andreae and Warren)) suggests that DMS production may be altered in response to changes in temperature and light. In short it is hypothesised that DMS production increases when marine algae produce more of one of its precursors, dimethylsulphoniopropionate (DMSP). The other DMS precursor, dimethylsulphoxide (DMSO), is omitted from the original hypothesis. It was suggested that DMSP upregulation may be driven by increases in temperature or light, causing subsequent increases in seawater concentrations of DMS. This DMS then fluxes from the oceans to the atmosphere, where it is oxidised to sulphate particles that act as cloud condensation nuclei (CCN), leading to increased cloud cover and promoting global/atmospheric cooling (Fig 1.3). However, if temperatures are lower, less DMS is produced and there is less cloud growth, resulting in net warming. The CLAW hypothesis therefore suggests that marine algal production of DMS potentially exerts a homeostatic control of earth's climate, in a biogenically - driven negative feedback loop. Whilst the hypothesis is still hotly debated, it has spawned a generation of research into its various facets, improving our knowledge of marine sulphur cycling. More recently, research has suggested that corals are a significant source of these compounds and do, in fact, affect cloud cover at local and regional scales (Deschaseaux et al. 2012).

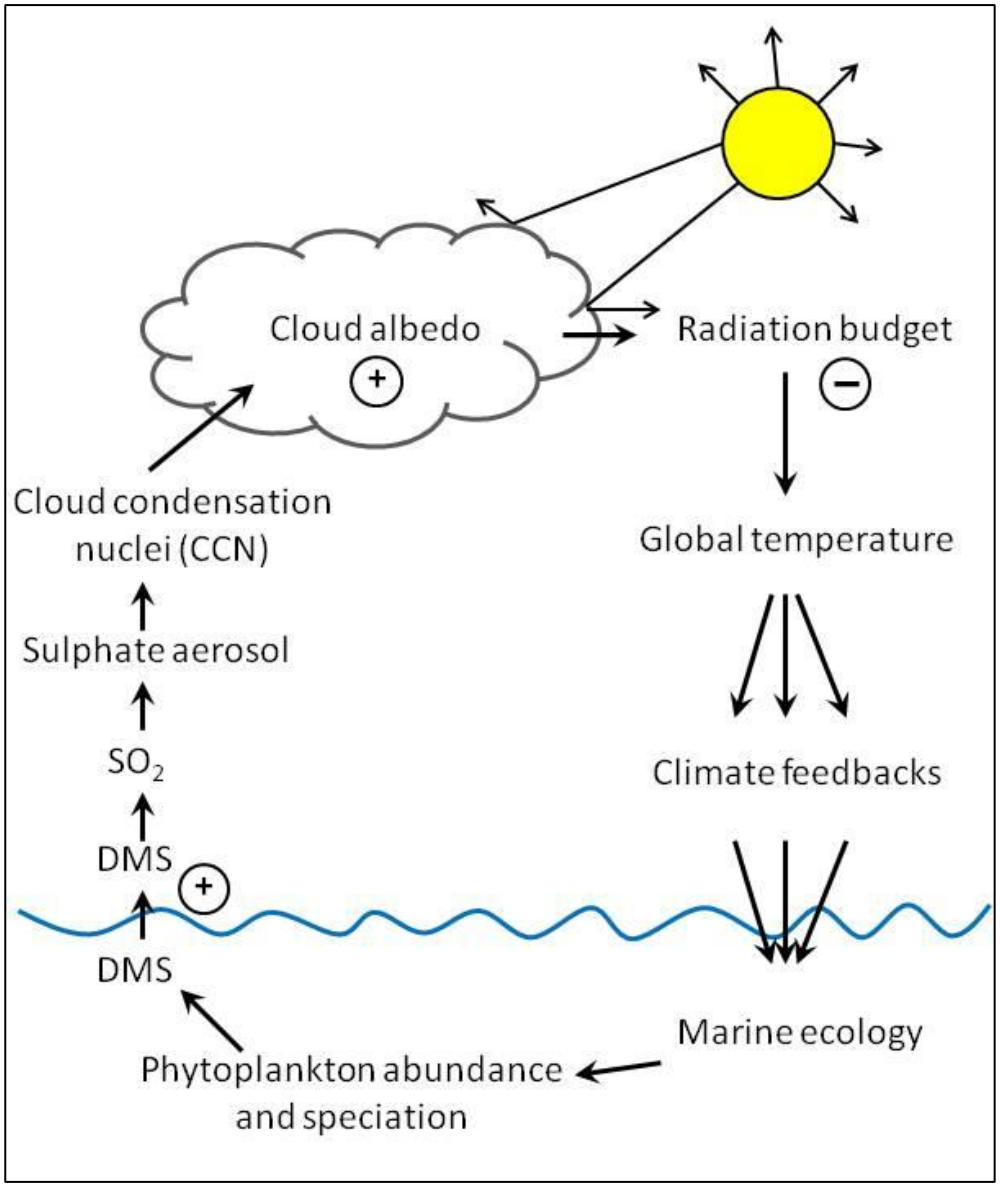


Figure 1.3 Conceptual diagram of the climate feedback loop proposed in the CLAW hypothesis, adapted from Charlson et al. (1987), in which positive and negative effects are marked by a + or a -

1.5 The production of dimethylated sulphur compounds in the marine environment

Dimethylsulphide (DMS) is the main source of marine sulphate aerosols and is the most abundant volatile sulphur species in seawater and accounts for the vast majority of oceanic sulphur emissions (Bates et al. 1992; Liss et al. 1997). DMS is mainly produced by either cleavage of dimethylsulphoniopropionate (DMSP) or reduction of dimethylsulphoxide (DMSO), both of which are produced by marine algae as part of the marine sulphur cycle

(Hatton et al. 1996, 2004; Stefels 2000). Collectively all three compounds are referred to as dimethylated sulphur compounds (DSC, Fig 1.4).

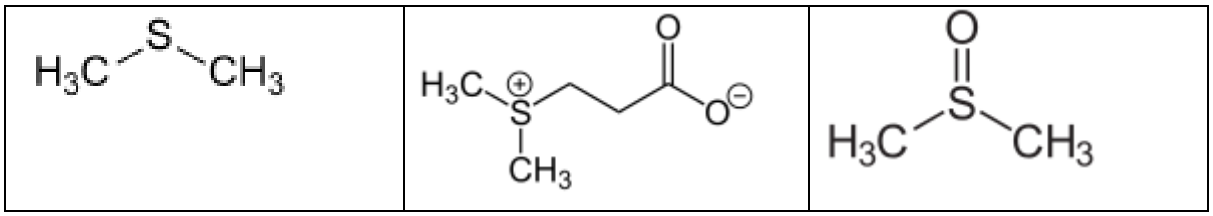


Figure 1.4 Skeletal diagrams of the structure of all three dimethylated sulphur compounds; DMS (left), DMSP (middle) and DMSO (right)

Research has shown that the high levels of atmospheric DMS measured over the Great Barrier Reef (GBR), Coral Sea, Gulf of Papua, Solomon Sea, and Bismarck Sea were correlated with areas of high coral reef biomass (Jones and Trevena 2005), suggesting that, globally, coral reefs were a potential source for atmospheric DMS emissions. Subsequent laboratory (Fischer and Jones 2012) and field (Broadbent et al. 2002; Fischer and Jones 2012; Swan et al. 2012a) studies established a relationship between corals and DMS production. These observations led to the hypothesis that coral derived DMS could influence local climate in the manner proposed by the CLAW hypothesis, making reefs a potentially significant control on climate. However, the CLAW hypothesis is severely limited in its application owing to the absence of the role of DMSO in marine biogeochemical cycling. Given that we now recognise DMSO as a significant compound in marine sulphur cycling (Hatton et al. 1996, 2004) and coral reef sulphur biogeochemistry (Deschaseaux et al. 2014b; Hopkins et al. 2016), it seems unlikely the mechanism underlying the CLAW hypothesis will hold true in reef systems.

1.6 Sources and producers of dimethylsulphoniopropionate (DMSP)

There is no known record of DMS being produced directly by any class of marine algae, rather it is produced as a breakdown product of two precursor compounds; dimethylsulphoniopropionate (DMSP) and dimethylsulphoxide (DMSO). It is believed that DMSP constitutes the predominant source of DMS (Liss et al. 1997) and is mostly found in photosynthetic organisms such as higher plants and various species of marine algae (Keller

et al. 1989; Malin and Kirst 1997; Stefels 2000). Amongst phytoplankton the highest per cell concentrations of DMSP are found in dinoflagellates, with concentrations ranging from 640 mM cell $^{-1}$ in dinoflagellates from seawater (Belviso et al. 1990), to 1082 mM cell $^{-1}$ in *Prorocentrum minimum* (Matrai and Keller 1994). However, subsequent studies have demonstrated that DMSP is produced by both the endosymbiotic microalgae (Broadbent et al. 2002; Steinke et al. 2011) and the host (Raina et al. 2013) in zooxanthellate corals.

1.7 Dimethylsulphoxide (DMSO), as a source and sink for DMS

DMSO is another precursor of DMS and was reported for the first time in the marine environment in 1980 (Andreae 1980; Andreae and Barnard 1983). Historically, DMSO was problematic to measure in seawater samples, not only because it is present in nanomolar concentrations, but also because it is readily soluble in water, is non - ionic and cannot be purged or steam - distilled (Harvey and Lang 1986). Therefore, for a long time the only source of DMSO in seawater was considered to be from the photooxidation of DMS in the euphotic zone of the water column (Shooter and Brimblecombe 1989). With the advent of more advanced analytical techniques (reviewed in Hatton et al. 2004), DMSO has now been shown to be present in seawater in concentrations often exceeding those of DMS (Hatton et al. 1996, 2004; Simó et al. 2000). Further studies have shown that bacteria could play a potentially important role in the oxidation of DMS to DMSO in seawater (Zeyer et al. 1987; Green et al. 2011; Hatton et al. 2012). More recent studies have demonstrated the presence of intracellular DMSO in phytoplankton (Simo et al. 1998a; Lee et al. 1999; Hatton and Wilson 2007) indicating that DMSO is also biogenically produced, possibly through DMS oxidation in the presence of reactive oxygen species (Sunda et al. 2002). DMSO is a highly effective antioxidant and DMS is less energetically costly to produce, so whilst the mechanism behind DMSO production is somewhat speculative, it is likely that it occurs in the presence of either light (photochemical oxidation) or bacteria (bacterial oxidation), with the net result being increased antioxidant capacity under times of cellular stress.

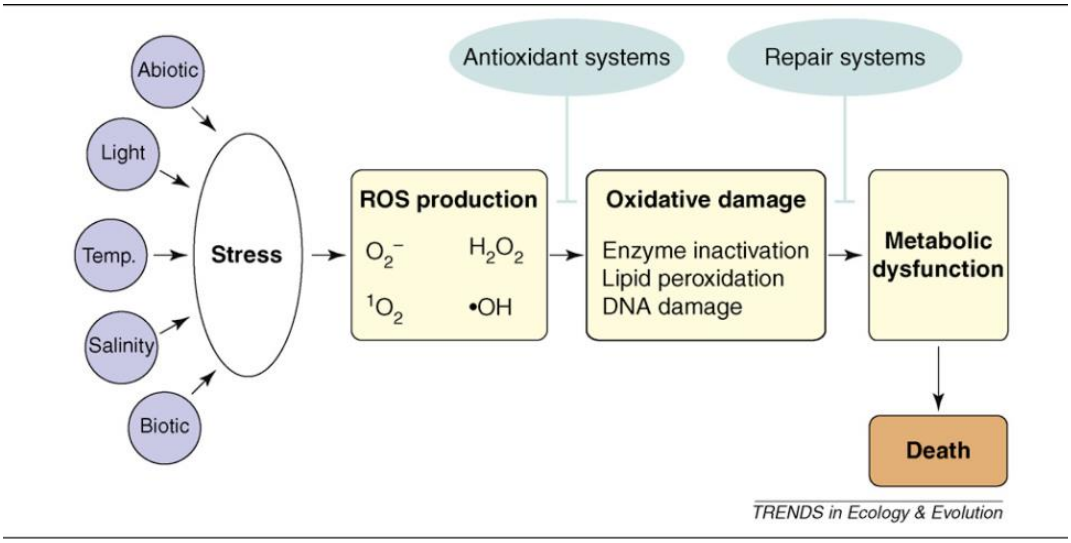
1.8 Functions of DMS, DMSP and DMSO

Seasonal and geographic trends have been reported for the distribution of all three dimethylated sulphur compounds (DSC), that suggest environmental factors such as temperature, pH, salinity and/or light availability could affect concentrations and production in the marine environment (Van Alstyne and Puglisi 2007; Vila-Costa et al. 2008). Further studies have reported that DMSP and DMS concentrations could directly be affected by salinity (Reed 1983; Vairavamurthy et al. 1985), temperature (van Rijssel and Gieskes 2002), pH (Archer et al. 2013; Arnold et al. 2013), and light/nitrogen availability (Keller et al. 1999; Harada et al. 2009). A variety of roles have been proposed for DSC; for example, DMSP and DMSO have been hypothesised to aid osmo - and thermo - regulation (Simo et al. 1998a; Lee et al. 1999; Stefels 2000), whilst other studies have shown that DMSP could act as a chemical defence for marine plankton and macroalgae against herbivores (Wolfe et al. 1997; Alstyne et al. 2001; Van Alstyne and Houser 2003). Conversely, DMSP and DMS have also been shown to act as chemo - attractants in a range of marine species (DeBose and Nevitt 2007; Seymour et al. 2010a), including the pathogenic coral bacteria, *Vibrio* (Garren et al. 2014). DMS and DMSP have even been shown to play a crucial role in fish and squid spawning during coral spawning events (Paul et al. 2008). Crucially in studies of all three compounds in coral reef ecosystems, some studies indicate that all three compounds may have an antioxidant function, helping organisms cope with high ultraviolet (UV) radiation by scavenging the harmful hydroxyl radical (Sunda et al. 2002; Vallina and Simó 2007; Deschaseaux et al. 2014b; Jones et al. 2014). This antioxidant role has led to a number of studies into DMSP under changing light and temperature conditions in coral reef environments, as it is believed to be linked to the mechanism involved in coral bleaching (Deschaseaux et al. 2014a; Jones et al. 2014).

1.9 The antioxidant function of DMS, DMSP & DMSO

In corals, reactive oxygen species (ROS) accumulation (Fig 1.5) is generally attributed to an increase in sea temperature; superoxide, hydrogen peroxide and OH radicals are the most commonly produced ROS. Accumulation of too many ROS can lead to a range of different types of cell damage, such as DNA damage and enzyme inactivation. Unchecked or without repair, metabolic dysfunction and cell death occurs.

Figure 1.5 A generalised sequence of stress (Baird et al. 2009)



Potentially toxic ROS are often removed by antioxidant systems. In marine algae (Sunda et al. 2002) intracellular DMSP production is increased in response to various oxidative stressors including ultraviolet radiation and hydrogen peroxide. In the presence of these ROS, DMSP, DMS and DMSP oxidise to DMSO (Fig 1.6), can further oxidise to methane sulphinic acid (MSNA).

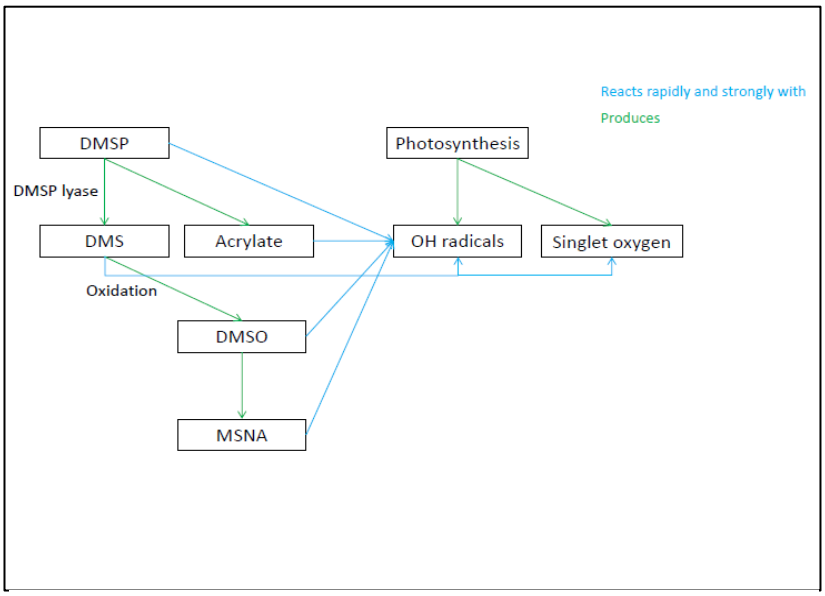


Figure 1.6 Diagram outlining the antioxidant cascade mechanism in marine algae.

Each sulphur compound (DMS, DMSP, DMSO, MSNA and acrylate) effectively "mop up" ROS in the algal cell, meaning production of one compound is a relatively energy efficient way to deal with oxidative stress. The importance of this mechanism for organisms living in high UV and high temperature environments, such as coral reefs, cannot be understated; all reef organisms are subjected to extremes of temperature and light, but in a nitrogen limited environment, production of glycine betaine is impossible. Its sulphur analogue, DMSP, however, is not and so production of DMSP confers a level of cellular protection that enables organisms to exist under conditions that would usually cause permanent harm.

1.10 Coral reef DMSP producing organisms

Dinoflagellates of the genus *Symbiodinium* (Fig 1.7) form mutualistic symbioses with a wide range of marine invertebrates including sponges, cnidarians and molluscs, as well as some protist hosts (Pochon et al. 2006). Initially it was thought that *Symbiodinium* was a single pandemic species, *Symbiodinium microadriaticum* (Freudenthal 1962). This was largely a result of the simple and almost featureless morphology of individual cells. We know now that the genus *Symbiodinium* is more diverse (> 400 species), as evidenced by numerous studies based on ultramorphology (Schoenberg and Trench 1980; Trench and Blank 1987), physiology (Iglesiasprieto and Trench 1994; Banaszak and Trench 1995), biochemistry

(Bishop and Kenrick 1980; Withers et al. 1982; Govind et al. 1990) and molecules (Baker 2003).

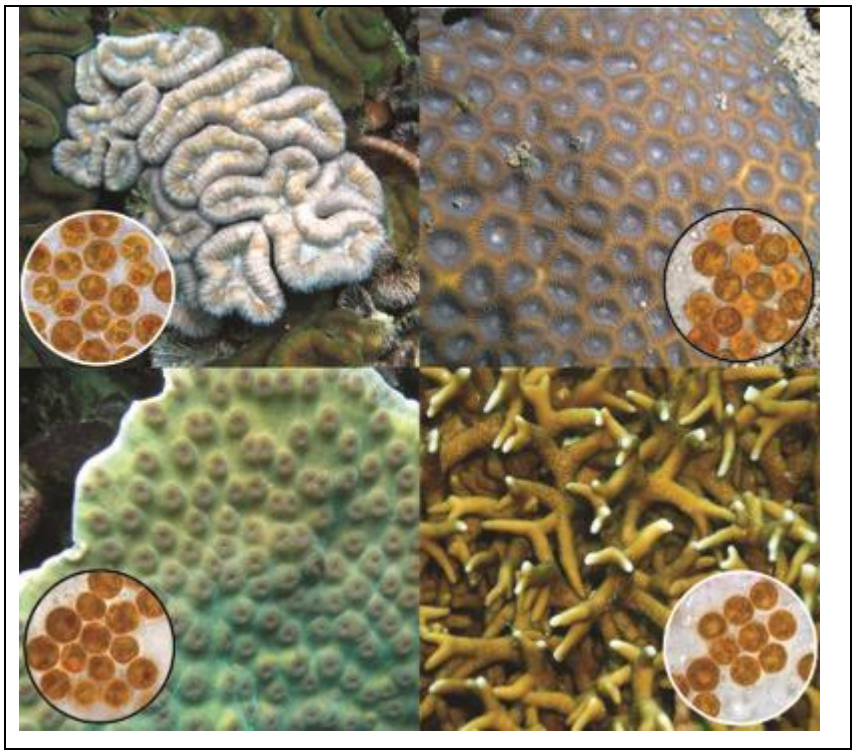


Figure 1.7 Four examples of Symbiodinum among corals. (LaJeunesse, 2012).

Nine phylogenetic lineages or clades (A-I) have now been distinguished, with the distribution of each clade following some broad geographic trends; the tropical Indo-Pacific is dominated by clade C, whilst clades A and B are more prevalent in the tropical Atlantic and temperate parts of the Indo-Pacific (Baker 2001; Rodriguez-Lanetty et al. 2001; LaJeunesse 2002; LaJeunesse et al. 2003, 2004a, 2004b; McClanahan et al. 2003; Fabricius et al. 2004; Chen et al. 2005b; Banaszak et al. 2006; Thornhill et al. 2006b, 2006a; Garren et al. 2006). Clade C is generally found at deeper depths (>7m) in all oceans, whilst clades A, B and D tend to be found at shallower depths (<7m) (Baker 2003; Rowan & Knowlton 1995; Rowan et al. 1997; Baker & Rowan 1997; LaJeunesse 2002).

From the perspective of coral reef sulphur cycling, *Symbiodinium* are a vital component of the reef, being capable of producing high intracellular concentrations of DMSP (Table 1.1)(Broadbent et al. 2002; Deschaseaux et al. 2014a). In fact, dinoflagellates associated with corals have been shown to be the main producers of this compound in the coral holobiont (Broadbent et al. 2002), however more recent research has shown that not only is

the coral animal also capable of producing DMSP in the absence of any symbiont *in hospite* (Raina et al. 2013), but non-eukaryotes are now known to produce DMSP (Curson et al. 2017). Reports of intracellular concentrations of DMSO in corals/zooxanthellae are limited (Deschaseaux et al. 2014b), but detectable levels of DMSO are present, albeit in slightly lower concentrations than DMSP.

Table 1.1 Summary of cellular DMSP concentrations reported in the literature for *Symbiodinium* species associated with corals and coral reef organisms. CV = cell volume. Values in bold were calculated based on the original published data in Yost & Mitchelmore (2009) by Deschaseaux et al. (2014) and published therein. Errors are standard deviation unless stated otherwise. Data sources are: 1) Broadbent & Jones (2002); 2) Deschaseaux et al. (2014); 3) Hill et al. (1995); 4) Steinke et al. (2011); 5) Van Alstyne (2006); 6) Yost et al. (2012).

Species/clade	***[DMSP] mmol L^{-1} CV***	***[DMSP] fmol $cell^{-1}$***	***Host origin***	***Original host***	***Common name (host)***	***Study***
Symbiodinium pilosum - clade A	-	164	GBR	Zonathus sociatus	Coral	1
Symbiodinium sp.	81	40	GBR	Lobophytum sp.	Coral	1
Symbiodinium sp.	72	43	GBR	Favites sp.	Coral	1
Symbiodinium sp.	36	21	GBR	Favites sp.	Coral	1
Symbiodinium sp.	7590	3831	GBR	Acropora palifera	Coral	1
Symbiodinium sp.	5968	2831	GBR	Acropora palifera	Coral	1
Symbiodinium sp.	1193	641	GBR	Acropora palifera	Coral	1
Symbiodinium sp.	673	436	GBR	Acropora palifera	Coral	1
Symbiodinium sp.	419	235	GBR	Acropora palifera	Coral	1
Symbiodinium sp.	356	171	GBR	Acropora palifera	Coral	1
Symbiodinium sp.	296	179	GBR	Pocillopora	Coral	1
Symbiodinium sp.	181	99	GBR	Pocillopora	Coral	1
Symbiodinium sp.	158	89	GBR	Pocillopora damicornis	Coral	1

D1	140 ± 16.6 SE	-	GBR	Acropora millepora	Coral	2
C1	220 ± 20.1 SE	-	GBR	Acropora tenuis	Coral	2
Symbiodinium sp.	-	117	Kaneohe Bay - Hawaii	Pocillopora damicornis	Coral	3
Symbiodinium sp.	-	77	Kaneohe Bay - Hawaii	Pocillopora compressa	Coral	3
Symbiodinium sp.	-	73	Kaneohe Bay - Hawaii	Montipora verrucosa	Coral	3
A2 - Mf	158 ± 3.8 SE	-	Florida	Montastrea faveolata	Coral	4
Symbiodinium sp.	-	950 ± 615	Guam - USA	Acropora cerealis	Coral	5
Symbiodinium sp.	-	425 ± 132	Guam - USA	Acropora valida	Coral	5
Symbiodinium sp.	-	417 ± 162	Guam - USA	Acropora digitifera	Coral	5
Symbiodinium sp.	-	310 ± 119	Guam - USA	Heliopora coerulea	Coral	5
Symbiodinium sp.	-	211 ± 230	Guam - USA	Leptastrea purpurea	Coral	5
Symbiodinium sp.	-	107 ± 39	Guam - USA	*Porites* cylindrica	Coral	5
Symbiodinium sp.	-	88 ± 28	Guam - USA	*Porites* rus, decumbent	Coral	5
Symbiodinium sp.	-	80 ± 22	Guam - USA	Pocillopora meandrina	Coral	5
Symbiodinium sp.	-	69 ± 24	Guam - USA	*Porites* rus, upright	Coral	5

Symbiodinium sp.	-	49 ± 19	Guam - USA	Psammocora digitata	Coral	5
Symbiodinium sp.	-	43 ± 19	Guam - USA	Pavona decusata	Coral	5
Clade A194	-	46.16 ± 29.27	Bermuda	*Porites* astreoides	Coral	6
Clade B184	-	24.32 ± 7.66	Bermuda	Diploria sp., Montastrea franksi	Coral	6
Clade C180	-	62.10 ± 43.5	Bermuda	Montastrea cavernosa	Coral	6
Clade A194	-	99.4 ± 44.8	Bermuda	*Porites* astreoides	Coral	6
Clade B184	-	80.0 ± 50.6	Bermuda	Diploria sp., Montastrea franksi	Coral	6
Clade C180	-	139 ± 54.1	Bermuda	Montastrea cavernosa	Coral	6
Clade A194	149.3 ± 49.0	-	Bermuda	*Porites* astreoides	Coral	6
Clade B184	195.2 ± 106.5	-	Bermuda	Diploria sp., Montastrea franksi	Coral	6
Clade C180	105.9 ± 42.3	-	Bermuda	Montastrea cavernosa	Coral	6

Table 1.2 Summary of DMSP concentrations across a range of coral reef taxa from reef sites around the world. Data show total DMSP concentrations (unless specific otherwise) measured in coral, macroalgal and other (mucus, pore waters etc.) samples according to site. Each study used a variety of DMSP normalisation indices; here, the same or similar indices have been used between studies for ease of comparison.

Sample	***DMSP (total) concentration***	***Site***	***Study***
Mucus ropes	44860 nM	Kelso Reef, GBR	Broadbent et al. 2004
Mucus ropes	2236 nM	One Tree Reef, GBR	Broadbent et al. 2004
Pore waters	105 nM	Nelly Bay Reef, GBR	Broadbent et al. 2004
Pore waters	480 - 900 nM	Kelso Reef, GBR	Broadbent et al. 2004
Pore waters	630 - 1200 nM	One Tree Reef, GBR	Broadbent et al. 2004
Coral mucus - Acropora	25443 nM	Kelso Reef, GBR	Broadbent et al. 2004
Coral mucus - P.damicornis	1226 nM	Kelso Reef, GBR	Broadbent et al. 2004
Coral mucus - A.formosa	19606 nM	Kelso Reef, GBR	Broadbent et al. 2004
Eggs - Acropora palmata	359 µmol per 100 uL eggs	Florida Keys	DeBose et al. 2015
Larvae - A. palmata	1.20 µmol per larva	Florida Keys	DeBose et al. 2015
Chlorophyta - Halimeda tuna	1.797 nmol cm^{-1}	One Tree Reef, GBR	Broadbent et al. 2002
Chlorophyta - H.sp	0.826 nmol cm^{-2}	Kelso Reef, GBR	Broadbent et al. 2002
Chlorophyta - H.sp	0.232 nmol cm^{-2}	Kelso Reef, GBR	Broadbent et al. 2002
Chlorophyta - Chlorodesmis fastigiata	0.024 µmol g^{-1} fw	One Tree Reef, GBR	Broadbent et al. 2002
Chlorophyta - Chlorodesmis spp.	0.009 µmol g^{-1} fw	Kelso Reef, GBR	Broadbent et al. 2002

Amphiroa sp.	12.6 & 24.9 mg S g^{-1}	Suleman Reef, Egypt	Burdett et al. 2013
Phaeophyta - Turbinaria	< 0.3 & > 0.5 mg S g^{-1}	Suleman Reef, Egypt	Burdett et al. 2013
	1.536 nmol cm^{-2}	One Tree Reef, GBR	Broadbent et al. 2002
	0.161 nmol cm^{-2}	Kelso Reef, GBR	Broadbent et al. 2002
Phaeophyta - Padina	0.989 nmol cm^{-2}	One Tree Reef, GBR	Broadbent et al. 2002
	2.472 nmol cm^{-2}	Nelly Bay Reef, GBR	Broadbent et al. 2002
	1.330 nmol cm^{-2}	Kelso Reef, GBR	Broadbent et al. 2002
Phaeophyta - Sargassum spp.	1.823 nmol cm^{-2}	Nelly Bay Reef, GBR	Broadbent et al. 2002
Rhodophyta - L.kotchyanum	2.061 nmol cm^{-2}	One Tree Reef, GBR	Broadbent et al. 2002
Rhodophyta - L.mollucense	2.955 nmol cm^{-2}	One Tree Reef, GBR	Broadbent et al. 2002
Laurencia *spp.*	*0.007* µmol g^{-1} fw	One Tree Reef, GBR	Broadbent et al. 2002
Spermatophyta			
Halodule wrightii	3.3 µmol g^{-1} fw	St Croix, US Virgin Islands	Dacey et al. 1994
Syringodium filiforme	0.1 µmol g^{-1} fw	St Croix, US Virgin Islands	Dacey et al. 1994
Thalassia testudinum - Non - epiphitized leaf	0.18, 0.21 µmol g^{-1} fw	St Croix, US Virgin Islands	Dacey et al. 1994
Thalassia testudinum - Epiphytized leaf	0.64, 1.4 µmol g^{-1} fw	St Croix, US Virgin Islands	Dacey et al. 1994
Thalassia testudinum -	2.9, 4.0 µmol g^{-1} fw	St Croix, US Virgin Islands	Dacey et al. 1994

Epihytized, moribund leaf			
Thalassia *testudinum* - *Rhizome*	*0.01* µmol g^{-1} fw	St Croix, US Virgin Islands	Dacey et al. 1994
Platygyra sinensis	0.355 nM mm^{-2}	GBR	Tapiolas et al. 2013
Echinopora spp.	0.467 nM mm^{-2}	GBR	Tapiolas et al. 2013
Acropora millepora	2.473 nM mm^{-2}	GBR	Tapiolas et al. 2013
Montipora spp.	0.092 nM mm^{-2}	GBR	Tapiolas et al. 2013
Seriatopora hystrix	0.362 nM mm^{-2}	GBR	Tapiolas et al. 2013
Pocillopora damicornis	0.333 nM mm^{-2}	GBR	Tapiolas et al. 2013
	126 nmol cm^{-2}	Nelly Bay Reef, GBR	Broadbent et al. 2002
	56 nmol cm^{-2}	Kelly Reef, GBR	Broadbent et al. 2002
	43 nmol cm^{-2}	One Tree Reef, GBR	Broadbent et al. 2002
Stylophora pistillata	0.774 nM mm^{-2}	GBR	Tapiolas et al. 2013
Porites spp.	0.271 nM mm^{-2}	GBR	Tapiolas et al. 2013
Merulina ampliata	0.042 nM mm^{-2}	GBR	Tapiolas et al. 2013
Pachyseris spp.	0.08 nM mm^{-2}	GBR	Tapiolas et al. 2013
Physogyra lichtensteini	1.048 nM mm^{-2}	GBR	Tapiolas et al. 2013
Fungia spp.	0.353 nM mm^{-2}	GBR	Tapiolas et al. 2013
Symphyllia recta	1.517 nM mm^{-2}	GBR	Tapiolas et al. 2013
Galaxea fascicularis	0.156 nM mm^{-2}	GBR	Tapiolas et al. 2013
Acropora aspera	97.4 nM cm^{-2}	Heron Island, GBR	Deschaseaux et al. 2014
Montastera cavernosa	20.2 nmol per µg chl a	Three Hill Shoals, Bermuda	Yost & Mitchelmore, 2010

Montastrea mirabilis	11.3 nmol per µg chl a	Three Hill Shoals, Bermuda	Yost & Mitchelmore, 2010
Porites astreoides	24.2 nmol per µg chl a	Three Hill Shoals, Bermuda	Yost & Mitchelmore, 2010
Montastrea franksi	15.0 nmol per µg chl a	Three Hill Shoals, Bermuda	Yost & Mitchelmore, 2010
Acropora formosa	330 nmol cm^{-2}	Nelly Bay Reef, GBR	Broadbent et al. 2002
	533 nmol cm^{-2}	Kelly Reef, GBR	Broadbent et al. 2002
Acropora palifera	3842 nmol cm^{-2}	Nelly Bay Reef, GBR	Broadbent et al. 2002
	3538 nmol cm^{-2}	One Tree Reef, GBR	Broadbent et al. 2002
Lobophytum sp.	70 nmol cm^{-2}	Orpheus Island, GBR	Broadbent et al. 2002
Acropora pulchra	34 nmol cm^{-2}	One Tree Reef, GBR	Broadbent et al. 2002

1.11 Algae

Of course, a coral reef is not exclusively composed of corals and investigations into DMSP production by algae in reef systems have also been conducted (Table 1.2, Broadbent et al. 2002; Burdett et al. 2013); Broadbent et al. (2002) reported that the dominant producers of DMSP amongst macroalgal species in reef ecosystems are the *Rhodophyta* (Broadbent et al. 2002). Additionally, Burdett et al. (2013) noted that water column DMS/P concentrations were highest over areas containing predominantly seagrasses and algae, rather than corals. This might seem, at first glance, counterintuitive as corals generally have higher intracellular concentrations of DMSP (Tables 1.1 & 1.2). However, total water column concentrations of DMS/P are subject to a variety of biotic and abiotic processes (summarised in Fig 1.5 and discussed later in this chapter).

1.12 Other producers

Sulphur compounds have not only been detected in coral, algae and reef waters on the GBR, but also in coral mucus, surface biofilms, sediment pore waters (Broadbent and Jones 2004), coral eggs and larvae (Debose et al. 2015). In fact, it would appear, based on the published values in Table 1.2, that corals are not the only significant source of DMSP in a reef

environment, and that the role of non - coral sources of DMSP is potentially understudied with respect to this compound. Furthermore, variable amounts of DMSP have been reported for a variety of corals (Table 1.2), but there is a distinct “skew” towards the Great Barrier Reef and nothing, so far has been reported for the Caribbean despite the meso - American Barrier Reef being the second largest barrier reef system in the world.

1.13 Marine biogeochemical cycling of DMS, DMSP and DMSO

The marine biogeochemistry of DMS/P/O is now recognised as a highly complex system involving a suite of biotic and abiotic factors (Fig 1.6), with key similarities and differences between DMS, DMSP and DMSO in terms of their production and removal.

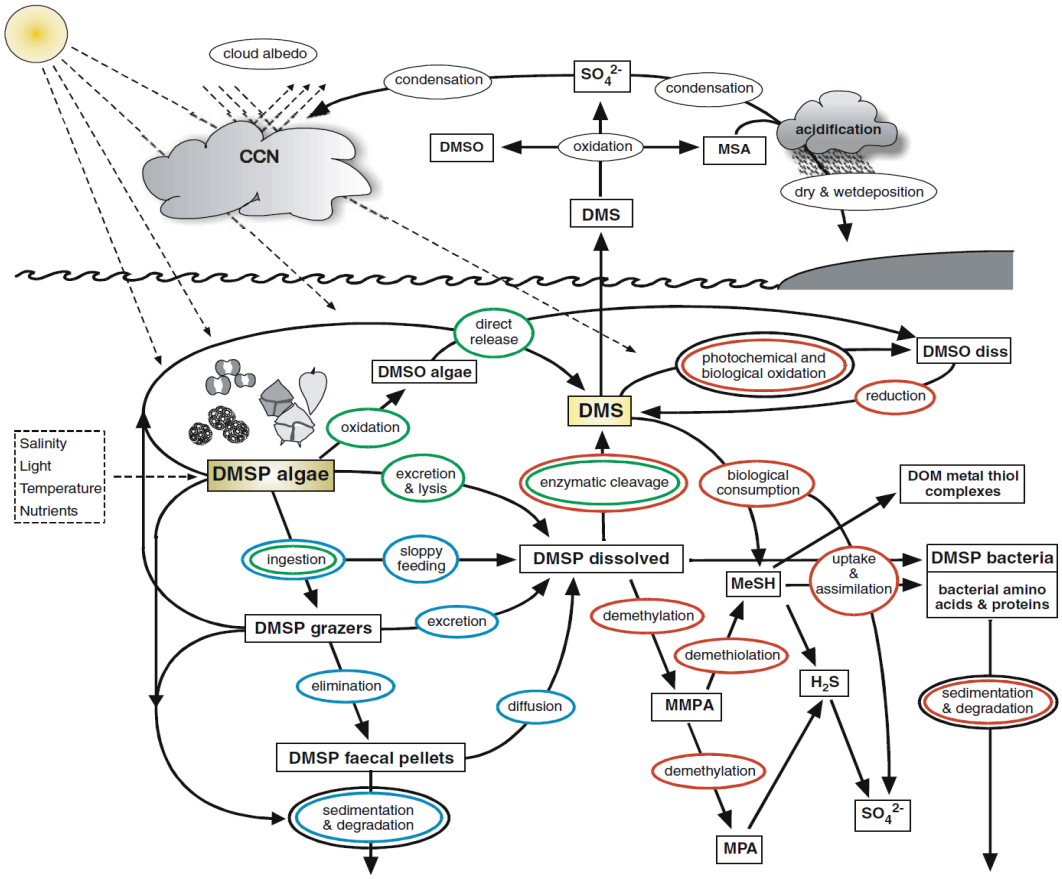


Figure 1.8 Schematic representation of the processes and pools involved in the marine biogeochemical cycling of DSC. The dominant role of functional groups in the different processes is indicated by coloured ellipses: green = phytoplankton; blue = zooplankton; red = bacteria; black = abiotic factors. Abbreviations are: CCN, cloud - condensation nuclei; DOM, dissolved organic material; DMSO, dimethylsulphoxide; MeSH, methanethiol; MPA, mercaptopropionate; MMPA, methylmercaptopropionate and MSA, methanesulphonic acid. Figure and explanatory text taken from (Stefels et al. 2007).

Whilst similar in terms of their cellular functions, key differences exist between DMSP and DMSO with respect to how they are cycled in the oceans. DMSP synthesis is exclusively *de novo* in the cells of marine algae (Stefels 2000; Yoch 2002), by the coral animal (Raina et al. 2013) or by bacteria (Curson et al. 2017). DMSO, on the other hand, can also be produced via biological/photochemical oxidation of DMS (Hatton et al. 1996, 2012; Simo et al. 1998a), thereby acting as both a source and sink for DMS. Due to its biochemical property as a dipolar aprotic hydroscopic substance, DMSO is known to permeate easily through membranes of healthy cells and into the surrounding seawater, where quantities are often higher than those of DMSP (Hatton et al. 1996; Hatton and Wilson 2007). Conversely, since cell membranes are hydrophobic and DMSP is a zwitterion (carrying both positive and negative charges), it is highly unlikely that DMSP will diffuse into surrounding seawater during normal metabolism (Dacey and Blough 1987) and is therefore found in much higher intracellular quantities than DMSO (Hatton and Wilson 2007), with the additional requirement that it must be released from the cell in order to enter seawater.

Both compounds occur in seawater in two fractions; a dissolved (extracellular) and particulate (associated with an algal/bacterial cell or a grazer) phase, with each fraction determined by what will (dissolved) or won't (particulate) pass through a filter; a pore size of 0.7 µm has been recommended (Keller et al. 1989; Kiene and Slezak 2006). Conversion of DMSPp (particulate) to DMSPd (dissolved) is accomplished via active exudation (Laroche et al. 1999), various forms of lysis (Stefels et al. 2007 and references therein) or grazing. Grazing can be by micro - (i.e bacteria, Belviso et al. 1990; Ledyard and Dacey 1994), meso - and macrozooplankton (Dacey and Wakeham 1986). DMSPp can also be transformed to the dissolved fraction following catabolism to DMS in the algal cell in the presence of the DMSP - lyase enzyme (Stefels and Boekel 1993; Stefels and Dijkhuizen 1996). Bacteria are crucial for the enzymatic cleavage of DMSP to DMS, and are also key consumers of these compounds, using them as carbon sources for energy and growth. It has been suggested that ~ 60 % of oceanic bacteria can participate in DMSP degradation (Moran et al. 2012), such that the dominant fate for ~ 75 % of dissolved DMSP is assimilation by bacteria (Kiene and Linn 2000). Once in the dissolved fraction, bacteria living on the coral or in seawater can cleave DMSPd to DMS, which is removed from marine waters by flux into the atmosphere, by oxidation into sulphate ions (SO_4^{2-}) or by biological (microbial) or photochemical

oxidation to dimethylsulphoxide (DMSO) in the presence of UVA/UVB light (Hatton 2002; del Valle et al. 2009). It is now hypothesised that DMS oxidation to DMSO may be the major pathway by which DMS is removed from surface waters (del Valle et al. 2009). Hatton (2002) found that in the northern North Sea, DMS photolysis is a significant removal pathway when compared with atmospheric ventilation and bacterial consumption, suggesting that photochemical oxidation to produce DMSO is a significant source for this compound. This is likely to be of even greater importance in coral reefs, where UV light levels are considerably higher.

1.14 Climate Change and Ocean Acidification

Anthropogenic activities such as deforestation and fossil fuel burning are proceeding at rates that outpace natural biogeochemical removal (sequestration) processes, such that concentrations of CO_2 in the Earth's atmosphere are increasing. Two significant consequences of this are increased sea surface temperature (SST) and ocean acidification (OA). Increasing SST has already been observed over most of the globe and is a direct result of elevated CO_2 levels altering the Earth's radiative balance and leading to a net warming of Earth's atmosphere. Oceanic uptake of anthropogenic CO_2 emissions is leading to an alteration in seawater carbonate chemistry, manifested as increasing $[H^+]$, falling $[CO_3^{2-}]$ and a decrease in surface seawater pH (ocean acidification - OA). OA and increasing SST present serious problems unique to marine environments, with uncertain consequences for marine DSC biogeochemistry.

1.15 Global environmental change and ocean acidification

In the atmosphere most carbon is in the form of carbon dioxide (CO_2), with minor contributions from methane (CH_4), carbon monoxide (CO) and other gases (Holmén 2000). CO_2 is a very important atmospheric gas that strongly influences the radiative balance of earth, as well as controlling oceanic carbonate equilibrium.

Whilst natural climate fluctuations occur over millennial timescales, modern climate change due to anthropogenic activities have proceeded more rapidly. Since the start of the

Industrial Revolution (1750), atmospheric CO_2 concentrations have steadily risen because of land use changes, burning of fossil fuels and cement production. Consequently, atmospheric concentrations of CO_2 are now at ~ 400 ppm, its highest level in 650,000 years. Importantly, the rate of change is one hundred times more rapid than at any other time during the last few hundred millennia of Earth's history (Raven et al. 2005a). It is this rate of change that distinguishes natural from anthropogenically forced climate change. For the purposes of this thesis, when talking about climate change we take it to mean anthropogenically forced climate change.

1.16 Global warming and increases to SST

The term global warming refers to the increases in average temperature of the air and sea (Fig 1.9) at the Earth's surface and is largely attributed to increased anthropogenic concentrations of various greenhouse gases (GHGs, IPCC 2013).

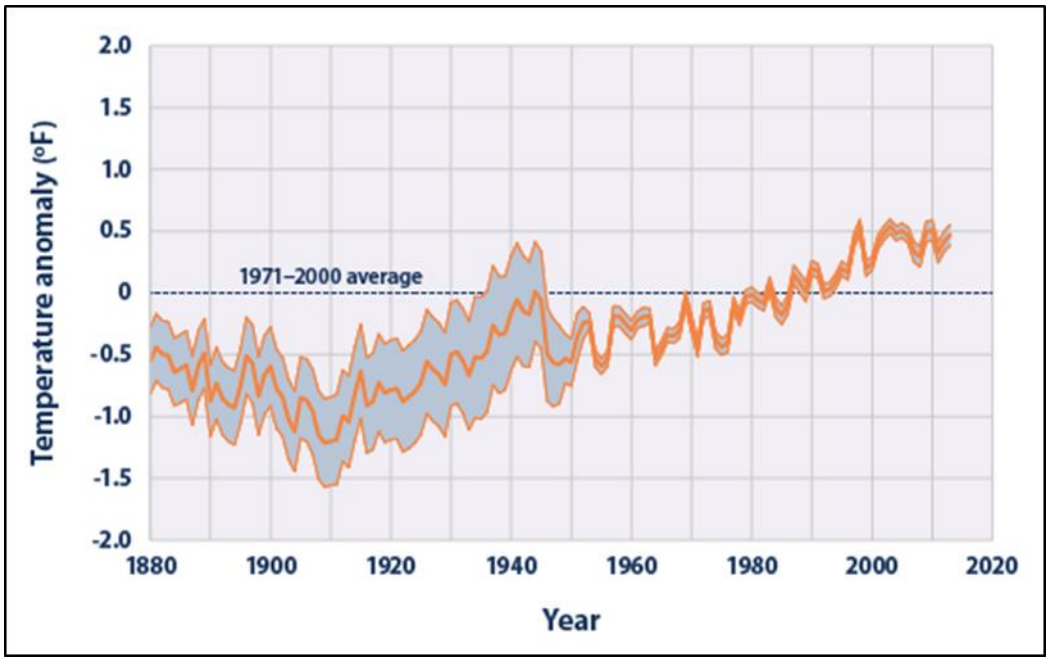


Figure 1.9 Average global sea surface temperature, 1880 – 2013. This graph uses the 1971 to 2000 average as a baseline for depicting change – changing the baseline will not alter the shape of the data over time. Shading shows the range of uncertainty in the data (NOAA 2014).

GHGs are important and essential to life on Earth; the greenhouse effect is the process by which absorption and emission of infrared radiation by GHGs in the atmosphere warm the lower atmosphere and surface. The major GHGs are water vapour, methane, ozone and CO_2 and it is now generally accepted that increased emissions of CO_2 are responsible for global warming (IPCC 2013).

The role of the oceans in mitigating this increase is significant; about 93 % of the excess heat energy stored by earth over the last 50 years is found in the ocean (Levitus et al. 2009; IPCC 2013). Depth averaged 0 - 700 m ocean temperature trends from 1971 to 2010 are positive over most of the globe and the global average warming over this period was 0.11°C per decade in the upper 75 m (Levitus et al. 2009; IPCC 2013). Whilst no long - term investigations have yet been conducted to understand how seawater DSC concentrations have changed over time, numerous studies have highlighted the relationship between increases in temperature and upregulation of DSC (see Green and Hatton 2014 and references therein).

1.17 Carbon flux to the oceans

Since 1750, approximately half of all anthropogenic CO_2 emissions have remained in the atmosphere, with the rest being removed from the atmosphere by sinks and stored in the natural carbon cycle reservoirs (IPCC 2013). Of these natural carbon reservoirs the oceans are critical and have, so far, absorbed around 30 % of all anthropogenic CO_2 (Sabine et al. 2004; Raven et al. 2005a). Furthermore, it is “virtually certain” that the increased storage of carbon by the ocean will exacerbate OA in the future under all projected scenarios (IPCC 2013). It has been suggested that the continued uptake of CO_2 will result in a doubling of surface ocean CO_2 partial pressure over the next 50 y (Kleypas et al. 2005a). Moreover, long term model projections show that the oceans are expected to absorb about 90 % of anthropogenic CO_2 over the next millennium (Kleypas et al. 2005). How changes in pCO_2 will affect DSC biogeochemistry remain uncertain, with few studies to have considered the relationship between pCO_2 and DSC (Burdett et al. 2013).

1.18 Future emissions scenarios

Between 1750 and 2011 total anthropogenic emissions of CO_2 were 555 ± 85 Pg C (1 PgC = 10^{15} g C); fossil fuel combustion contributed 375 ± 30 Pg C (IPCC 2013). A variety of different emissions scenarios for the coming century have been proposed, but even the most optimistic scenario (RCP2.6,IPCC 2013) still forecasts fossil fuel emissions globally of 140 – 410 Pg C. Less optimistic forecasts using the CMIP5 model imply cumulative fossil fuel emissions of between 780 - 1685 Pg C, with a "very high confidence" that ocean carbon uptake of anthropogenic CO_2 emissions will continue under all scenarios (IPCC 2013). It is currently unclear how increases in CO_2 emissions and seawater pCO_2 will impact marine DSC biogeochemistry, making future projections/forecasts extremely difficult.

1.19 Ocean acidification

The mechanism by which ocean acidification (OA) operates lies in the buffering capacity of the oceans (see Chapter 2 for a more detailed explanation); when CO_2 dissolves in seawater it forms carbonic acid, part of which is neutralised by the buffering effect of seawater but most of which serves to increase the acidity (Raven et al. 2005a). Over the past 250 years CO_2 levels have increased by nearly 40 % (Fig 1.10), resulting in a drop in average ocean pH of 0.1 units (Caldeira and Wickett 2003; Doney et al. 2009) and is expected to decrease a further 0.3 - 0.4 units (Orr et al. 2005) if atmospheric CO_2 concentrations reach 800 ppm.

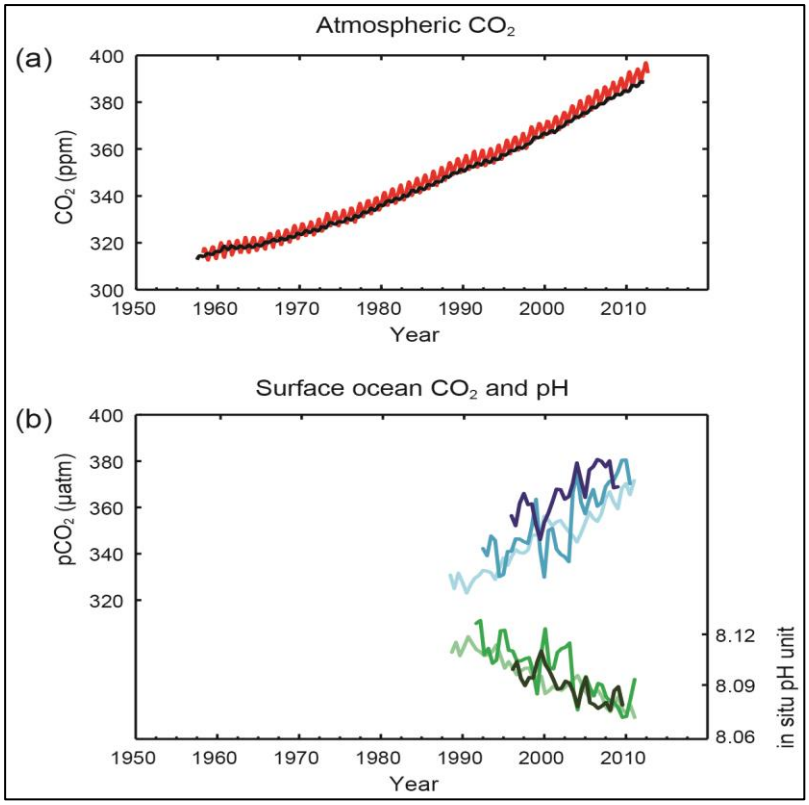


Figure 1.10 Multiple observed indicators of a changing global carbon cycle: (a) atmospheric concentrations of carbon dioxide (CO2) from Mauna Loa (19°32'N, 155°34'W – red) and South Pole (89°59'S, 24°48'W – black) since 1958; (b) partial pressure of dissolved CO2 at the ocean surface (blue curves) and in situ pH (green curves), a measure of the acidity of ocean water. Measurements are from three stations from the Atlantic (29°10'N, 15°30'W – dark blue/dark green; 31°40'N, 64°10'W

There are a range of studies to have investigated the effects of OA on DMS/P production in algal cells and the water column, however results are inconsistent and often conflicting. For example, Archer et al. (2014) reported decreased DMS production that was coupled with increased DMSP concentrations in a mesocosm experiment in Norway. In direct contrast with this, results from shipboard bioassays conducted in the north Atlantic showed increased DMS concentrations coupled with decreased DMSP concentrations under OA conditions (Hopkins and Archer 2014). Clearly, further experiments are required so that more definitive conclusions may be drawn regarding the future of the global sulphur cycle in the context of increasing ocean acidity.

1.20 Impacts of climate change on coral reef sulphur biogeochemistry

It is now generally accepted that increased emissions of CO_2 are responsible for global warming (IPCC 2013). A variety of different CO_2 emissions scenarios for the coming century have been proposed, but even the most optimistic of those (RCP2.6, IPCC 2013) still forecasts increased fossil fuel emissions globally with increases in SST of ~ 2°C by the end of the century. The role of the oceans in mitigating this increase is significant; about 93 % of the excess heat energy stored by earth over the last 50 years is found in the ocean (Levitus et al. 2009; IPCC 2013). In short, the oceans are getting warmer everywhere and this is having serious and deleterious effects on coral reefs – this subject has been covered in multiple papers and will not be covered again here (see Hoegh - Guldberg et al. 2007; Carpenter et al. 2008 & Lesser 2011 and references therein for detailed synopses). Whilst elevated temperatures are not well correlated with increased atmospheric DMS concentrations, there have been several studies to investigate the effects of elevated temperature on DMSP concentrations in *Symbiodinium*/corals/coral reefs, which have shown increases in DMSPp and seawater total DMSP (DMSP; DMS + DMSP) concentrations (McLenon and DiTullio 2012; Burdett et al. 2013; Deschaseaux et al. 2014b). These findings are in line with other studies that have shown DMSP upregulation in response to elevated temperature in a wide range of algal taxa (Stefels 2000; van Rijssel and Gieskes 2002; Spielmeyer and Pohnert 2012a). Temperature is one of the main stressors associated with coral bleaching (Douglas 2003), which is known to be intimately linked with the build - up of reactive oxygen species (ROS) in the coral holobiont (Lesser 1997). As a protective mechanism against oxidative damage, a wide range of antioxidants, including DMSP and DMSO, are produced in corals (Suzanne and Deschaseaux 2013; Jones et al. 2014). The impacts this has on the wider coral reef are still being investigated, but initial research suggests that increasing SSTs lead to increased production of DMSP/O by corals, with greater concentrations of these compounds occurring in reef waters and with potentially increased flux of DMS to the atmosphere (Fischer and Jones 2012; Deschaseaux et al. 2014b; Jones et al. 2014; Jones and King 2015).

1.21 Aims of this research

We know that DMSO is a highly effective antioxidant, and can act as a source and a sink for DMS, however to date, there is very little data on DMSO in coral reef systems or for non - coral reef taxa, coral zooxanthellae or the coral holobiont (Broadbent and Jones 2006; Deschaseaux et al. 2014b). Similarly, several studies have investigated the potential effects of OA on DMS/P production (Burdett et al. 2012; Archer et al. 2013; Arnold et al. 2013; Hopkins and Archer 2014), however very little data exists for the impacts of ocean acidification on DMS/P production by corals or in coral reef systems and no data exists anywhere for the impacts of OA on DMSO production in any organism or system. Mesophotic reefs are, owing to accessibility issues, equally understudied in many fields, but certainly with respect to dimethylated sulphur compounds, for which no data has yet been published for either seawater or tissue concentrations of DMSP or DMSO.

Thus, the over - arching goal of this research was to improve our current understanding of sulphur dynamics in coral reefs. Specifically, the research objectives for this study were:

1. To better understand the spatial distribution of seawater DMSP and understand biotic and abiotic drivers or seawater DMSP variability
2. To provide novel and unique data that facilitates a better understanding of the role of mesophotic reefs with regard dimethylated sulphur cycling
3. To illustrate the importance of DMSO in coral reef environments, and provide much - needed data on tissue concentrations of this compound
4. To augment the current data on the impacts of ocean acidification on DMSP/O production and cycling with novel data for corals and coral reef ecosystems

Based on knowledge gained to date, it is highly likely that climate change will drive changes to reef ecology, specifically from a coral dominated assemblage to a macroalgal/sediment dominated assemblage and will impact DMSP/O production and cycling in reef waters as climate change progresses. This thesis aims to test the following hypotheses:

1. Increased temperature and decreases in oceanic pH will drive increases in cellular DMSP/O production by reef organisms
2. Temperature will exert a greater effect on production of DMSP/O, whilst changes in pH will affect DMSP/O production in reef waters but due changes in community ecology rather than increases in production at the cellular level
3. Corals and their symbionts are not the main drivers of seawater DMSP in shallow reef systems, rather it is the presence/absence of macroalgae and seagrasses that drives seawater DMSP levels
4. In contrast to (3) above, corals and their symbionts are the main drivers of seawater DMSP in mesophotic reefs, which are understudied but significant components of coral reef sulphur production
5. Cellular production of DMSP/O decreases with depth owing to more stable conditions and the absence of the environmental stressors (temperature, light) that drive DMSP/O production in shallow water reefs, driving the need for the antioxidant cascade

2 Methods and Techniques

2.1 DMSP and DMSO analysis

Since the discovery of DMSP and DMS (Haas 1935; Challenger and Simpson 1948), a variety of methods for the analysis of these compounds have been developed. These include thin layer chromatography (Greene 1962) with mass spectrometry (Hanson et al. 1994), high performance liquid chromatography (Gorham 1986; Colmer et al. 2000) with UV detection (Wiesemeier and Pohnert 2007), nuclear magnetic resonance (Ansede et al. 2001), capillary electrophoresis (Zhang et al. 2005) and gas chromatography with mass spectrometry (Zhou et al. 2009). The most widely used method for analysing DMSP/DMS uses cold alkaline hydrolysis to cleave DMSP to DMS, which is then measured using gas chromatography and was initially proposed by Turner et al. (1990). This method has a proven record in the literature. Thus, this technique was adopted through this research for quantification of DMSP in all samples.

Analysis of DMSO in seawater samples, however, originally proved problematic not only because it is present in nanomolar concentrations, but also because it is readily soluble in water, is non - ionic and cannot be purged or steam distilled (Harvey and Lang 1986). Therefore, research has focussed on the development of indirect methods in which DMSO is reduced to DMS (Andreae 1980; Anness 1981; Kiene and Gerard 1994; Simó et al. 1996). Whilst such reduction methods demonstrate a greater sensitivity and are suitable for saline solutions, they are subject to some interference; the sample preparation technique reported by Andreae (1980) involved the addition of sodium borohydride ($NaBH_4$) or chromium II chloride (Cr_2Cl) as a reducing agent. However, the DMS yield by Cr_2Cl was only 42 % of the expected level. Additionally, the accuracy of the $NaBH_4$ method was compromised by the assumption that all DMSO produced originated from DMSO, even though it had already been shown that $NaBH_4$ could initiate the conversion of DMSP to DMS and acrylic acid (Challenger and Simpson 1948; Simó et al. 1998; Hatton et al. 2004). One method that has shown sufficient sensitivity, precision and accuracy whilst also being suitable for marine samples is an enzyme - linked method in which DMSO is reduced to DMS, facilitated by the DMSO reductase enzyme (Hatton et al. 1994). Despite the reported

problems with several reduction methods, many of them are still widely used globally and there remains no general consensus in choice of analytical method. Because of its reported precision and accuracy, as well as its ease and safety of use, the enzyme - linked method was adopted throughout this research for quantification of DMSO in all samples.

2.2 Preparation of DMSO reductase

DMSO is used by a variety of bacteria as an electron acceptor in anaerobic respiration (Barrett and Kwan 1985). In the photosynthetic bacterium, *Rhodobacter capsulatus*, the reduction of DMSO is catalysed by the water - soluble enzyme DMSO reductase (DMSOr, McEwan et al. 1991), which is found in the periplasmic space (McEwan et al. 1991). The procedure for obtaining DMSO reductase follows 7 steps: Grow *R.capsulatus* cells; harvest cells; periplasmic fractionation; 50 - 75 % ammonium sulphate cut; dialysis; phenyl sepharose column; and, finally, quality tests.

2.2.1 Grow cells

R.capsulatus (strain H123, kanamycin resistant mutant) was grown anaerobically in fully filled stoppered vessels in front of light banks at 30°C in the presence of 30 mM DMSO – the growth medium was based on RCV medium as described in Weaver et al. (1975).

RCV growth medium (per L):

- 50 ml super salts solution
- 10 ml 10 % $(NH_4)_2 SO_4$ solution
- 2.2 ml propionic acid
- 15 ml 0.64 M $K_2 HPO_4$ pH 6.8.

Super salts solution (per2 L):

- 4.8 g $MgSO_4.7 H_2 O$
- 0.96 g $FeSO_4$
- 0.8 g Na_2 EDTA
- 0.04 g Thiamine
- 3 g $CaCl_2.2 H_2 O$
- 40 ml Trace element solution

Trace element solution (per L):

- 2.8 g H_3BO_3
- 1.6 g $MnSO_4.H_2O$
- 0.75 g $NaMoO_4$
- 0.24 g $ZnSO_4.7\ H_2O$
- 0.04 g $Cu(NO_3)_2.3\ H_2O$

30 mM filter (Whatman ™, 0.60 µM PVDF) sterilised DMSO was added to the RCV medium after autoclaving.

Cells were grown initially from - 80°C stocks in 25 ml McCartney bottles in the presence of 20 µl of 20 mg/ml kanamycin to ensure only strain H123 grows. The large - scale incubation was conducted in stages: 25 ml McCartney → 250 ml medical flask → 2 L medical flask → 25 L carboy with magnetic stirrer.

2.2.2 Harvest cells

Cells were harvested in late exponential phase using cross flow filtration (0.1 µM pore size), leaving about 1 - 2 L of deep orange/brown cell suspension. This suspension was centrifuged in a GSA rotor at 8000 rpm at 4°C for 15 - 20 mins, and the pellet washed once more in 50 mM Tris buffer (pH 8.0) and recentrifuged as before.

2.2.3 Periplasmic fractionation

The aim of this stage is to open the cells to release the protein contents contained within the periplasmic space. The pellet was resuspended in 20 ml STE (sucrose - Tris - EDTA) buffer per gram wet weight of cells. From 12 L, 19 g wet weight of cells were collected. STE buffer was composed of 0.5 M sucrose, 50 mM Tris pH 8.0, 1.5 mM EDTA. Freshly prepared lysozyme stock (10 mg ml^{-1}) was added to give a final concentration of 600 µ g ml^{-1}, after which the solution was incubated for ~ 45 mins at 30°C with occasional swirling. The solution was then centrifuged at 8000 rpm, 4°C for 15 - 20 mins to give a clear orange

supernatant, which contains the periplasm and DMSOr. The supernatant was then recentrifuged at 8000 rpm, 4°C to remove any remaining sphaeroplasts. After this stage it is imperative to keep the periplasmic solution cold.

2.2.4 Ammonium sulphate cut

29.5 g grade III ammonium sulphate was added per 100 ml of periplasmic solution, which was then left stirring at 4°C for 30 mins (50 % ammonium sulphate cut) and then centrifuged at 8000 rpm for 15 - 20 mins. The supernatant was then collected, and a 75 % ammonium sulphate cut was performed by adding 16.1 g grade III ammonium sulphate per 100 ml. The solution was then left stirring at 4°C for 30 mins and centrifuged at 8000 rpm for 15 - 20 mins and the pellet collected.

2.2.5 Dialysis

The pellet was resuspended in 50 mM Tris pH 8.0 and 1.5 mM EDTA and dialysed against 2 mg/ml L 50 mM Tris buffer pH 8.0 at 4°C overnight.

2.2.6 Phenyl sepharose column and hydrophobic interaction chromatography

The purpose of this stage is to separate the various proteins contained in the periplasm. A phenyl sepharose fast flow resin column (200 ml) was used because it causes a greater range of hydrophobic proteins to travel more slowly, with less hydrophobic ones eluting from the column more quickly. As a hydrophilic solvent, DMSO elutes at ~ 50 - 75 mins.

The column was first equilibrated in $(NH_4)_2 SO_4$ in 50 nM Tris pH 8 (approx. 800 ml), after which the dialysed sample was made up to 15 % with $(NH_4)_2 SO_4$ and loaded onto column. The sample was loaded using a syringe pump at 5 ml min^{-1} and was washed with equilibration buffer at 5 ml min^{-1}. A gradient of 15 - 0 % $(NH_4)_2 SO_4$/Tris buffer pH 8 gradient (300 ml) was applied and run at 65 ml hr^{-1} at 5 ml min^{-1}. DMSO elutes at 4 % after which

the peak fractions were collected and concentrated over a PM10 Amicon membrane to yield ~ 5 ml of dark brown solution.

2.2.7 Assurance checks:

Two checks are necessary once DMSO has been extracted; concentration of DMSOr is checked using a Bradford's Protein Assay, and the activity of the enzyme is checked using a methyl viologen assay.

2.2.7.1 BCA protein assay

Protein standards were prepared (0.2 - 1.5 mg ml $^{-1}$ protein) from BSA stock (200 mg ml $^{-1}$) diluted in 50 mM pH 8.0 Tris - HCl buffer. 100 µl of each standard was pipetted into clean test tubes in a 1:100 dilution, to which 3 ml Bradford's reagent was added and the tubes vortexed gently. After 45 mins incubation absorbance was measured in duplicate at 595 nm. Absorbance was plotted against each dilution to create a standard curve, after which the process was repeated, and the standard curve was generated. The absorbance of DMSOr in a 1:10 dilution at 595 nm was measured at a wavelength of 0.0544, and using the absorbance curve from the standards the protein content of DMSOr was determined to be 67.2 mg ml $^{-1}$.

2.2.7.2 Methyl viologen assay

As methyl viologen is readily auto - oxidisable, assays were performed under anaerobic conditions. Degassed 50 mM Tris - HCl pH 8.0, 500 nM methyl viologen and 2 µl of DMSOr were added to a 1 cm^3 round - topped glass cuvette. A rubber Suba Seal (Aldrich Chemical Co) was used to stopper the cuvette and the headspace was sparged with nitrogen (OFN grade) via hypodermic needles for ~ 3 mins. A small aliquot of sodium dithionite dissolved in water was then added using a Hamilton syringe until a deep blue colouration developed, due to the reduction of the methyl viologen. The absorbance at 600 nm was monitored using a single beam spectrophotometer and chart recorder, and the trace allowed to settle to a minimal rate of drift. The reaction was initiated by the addition of 50 nM DMSO and the

rate of the reaction monitored by the decrease in absorbance at 600 nm. The rate was calculated using Beer's equation:

$$A = \varepsilon L C \qquad \text{(Equation 1)}$$

Where:

A = absorbance change per min

ε = molar extinction coefficient (methyl viologen at 600 nm = 13)

L = pathlength (equal to 1 cm)

C = concentration (2 µL)

Therefore C = A/ε

A change in absorbance of 1.3871 in 30 s was recorded, so:

A = 1.3871/0.5 = 2.7742

Then C = A/ ε so (1.3871/0.5)/13 = 0.2134 µmol methyl viologen oxidised per min for 2 uL (1/10 dilution).

Thus, per ml of sample = 0.2134 * 10 * 500 (from 2 µl to 1000 µl) = 1067 µmol methyl viologen oxidised per min. A total of 3.5 ml DMSOr was extracted in total = 3734.5 µmol methyl viologen oxidised per min and 100 mg of DMSOr from the Bradford Assay, so the specific activity = 37.34 µmol methyl viologen oxidised per min.

2.3 Gas chromatography method and operational settings

DMSP was measured as DMS in a 1:1 ratio (Turner et al. 1990) using a SRI - 8610 C gas chromatograph (GC). The GC was fitted with a 15 m 5.0 U MXT - 1 capillary column and a sulphur - specific FPD detector. The operational settings for the GC were N_2 carrier gas air pressure; 8 psi, air pressure; 2 psi, hydrogen air pressure; 27 psi, injection port and oven temperature; 45°C and flame photometric detector (FPD) temperature; 150°C. DMSP and DMS concentrations in the oceans are usually very low (< 2.8 nM,Kiene & Slezak 2006) so it

is necessary to pre - concentrate the DMS generated from the sample to allow quantification by the GC - FPD method. This is achieved by using a cryogenic purge and trap system (Fig 2.1), after which the square - root of peak area was linearly related to DMS concentration, permitting DMSP quantification. The GC was calibrated using a series of DMSP standards in deionised water.

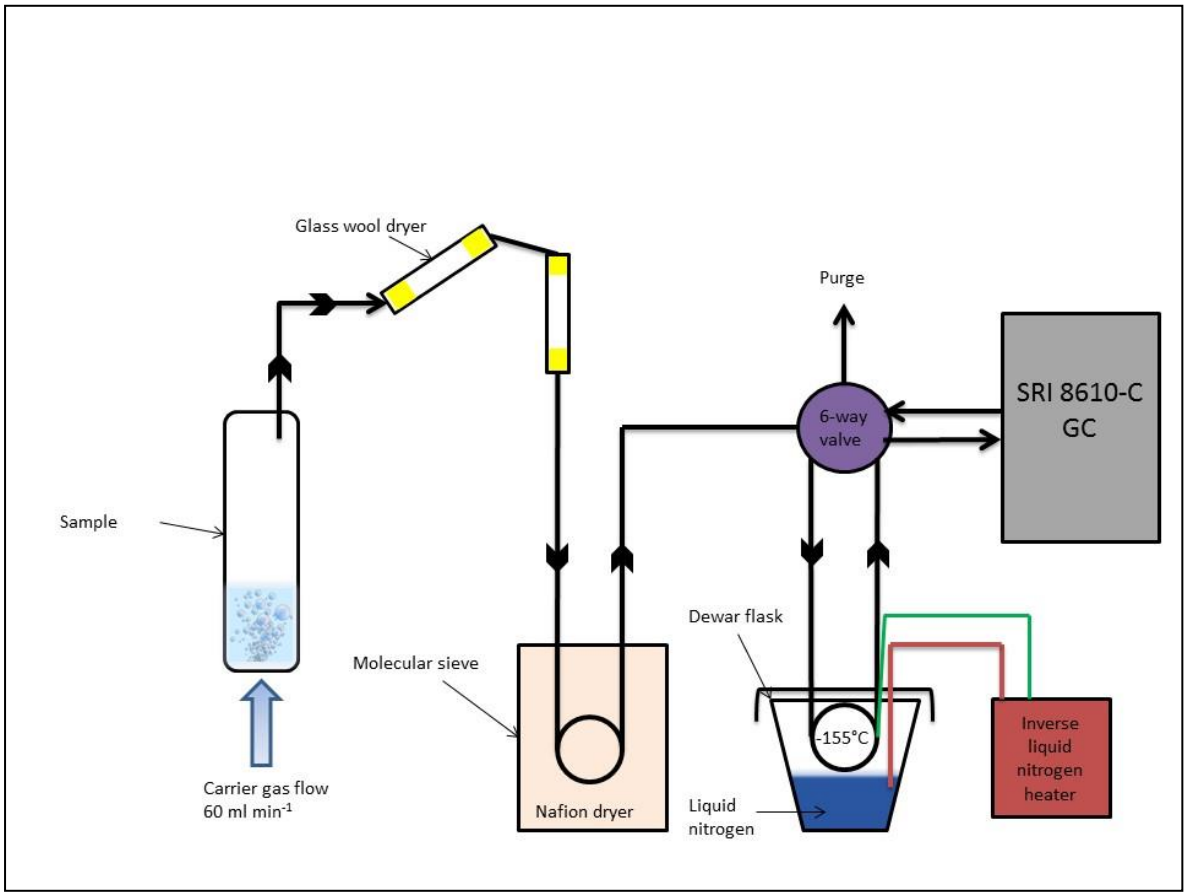


Figure 2.1 Diagram of the purge and trap system used throughout this project

2.3.1 Standard preparation and calibrations

DMS concentrations in samples were quantified through the construction of standard calibration curves (Fig 2.2). DMSP crystals were supplied by Research Plus Inc and aliquots of standards were prepared by Dr Heidi Burdett; crystals were dissolved in autoclaved Milli - Q water using a sterile preparation technique to yield a DMSP standard solution of known concentration. Aliquots of the standard were stored in 1 ml volumes at - 20°C until required.

Standards from the same batch of DMSP crystals were used throughout this research. The precision (coefficient of variation of replicate standards) was always better than ± 4 %.

2.3.2 Liquid calibrations

Aliquots of DMSP standards of varying concentrations (0.48 µg S ml $^{-1}$ to 47.5 mg S ml $^{-1}$) were used to create a working stock solution of DMSP. Liquid standard stock was prepared by diluting DMSP standard stock in 250 ml Milli - Q water. This DMSP solution was kept up to one week in the dark at 4°C. Five replicates of varying standard concentrations were analysed in triplicate using the purge - cryotrap system by adding 1 ml 10 M NaOH to the vial with the standard to achieve a standard calibration curve (Fig 2.2). Calibrations were conducted such that all samples analysed fitted on the calibration line.

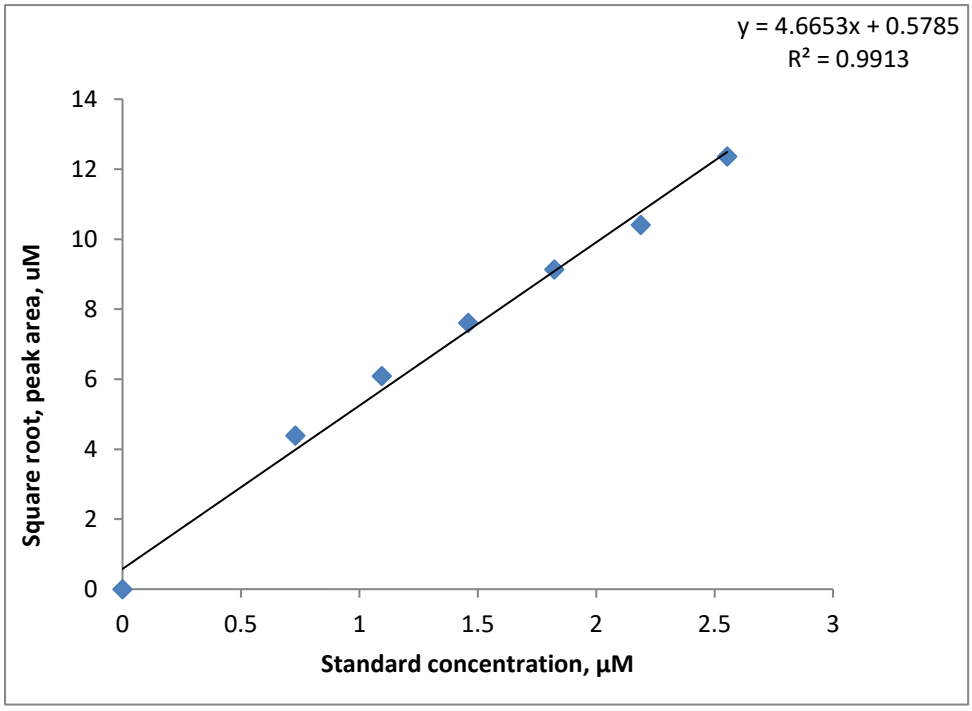


Figure 2.2 Example calibration curve of liquid samples. Mean coefficient of variation (%) of all replicates (n = 13) = 1.78 %.

2.3.3 Headspace calibrations

Intracellular DMSP/O concentrations in coral tissue and macroalgal/seagrass samples were high enough to quantify DMS by direct injection of equilibrated headspace rather than using the cryotrap. Headspace standards were prepared using 10 ml Wheaton™400 crimp top serum vials. DMSP stock solution was diluted with autoclaved Milli - Q water (total volume 1000 µl) to achieve a range of headspace standards. This was added to vials with 1000 µl 10 M sodium hydroxide (NaOH) to give a final volume of 2000 µL (equal to the sample preparation) and quickly crimped shut with Pharma - fix septa (Discovery Sciences). Standards were left in the dark overnight to ensure DMSP was fully hydrolysed to DMS and DMS had equilibrated between the NaOH solution and vial headspace. Triplicate injections of 100µl of headspace from each standard was injected directly into the GC using a gas tight syringe fitted with a 24 - gauge needle, and then plotted to achieve a standard calibration curve (Fig 2.3).

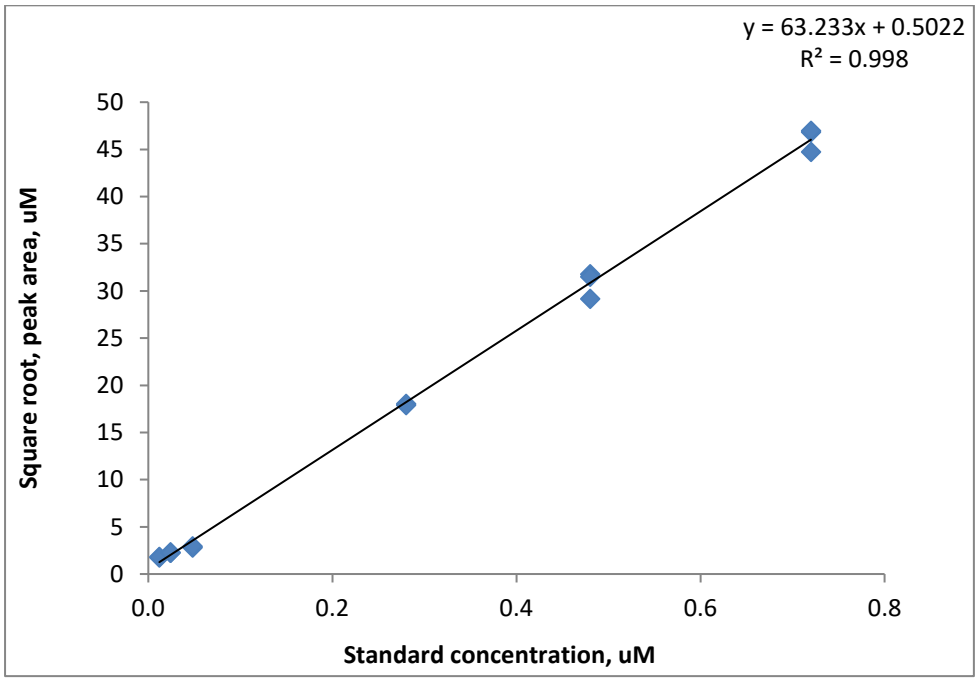


Figure 2.3 Example calibration curve of headspace samples. Mean coefficient of variation (%) of all replicates (n = 12) = 2.69 %.

2.3.4 DMSO

DMSO was analysed in tissue samples and samples were measured using the reductase enzyme method (Hatton et al. 1994); 1 ml 500 mM Tris – HCl pH 8.0 was added to each 1 ml tissue homogenate sample and neutralised using 50% HCl or 10 M NaOH to pH 7.0. Samples were measured after DMSPt, so all DMS/P will already have been removed. However, to be certain there was absolutely no accumulation of DMS/P during the preparation process, samples were then fast - purged for 4 min to remove any DMS/P. Where samples could not be analysed within 24 h of preparation, they were frozen (- 20°C) until analysis could be completed.

To 100 ml of DI water, 50 µL purified DMSO reductase (prepared as above), 0.026 g flavin mononucleotide (FMN) and 30 nM (1.116 g) ethylenediaminetetraacetic acid (EDTA) was added. To each prepared 1 ml sample, 2 ml of FMN solution was added and the vials crimp sealed with PTFE lined caps. Samples were then purged for 1 hour in front of 3 x 60 W lights. Light acting on the FMN solution forms radicals that reduce FMN to $FMNH_2$, which acts as an electron donor to DMSO reductase, catalysing the reduction of DMSO to DMS (Hatton et al. 1994). The resulting DMS was then analysed using headspace injection in the same manner as DMSP. Because DMSO, like DMSP, is measured as DMS in a 1:1 ratio, calibrations for DMSO are conducted in the same manner as DMSP.

2.3.5 The purge and trap system

DMSP, DMSO and DMS concentrations in the oceans are usually very low (< 2.8 nM, Kiene & Slezak 2006) making it necessary to pre - concentrate DMS generated after the preparation of samples to allow quantification by the GC - FPD method. This is achieved by using a cryogenic purge and trap system (Turner et al. 1990), in which samples are purged with nitrogen gas (60 ml min^{-1}) to liberate any DMS, which is then transported through two drying stages (glass wool and a molecular sieve) before reaching the cryotrap. The cryotrap is a loop of PTFE tubing maintained at ~- 155°C using liquid nitrogen (LN_2) in a thermostatically controlled dewar. This rapidly cools DMS, allowing it to be trapped in the tube. The time taken to purge samples varies according to sample size and method of

preparation used; DMSO samples require sufficient time for the enzyme reaction to occur and 5 ml water samples were purged for 15 min, whereas 1 ml tissue samples were purged for 10 min, whilst 7 min was sufficient for 5 ml samples being analysed for DMSP content.

The 6 - way valve (Fig 2.4) has two distinct modes for loading or injecting a sample. In the loading mode the purge tube and trap are linked, ensuring any DMS gas remains in the closed system, whilst in the inject mode the trap and GC are linked. Following purging, the valve was switched to divert the flow from the trap into the GC; the cryotrap temperature was rapidly increased to ~ 100°C by transferring it into boiling water for ~ 15 seconds, which strips all DMS from the trap and transports the sample to the GC for detection by the FPD detector.

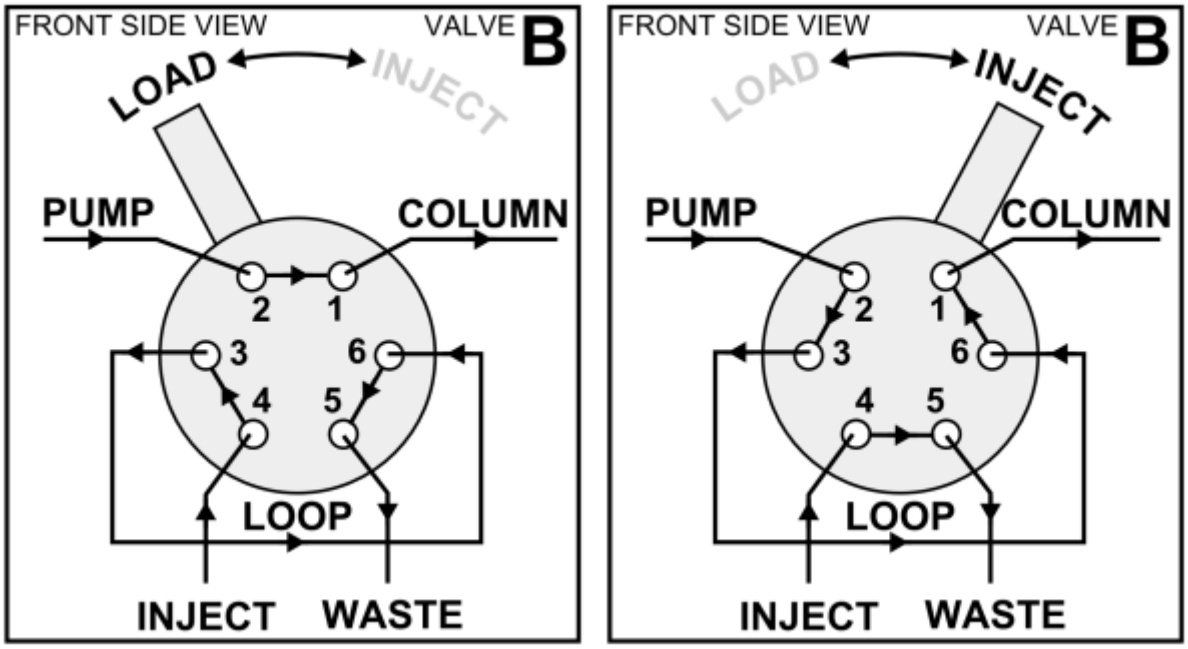


Figure 2.4 The internal mechanism of the 6 - way valve used for injecting samples.

2.4 Sampling strategy

2.4.1 Total DMSP/O

A pilot study was conducted prior to any sampling to determine the best sampling strategy. DMSP in seawater occurs in two fractions; dissolved (extracellular) and particulate (associated with an algal/bacterial cell or a grazer), with each fraction determined by what will (dissolved) or won't (particulate) pass through a 0.6µm filter (Kiene and Slezak 2006). Total DMSP ($DMSP_t$) comprises both the dissolved and particulate fractions as well as seawater DMS (DMS_w). DMS_w comprises a significant fraction of seawater dimethylated sulphur (Curran et al. 1998) but is not produced directly by marine algae, as far as is known, rather, it is a degradation product of DMSP. The current methodology for sampling DMSP and its fractions involves the filtration and acidification/basification of water samples (Kiene and Slezak 2006), however visual observations when using this technique suggested there may be issues with the precision and accuracy of analyses. Specifically, when passing samples through a filter, there was visual evidence of loss of the sample and there was a slight delay in crimping the vial, which could contribute to further loss of the sample through outgassing.

Trial incubations using the experimental corals in the coral culturing laboratory at St Andrews University were conducted using the method outlined in Kiene & Slezak (2006). Sample vials, reagent and lids were all weighed prior to the addition of the seawater sample. Vials were weighed again, following addition and sealing of the sample and the difference in weight between the two, with differences in density between seawater and freshwater accounted for, was used as the total volume of water in the vial. Samples were analysed, and a value was calculated based on the weight of the sample in the vial and also based on an assumed value of 5 ml per vial. Samples were taken at the beginning and end of an incubation. The standard error of all samples, corrected for weight and based on an assumption of 5 ml per vial, were calculated (Table 2.1).

Table 2.1 Summary of error statistics for pilot data sampling test, with uncorrected (sample volume assumed to be 5 ml) and corrected (sample water weighed) standard errors given for each sampling technique.

T1 averages	*Std error Uncorrected*	*Std error Corrected*
DMSPd	0.173	0.423
DMSPp + d	0.177	0.194
DMSPt	0.154	0.150
T2 averages		
DMSPd	1.1887	1.4915
DMSPp + d	0.1898	0.2032
DMSPt	0.1193	0.0913

Samples that were weighed for DMSPt consistently exhibited the most precise results, so this method was adapted for all samples taken as part of this research.

There was no significant difference in the accuracy of samples of weighed or assumed volume tests (two - tailed Welch's t - tests: $p = 0.47$). Thus, samples throughout this study were sampled using a calibrated pipette (calibrated every 6 months) and sampling for total DMSP/Ot (i.e. particulate and dissolved DMSP, as well as seawater DMS). Further references to DMSP/O hereafter refer to total DMSP, unless otherwise stated. Tissue samples were also sampled for total DMSP/O however since DMS concentrations within algal cells were found to be negligible compared to the concentrations of DMSP/O (Burdett, pers. comm), and so this was disregarded as a significant factor.

2.5 Seawater dissolved inorganic carbon (DIC), total alkalinity (TA) and carbonate equilibrium

2.5.1 The carbonate system of seawater

The carbonate system of seawater consists of three main inorganic forms: CO_2 (aq), HCO_3^- and CO_3^{2-}. A fourth form, carbonic acid (H_2CO_3) also exists but is normally represented by CO_2 (aq) as its concentrations are minimal (~ 0.3 %) at pH levels currently seen in seawater (Fig 2.8, Zeebe & Wolf - Gladrow, 2001). The carbonate system is related via a series of chemical equilibrium reactions that change in accordance with temperature, pressure and salinity (see equations 2 - 4). As atmospheric levels of CO_2 increase, the carbonate system of

the ocean shifts to re - establish equilibrium. Atmospheric CO_2 is absorbed by surface seawater and is in thermodynamic equilibrium according to Henry's Law. Dissolved CO_2 forms a weak acid (H_2CO_3) which rapidly dissociates to form HCO_3^- and a proton (H^+). The HCO_3^- also dissociates to form CO_3^{2-} and another H^+; this H^+ formation lowers the pH to make the water less alkaline in a process known as ocean acidification (OA).

$$CO_2\,(aq) + H_2O \leftrightarrow H_2CO_3 \qquad \text{(Equation 2)}$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \qquad \text{(Equation 3)}$$

$$HCO_3 \leftrightarrow CO_3^{2-} + H^+ \qquad \text{(Equation 4)}$$

The buffering capacity of seawater is quantified by the Revelle factor which is a measure of how the partial pressure of CO_2 in seawater changes for a given change in DIC (Sabine *et al.,* 2004). The Revelle factor means that a doubling in atmospheric CO_2 only results in a 10 % change in DIC (provided temperature and other factors remain the same). The buffering capacity of seawater is due to the presence of CO_3^{2-}, which reacts with CO_2 and H_2O to form 2 HCO_3^- (see equations 2-4).

However, despite the buffering from CO_3^{2-}, the acidity of seawater still increases slightly as some of the HCO_3 dissociates to form CO_3^{2-} and H^+. Current rates of change in CO_2 (and consequently H^+ production) exceed the natural geological scales of buffering; historically (over the last 50 million years) ocean mixing over longer time scales has been able to buffer the seawater chemistry via interactions with carbonate - sediment. As the oceans absorb more CO_2, their ability to buffer any changes in seawater chemistry are reduced (Zeebe & Wolf - Gladrow, 2001).

2.5.2 Total Alkalinity

Key to understanding seawater carbonate chemistry is the concept of total alkalinity (TA, Fig 2.5), which is defined by Dickson (1981) as:

"the number of moles of hydrogen ion equivalent of excess proton acceptors with a dissociation constant K≤ 10 - 4.5 over proton donors (acids K > 10 - 4.5) in 1 kg of sample" (Dickson, 1981)

And is thus represented by the equation:

$$TA = [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH] + [HPO_4^{2-}] + [H_3SiO_4^-] + [NH_3] + [HS^-] - [H^+]_F - [HSO_4^-] - [H_3PO_4] \quad \text{(Equation 5)}$$

Where $[H^+]_F$ = free concentration of H^+

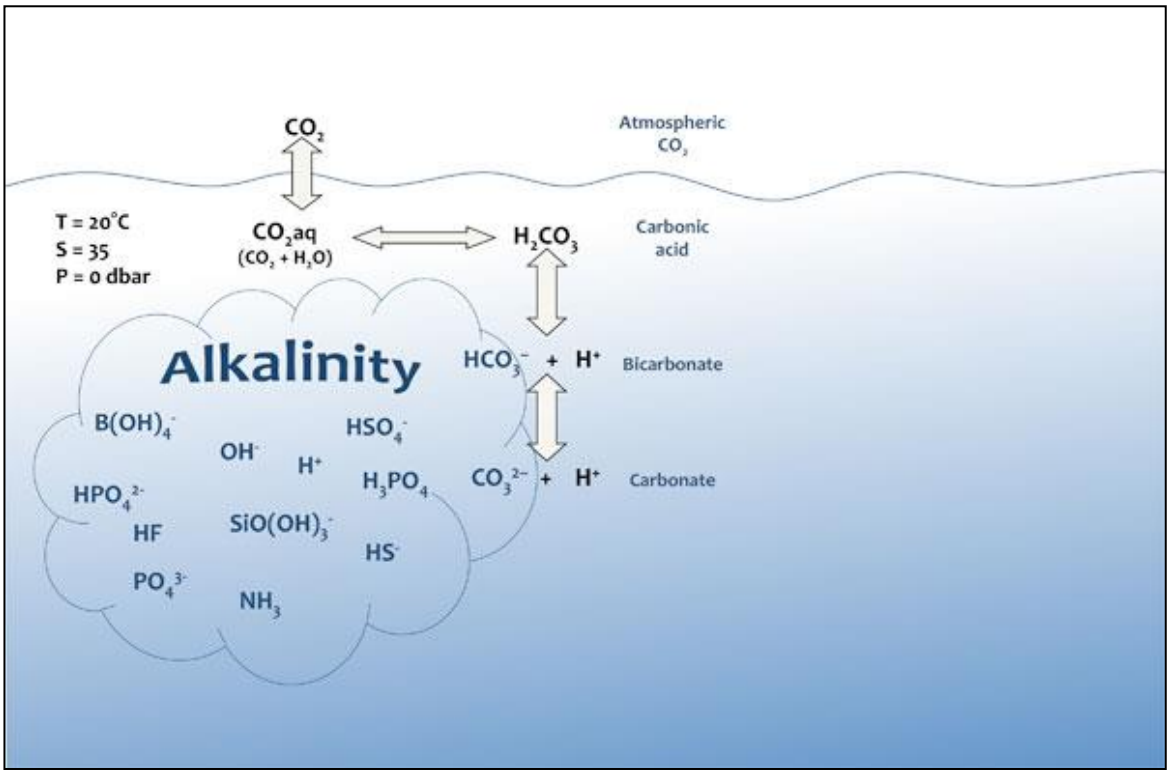


Figure 2.5 Schematic of the carbonate system species in seawater and some of the equilibrium reactions that occur among them (WHOI 2012)

TA is thus especially useful for assessing the saturation state of calcium carbonate in the ocean. Seawater TA buffers changes in ocean pH because it includes so many different acid - base pairs, which lessen the fall in pH. Because seawater can buffer itself against changes in CO_2, TA remains constant as the net reaction produces equivalent numbers of proton donors (H^+) and proton acceptors (HCO_3^-, CO_3^{2-}).

2.5.2.1 Measurement principle

Whilst various methods and equipment are available for determining TA, analyses for this project were performed on a Metrohm 848 Titrino Plus (Fig 2.5) using an open - cell potentiometric titration (Dickson et al., 2007). The setup for potentiometric measurements consists of an indicator and a reference electrode; the indicator electrode provides a potential that depends on the composition of the sample solution, whilst the reference electrode supplies a potential which is independent. The set - up used in this study employs a combined electrode, in which both electrodes are contained in the same shaft. The end point is determined by measuring the potential difference in current (which after calibration is pH) after the successive addition of known increments of acid titrant.

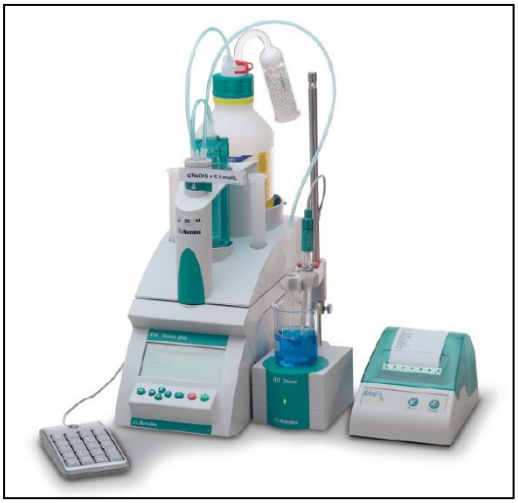


Figure 2.6 TA analyser used for all TA sampling

The probe was calibrated daily against three buffers of known pH values, 4, 7 and 10; where measured values were deemed to have drifted too far, the buffers were replaced and the

calibration restarted. Samples of ~ 10 - 30 ml were weighed and the weight noted; after calibrating the pH probe (Metrohm Aquatrode Plus with Pt 1000), the pH probe, hydrochloric acid dosing tip and a metal stirrer bar were placed in the sample on the stir - plate and titrated with a solution of standardised 0.1 M HCl. The precision of duplicate vials did not deviate by more than 7 µeq kg^{-1}. Where precision for two samples was outside this, a third vial was analysed (where available) on the same day and calibration, and the closest two values were used to calculate a mean. Subsequent analysis was conducted using Gran titration (Gran et al. 1950)

2.5.2.2 Hydrochloric acid standardisation and sodium carbonate standard preparation

Hydrochloric acid had previously been standardised (C. Cole, University of St Andrews), thus standards made during this study were used to assess instrument/titrant drift. Three concentrations of standard solution of sodium carbonate were prepared, 0.5 mM, 1 mM and 1.25 mM on two separate occasions during this study. Appropriate weights (Table 2.2) of reagent grade sodium carbonate were placed in a drying oven at 280 °C for > 2 h, after which they were added to1 L 0.3 M sodium chloride solution. Sodium chloride solution was prepared by adding 61.60 g NaCl to DI water to give a final volume of 3.5 L.

Table 2.2 Weights and final concentrations of both sodium carbonate standard solutions prepared for TA analysis during this research.

Standard concentration (mM)	1: Final Mass (mg)	1: Final Concentration (mM)	2: Final Mass (mg)	2: Final Concentration (mM)
0.5	53.144	0.501	53.498	0.505
1.0	105.958	1.000	106.300	1.003
1.3	132.287	1.248	131.968	1.245

The alkalinity for each standard was calculated using the final concentrations (Table 2.2) and this was plotted against the measured endpoint of each titration (Figure 2.7); the coefficient of variation (%) between replicate concentrations was always < 0.3 %.

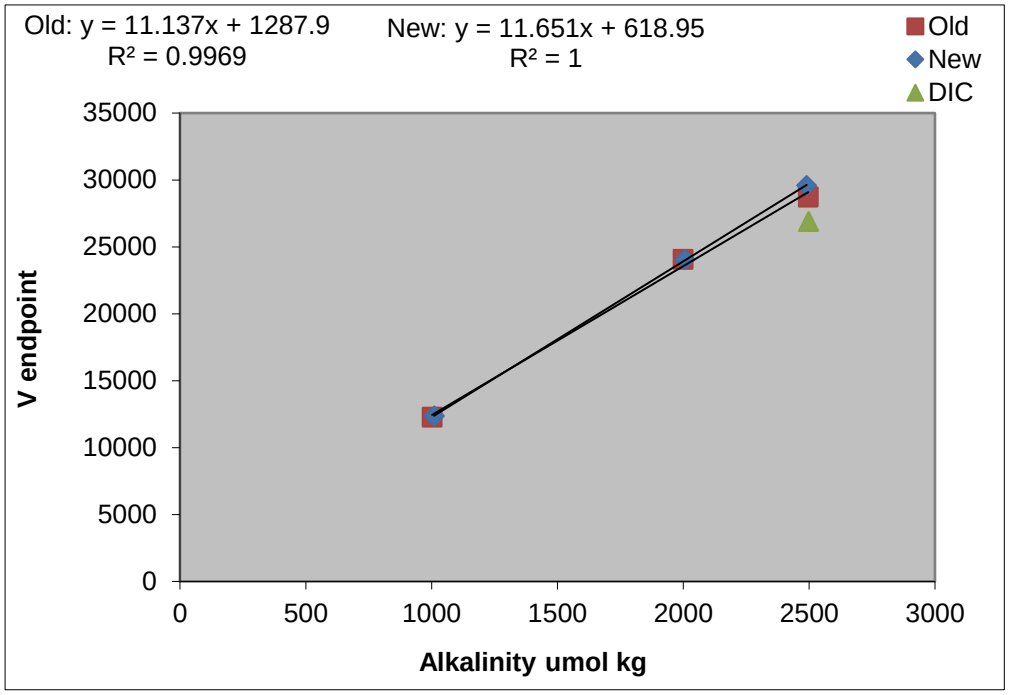


Figure 2.7 Endpoint determination of two sodium carbonate standard solutions with the first set of standards (old) and second set of standards (new) shown together. Dickson CRM was also measured (DIC) and the prepared standards were in good agreement with the known TA of the CRM.

Standards were stored in polyethylene bottles that were further sealed with parafilm and stored in plastic bags to prevent incursion of CO_2. Replicate vials of one standard concentration were run at random points during each day of analysis to account for instrumental/titrant drift.

2.5.2.3 Analyses and Gran titration

To measure alkalinity, a seawater sample was titrated with acid to an endpoint at which carbonate was converted to bicarbonate and bicarbonate was converted to carbonic acid:

$$H^+ + CO_3^{2-} \rightarrow HCO_3^- \quad \text{(Equation 6)}$$

$$H^+ + HCO_3^- \rightarrow H_2CO_3 \quad \text{(Equation 7)}$$

In seawater, this endpoint occurs at about pH = 4.2, however this value will vary according to the buffers present in a solution. To be certain that titrations achieved maximum accuracy and encompassed the full range of alkalinities typically exhibited in coral reef seawater, the Gran titration method was used in this study. Gran titration relies on a mathematical evaluation of the second equivalence point of carbonate titration in seawater using the most stable part of the titration curve (i.e., the part beyond the equivalence point on the low pH side). Essentially, the Gran method linearizes the titration curve by means of a simple function:

$$F = (v + V_0) \times 10^{E/A} \quad \text{(Equation 8)}$$

where:

F = Gran factor,

v = volume of acid added to the solution in the titration vessel,

V_0 = original volume of the sample,

E = EMF (millivolts) at v, and

A = slope of electrode determined during the electrode calibration.

To ensure the Gran method works optimally, titrations should be carried out to pH values well below the final endpoint, which during this study was set at pH 3.0. Data were processed using TiBase™ (Version 1, 2009, Metrohm AG Switzerland) and the results from that day's calibration. Where the precision between any two replicate vials exceeded 7µeq kg^{-1}, a third replicate was analysed.

2.5.3 Dissolved inorganic carbon

CO_2 obeys Henry's Law, meaning that the concentration of CO_2 in the surface ocean (pCO_2) is virtually equal to partial pressure of CO_2 in the atmosphere (pCO_2). Subsequently, dissolution of CO_2 in seawater is favoured and results in the production of carbonic acid (H_2CO_3), which rapidly dissociates to produce bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions, as well as increasing the concentration of hydrogen ions (H^+).

$$CO_2 + H_2O <-> H_2CO_3 <-> HCO_3^- + H^+ <-> CO_3^{2-} + 2H^+ \quad \text{(Equation 9)}$$

DIC is the collective term for the carbon species in Equation 9, and their proportions in seawater are characteristic of the pH of that seawater. DIC acts as a natural buffer to the addition of hydrogen ions; if an acid is added to seawater, the additional hydrogen ions react with carbonate ions and convert them to bicarbonate (Raven et al. 2005a). Thus, the amount of CO_2 entering the oceans, fundamentally alters the proportions of each species found and thus the oceanic pH.

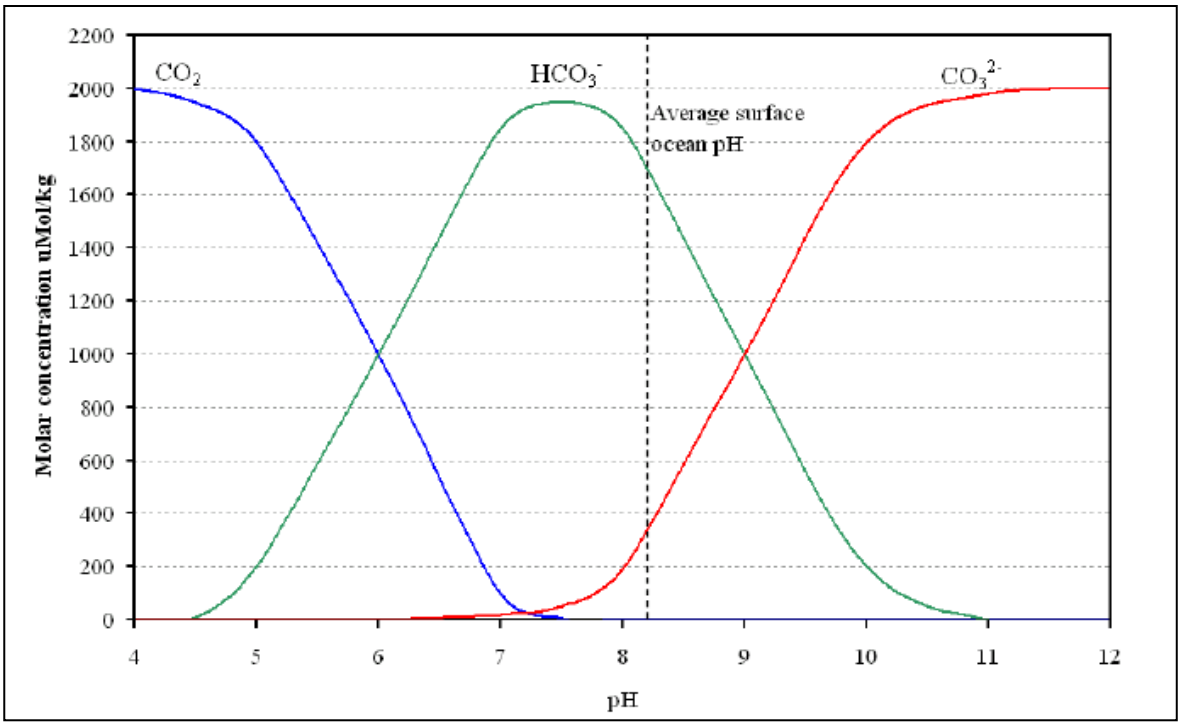


Figure 2.8 Relationship between pH and dominant DIC species in seawater (adapted from Zeebe & Wolf - Gladrow 2001)

2.5.3.1 Measurement principle

DIC was measured using a LI - 7000 CO2 differential, non - dispersive, infrared gas analyser (Apollo SciTech; AS - C3, Fig 2.9). The analyser consists of a solid state infra - red CO_2 detector (LI 7000), a mass - flow - controller to control the carrier gas flow precisely, and a digital pump for transferring accurate amounts of reagent and sample.

Figure 2.9 DIC analyser used for all samples during this project.

Upon addition of a strong acid (phosphoric acid) the carbon species in Equation 10 are converted to CO_2. The resulting CO_2 gas is purged from the water sample by the nitrogen (N_2) carrier gas, through an electronic cooling system (~ 3°C) that reduces water vapour. The concentration of dried CO_2 gas is then measured with the LI - 7000 CO_2 analyser; it uses a dichroic beam splitter and two separate detectors to measure infrared absorption by CO_2 and H_2O in the same gas stream. The total amount of CO_2 in the sample is quantified as the integrated area under the concentration - time curve.

Samples were calibrated (Fig 2.10) against a natural seawater certified reference material (CRM; A. Dickson, Scripps Institution of Oceanography), which had a known value of 2033.26

µmol kg $^{-1}$. Internal reproducibility was calculated from the standard deviation of 8 replicate measurements of a single sample (σ/√n) and was always < 0.1 %. Reproducibility of samples was always < 0.2 % and analytical times were ~ 40 mins per sample.

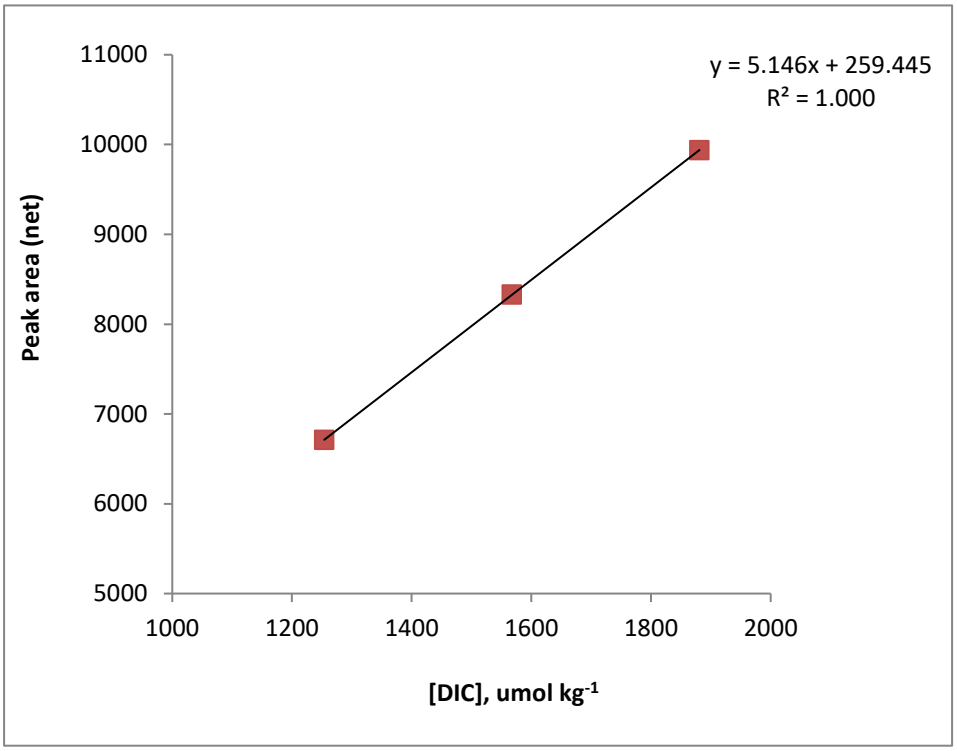


Figure 2.10 Example calibration curve for seawater DIC samples analysed during this study. The standard error for each point on the calibration was always < 0.1 %.

2.5.4 Calculation of remaining carbonate chemistry parameters

The saturation state (Ω) describes the thermodynamic potential for calcium carbonate to precipitate or dissolve, thus a reduced saturation state decreases the ability of many coral species to produce their carbonate skeletons (Anthony et al. 2008a). Therefore, temperature, salinity, TA and DIC and salinity were measured during all sampling in this study enabling the calculation of the remaining carbonate chemistry parameters using CO2SYS (Pierrot et al. 2006). The dissociation constants, K_1 and K_2, of Cai & Wang were used, with refit by Dickson & Millero (1987) and [KSO_4] using Dickson (1990) for all analyses.

3 Effects of changes in seawater pCO_2 and temperature on dimethylated sulphur production by *Porites* corals

3.1 Introduction

Although climate fluctuations have affected the Earth's atmosphere since its origin, rising anthropogenic greenhouse gas concentrations have amplified and accelerated global warming since the start of the industrial revolution (Abram et al. 2016). Specifically, rising atmospheric CO_2 concentrations mean marine ecosystems are predicted to experience increases in sea surface temperatures (SSTs), whilst concomitant increases in seawater [pCO_2] are leading to decreases in pH in a process known as ocean acidification (Barker and Ridgwell 2012). Anthropogenic activities such as these have caused a net decline in global coral reefs of 19 %, with an additional 35 % at risk (Wilkinson 2008). Although coral reefs represent only 0.2 % of the world ocean area (Spalding et al. 2001) they are marine ecosystems of great environmental and financial importance with more than 500 million people reliant on them for food and other resources (Hoegh-Guldberg 2011). The three - dimensional structure of coral reefs and their high productivity provide a habitat and a source of food and nutrients for 25 % of all marine biodiversity, including many thousands of species of invertebrates, fish, reptiles, birds and marine mammals (Harrison and Booth 2007). It is predicted that coral reefs will transition to a state of net dissolution (Eyre et al. 2014, 2018) before the end of the century, thereby jeopardising the organisms reliant on them.

Porites spp. corals are a particularly important reef building species in Indo-Pacific (>140 species) and Caribbean (4 species) reef systems, although there is no species in common between the two areas (Pichon 2011). *Porites* species are found in almost all reef habitats on the seaward reef front, the back reef and the reef flat (Pichon 2011). The genus includes large massive species, which are often a dominant component of back reef coral fauna, but also encrusting and branching morphologies (Pichon 2011). *Porites* corals are very tolerant to extremes of seawater temperatures (15 - >32°C) and there is evidence to suggest that *Porites* corals will, at least initially, fare better under climate change conditions (Lough and Barnes 2000).

Dimethylsulphoniopropionate (DMSP) and dimethylsulphoxide (DMSO), collectively DMSP/O, are two biogenically - derived dimethylated sulphur compounds that are particularly abundant in coral reefs (Hill et al. 1995; Broadbent et al. 2002; Broadbent and Jones 2004, 2006; Jones et al. 2007; Burdett et al. 2013), being found in the corals (Broadbent et al. 2002; Fischer and Jones 2012; Yost et al. 2012), macroalgae (Broadbent et al. 2002; Burdett et al. 2013) and coralline algae (Kamenos et al. 2008). At the reef ecosystem level, DMSP has been implicated in bacterial chemo - attraction (Garren et al. 2014), whilst the degradation product of DMSP/O, dimethylsulphide (DMS) is widely recognised as a major vector of sulphur from oceanic to terrestrial habitats and has been implicated in cloud formation and climate control (Charlson et al. 1987).

DMSP and DMSO are produced by virtually all known classes of marine algae and perform a range of biological functions, but are generally thought to act as compatible solutes, aiding in osmoprotection (Welsh 2000) and cryoprotection (Karsten et al. 1996a). Of increasing interest with respect to corals, however, is the role of DMSP and its cleavage products (including DMS and DMSO) in an antioxidant system that readily scavenges harmful reactive oxidants from cells (Sunda et al. 2002). Subsequent studies have suggested that the ratio of DMSO to DMSP can therefore act as an indication of stress, with higher ratios resulting from increased cellular stress (Husband and Kiene 2007; McFarlin and Alber 2013). The intimate link between increasing SSTs and coral bleaching incidences (Lesser 1997; Douglas 2003) has led to the hypothesis that DMSP/O production by the coral holobiont may also be increasing in response to temperature stress. Consequently, there are multiple studies investigating the coral tissue concentrations of DMSP (Broadbent et al. 2002; Yost and Mitchelmore 2010; Yost et al. 2012; Tapiolas et al. 2013), but very few have also considered DMSO (Deschaseaux et al. 2014b) and there are no studies considering the ratio DMSO:DMSP. Whilst studies have examined the effect of temperature on DMSP/O production in coral tissue (Deschaseaux et al. 2014b), no effort has focussed on production of these compounds in response to different seawater pCO_2 either in isolation or in combination with other environmental stressors. *Porites* corals are also an understudied model species in this field of research, despite their potential importance in governing reef structures under climate change conditions.

3.2 Aims of this study

Whilst there are studies assessing how rates of DMSP production in seawater are affected by temperature (Fischer and Jones 2012; Jones et al. 2014), there are no reports on the effects of changing seawater pCO_2 in tropical corals. Instead, research has focussed on temperate marine systems with variable results; some studies report decreased DMSP production under elevated CO_2 conditions (Avgoustidi et al. 2012; Hopkins and Archer 2014), whilst others report increased production (Archer et al. 2013). Changes to seawater DMSP are likely to have wide ecosystem impacts given the role of this compound in the microbial food web (Garren et al. 2014), herbivory (DeBose and Nevitt 2007) and as an anti - grazing defence (Wolfe et al. 1997). To address some of this lack of knowledge, the effects of different seawater pCO_2 levels in combination with two different temperatures on both coral tissue DMSP/O content and rates of DMSP production in seawater were investigated in *Porites* corals.

3.3 Hypotheses

1. We hypothesised that tissue DMSP/O concentrations and seawater DMSP would both increase under elevated temperatures.
2. However, given the knowledge that Porites corals fare better under extremes of temperature, we hypothesised that temperature would not exert any significant effect on the DMSP:DMSO ratio
3. Considering the inconsistent findings into the effects of seawater pCO_2 changes on tissue/seawater DMSP/O production, it is difficult to suggest whether higher seawater pCO_2 would favour increased tissue DMSP/O and increased seawater DMSP. However, we hypothesised that if seawater pCO_2 did have any effect, this would be evident as an increase in the DMSO:DMSP ratio.
4. Given that DMSP/O production is principally carried out by coral symbionts, we also hypothesised that increases in DMSP/O production would occur independently of changes to the algal biomass (determined by chl – a content and/or numbers of

cells) associated with each coral colony, i.e. changes are a result of upregulation, not increases to the numbers of algal cells or algal cell size

3.4 Methods

We tested the impact of variations in seawater pCO_2 and temperature on seawater DMSP production and tissue concentrations of DMSP and DMSO in *Porites* spp. corals.

3.4.1 Experimental design

Heads from *Porites* spp. corals were harvested from a reef in Fiji by a collector and imported to the UK. Each coral head was further divided into smaller sub - colonies (each > 8 cm diameter) to produce smaller experimental colonies. Colonies were cultured in 21 L purpose - built aquarium system (Fig 3.1) constructed of low CO_2 permeability materials designed to control temperature, salinity and dissolved inorganic carbon (DIC) system parameters within narrow limits (Cole et al. 2016).

At the start of the experiment, the reservoir seawater was ~ 80–85 % fresh artificial seawater (Red Sea Salt, Red Sea Aquatics, UK) diluted with local seawater. 10–15 L of seawater was usually removed from each reservoir each week (during removal of microalgae from the tank surfaces and coral colonies) and was replaced with fresh artificial seawater. No seawater replacement occurred during the experimental periods. Seawater was recirculated from high density polyethylene reservoirs containing ~ 900 litres of seawater. The reservoirs were bubbled (at 10 L min^{-1}) with gas mixes set to reach the target seawater pCO_2 compositions.

Corals were maintained prior to the experimental phase under LED lighting (Maxspect R420 R 160 w - 10000 k) on a 12 h light/12 h dark cycle, with wavelength settings configured so that photosynthetically - active radiation (PAR) intensity at coral depth was ~ 300 µmol photons m^{-2} s^{-1}. Corals were fed weekly with rotifers.

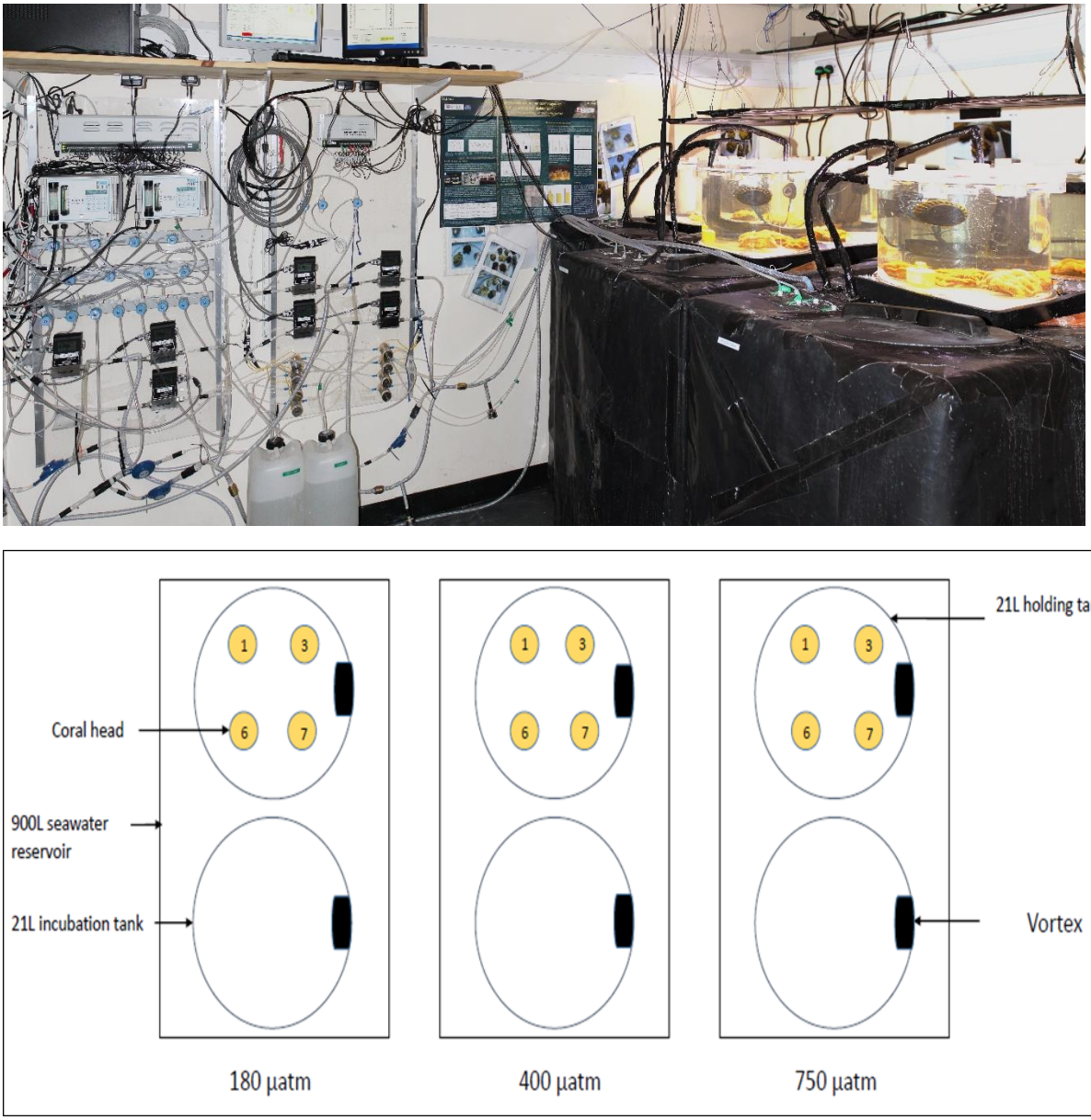


Figure 3.1 Top – photograph of the experimental coral lab set - up at the University of St Andrews. Bottom - diagrammatic representation of coral experimental design.

Corals were cultured at 3 different pCO_2 concentrations of 180 ppm CO_2 (the CO_2 atmosphere during the last glacial maximum), 400 ppm (the present day) and 750 ppm (projected by the end of the present century). The same coral individuals were cultured at 28°C and then at 25°C. Temperature treatments had been previously selected as part of another study, however given that numerous studies have shown increased algal DMSP/O production in response to even minor changes in temperature (see Green and Hatton 2014 and references therein), and that the primary aim here was to assess the coral response to combined pCO_2 and temperature changes, these temperature levels were not considered to be a detrimental

factor. After import into the aquarium, corals were maintained at ambient pCO_2 conditions for 2 months, adjusted to pCO_2 treatment conditions over 1 month and then acclimated at these final treatment pCO_2 for 4 months at 28°C. This was followed by a 4 - week experimental period in which seawater DMSP production was measured in each individual coral on 2 occasions. Seawater temperatures were then reduced to 25°C over a period of 4 weeks and then acclimated at this temperature for another month. This temperature reduction had previously been determined as part of the experimental design for another study that was running in conjunction with this one. Seawater DMSP production was again measured in each coral twice over a 4 - week period.

3.3.2. Seawater pCO_2 control

The present - day treatment was bubbled with untreated ambient air, while high - and low - CO_2 treatments were bubbled with ambient or low - CO_2 air, respectively, combined with high purity CO_2 (Foodfresh, BOC, UK). Flow rates of air and CO_2 were regulated by high - precision mass flow controllers (SmartTrak 50 Series, Sierra USA) controlled by purpose - written MATLAB® programs. Low - CO_2 air was produced by bubbling an ambient air stream through a caustic solution (0.9 M NaOH and 0.1 M $Ca(OH)_2$) and rinsing it by bubbling through deionised water. The [CO_2] of this air was monitored every 2 h by automated non - dispersive infra - red CO_2 analysers (WMA04, PP systems, USA) and ranged from 20 – 100 ppm CO_2 depending on the age of the caustic solution. The [CO_2] of the low, ambient and high CO_2 gas streams (after addition of any CO_2) was monitored automatically 3 - 4 times per day and were 180 ± 3, 400 ± 5 and 761 ± 6 ppm (mean ± 1σ) over the experimental period. The total alkalinity (TA) of the culture seawater was maintained by additions of 0.6 M Na_2CO_3 and a mixture of 0.58 M $CaCl_2$ and 0.02 M $SrCl_2$ by 200 µl volume solenoid diaphragm pumps, evenly spaced over a 24 h period, controlled by a custom - written MATLAB® dosing control program. Addition rates were adjusted to maintain TA within narrow limits (± ≤14 µmol kg^{-1}); variations of this magnitude have little effect on seawater carbonate chemistry (~ 0.002 pH units, ~ 0.6 % [DIC]) at constant seawater pCO_2.

3.3.3. Monitoring seawater parameters

Seawater temperature was measured hourly (TinyTag Aquatic, Gemini Data Loggers, UK) and salinity was measured at least weekly (Thermo Orion 5 - star conductivity meter) and calibrated to NIST conductivity standards. TA and DIC analyses were carried out principally by Dr Catherine Cole of the University of St Andrews. TA was measured by automated Gran titration (Metrohm, 888 Titrando) weekly during the acclimation and twice daily on 4 days of each week in the experimental periods. Precision of duplicate ~ 30 ml TA analyses was typically ± 2 µeq kg^{-1}. Between days, the precision of multiple measurements of synthetic Na_2CO_3 was consistently ± 3 µeq kg^{-1} (1σ, n = 14).

DIC was measured weekly in each reservoir during the experimental periods (LI - 7000 CO_2 differential, non - dispersive, infrared gas analyser, Apollo SciTech; AS - C3). Samples were calibrated against a natural seawater certified reference material (CRM; A. Dickson, Scripps Institution of Oceanography). Internal reproducibility was calculated from the standard deviation of 8 replicate measurements of a single sample (σ/√n) and was always < 0.1 %. Multiple measurements of the CRM were analysed as unknown samples over the 4 - week period to check the calibration, and these were in good agreement with the certified value (unknown = 2019 ± 6 (1σ) µmol kg^{-1}, n = 4; CRM = 2014 µmol kg^{-1}).

3.4.2 Experimental incubations

During the experimental period, each individual coral head was isolated in a 21 L Perspex tank for 5 h (in the light) or 7 h (in the dark) every week. Prior investigations by Dr Catherine Cole and Dr Nicola Allison had demonstrated these times would enable even minor physiological and growth changes to be measured in the dark. Since seawater DMSP concentrations were to be normalised to hourly rates, this was not considered to be a detrimental factor. Net photosynthesis and respiration rates were estimated from measurements of dissolved oxygen (DO_2, Thermo Orion 5 - star meter with RDO sensor) at the start and end of each incubation (Schneider and Erez 2006). Net photosynthesis was defined as the production of oxygen in the light and respiration as the consumption of oxygen in the dark. Gross photosynthesis was calculated as net photosynthesis minus respiration. Seawater

concentrations of DO_2 typically increased by up to * 80 µmol l^{-1} (from * 200 µmol l^{-1}) in the light and decreased by up to ~ 40 µmol l^{-1} in the dark (Cole et al. 2018). The precision of repeat DO_2 measurements was always better than 0.3 %. Seawater was sampled for total DMSP at the start and end of each incubation; DMSP includes dissolved and particulate (i.e intracellular or associated with grazers etc.) DMSP and dissolved dimethyl sulphide (DMS). Replicates (n = 5) of 5 ml seawater volumes were sampled by pipette, alkalinised with 1 ml of 10 M NaOH in 10 ml crimp top vials and immediately sealed with PTFE lined gas tight crimp top caps. NaOH preserves the samples and cleaves DMSP to DMS and acrylic acid (Turner et al. 1990). Samples were left for > 24 h prior to analysis to facilitate full conversion of DMSP to DMS, which was then measured by gas chromatography. Seawater DMSP did not change significantly in control incubations when no coral was included in the tanks and we infer that seawater DMSP production or consumption in the experimental incubations (including coral) reflects the activity of the coral holobiont in the experimental setup. Changes in seawater [DMSP] were normalised to the coral surface area (cm^2) and per unit area of time (h^{-1}).

3.4.3 DMSP seawater analysis

Seawater DMSP samples were pipetted into 10 ml crimp top vials, to which 1 ml 10 M NaOH was added and immediately sealed with PTFE lined gas tight crimp top cap. Samples were analysed as per the method outlined in section 2.3.

3.4.4 Coral tissue extraction

At the end of each one - month temperature treatment, two or more (where possible) coral heads for each pCO_2 treatment were sacrificed for tissue analysis (750 ppm - 4 colonies, 400 ppm – 2 colonies, 180 ppm – 3 colonies). Each head was cut using a rock saw into multiple fragments, immediately flash frozen in liquid nitrogen and stored in polythene bags at – 80°C until analysis could be conducted. However, it was necessary to preserve as many coral heads as possible for the second half of the experiment and to leave enough fragments for other analyses. Single fragments were analysed from each colony (750 ppm $C0_2$, n = 4), two colonies (400 ppm $C0_2$, n = 2) and 3 colonies (180 ppm $C0_2$, n = 3) at the end of the 28°C experiment. At the end of the 25°C experiment a maximum of two fragments per colony were analysed

(180 & 400 ppm $C0_2$, n = 7, 750 ppm $C0_2$, n = 8). Coral tissue and zooxanthellae were extracted using the airbrushing method, which has previously been identified as the most effective manner in which to extract DMSP (Deschaseaux et al. 2013). Nubbins were placed in 15 ml of deionised water in a small polythene bag into which an artist's airbrush tip was inserted and the bag gripped such that no water could escape during airbrushing. Once a reasonable seal had been achieved, coral tissue removed by blasting it from the skeleton into the surrounding DI water using the airbrush, which was connected to a nitrogen cylinder. Tissue was homogenised using a vortex mixer and centrifuged at 3000 rpm for 10 min to remove skeletal tissue after which the supernatant was subsampled for DMSP/DMSO and chl a. The supernatant was then centrifuged twice at 3000 rpm for 10 min to yield a pellet containing algal cells, enabling cell counts to be completed.

3.4.5 Chl – a

4.5ml of 90% acetone was added to 3 ml of supernatant from each coral nubbin and samples were frozen at -20°C for 48 h. The extractant was decanted in triplicate into 1 ml cuvettes and absorbances measured at 630, 647, 664 and 750 nm (turbidity correction). Spectrophotometric analyses were conducted in a dimly lit and cold room to prevent degradation of chl a in samples. The spectrophotometer was zeroed using the same 90% acetone used for the extractions at the start of the run, and every five samples thereafter.

3.4.6 Cell counts

The pellet was resuspended in 3 ml deionised water, vortexed and sonicated. *Symbiodinium* cells were counted on a Neubauer Haemocytometer (Marienfeld Laboratory Glassware); replicate (x5) analyses of 20 uL of sample were counted under a compound microscope (Zeiss Ltd) at x40 magnification. Errors in the range of 20 - 30 % are common using this method and the precision ranged from 5 - 29 % (Bastidas 2017).

3.4.7 Tissue DMSP and DMSO analysis

From the homogenised supernatant, triplicate vials were prepared for tissue DMSP analysis; 1 ml of sample was added to a crimp top vial and 1 ml 10 M NaOH added and the vial immediately sealed. Samples were left for > 12 h to facilitate the conversion of DMSP to DMS (Turner et al. 1990), which was then measured using triplicate headspace injections directly into the GC. The GC was calibrated and operated as per the details in Chapter 2.

Once samples had been analysed for tissue DMSP they were prepared for tissue DMSO analyses using the reductase enzyme method (Hatton et al. 1994); 1 ml 500 mM Tris – HCl pH 8.0 buffer was added to each sample and neutralised to pH 7.0. Samples were measured after DMSP, but to be certain there was absolutely no accumulation of DMS/P during the preparation process, samples were then fast - purged for 4 min to remove any DMS/P. Where samples could not be analysed within 24 h of preparation, they were frozen (- 20°C) until analysis could be completed.

To prepare DMSO samples for analysis 50 µL purified DMSO reductase (prepared in house using the method of McEwan et al. 1991), 0.026 g flavin mononucleotide (FMN) and 30 nM (1.116 g) ethylenediaminetetraacetic acid (EDTA) was added to 100 ml DI water to create FMN solution, which was then fast purged in front of lights for 5 min. To each prepared 1 ml sample, 2 ml of FMN solution was added and the vials crimp sealed with PTFE lined caps. Samples were left in front of 3 x 60 W light bulbs; light acting on the FMN solution forms radicals that reduce FMN to $FMNH_2$, which acts as an electron donor to DMSO reductase thereby catalysing the reduction of DMSO to DMS (Hatton et al. 1994). The time taken for this reaction to occur has previously been shown to take 15 min (Hatton et al. 1994); samples were left in front of the lights for 1 h after which they were left overnight to allow the headspace to equilibrate prior to analysis by headspace injection (see Chapter 2). Because DMSO, like DMSP, is measured in a 1:1 ratio, calibrations were performed as per the method outlined for DMSP.

3.4.8 Statistical analyses

All statistical analyses were conducted using R statistical software (R Development Core Team 2013); where the residuals of the data were not normally distributed (this was assessed visually), log transformations were performed and the log - normal data were used instead. ANOVA models were used for all data, of which there are three types (Types I – III). The key differences between types I – III ANOVA lies in the way the model deals with individual factors. Type I ANOVA is "sequential", meaning that the factors are tested in the order they are listed in the model. Because of the sequential nature and the fact that the two main factors are tested in a particular order, this ANOVA model will give different results for unbalanced data depending on which main effect is considered first. Type III, by contrast, is "partial" and tests for the presence of a main effect after the other main effect and interaction. This approach is therefore valid in the presence of significant interactions and is considered more a more robust model choice for unbalanced data. Type II ANOVA is similar to type III, except that it preserves the principle of marginality. This type tests for each main effect after the other main effect, but not in light of the interaction term. If there is indeed no interaction, then type II is statistically more powerful than type III (Langsrud 2003).

Seawater DMSP: Welch's two sample t - tests were conducted to assess if there were significant differences in seawater DMSP between sampling weeks for each coral head and to assess whether seawater [DMSP] were significantly different at the start and end of each incubation. Interactions using type II were significant, therefore type III ANOVA models were used to assess the significance of temperature, light/dark regime and pCO_2 on seawater DMSP production and interactive effects.

Tissue DMSP/DMSO: No significant interactive effects were observed, so type II ANOVA models were used to assess which factors (temp, pCO_2) significantly affected tissue DMSP/DMSO when normalised to different indices (chl a, surface area, cellular).

The residuals of all data were checked for normality (Shapiro-Wilks test) and homogeneity of variance (Bartlett test), however, no violations of either assumption were noted.

Throughout this chapter (and beyond) significant results, with $\alpha \leq 0.10$, are included intentionally. This has been done principally because not only is there is no authoritative reference for using $\alpha \leq 0.05$ as a significance level, but also because the sample sizes used throughout this study are small and setting an arbitrary cut off might exclude results that might otherwise be of interest. Thus, highlighting any results that are significant at 0.10 may help inform future research efforts.

3.5 Results

3.5.1 Chl a and cell counts

There was no effect of either pCO_2 ($p = 0.78$) or temperature ($p = 0.37$) on the number of cells per cm^2 of coral. pCO_2 had no effect on [chl a] when normalised to either surface area ($p = 0.83$) or per cell ($p = 0.85$), nor did temperature for either surface area ($p = 0.94$) or cellular ($p = 0.35$) normalisation indices. Thus, increases to DMSP/O production in seawater and tissue occur independently of any changes to algal cell numbers or cell size.

3.5.2 Tissue DMSP and DMSO

In line with our original hypothesis, concentrations of both compounds increased with increases in seawater temperature. Concentrations of DMSP (Fig 3.2) per cell ranged from 14.96 – 143.27 fmol cell $^{-1}$ at 25°C and increased to 239.91 – 844.49 fmol cell $^{-1}$ at 28°C. DMSO concentrations per cell ranged from 19.46 – 150.83 fmol cell $^{-1}$ at 25°C and increased to 48.89 – 891.67 fmol cell $^{-1}$ at 28°C.

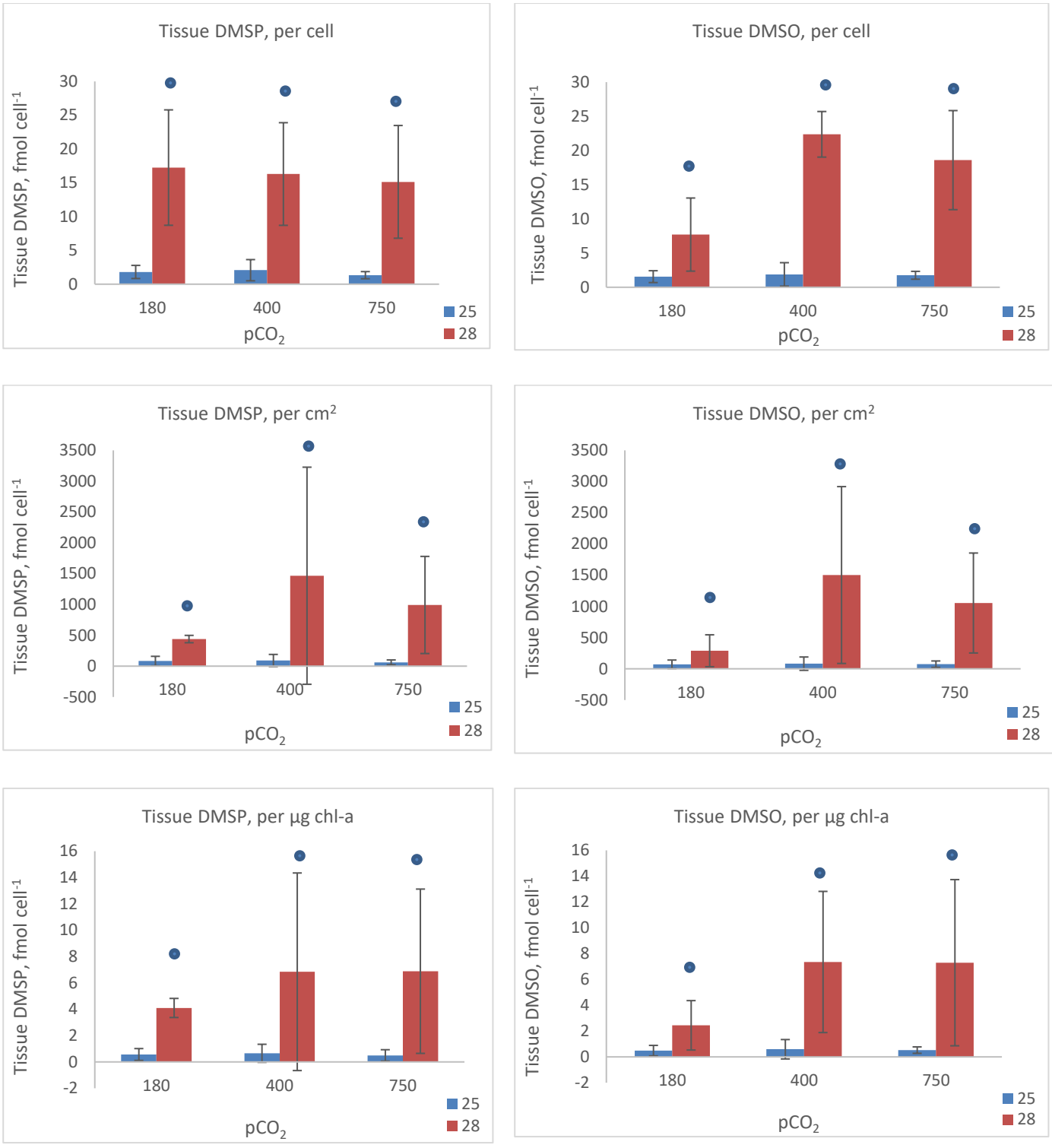


Figure 3.2 Summary of tissue DMSP (left hand graphs) and DMSO (right hand graphs) concentrations normalised to per cell, chl a and areal indices, for both temperature treatments and all pCO_2 treatments. Error bars represent the standard deviation of tissue concentrations between multiple fragments in each pCO_2 treatment: 28 °C: 750 ppm, n = 4, 400 ppm, n = 2 and 180 ppm, n = 3. 25 °C 180 & 400 ppm, n = 7, 750 ppm, n = 8. Significant differences between temperature treatments are indicated with a dot over the appropriate bar.

There was no evident pattern of tissue concentrations of DMSP or DMSO in response to different pCO_2 treatments, however there are consistently greater concentrations of both compounds in response to temperature and this was independent of changes to algal biomass, which did not increase in response to changes to temperature/pCO_2. This was found to be significant (Table 3.1), as originally hypothesised, with temperature having a significant effect on tissue concentrations for both compounds irrespective of normalisation index.

Table 3.1 Summary of ANOVA analyses for DMSP and DMSO for each normalisation index. Significant values ($\alpha < 0.05$) are highlighted in bold and significant at $\alpha < 0.10$ values are italicised.

Normalised index	***pCO_2***	***Temperature***	***Interaction***
DMSP chl a	0.73	**< 0.001**	0.48
DMSO chl a	0.34	**< 0.001**	*0.10*
DMSP cm $^{-2}$	0.43	**< 0.001**	0.11
DMSO cm $^{-2}$	0.21	**< 0.001**	**0.03**
DMSP cell $^{-1}$	0.83	**< 0.001**	0.91
DMSO cell $^{-1}$	**0.02**	**< 0.001**	**< 0.001**
DMSO:DMSP	0.15	0.73	0.56

Also evident was the significant effect of pCO_2 treatment on DMSO when normalised to cellular levels, however there was no effect of pCO_2 for any other normalisation index, or for DMSP. In contrast to our original hypothesis, there was no effect of pCO_2 or temperature on the DMSO:DMSP ratio, which did not significantly change in response to either variable. However, the interactive effect of pCO_2 with temperature did significantly affect concentrations of DMSO when normalised to areal ($p = 0.003$), cellular ($p = 0.10$) and chl a ($p < 0.001$) indices.

3.5.3 Seawater DMSP flux

Seawater [DMSP] at the start of the incubations (1.8 to 4.7 nmol) was considerably lower than at natural coral reef sites (15 – 24 nmol, Burdett et al. 2013, Jones et al 2006). Significant net DMSP production in seawater was observed in 16 of the 24 incubations in the light at 28°C but in only 3 of the 24 incubations in the light at 25°C (Table 3.2).

Table 3.2 Summary of changes in seawater [DMSP] during coral incubations. Rates are nmol S cm^2 h^{-1}. Values in bold indicate significant net seawater DMSP production/removal (Welch's two - tailed t - test, $p > 0.05$). Boxes are colour coded; red = lowest values (net uptake), green = highest values (net production).

	180 ppm		180 ppm		400 ppm		400 ppm		750 ppm		750 ppm	
Coral colony	Light		Dark		Light		Dark		Light		Dark	
25°C	Wk1	W2	Wk1	W2	Wk1	W2	Wk1	W2	Wk1	W2	Wk1	Wk2
Porites colony 1	**-0.067**	**0.022**	0.019	**0.066**	-0.038	0.002	0.03	0.008	-0.02	0.034	**0.045**	0.012
Porites colony 2	-0.03	-0.034	0.008	**0.04**	-0.02	0.006	-0.001	**0.056**	0.043	-0.011	**0.057**	0.036
Porites colony 3	0.082	0.006	**0.119**	**0.115**	0.041	**0.08**	0.024	**0.1**	0.034	-0.016	**0.042**	0.056
Porites colony 4	0.031	0.016	**0.029**	**0.077**	0.064	**0.028**	**0.06**	**0.079**	0.014	0.01	**0.018**	0.044
28°C												
Porites colony 1	**0.072**	0.098	**0.072**	**0.043**	**0.052**	**0.075**	**0.032**	0.026	**0.011**	**0.03**	0.012	0.028
Porites colony 2	**0.027**	**-0.032**	**0.016**	0.009	0.006	**0.029**	**0.061**	**0.024**	0.039	-0.011	**0.132**	0.038
Porites colony 3	**0.072**	**-0.02**	-0.001	-0.002	**0.066**	0.045	0.03	**0.039**	**0.108**	**0.019**	**0.023**	0.033
Porites colony 4	**0.049**	0.005	0.018	**0.034**	**0.147**	**0.06**	**0.053**	**0.07**	**0.044**	**0.014**	**0.058**	0.044

Significant net production of seawater DMSP was observed in 17 of the 24 incubations in the dark at both temperatures. Significant net removal of seawater DMSP was observed in 3 incubations, all in the light at 180 ppm seawater pCO_2. The means for all colonies in each pCO_2 treatment, temperature and light/dark incubation were calculated (Fig 3.3) so that any patterns in seawater DMSP production between treatments and the light/dark incubations could be ascertained.

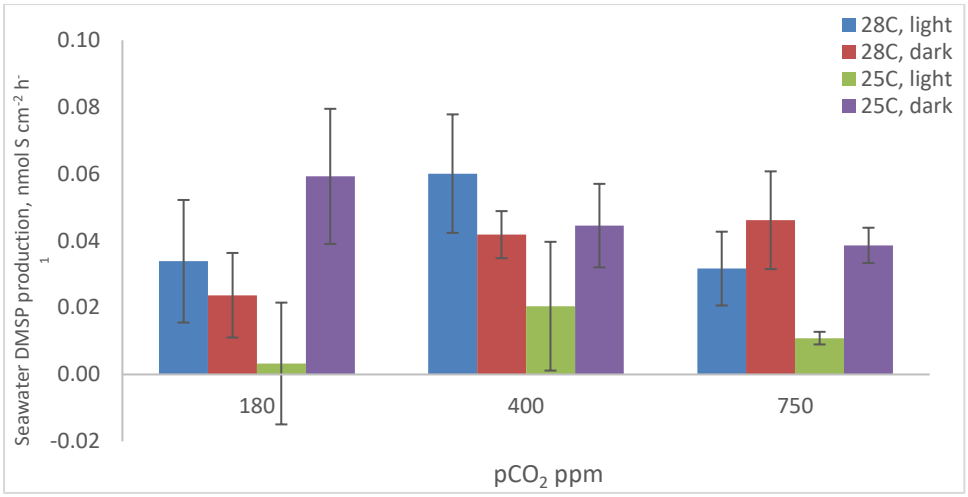


Figure 3.3 Mean (n = 4) seawater DMSP production by *Porites* spp. in response to changing temperatures, pCO_2 treatments and in light/dark incubations. Error bars represent the standard error of seawater DMSP production per colony averaged over both weeks of sampling.

We originally hypothesised that increased temperature would result in increases to seawater DMSP, but that pCO_2 would not necessarily exert the same effect. However, the effect of pCO_2 on seawater DMSP production was significant at $\alpha < 0.10$ (ANOVA, $p = 0.10$), similarly at $\alpha < 0.10$, temperature significantly affects seawater DMSP production (ANOVA, $p = 0.06$). The effect of light/dark on seawater DMSP production was highly significant (ANOVA, $p < 0.001$). However, interactive effects between pCO_2 (ANOVA, $p = 0.10$) and light/dark (ANOVA, $p = 0.01$) with temperature were noted. Given the significant effect of temperature on seawater DMSP production, the interactive effects between pCO_2 and light/dark with temperature suggests that in isolation pCO_2 exerts no significant effect on DMSP production, rather it is dependent on seawater temperature.

To assess whether seawater DMSP production was driven by metabolic processes, rather than in response to the experimental conditions, we investigated whether gross photosynthesis (GP) or respiration were related to seawater DMSP flux (Fig 3.4). GP rates were not significantly different between temperature treatments; GP was identical for both 25°C and 28°C (2.88 µmol DO_2 cm^{-2} h^{-1}), whilst respiration rates were marginally lower at 25°C (- 0.7 µmol DO_2 cm^{-2} h^{-1}) than at 28°C (- 0.8 µmol DO_2 cm^{-2} h^{-1}) these differences were not significant (Two - tailed t - test, $p = 0.14$).

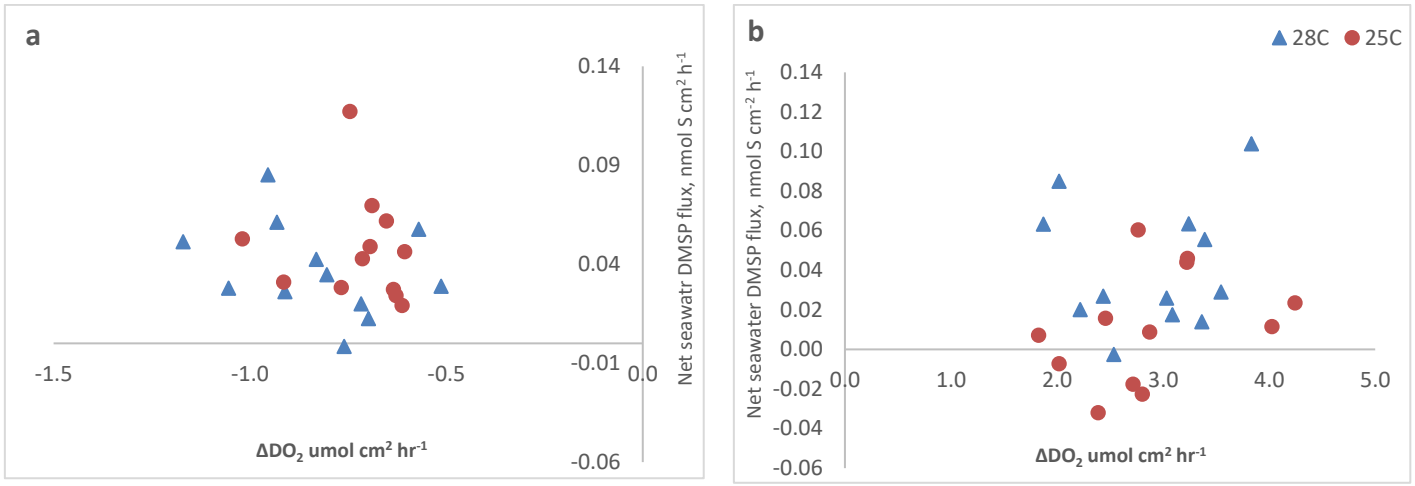


Figure 3.4 Net seawater DMSP flux as a function of a) gross respiration and b) gross photosynthesis for all coral colonies in all seawater pCO_2 treatments separated by temperature.

Control incubations showed no significant change in DO_2 in the absence of a coral colony. Linear models with ANOVA were conducted to assess the relationship between photosynthesis/respiration, temperature and pCO_2. A significant effect of both temperature ($p = 0.02$) and pCO_2 ($p = 0.08$) was observed for respiration, however no effect of either variable was evident for photosynthesis. Linear models showed that there was no evidence of a relationship between seawater DMSP production and photosynthesis. There were weak and non-significant correlations in the light at 25°C ($R^2 = 0.13$, $p = 0.25$, Pearson's = 0.36) and 28°C ($R^2 = 0.009$, $p = 0.76$, Pearson's = 0.10), and in the dark at 25°C ($R^2 = 0.01$, $p = 0.71$, Pearson's = - 0.12) and 28°C ($R^2 = 0.10$, $p = 0.32$, Pearson's = - 0.31). Thus, results suggest that seawater DMSP flux does not appear to be related to photosynthetic/respiration rates.

3.6 Discussion

3.6.1 Comparison of tissue DMSP and DMSO concentrations with other regions

Areal concentrations of tissue DMSP in massive *Porites* spp. were ~ 10 times higher at 28°C than at 25°C (Figure 3.2). This data is broadly comparable to previous reports for *Porites* spp. (174 – 420 nmol cm $^{-2}$, Hill et al. (1995); 27 nmol cm $^{-2}$ (DMSP only), Tapiolas et al. (2013); 21.5 – 67.4 nmol µg chl a $^{-1}$, Yost et al. (2010)), however the range of the current data exceed those of any datasets previously reported in the literature. Variation between this data and previous findings could be attributed to variable DMSP concentrations between different Poritid coral species; Hill et al. (1995) investigated *Porites compressa*, whilst Yost et al. (2010) reported values for *Porites astreoides*. Similarly, subtle changes between methodologies used could affect results. For example, when compared to the same extraction, temperature and normalisation index, DMSP concentrations at 25°C (0.78 - 20.93 nmol µg chl a) were lower than those reported by Yost et al. (2010). Another potentially significant source of variation between current results and the literature is likely to come from the geographic location of experimental corals; the current study and those of Hill et al. (1995) and Tapiolas et al. (2013) were based in the Indo - Pacific, however Yost et al. (2010) studied Bermudian corals. Corals from each of these regions are likely to host genetically distinct symbionts, which could result in changes to DMSP production (Baker 2003; Steinke et al. 2011; Borell et al. 2016).

Production of DMSP in the coral holobiont is chiefly carried out by *Symbiodinium*, which are known to produce variable amounts of DMSP (Steinke et al. 2011). Since the distribution of *Symbiodinium* is known to vary geographically, corals from different regions could potentially harbour different *Symbiodinium* communities (Baker 2003), thereby altering DMSP production by the holobiont. Current data was within the range of values for tissue DMS/P previously reported for *Acropora* spp., with concentrations ranging from 17 – 2200 nmol cm $^{-2}$ (156 - 690 nmol cm $^{-2}$ Broadbent et al. 2002; 247 nmol cm $^{-2}$ Tapiolas et al. 2013; 71 - 2200 nmol cm $^{-2}$ Deschaseaux et al. 2014; 190 - 166 Jones and King 2015). The similarities in concentrations of DMSP produced by different coral species is interesting; with variable concentrations produced by different coral symbionts, it is perhaps more

reasonable to expect DMSP concentrations to be significantly different between species, which in a sense it is. Whilst the values reported in this study are within the range of values reported from other reefs, the overall range of those values is remarkably broad. Thus, it seems likely that the range of potential DMSP production by corals is highly variable. However, subtle differences in experimental design also need to be acknowledged; for example, Deschaseaux et al. (2014) and Jones & King (2015) employed a range of temperature treatments (27.5 - 32°C), with temperature increases happening over < 7 - 15 d, which is markedly different to the one - month temperature decrease from 28°C to 25°C used here. Furthermore, unlike the other studies mentioned here, Tapiolas et al. (2013) measured DMSP in isolation, i.e. they did not measure total DMSP, which also includes DMS.

Whilst reported values for coral tissue DMSO are scarce, the current data are also in the range of values reported by Deschaseaux et al. (2014), who reported values ranging from 13.4 ± 2.17 fmol cell $^{-1}$ (control corals at 28°C) to 801 ± 150 fmol cell $^{-1}$ (elevated temperature at 31°C).

3.6.2 The effect of pCO_2 on tissue concentrations of DMSP/O and seawater DMSP flux

pCO_2 (Fig 3.2, Table 3.1) was shown to significantly affect tissue [DMSO] but only when normalised to tissue cellular concentrations, with interactive effects between temperature and pCO_2 for all normalised indices. There was no effect of pCO_2 on tissue [DMSP] when normalised to any index, however seawater production of DMSP was significantly affected by pCO_2.

Organisms generally acclimate to oxidative stress, such as that caused by CO_2 limitation, by up - regulation of antioxidants (Sunda et al. 2002; Bucciarelli and Sunda 2003). Thus, it was expected that corals subjected to the lower pCO_2 treatment would exhibit higher concentrations of both compounds. However, this was not the case as cellular concentrations of DMSO (Fig 3.2) were lowest in the 180 ppm CO_2 treatment than in the other pCO_2 treatments. To date, we know of no studies to have previously considered the effects of changing pCO_2 concentrations on algal/coral production of DMSO. However, several studies have linked high CO_2 concentrations to increased cellular DMSP in cold water

corals (Burdett et al. 2014), some phytoplankton (Spielmeyer and Pohnert 2012b; Arnold et al. 2013) and macroalgae (Burdett et al. 2012, 2013). Spielmeyer and Pohnert (2012) noted enhanced DMSP production in a non - calcifying strain of *Emiliana huxleyi*, suggesting the increase in cellular DMSP could be attributed to increased photosynthetic rate. As a potentially derived product of DMSP, there is also likely to be a relationship between DMSO production and photosynthesis. Thus, reduced photosynthetic activity under low CO_2 conditions, which are not low enough to induce oxidative stress, could lead to the lower cellular DMSO concentrations observed in this study. Tissue DMSP concentrations, however, were not found to be affected by pCO_2 suggesting either that production of these two compounds may not be as intimately linked as previously thought, or that the threshold for observing an effect is either much higher or lower than the range of pCO_2 levels used here. If production of DMSO is derived and therefore dependent on production of DMSP, an effect of pCO_2 on cellular concentrations would be evident. These results suggest that DMSO synthesis is, potentially, independent of DMSP production, which is supported by previous studies that have detected *de novo* DMSO production in a range of microalgal taxa (Simo et al. 1998b; Hatton and Wilson 2007).

There was no significant effect of either pCO_2 or temperature on the tissue ratio of DMSO:DMSP, and this result is worthy of discussion. DMSP and its breakdown products (dimethylsulphide (DMS), acrylate, DMSO and methanesulphonic acid (MSNA)) can readily scavenge hydroxyl radicals and other reactive oxygen species during cellular stress (Sunda et al. 2002). Upon reacting with reactive oxygen species, DMS and DMSP are oxidized to form DMSO. Therefore, when algal cells are subject to increased oxidative stress, DMSO concentrations should increase and even exceed those of DMSP, resulting in a higher DMSO:DMSP ratio. This is supported by other studies in the literature in which increases in this ratio are linked to cellular stress in *Spartina* spp. (Husband and Kiene 2007; McFarlin and Alber 2013), with several studies showing that elevated temperatures lead to increases in coral tissue DMSP (Jones et al. 2014; Jones and King 2015) and DMSO (Deschaseaux et al. 2014b). It is possible that the corals used in this experiment were not subjected to sufficient levels of cellular stress that would lead to an increase of the DMSO:DMSP ratio. The lack of significant decreases in either number of cells or in chl a concentration in response to changes in either temperature or pCO_2 would seem to support this suggestion. This

represents an interesting result, supporting the original hypothesis of this chapter, that *Porites* corals are likely to fare better under projected climate change scenarios than previously thought.

3.6.3 The effects of temperature and light on tissue and seawater DMSP production

Increasing seawater temperatures from 25°C to 28°C significantly increased tissue concentrations and seawater flux of DMSP. Multiple studies report large increases in tissue [DMSP] in response to short - term seawater temperature increases of 2 - 5°C, which are associated with at least partial bleaching of the coral (Raina et al. 2013; Deschaseaux et al. 2014b). However, tissue [DMSP] normalised to area was not significantly affected by seasonal temperature variations up to ~ 6°C (Jones et al. 2014; Jones and King 2015).

Current data fall between these two extremes. A large increase in tissue [DMSP] was observed but this was not associated with any visible evidence of coral bleaching, nor were there differences in chl a concentrations between the two temperatures. Deschaseaux et al. (2014) also reported increases in coral tissue DMSP production under elevated temperature that occurred with no significant difference in chl a between control and temperature treated corals. However, the authors did note a significant decrease in symbiont density suggesting that bleaching did occur, at least partially. Coral tissue DMSP production may be increased at high temperatures to mitigate against enhanced oxidative stress and accumulation of tissue reactive oxygen species (Lesser 1997; Sunda et al. 2002; Jones et al. 2014). At higher temperatures, however, Fischer & Jones (2012) reported decreased tissue [DMSP], decreased zooxanthellae density and increased chl a. These results suggest that high tissue [DMSP] has a source other than the symbiont. One such source could be coral mucus, which has been shown to contain the highest concentrations of DMSP in the marine environment (Broadbent and Jones 2004) and production increases under increasing temperature (Sawall et al. 2015). This mechanism could also account for the increased flux of DMSP to seawater.

There was an approximate 300 % increase in DMSP seawater flux in the light from 25°C to 28°C, which has been supported by other studies in the literature (Fischer and Jones 2012;

Jones et al. 2014). Fischer & Jones (2012) reported ~ 6000 % increases in seawater DMSP flux, whilst Jones et al. (2014) reported more modest 45 % increases under elevated temperature conditions. Again, current data lie between these two extremes. Seawater DMSP flux increased in response to temperature increases, but this pattern was not found in night - time incubations. It has previously been suggested that increased cellular DMSP production can alleviate the effects of oxidative stress via an antioxidant cascade mechanism (Sunda et al. 2002). However, since we used LED lights that did not emit UVA/UVB light, changes in [DMSP] concentrations between light and dark incubations could not be driven by photolytic removal processes and must be driven by other factors.

Previous studies have estimated that coral expel ~ 10 % of their algae each day (Broadbent and Jones 2006), with Fischer & Jones (2012) suggesting that zooxanthellae expulsion could account for increased seawater DMSP flux. Since we observed increased tissue DMSP at the higher temperature and increased seawater DMSP, it is possible that the higher seawater DMSP concentrations were driven by increased particulate DMSP in seawater caused by zooxanthellae expulsion.

3.6.4 Implications for coral reefs

Increasing evidence of climate change has been found and its continuation is now considered inevitable. Current forecasts for global oceanic warming are between 0.6 - 2.0°C by the end of the century, and that atmospheric CO_2 levels will continue to increase resulting in atmospheric CO_2 levels of ~ 900 ppm (RCP 8.5, IPCC 2013).

This study demonstrated that temperature, pCO_2 and light all affect tissue and seawater production of DMSP and DMSO, both in isolation and in interaction with each other. Furthermore, the current data shows that *Porites* corals produce less seawater DMSP than *Acropora* corals. Despite being recognised as major structural reef builders (Pichon 2011), results indicate that the overall contribution of *Porites* to the DMSP pool in reef waters is probably much lower than that of other coral species, such as *Acropora*. However, *Porites* corals are recognised as being fairly resilient to changes in ocean pH and CO_2 levels, with reports suggesting they will be a “winner” under climate change forecasts (Fabricius et al.

2011) and the data presented here supports this. There was no significant increase in DMSO:DMSP caused by either changes to pCO_2 or increased temperature, suggesting that the corals in our experiment were not stressed. This suggests that under climate change forecasts, *Porites* will fare better than other higher DMSP producing, coral species. Thus, the data presented here supports our original hypothesis that *Porites* corals will fare better under climate change conditions. By extension, these data suggest that coral - derived DMSP in reef waters is likely to decrease as hard coral communities shift to *Porites* dominated assemblages.

We suspect that different zooxanthellae assemblages associated with each coral drive variability in tissue and seawater concentrations of DMSP, which has potentially significant ramifications for coral reef ecosystems. Studies have shown that DMSP in coral tissue can act as a chemo - attractant for pathogenic coral bacteria (Garren et al. 2014) and *Porites* colonies that produce more tissue DMSP than a neighbouring colony could be more susceptible to infection, exacerbating the stress effects of climate change.

3.6.5 Conclusions

Porites corals are likely winners under climate change forecast scenarios, but this is likely to mean decreased coral derived seawater DMSP concentrations. As coral reef communities shift to a macroalgal dominated assemblage, the resilience of *Porites* corals to increasing temperatures could ensure their survival, preserving an element of “coral” in coral reefs of the future. However, their survival and resilience may be short-lived; increased production of DMSP and a reduced coral community could exacerbate the threat from pathogenic bacteria, which could be fatal to surviving colonies although further research would be required to quantify the severity of this threat.

4 Controls on spatio - temporal variability of shallow reef seawater DMSP

4.1 Introduction

The previous study highlighted the importance of increases in pCO_2 and temperature on coral production of DMSP and DMSO, which resulted in increased concentrations of DMSP in the surrounding water. However, the conditions to which corals were subjected were tightly controlled. Wild corals, in a typical reef setting, are subject to an array of variable conditions and despite the name, coral reefs are not solely comprised of corals, but a wide range of different taxa including sponges, coralline algae, soft corals and macroalgae. In addition, it is known that there is spatial heterogeneity in carbonate and DMSP chemistry across the reef topography (Zhang et al. 2012, Burdett et al. 2013). Therefore, a study was designed to investigate what factors drive spatial variability of coral reef DMSP in a Caribbean reef system, which have hitherto been unstudied with respect to DSC. Abiotic and biotic variables were considered over two summer seasons, to ascertain what the current drivers of seawater DMSP were. Only when we understand contemporary reef sulphur cycling, can we forecast the likely impacts of climate change on DSC in reef systems.

Average oceanic pH has begun to decrease from pre - industrial levels as atmospheric carbon dioxide increases and CO_2 dissolves into the oceans (see Chapter 2) in a process known as ocean acidification (OA). Whilst the physiological effects (e.g. calcification and growth rates) of reduced pH on marine calcifiers is relatively well understood, the impact on the marine sulphur cycle has not been well researched.

Perhaps one of the most vulnerable and charismatic marine ecosystems vulnerable to pH decline are coral reefs. Coral reef ecosystems are vulnerable to OA and climate change induced ocean warming (Hoegh-Guldberg et al. 2007a) with a range of effects on the ecosystem (Fabricius et al. 2011). Specifically, increases in oceanic CO_2 will reduce the marine aragonite and calcium carbonate saturation states (ΩAr and ΩCa respectively). The saturation state (Ω) describes the thermodynamic potential for calcium carbonate to precipitate or dissolve, thus a reduced saturation state decreases the ability of many coral species to produce their carbonate skeletons (Anthony et al. 2008a). This has led to the

suggestion that coral reefs will be replaced by a fleshy macroalgal/seagrass dominated assemblage (Dubinsky and Stambler 2011). Also of interest is the Revelle factor, also known as the buffer factor, which represents the ratio of instantaneous change in carbon dioxide (CO_2) to the change in total dissolved inorganic carbon (DIC), and is a measure of the resistance to atmospheric CO_2 being absorbed by the ocean surface layer. The lower the Revelle factor, the larger the buffer capacity of seawater, which makes this parameter of particular interest when assessing the impact of climate change on marine systems. With respect to DSC, the Revelle factor may represent a source of stress to corals, with higher factors resulting in increased DMSP production, although there is no evidence to support this as yet.

Dimethylsulphoniopropionate (DMSP) is a sulphur compound produced by many marine algae, with a number of cellular functions having been described for this compound in marine algae (Karsten et al. 1996b; Kirst 1996; Sunda et al. 2002; Van Alstyne and Puglisi 2007; Seymour et al. 2010a). In tropical reef environments, DMSP may be particularly important as an antioxidant, grazing deterrent and/or compatible solute to aid in osmoregulation. Further research has also suggested that DMSP may play a role in improving tolerance to variable carbonate chemistry conditions (Burdett et al. 2012), thus its production is possibly also linked to cellular stress.

Spatial heterogeneity in carbonate chemistry has been observed in coral reef systems, with reef flats and lagoons more variable than the reef - front due to differences in benthic community composition and the physical (i.e. wave action) context (Kleypas et al. 2011; Gray et al. 2012; Zhang et al. 2012). Indeed, coral reefs are characterised by extreme natural variations in carbonate chemistry, reflecting diurnal cycles driven by biological and physical processes. Research has also shown that macroalgae and seagrasses are prolific producers of tissue DMSP (Broadbent et al. 2002; Burdett et al. 2013; Borges and Champenois 2015). Thus, it logically follows that spatial heterogeneity exists with respect to seawater DMSP and indeed this has been suggested in a previous report (Burdett et al. 2013), although there remains scope for further investigation.

4.2 Aims of this study

Whilst spatiotemporal variability of DMSP in coral reefs has previously been investigated (Broadbent and Jones 2006; Burdett et al. 2013), no such study exists for the Caribbean, where coral reef ecology is distinct from the Red Sea or Great Barrier Reef systems. Furthermore, of the two studies to have investigated spatial variability in seawater DMSP, neither presented data in relation to the underlying benthic ecology and although a relationship between higher seawater DMSP and seagrass dominated assemblages was suggested by Burdett et al. (2013), the authors stated that further work would be required to fully investigate this relationship. In order to fill the gaps in the current knowledge regarding biogeochemical cycling of DMSP, this study assessed which factors, abiotic and biotic, affected the natural spatiotemporal variability of coral reef seawater DMSP at two sites in the Caribbean over two years.

4.3 Hypotheses

1. Concentrations of seawater DMSP are dependent on the underlying reef ecology, with higher concentrations occurring in areas of high DMSP producing organisms (i.e. seagrass)
2. Seawater DMSP exhibits spatial variability that is driven by abiotic factors; light, depth, seawater mixing, temperature and pH. Areas of increased temperature and light will exhibit higher DMSP concentrations, similarly shallower areas will act to "trap" DMSP through reduced mixing and seawater concentrations will be higher.
3. Because of 1 & 2, corals are not the primary DMSP producing organisms in coral reef settings. We expect therefore, that seawater DMSP concentrations in deeper reef habitats will be lower, which is driven by decreased coverage of DMSP producing organisms, lower light/temperature and increased mixing.
4. There is temporal variability in seawater DMSP concentrations, with highest concentrations occurring late in the afternoon and first thing in the morning, when DMSP production rates increase due to increased grazing pressure (morning) and temperature (afternoon)

4.4 Methods

4.4.1 Research sites

Two sites were studied in 2015 and 2016; Coral View and Little Bight. Both sites are located on the island of Utila, within the Honduran Bay Islands on the southern end of the Meso - American Barrier Reef (Fig 4.1).

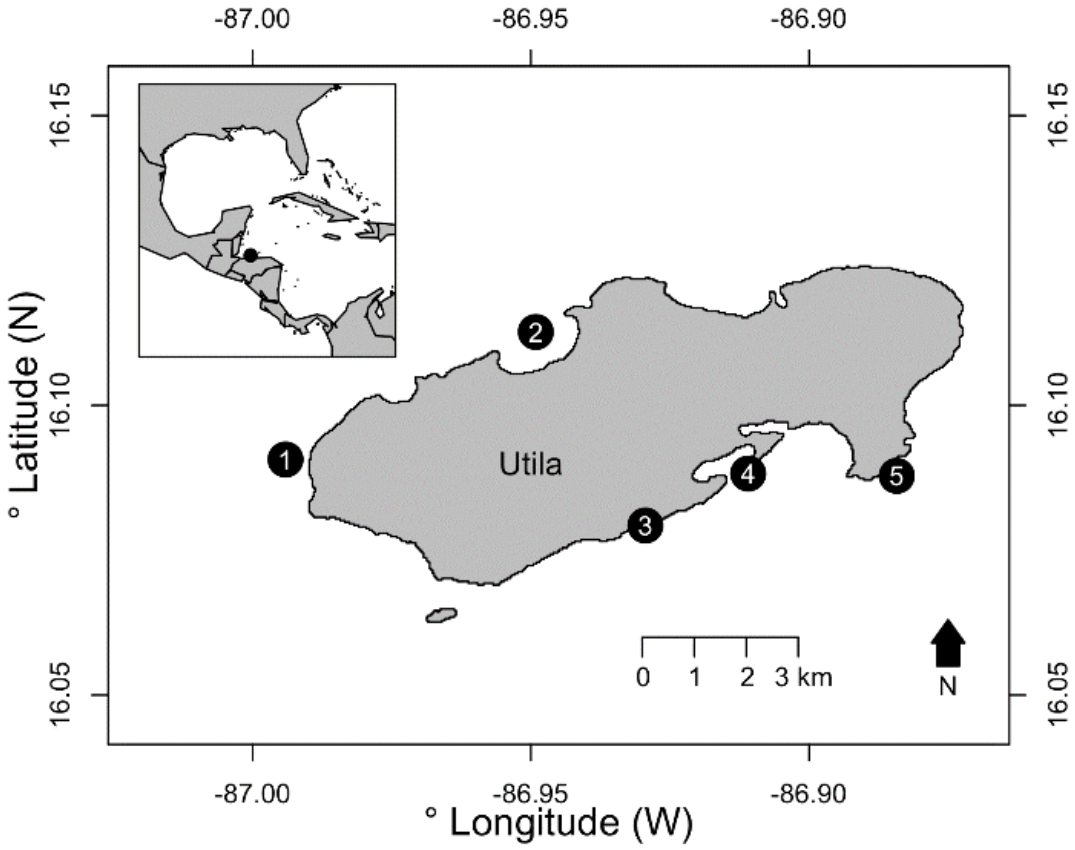


Figure 4.1 Map showing the location of Utila Island (Honduras) on the southern end of the Meso - American Barrier Reef, with the location of all research sites used during this study. Coral View (4) and Little Bight (3) are the focus of this chapter and details are included below, however all dive sites are shown. Dive sites are listed with GPS coordinates and abbreviations starting left in WGS84 format: (1) Raggedy Cay (RC: N 16.09065964, W - 86.9941015), (2) The Maze (TMA: N 16.11266214, W - 86.94911793), (3) Little Bight (LB: N 16.07926302, W - 86.92942222), (4) Coral View (CV: N 16.08823274, W - 86.91094506), (5) Rocky Point (RP: N 16.08784039, W - 86.88423403). The dive site map was sourced with permission from Laverick et al. (2017).

Both sites are south facing and characterised by a spur and groove reef structure (Fig 4.2); spur and groove formations are found on the fore reefs of many coral reefs worldwide and are primarily present in wave-dominated environments (Storlazzi et al. 2003; Rogers et al. 2015).

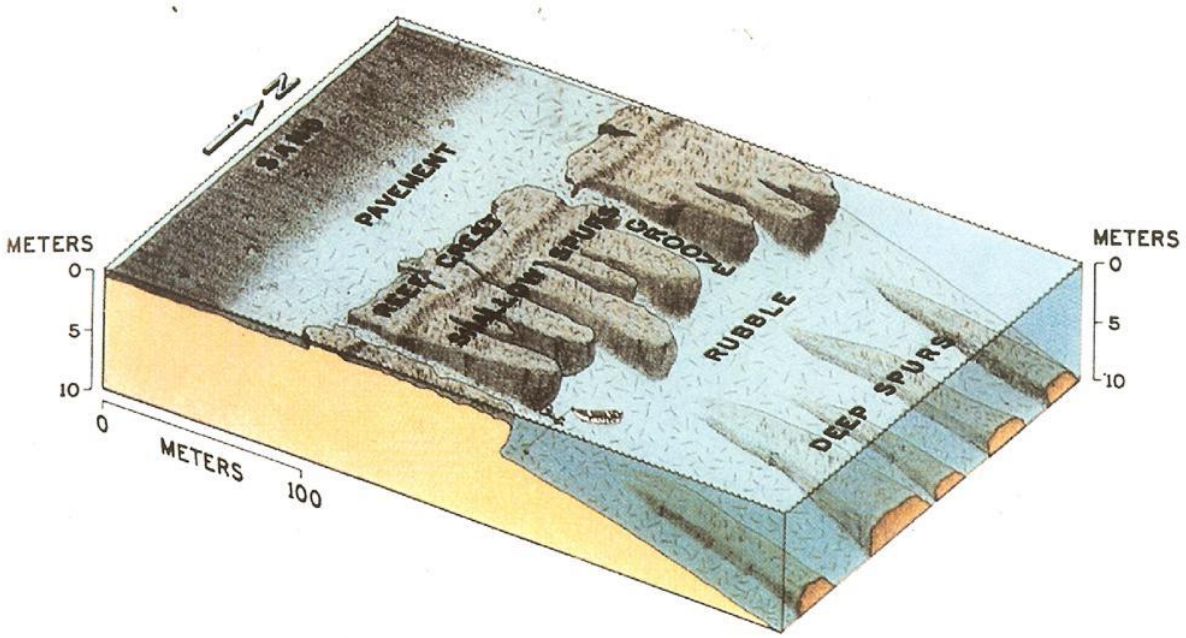


Figure 4.2 Diagram illustrating the typical structure of a spur and groove reef (University of South Florida 2009).

Spur and groove formations have typical scales of: spur height 0.5 to 10 m, alongshore wavelength 5 to 150 m, width of groove 1 to 100 m, and found in depths from 0 to 30 m. Visual observations of the spur and groove formations at both reef sites used in this study are within the parameters described for typical spur and groove formations.

Three spurs and the corresponding grooves were sampled in 2015 (Fig 4.3) at both Coral View and Little Bight, whilst one spur was sampled in 2016 at each site and is the spur labelled 1 in Fig 4.3.

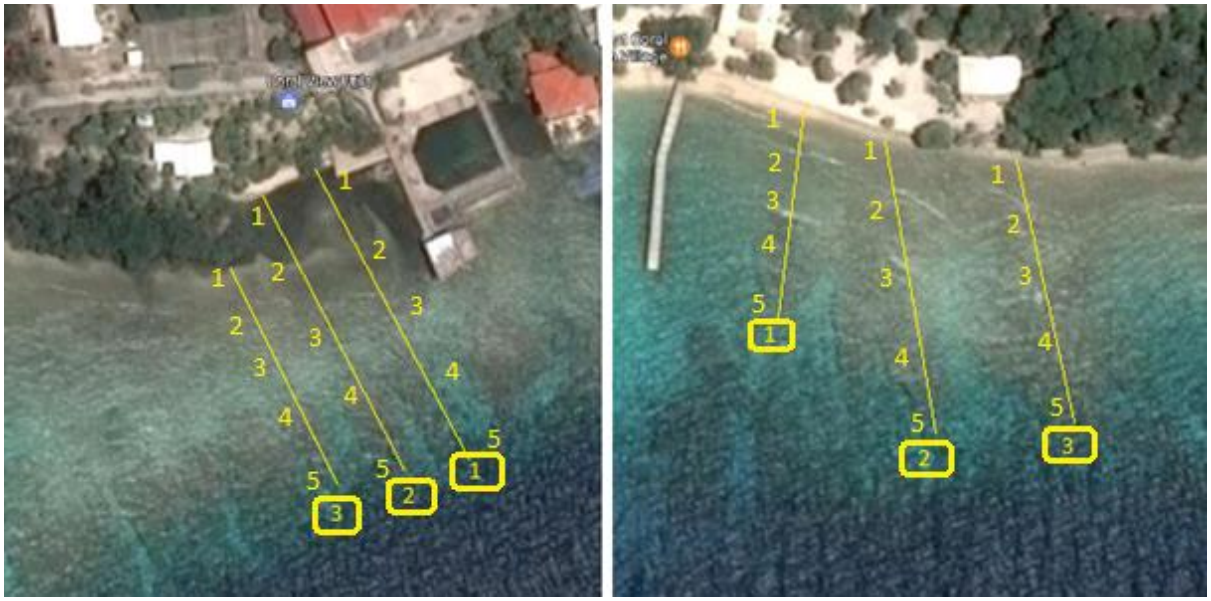


Figure 4.3 Aerial view of Coral View (left) and Little Bight (right) reef sites; both sites are characterised by a spur and groove formation, with the spurs identified as being the portion of the reef under each yellow line. Sampling locations for each spur are indicated by numbers; spurs are indicated by boxed numbers

Spurs at each site were measured during sampling and the accuracy confirmed using the measuring capability of Google Earth™; a transect line was plotted over each spur and run to the end of the spur, the distance was noted, and this was repeated several times. Distances measured on Google Earth™ were within 5% of the actual measured distance, thus confirming the accuracy of field - based measurements. Sampling locations (Fig 4.3) were sited as equidistantly down the length of each spur - groove pair as possible. The boundaries to identify the back and mid reef locations (Table 4.1) were based on visual observations of the benthic cover, whilst the reef front was always measured from the beginning of each spur. Grooves preceded spurs, such that the groove for spur 1 at Coral View is the groove to the right of the yellow line for Coral View, whilst the groove for spur 1 at Little Bight is to the left of the line for spur 1 at Little Bight (Fig 4.3).

Table 4.1 Summary of back, mid and front reef lengths for each of the three spurs (and grooves) used in 2015. Spur 1 only was sampled in 2016 and sampling locations were identical to 2015; measurements made during sampling in 2016 showed no difference in spur length between years.

	Coral View			***Little Bight***		
	Spur 1 (m)	Spur 2 (m)	Spur 3 (m)	Spur 1 (m)	Spur 2 (m)	Spur 3 (m)
Back	39.80	42.8	31.5	22.1	21.3	26
Mid	11.80	11.5	9.2	10.7	13	12.9
Front	35.4	33.3	32.7	27.3	42.4	40.5
Total length	87.00	87.60	73.40	60.10	76.70	79.40

4.4.2 Water sampling – 2015

Samples were taken at five equidistant points along the length of three spurs and the three corresponding grooves, at two different locations, Coral View and Little Bight (Fig 4.1). Sampling was conducted on three randomly chosen days, and owing to logistical constraints, the sampling time was different between and within sites on each day. Samples were retrieved in 1 Litre Flexi Water Bottles (Mountain Warehouse™) and returned to shore for subsampling of dissolved inorganic carbon (DIC), total alkalinity (TA) and DMSP. DIC and TA samples were decanted using Tygon tubing from the bottom of the bag into 12 ml borosilicate glass vials and were immediately fixed with 10 µL of HgCl and sealed. DMSP samples were pipetted into 10 ml crimp top vials, to which 1 ml 10 M NaOH was added and immediately sealed with PTFE lined gas tight crimp top caps. All samples were analysed as per the methods described in section 2.5.2 (TA), section 2.5.3 (DIC) and section 2.3 (DMSP).

4.4.3 Temperature and light measurements - 2015

Temperature was measured using Gemini TinyTag TGI - 3080 data loggers at two points (corresponding with points 3 and 5, Fig 4.2) along spur at the house reef site (Coral View). This site was chosen as the loggers would be less likely to be tampered with whilst deployed; loggers were left in place for two days and were set to record temperature every 30 min.

Photosynthetically active radiation (PAR) was recorded using Odyssey Integrating PAR Sensors (Dataflow Systems PTY Limited), which were deployed with the temperature loggers as above. Sensors were left in place for 4 days so that variability owing to changes in cloud cover or wind (which had not noticeably affected temperature) could be accounted for. Loggers were set to record every 15 min.

4.4.4 Ecological sampling - 2015

The ecologies of all three spurs and grooves at both shallow reef sites were assessed using point intercept transects, with measurements made every 0.2 m. Initial measurements were recorded using a dive slate and by swimming along a tape measure, however owing to logistical constraints the majority were recorded by filming the transect (and tape measure) and analysing the video footage retrospectively. Organisms were identified to family level or higher where possible and the major categories were defined thus:

- Hard coral
- Soft coral (gorgonians)
- Seagrass
- *Chlorophyta*, *Rhodophyta* and *Phaeophyceae*
- All encrusting pink/red algae were classified as crustose coralline algae (CCA)
- Turf algae
- Sand/pavement/rubble
- Unknown

Transect data was collated for every 5 m section of video section and the percentage occurrence of each category within each 5 m section was calculated.

4.4.5 Water sampling – 2016

Samples were taken from the back, mid and front part of a spur (Fig 4.1) at Coral View and Little Bight reef sites, on Utila Island, Honduras. Both sites were sampled at identical and equally spaced times of randomly chosen days during July 2016 (8.00 am, 10.45 am, 1.30 pm and 4.15 pm) and water samples were collected in polypropylene Azlon bottles. Each sample was returned immediately to shore and samples were aliquoted in the specific order: temperature/dissolved oxygen/salinity, followed by carbonate chemistry with DMSP samples taken last. Two samples of ~ 30 ml were decanted immediately into two separate falcon tubes for measurement of dissolved oxygen (DO_2), temperature and salinity. Water was then decanted using Tygon™ tubing from the bottom of the Azlon™ bottle into 12 ml borosilicate glass vials and were immediately fixed with 10 µL of HgCl and sealed. DMSP samples were pipetted into 10 ml crimp top vials, to which 1 ml 10 M NaOH was added and immediately sealed with PTFE lined gas tight crimp top caps. All samples were analysed as per the methods described in section 2.5.2 (TA) and section 2.5.3 (DIC). Carbonate chemistry data for DIC and TA, as well as temperature and salinity, were inputted into CO2SYS as per the details in section 2.5.4 so that the remaining carbonate chemistry parameters (i.e. pH, Revelle factor, aragonite and calcite saturation states) could be calculated.

4.4.6 Dissolved oxygen, temperature and salinity – 2016

Samples were measured for DO_2, temperature and salinity using a Thermo Orion 5 - star meter with RDO sensor. The DO_2 probe was placed in the water sample and the screen continually refreshed until the saturation level remained constant. The probe was calibrated daily prior to any measurements being taken. Salinity was measured using a Thermo Orion 5 - star conductivity meter and calibrated to an unknown seawater sample, which was then calibrated retrospectively against NIST conductivity standards retrospectively (St Andrews). After each measurement, all probes were rinsed thoroughly in DI water, padded dry and the

DO probe was returned to its storage case (which consisted of a sealed chamber with a wetted sponge to prevent drying of the measurement tip).

4.4.7 Ecological surveys – 2016

The length of each spur and the width at various points along its length was measured using a measuring tape and then confirmed using Google Earth. High resolution underwater photographs were taken and either a quadrat of known size or a measuring tape was included in each photograph for scale. Photographs were taken in an approximate belt transect formation equilaterally (or as close as possible) down the length of one spur at Coral View and one at Little Bight. Images were processed using CPC software version 4.1 (Kohler and Gill 2006); and the known scale from either the quadrat or measuring tape and a 40 point overlay was added (Fig 4.4).

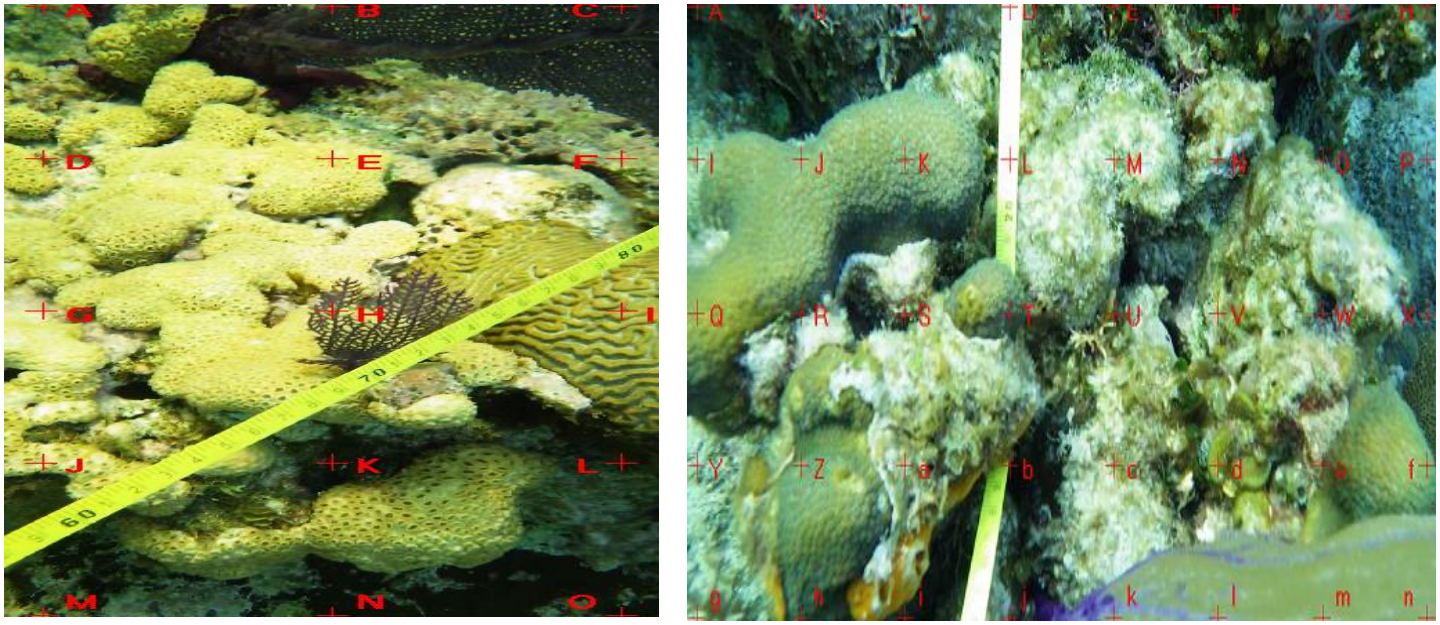


Figure 4.4 Example CPC files of images from the field (2016), illustrating the point - overlays, and scale and quality of benthic images used in assessing the coverage at each site.

At each overlay point, the benthic type was identified to species level where possible using the Caribbean Reef Coral Identification guide (Humann 1992), or, where there was uncertainty, identification was to Genus. The data from each image were imported into

Excel with each column of data representing one image. The distance from shore was included with the data from each image, which was 'binned" according to distance from shore and the % cover data for each distance was calculated by averaging the % covers of each benthic type in all the images for that distance bin.

A broader benthic substratum percentage cover regime was adopted to map the major benthic producers of DMSP. The major categories were defined thus:

- Hard coral
- Soft coral
- Coralline algae
- Sand, pavement, rubble (SPR)
- Seagrass
- Rhodophyta
- Phaeophyta
- Chlorophyta
- Turf algae
- Unknown

Where the tape, quadrat or a hand obstructed the view, this was recorded as "Tape/wand/shadow" and did not count toward the % cover calculations.

4.4.8 Statistical analyses

Linear mixed models were applied separately to the Coral View and Little Bight 2015 datasets to assess the effects of abiotic/biotic parameters on seawater DMSP at each site. c

Model <- lme(fixed = dmsp ~ var1+ var2+…, random = list(~ 1|day/time)), na.action = na.omit, data = dataset)

Anova was run on the model output using the "anova.lme" function.

The 2016 fieldwork data was analysed differently, owing to the nature of the experimental design. Mixed models were conducted for all abiotic parameters whilst controlling for day, which was included as a random factor. Thus, the model input was:

model <- lme(fixed = dmsp ~ var1+ var2+…, random = ~ 1|day, na.action = na.omit, data = dataset)

Anova was conducted on the model output using the anova.lme function.

To assess which factors affected benthic ecology in 2016, linear models were run on each benthic type, with temperature, distance from shore and salinity as independent variables. The general model output was thus:

model <- lm(benthictype ~ temp*distance*salinity, na.action = na.omit, data = dataset)

Anova was conducted on the model output using the base 'anova' function to test for interactions.

Mixed models were used to assess which benthic types significantly affected seawater DMSP in 2016, whilst controlling for time of day within day by including them as random effects. The general model formula for each benthic type was thus:

model <- lme(fixed = dmsp ~ benthictype + var1+ var2 etc…, random = ~ 1|day/time, data =dataset, na.action = na.omit)

Anova was conducted on the model output using the 'anova.lme' function.

Models were run for dissolved inorganic carbon and total alkalinity using the same model formula as described above, but with depth, distance from shore, location (spur/groove), hard coral and SPR as the independent variables:

model <- lme(fixed = carbonate parameter ~ var1+ var2+…, random = ~ 1|day, na.action = na.omit, data = dataset)

Mixed models for dissolved oxygen were run on the 2016 data, using the method described above for seawater DMSP. Analysis of variance analysis was conducted on the model output:

model <- lme(fixed = dissolved oxygen ~ var1+ var2+…, random = ~ 1|day, na.action = na.omit, data = dataset)

4.5 Results

4.5.1 Factors influencing the variability of water column DMSP in 2015

There were significant differences between water column seawater DMSP between each reef site (Coral View and Little Bight, t - test, p = 0.04), with values ranging from 6.7 – 20.9 nmol DMSP at Coral View and 8.86 – 47.21 nmol DMSP at Little Bight (Fig 4.5)

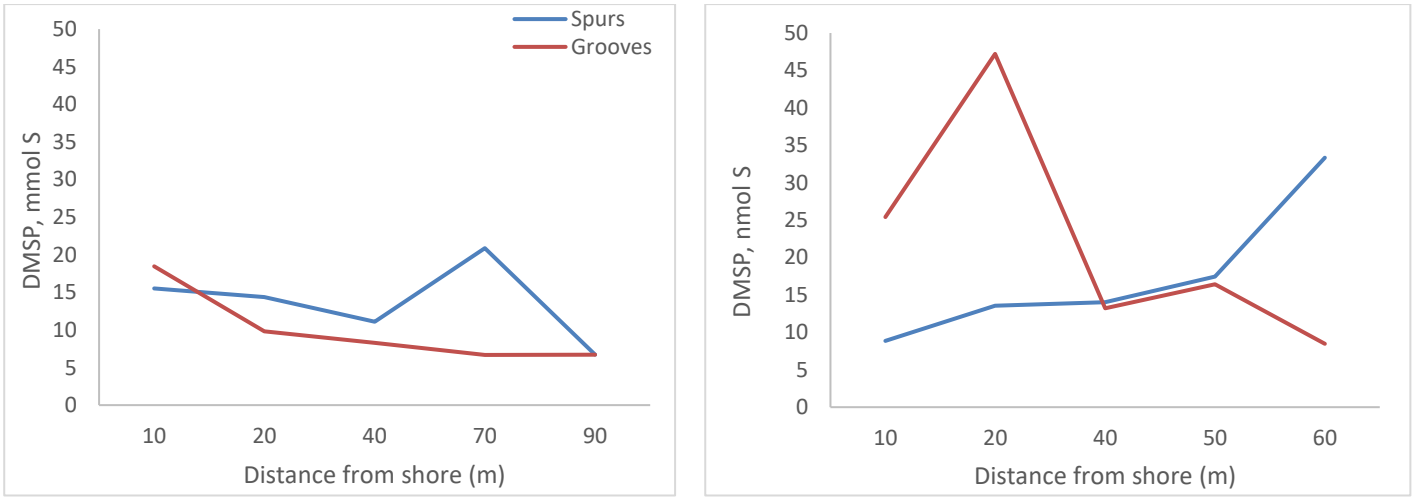


Figure 4.5 Seawater DMSP concentrations in the spurs and grooves at Coral View (left hand graph) and Little Bight (right hand graph) as a function of distance from shore (x - axis).

There was a significant trend (Table 4.2) of decreasing seawater DMSP with distance from shore at Coral View and this pattern was also evident in the grooves at Little Bight, however seawater DMSP increases with distance from shore in the spurs at Little Bight. Significant differences in seawater DMSP between spurs and grooves at Coral View were noted (Welch's two - sample t - test, $p = 0.04$), however there were no differences at Little Bight (Welch's two - sample t - test, $p = 0.50$).

Linear mixed models were conducted to examine which factors significantly affected the spatial distribution of seawater DMSP at both Coral View and Little Bight (Table 4.2).

Table 4.2 Summary of mixed model output for Coral View and Little Bight showing factors that significantly affected seawater DMSP concentrations. Significant values are highlighted in bold.

Factor	*Coral View*	*Little Bight*
Temperature	**0.02**	n/a
Light	0.25	n/a
Distance from shore	**0.0008**	0.99
Seagrass	0.89	n/a
Turf algae	**0.01**	0.46
Crustose coralline algae	**0.008**	0.11
Hard coral	0.50	0.22
Soft coral	**0.005**	**0.04**
Sand, pavement, rubble (SPR)	**< 0.001**	0.86

Temperature significantly affected the seawater DMSP distribution at Coral View, however temperature was not measured at Little Bight. The presence of soft coral significantly affects seawater DMSP at both sites and was the only factor that was measured to affect seawater DMSP at Little Bight. Distance from shore, whilst a significant factor at Coral View, did not significantly affect DMSP at Little Bight. Similarly, the presence of turf algae, coralline algae and sand/pavement/rubble all affect seawater DMSP at Coral View, but not at Little Bight.

4.5.2 Factors influencing the variability of water column DMSP 2016

Seawater DMSP concentrations are variable across both reef sites with concentrations at Coral View ranging from 7.2 – 118.3 nmol DMSP, and from 4 – 21.5 nmol DMSP at Little Bight. The mean (n = 4) DMSP value across the four sampling days was calculated and the temporal trends plotted (Fig 4.6) for the back, mid and front reef locations at both Coral View and Little Bight reef sites.

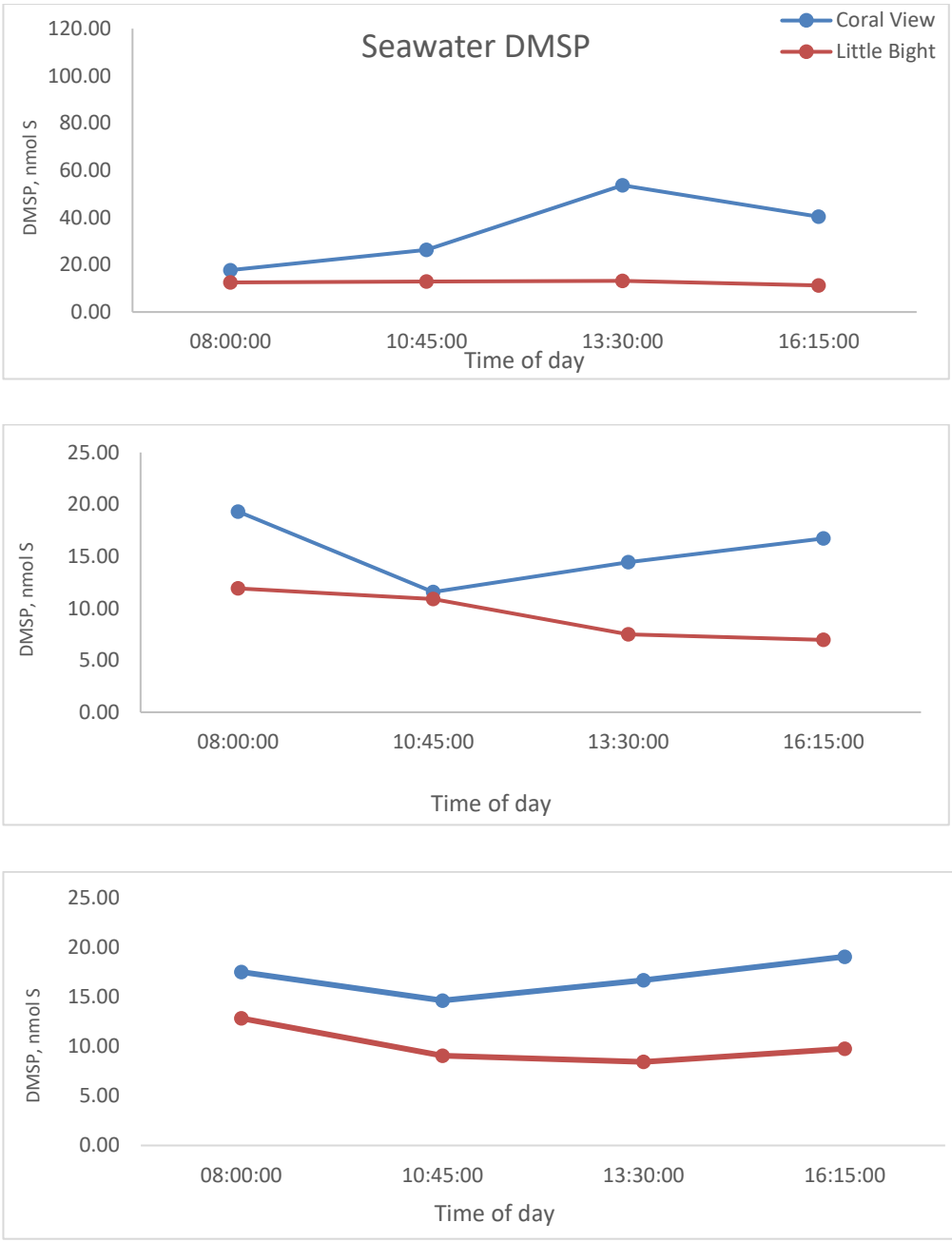


Figure 4.6 Mean (n = 4) daily variability in seawater DMSP at the back (top), mid (middle) and front (bottom) reef locations for Coral View and Little Bight reef sites. Note the different y - axis for the back - reef (top) graph.

Overall, seawater DMSP was significantly higher at Coral View (mean 22.338 nmol DMSP, n = 48) than at Little Bight (mean 10.610 nmol DMSP, n = 48) across all reef locations (Welch's t - test, p =< 0.001). The back - reef seawater DMSP concentration generally increased throughout the day at Coral View, however at Little Bight seawater DMSP was more constant throughout the day. The highest seawater DMSP concentrations (53.6 nmol DMSP,

n = 4) occurred at 1.30 pm in the back reef at Coral View. Seawater DMSP decreased at 10.45 am in the mid - and front - reef locations at Coral View and Little Bight at 10.45 am, with concentrations higher both before and after. There is a trend toward higher seawater DMSP concentrations at the beginning of the day in the mid - and front - reef locations at both sites, but this pattern was not evident in the back reef and was less pronounced at Little Bight. The data were also plotted so that spatial variability could be elucidated (Fig 4.6).

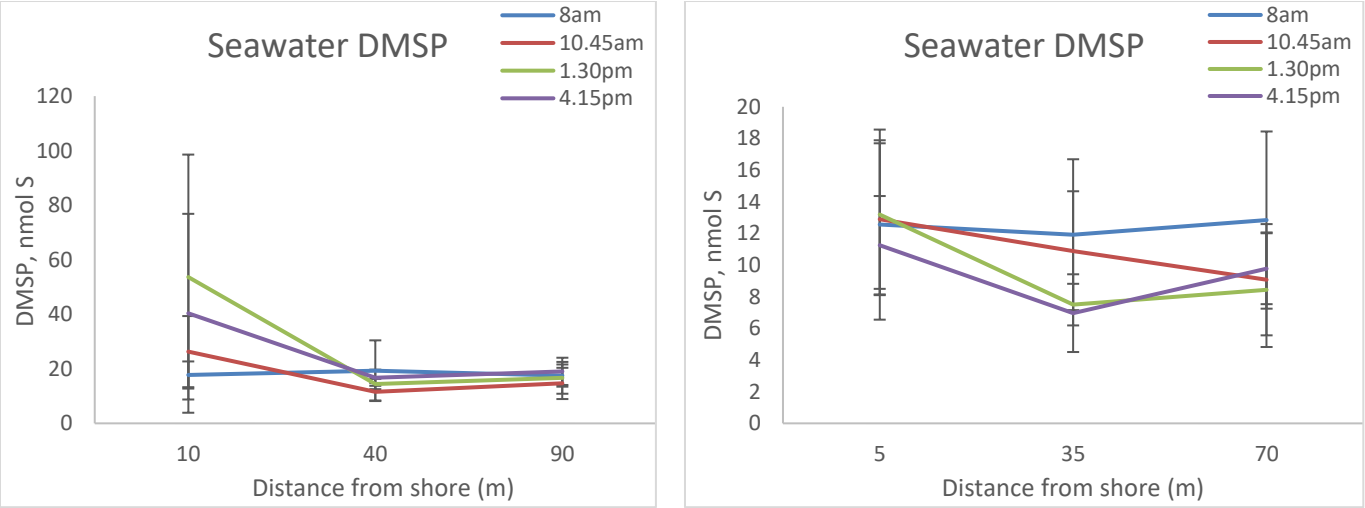


Figure 4.7 Mean daily (n = 4) DMSP variability at Coral View (left hand graph) and Little Bight (right hand graph) with increasing distance from shore (x - axis). Times of day are indicated in the legend. Note the different scales on the y-axes. Error bars are standard deviations.

Spatial variability was evident at Coral View (Fig 4.7) where higher concentrations were found in the back - reef environment, decreasing with increasing distance from shore. Seawater DMSP concentrations at Little Bight, also exhibited variability with increasing distance from shore although this variability was less pronounced when compared to Coral View. Mixed models were analysed using ANOVA to assess which abiotic factors affected seawater DMSP concentrations at each reef site, with the model p - value outputs presented (Table 4.3).

Table 4.3 Summary of ANOVA output for spatiotemporal and physicochemical parameters on seawater DMSP levels**.**

Factor	*Coral View*	*Little Bight*
Temperature	**< 0.001**	0.73
Distance	**0.05**	**0.002**
Salinity	**0.04**	0.76
Dissolved oxygen	*0.07*	0.86
Tide	0.73	0.83
Wind speed	*0.07*	**0.007**
pH	**0.003**	**0.009**
Time of day	**0.05**	**0.04**
Revelle factor	**0.001**	*0.10*

Temperature significantly affected seawater DMSP at Coral View ($p < 0.001$) but not at Little Bight ($p = 0.73$), similarly salinity affected seawater DMSP at Coral View but not at Little Bight (Coral View $p = 0.05$, Little Bight $p = 0.76$). Distance from shore, time of day and pH all significantly affected seawater DMSP at both sites. There was a suggestion that wind speed affected seawater DMSP at both sites but was only significant at Little Bight ($p = 0.007$). Similarly, the Revelle factor significantly affected DMSP at Coral View ($p = 0.001$) but was only significant if $p < 0.10$ at Little Bight ($p = 0.10$).

4.5.3 Carbonate chemistry

4.5.3.1 Variability in carbonate chemistry between and within reef sites 2015

DIC in the spurs at Coral View ranged from 983 – 2014 µmol kg^{-1} (mean 1762.27 µmol kg^{-1}, n = 20) and 1085.26 – 2020.98 µmol kg^{-1} in the grooves (mean 1788.42 µmol kg^{-1}, n = 18) (Fig 4.7). TA values range from 1740 - 2378 µmol kg^{-1} in the spurs at Coral View (mean 2263.43 µmol kg^{-1}, n = 15), and from 2270 – 2325 µmol kg^{-1} in the grooves (mean 2218.31 µmol kg^{-1}, n = 18) (Fig 4.7). Seawater DIC in the spurs at Little Bight was less variable, ranging from 1637.90 – 1995.18 µmol kg^{-1} (mean 1844.02 µmol kg^{-1}, n = 18) and 1731.45 – 2146.65 µmol kg^{-1} in the grooves (mean 1910.92 µmol kg^{-1}, n = 20). Although there are

significant differences in seawater DIC between Coral View and Little Bight (Welch's t - test, $p = 0.03$) and seawater TA (at $p < 0.10$, Welch's t - test, $p = 0.07$), seawater DIC and TA concentrations are not significantly different between grooves and spurs within the same site.

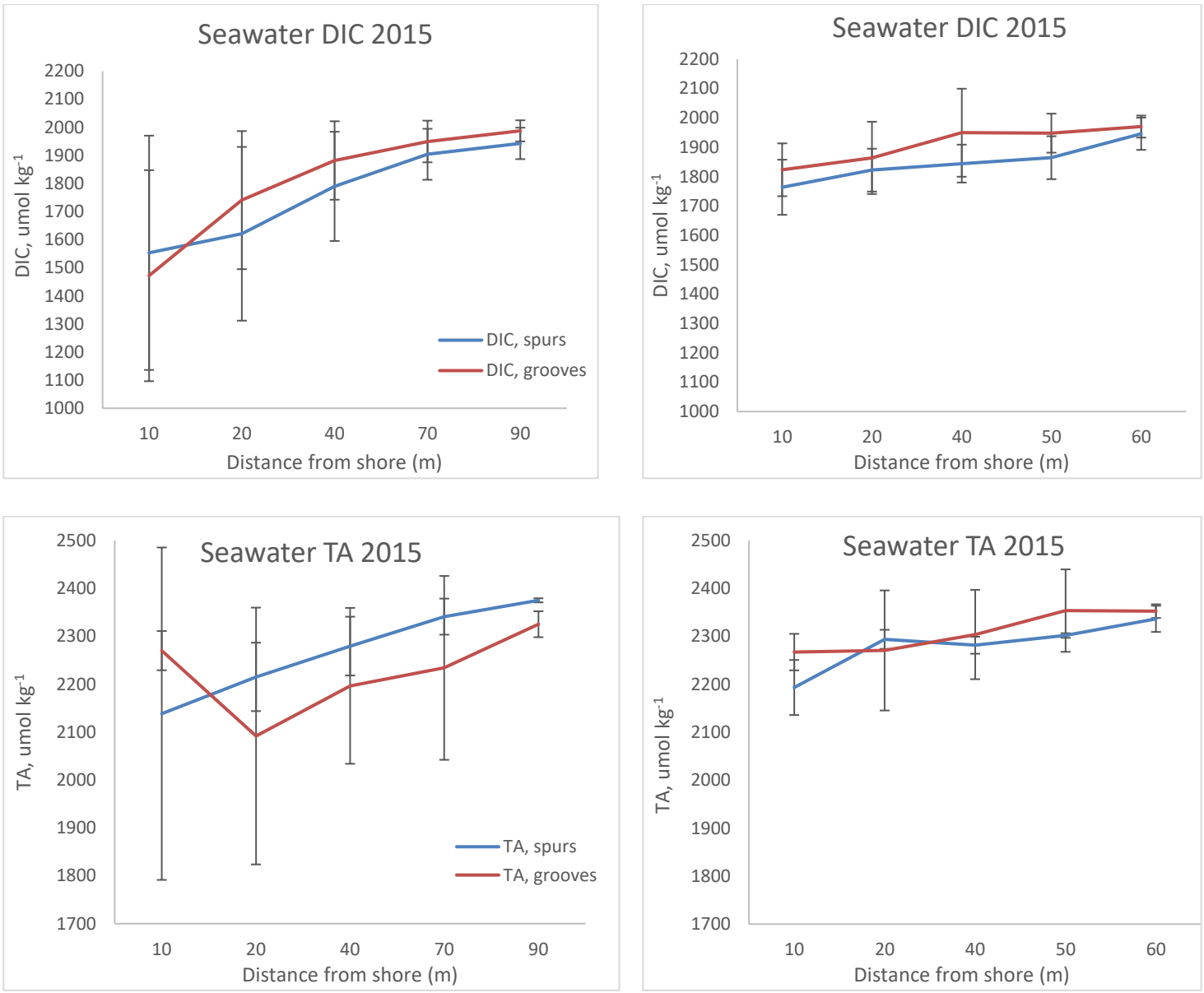


Figure 4.8 Seawater DIC (top graphs) and TA (bottom graphs) for Coral View grooves and spurs (left hand graphs) and Little Bight grooves and spurs (right hand graphs) with increasing distance from shore. Distance from shore is on the x - axis in all graphs. Note the different y – axes for the top and bottom graphs. Error bars are standard deviations; standard deviations are calculated using the mean from all sampling days (n = 2) and grooves/spurs (n = 2) at each sampling location (distance from shore).

In general, both TA and DIC increased with increasing distance from shore, in both the grooves and spurs and at both reef sites. Mixed models were used to assess which factors most affected seawater carbonate chemistry in 2015 (Table 4.4).

Table 4.4 Summary of mixed model output for seawater carbonate chemistry at Coral View and Little Bight. Significant values are highlighted in bold.

Factor	*Coral View DIC*	*Little Bight DIC*	*Coral View TA*	*Little Bight TA*
Location (spur/groove)	0.84	**0.02**	0.54	0.21
Distance from shore	**< 0.001**	**< 0.001**	**0.003**	**< 0.001**
Hard coral	0.71	0.82	0.57	0.57
Soft coral	0.40	0.93	0.64	0.19
Seagrass	0.31	N/A	0.79	N/A
CCA	0.52	0.69	0.28	0.86
Phaeophyta	0.87	0.65	0.86	0.78
Chlorophyta	0.69	0.84	0.26	0.52
SPR	0.68	0.90	0.50	0.36

Distance from shore was a significant factor in determining seawater DIC and TA at both sites, however there was no effect of benthic ecology on seawater carbonate chemistry.

4.5.3.2 Variability in carbonate chemistry between and within reefs 2016

Seawater DIC was significantly different between Coral View and Little Bight (Welch's t - test, p 0.02), as was seawater TA (Welch's t - test, p = 0.01). DIC concentrations at Coral View range from 1157.35 - 2026.42 µmol kg^{-1} (mean 1779.66 µmol kg^{-1}, n = 45) but were less variable at Little Bight, where concentrations range from 1579.61 – 2052.45 µmol kg^{-1} (mean 1886.53 µmol kg^{-1}, n = 45). TA at Coral View ranges from 1979.50 – 2419 µmol kg^{-1} (mean 2303.85 µmol kg^{-1}, n = 48) and from 2177 – 2427 µmol kg^{-1} (mean 2355.25 µmol kg^{-1}, n = 48) at Little Bight.

The means of each of the four sampling days were combined and the daily trends and temporal variability in carbonate chemistry for each presented (Fig 4.9).

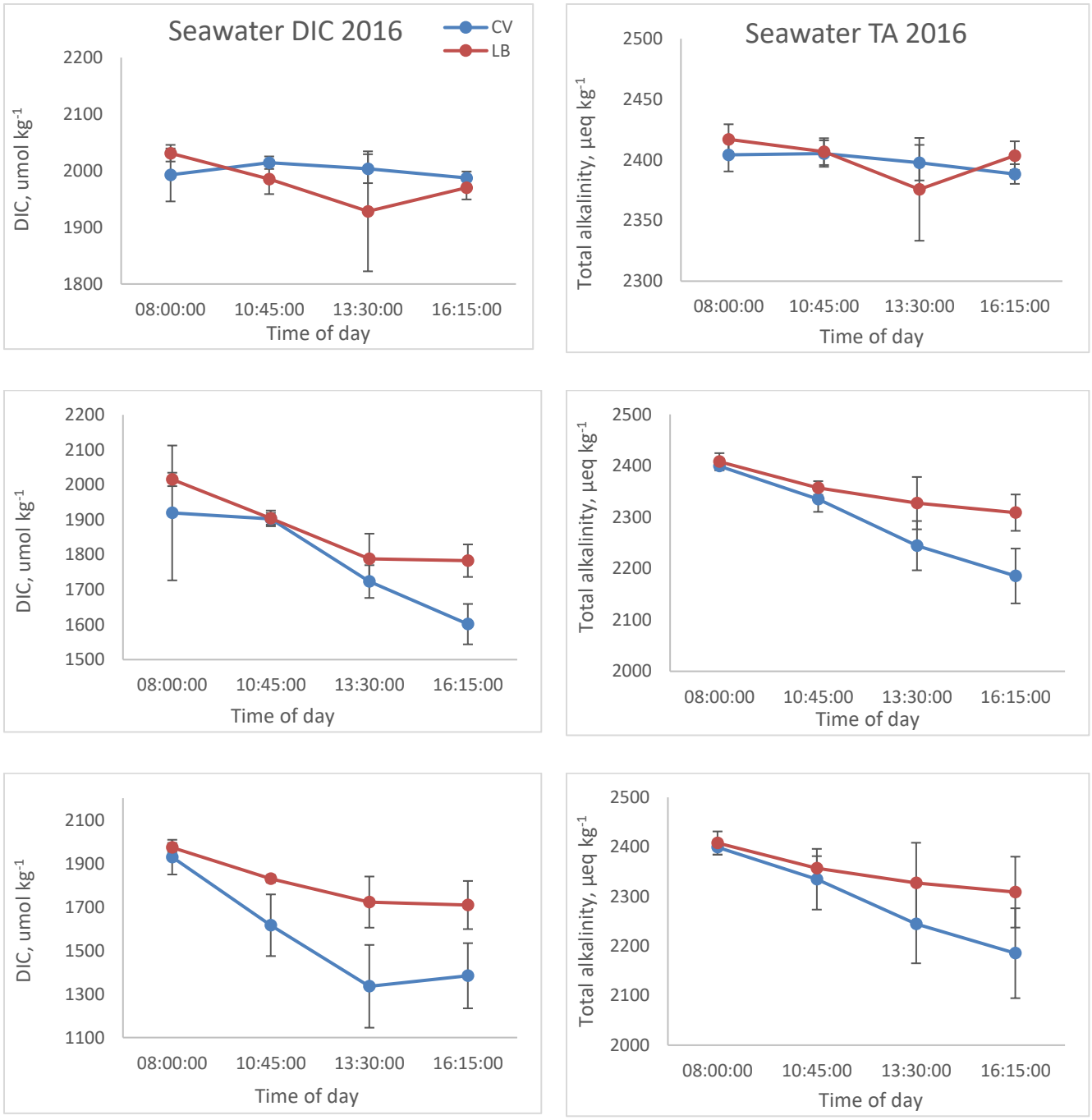


Figure 4.9 Mean daily values (n = 4) for DIC (left hand graphs) and TA (right hand graphs) at the front (top), mid (middle) and back (bottom) reef sites at Coral View (CV, blue) and Little Bight (LB, red). Error bars are standard deviations. DIC units are µmol kg $^{-1}$ and TA units are µeq kg $^{-1}$. Note the different y – axes for all graphs. Time of day is on the x axis.

Both TA and DIC decreased during the day, with highest concentrations in the morning; this pattern was less evident in the reef - front. The back - reef environment exhibited the strongest temporal trend, with TA and DIC decreasing appreciably throughout the day. Changes to the calcium carbonate saturation state (ΩCa) throughout the day were also investigated (Fig 4.10) with respect to each portion of the reef (front, mid & back) to identify the key trends.

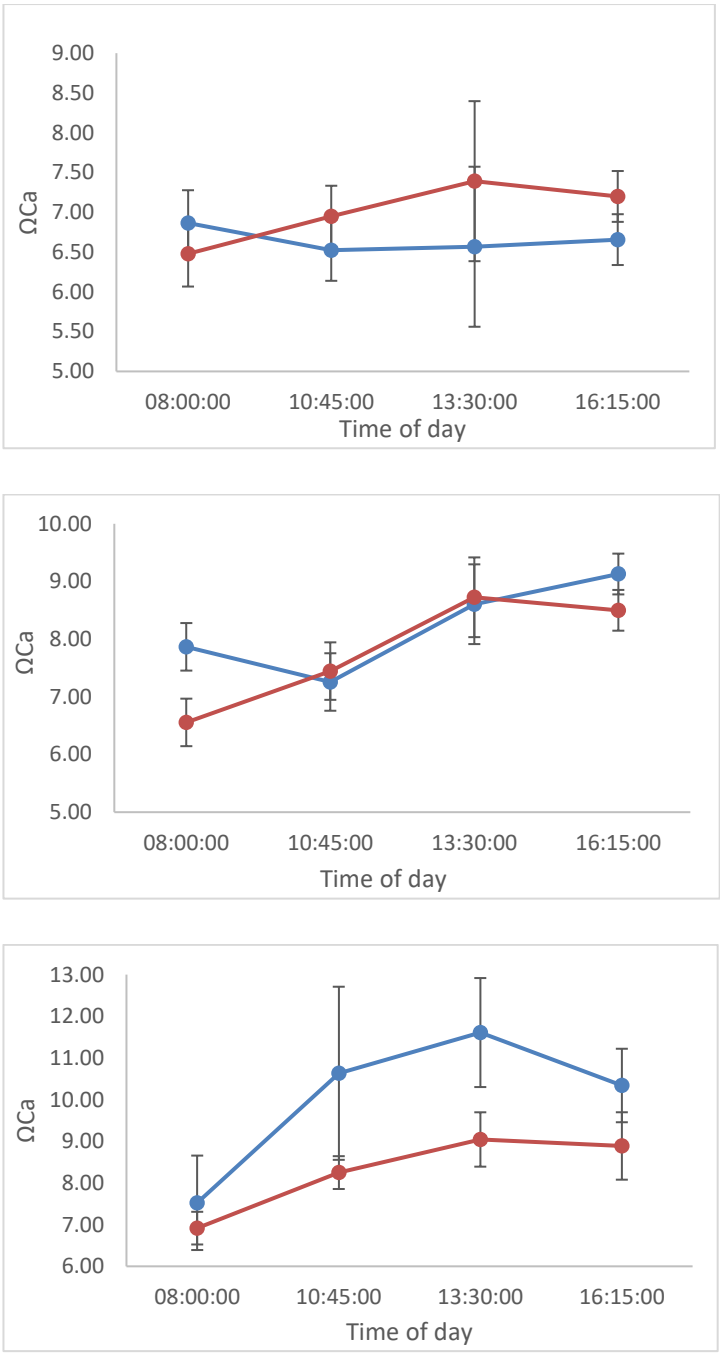
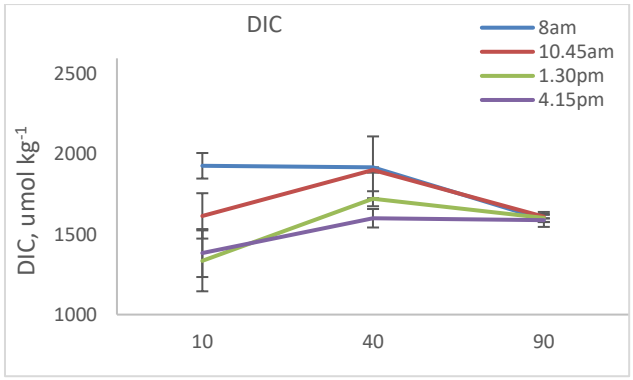
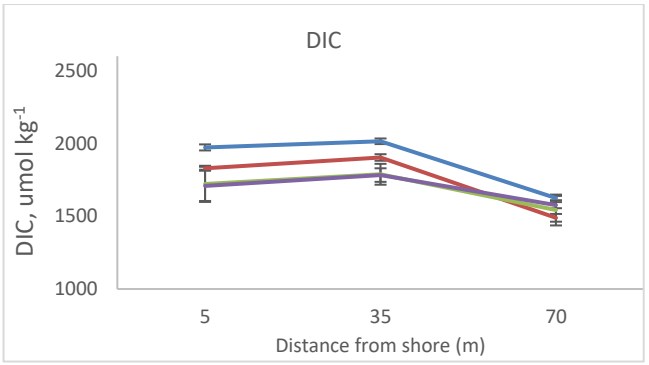


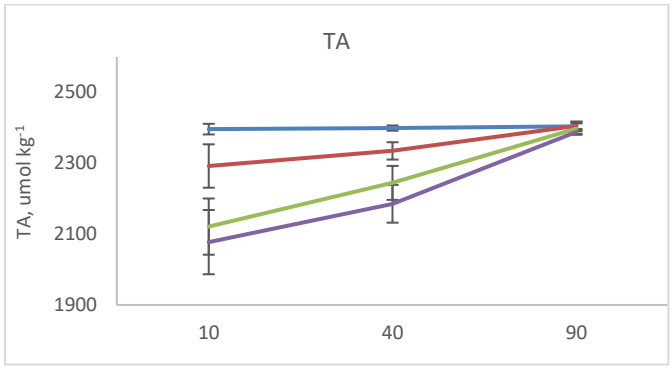
Figure 4.10 Mean daily values (n = 4) for ΩCa (y - axis) for the front (top), mid (middle) and back (bottom) reefs at Coral View (CV, blue) and Little Bight (LB, red). Error bars are standard deviations. Time of day is on the x axis. Note the different y - axes.

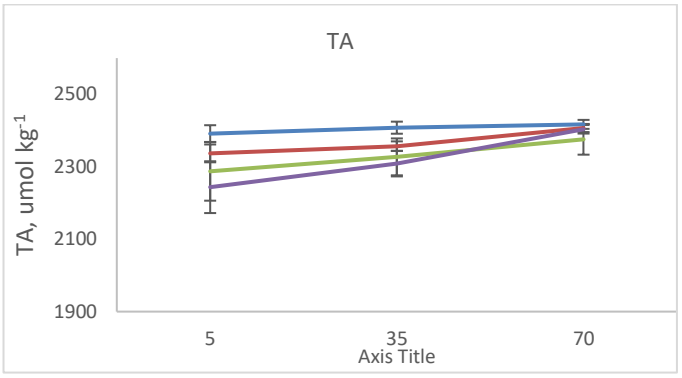
The back reef at both sites was characterised by increasing ΩCa throughout the day and a decrease towards the end of the day; this trend is more pronounced at Coral View than at Little Bight. ΩCa in the mid - reef environment increased throughout the day and, whilst Little Bight exhibited a decrease toward the end of the day, this was not the case at Coral

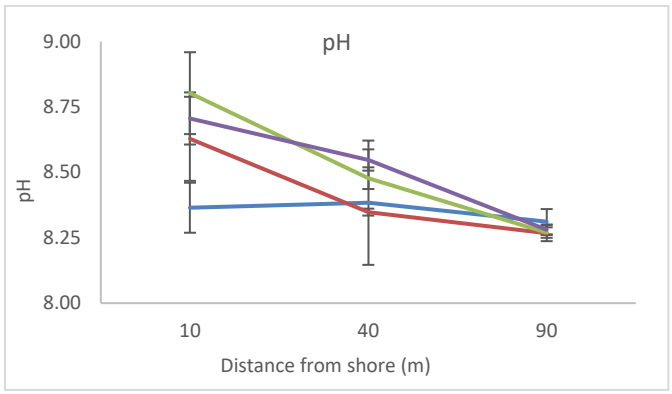
View. ΩCa in the reef - front at both sites was less variable throughout the day and characterised by overall lower values of ΩCa than at the mid - and back - reef environments. The data were also plotted so that spatial variability could be investigated (Fig 4.11).

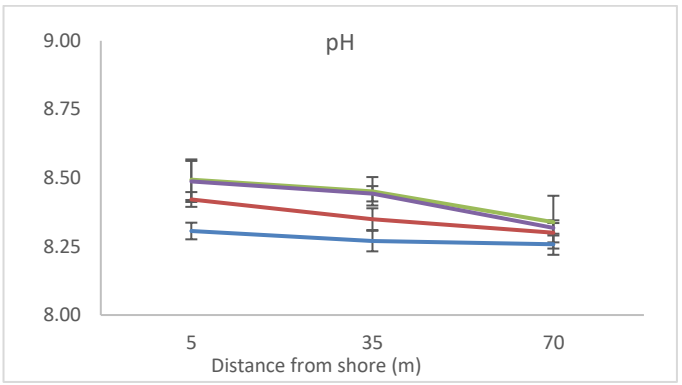


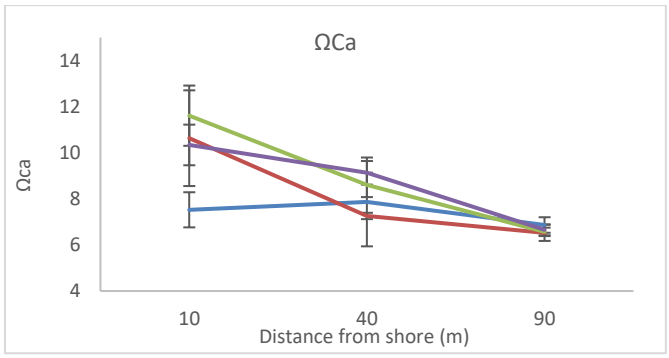












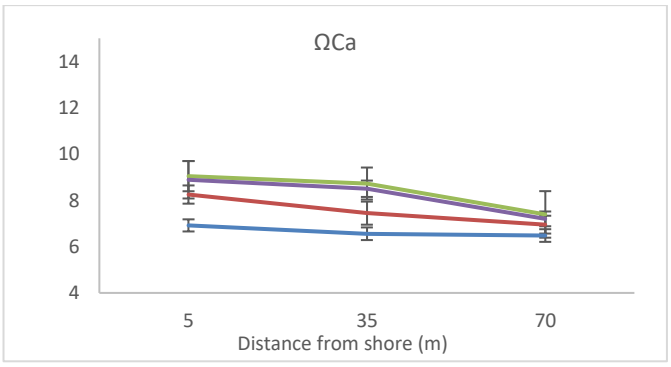


Figure 4.11 Mean daily (n = 4) variability for dissolved inorganic carbon (DIC), total alkalinity (TA), pH and the calcium carbonate saturation state (ΩCa) at Coral View (left hand graph) and Little Bight (right hand graph) with increasing distance from shore (x - axis). Times of day are indicated in the legend. Error bars are standard deviations. Note the different y - axes for different carbonate chemistry parameters.

There is a general trend for decreasing DIC, pH and ΩCa with increasing distance from shore at both sites, whilst TA increases. Furthermore, variability in all parameters at Coral View was more evident than at Little Bight, with the greatest variability occurring in the back - reef and decreasing with increasing distance from shore. Thus, the back - reef environment at both reef sites was characterised by more variable carbonate chemistry, whilst the reef - front was more stable.

Mixed models were conducted to assess which variables significantly affect the carbonate chemistry at the Coral View and Little Bight reefs sites (Table 4.5).

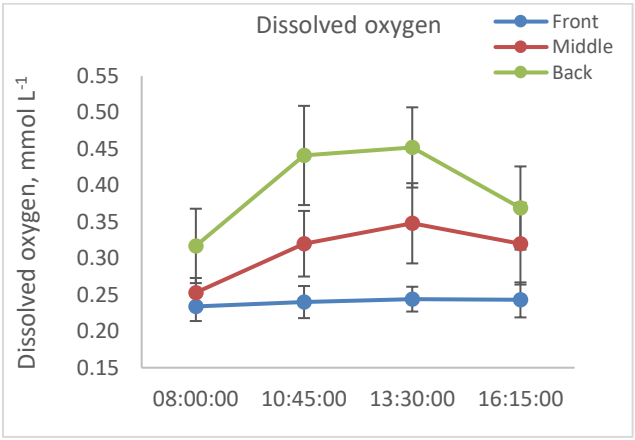
Table 4.5 Summary of ANOVA output for the influence of abiotic variables on seawater carbonate chemistry at both reef sites. Significant values are highlighted in bold.

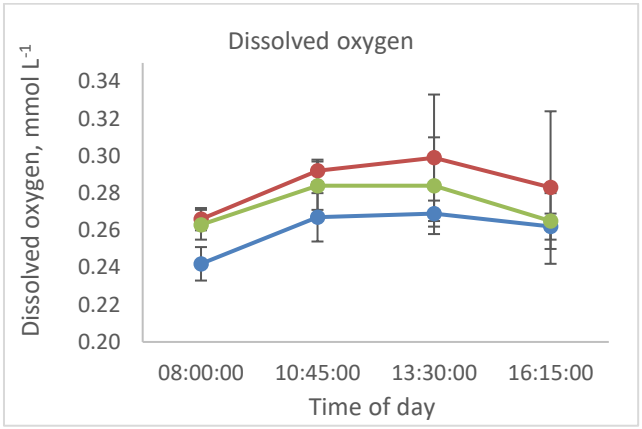
	Coral View DIC	*Coral View TA*	*Little Bight DIC*	*Little Bight TA*	*Coral View ΩCa*	*Little Bight ΩCa*
Time of day	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.006**	**< 0.001**
Distance from shore	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Temperature	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.58

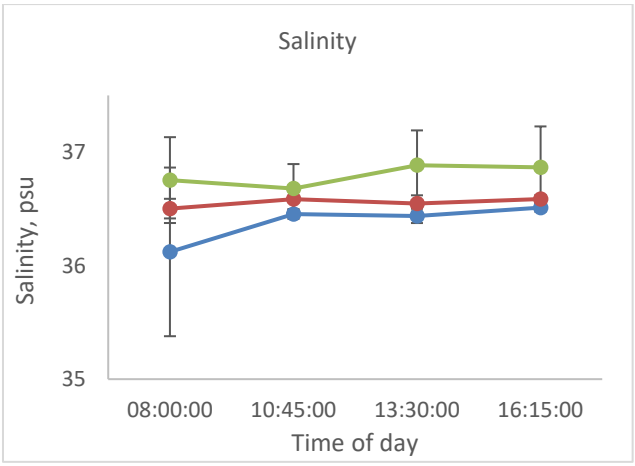
Distance from shore, temperature and time of day all significantly affected DIC and TA at both Coral View and Little Bight ($p < 0.001$). ΩCa was significantly affected by time of day and distance from shore at both sites. However, whilst temperature significantly affects ΩCa at Coral View, it has no effect at Little Bight.

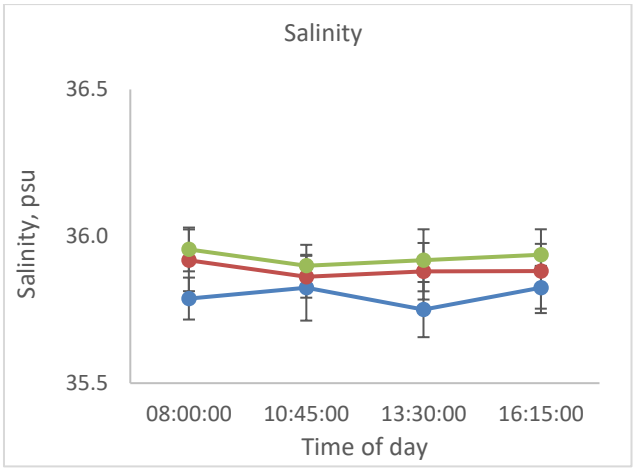
4.5.4 Spatiotemporal variability in temperature, salinity and dissolved oxygen 2016

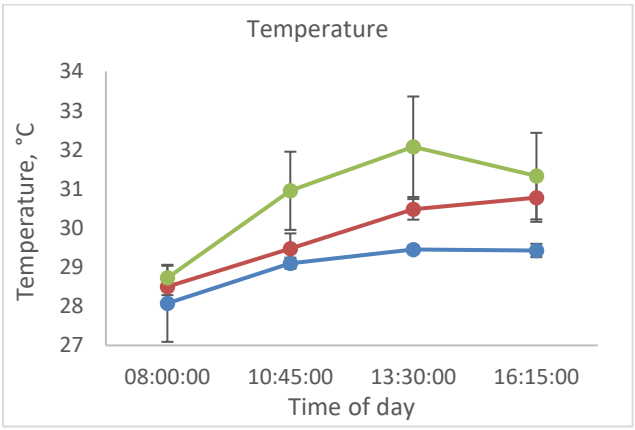
The spatiotemporal variability of physicochemical properties (salinity, temperature and dissolved oxygen) within and between sites was compared (Fig 4.12). Temperature at Coral View was more variable than Little Bight, ranging from 26.6 – 33.8°C compared with 28.9 – 32.5°C. There was also less variability in the observed salinity range at Little Bight (35.6 - 36.1 psu) compared with Coral View (34.5 - 36.9 psu), and dissolved oxygen (Coral View 0.205 – 0.524 mmol L^{-1}, Little Bight 0.236 - 0.345 mmol L^{-1}).











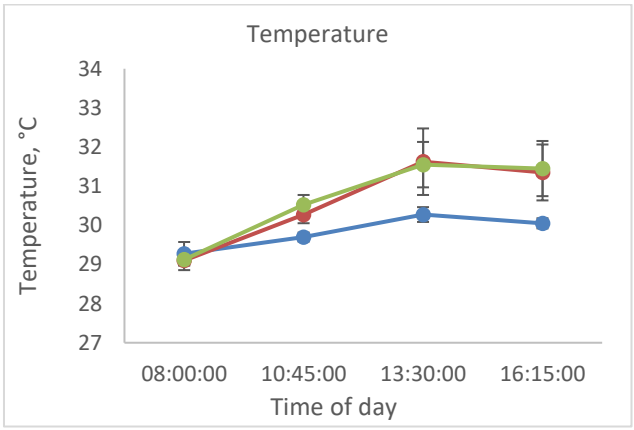


Figure 4.12 Mean values (n = 4) across sampling days for dissolved oxygen, salinity and temperature for each sampling time (y - axis) at both Coral View (left hand graphs) and Little Bight (right hand graphs) reef sites. Error bars represent standard deviations. Note the different y - axes for dissolved oxygen and salinity between sites.

Values for dissolved oxygen, temperature and salinity at the reef front were consistently lower when compared with the mid - and back - reef locations at both sites, also showing the least amount of variability over the course of a day. Dissolved oxygen was more variable in the back of the reef at Coral View, with the least variability in the front of the reef. This pattern was mirrored at Little Bight, but the magnitude of the variability was not as pronounced.

Both sites demonstrate limited variability in salinity between the front, mid and back reef, and there was less variability in salinity at Little Bight over the course of the day, when compared with Coral View. Similarly, temperature variability between reef locations was more pronounced at Coral View than at Little Bight. Both sites exhibit a general increase in temperature throughout the day, and Coral View exhibits greater variability in temperature between back -, mid - and front reef locations. The mid - and back - reef locations at Little Bight, however, were nearly identical and follow the same diurnal increase. The reef - front at both sites was noticeably cooler than the other reef locations. Overall, the reef - front was characterised by a cooler and more stable physicochemical environment, whilst the back - and mid - reefs were warmer and shallower. Furthermore, conditions at Coral View were more variable both between reef locations and over the course of the day, whilst Little Bight was characterised by less spatiotemporal variability for salinity, dissolved oxygen and temperature. Visual observations made during this study indicate that the back - reef environment at Coral View is visibly much shallower than the back reef at Little Bight, which is likely to reduce any mixing potential with cooler waters from the reef front. Paired t - tests confirmed that all variables were significantly different between Coral View and Little Bight; dissolved oxygen ($p = 0.0005$), salinity ($p = 0.00002$) and temperature ($p = 0.04$).

Mixed models were used to assess the factors that affect temperature, salinity and dissolved oxygen at both reef sites. Distance from shore, time of day, windspeed and tide were all included as fixed factors, whilst day was included as a random factor (Table 4.6)

Table 4.6 Summary of ANOVA output for the influence of abiotic variables on seawater dissolved oxygen, temperature and salinity at both reef sites. Significant values are highlighted in bold.

	Distance from shore	*Time of day*	*Windspeed*	*Tide*
Coral View DO_2	**< 0.001**	**< 0.001**	0.38	0.51
Little Bight DO_2	0.15	**< 0.001**	0.19	**0.02**
Coral View salinity	0.76	0.18	0.41	0.55
Little Bight salinity	**0.001**	0.34	0.32	0.99
Coral View temp.	**0.004**	**< 0.001**	0.70	0.53
Little Bight temp.	**< 0.001**	**< 0.001**	0.31	0.43

Time of day significantly affected seawater dissolved oxygen and temperature at both sites but has no effect on salinity at either site. Distance from shore significantly affected seawater dissolved oxygen at Coral View (p =< 0.001) but not at Little Bight (p = 0.15). A reverse trend was noted for the effect of distance from shore on salinity, which is significant at Little Bight (p = 0.001) but not at Coral View (p = 0.76). Temperature at both sites was significantly affected by distance from shore. Windspeed did not significantly affect any variable at either site and tide only affected seawater dissolved oxygen at Little Bight (p = 0.02). T - tests were conducted to assess whether there were significant differences in mean daily temperature between sites, however none were found (p = 0.29).

4.5.5 Reef ecology at Coral View and Little Bight 2015

Mean percentage cover of benthic typology for spurs and grooves were plotted with increasing distance from shore for both reef sites (Fig 4.13).

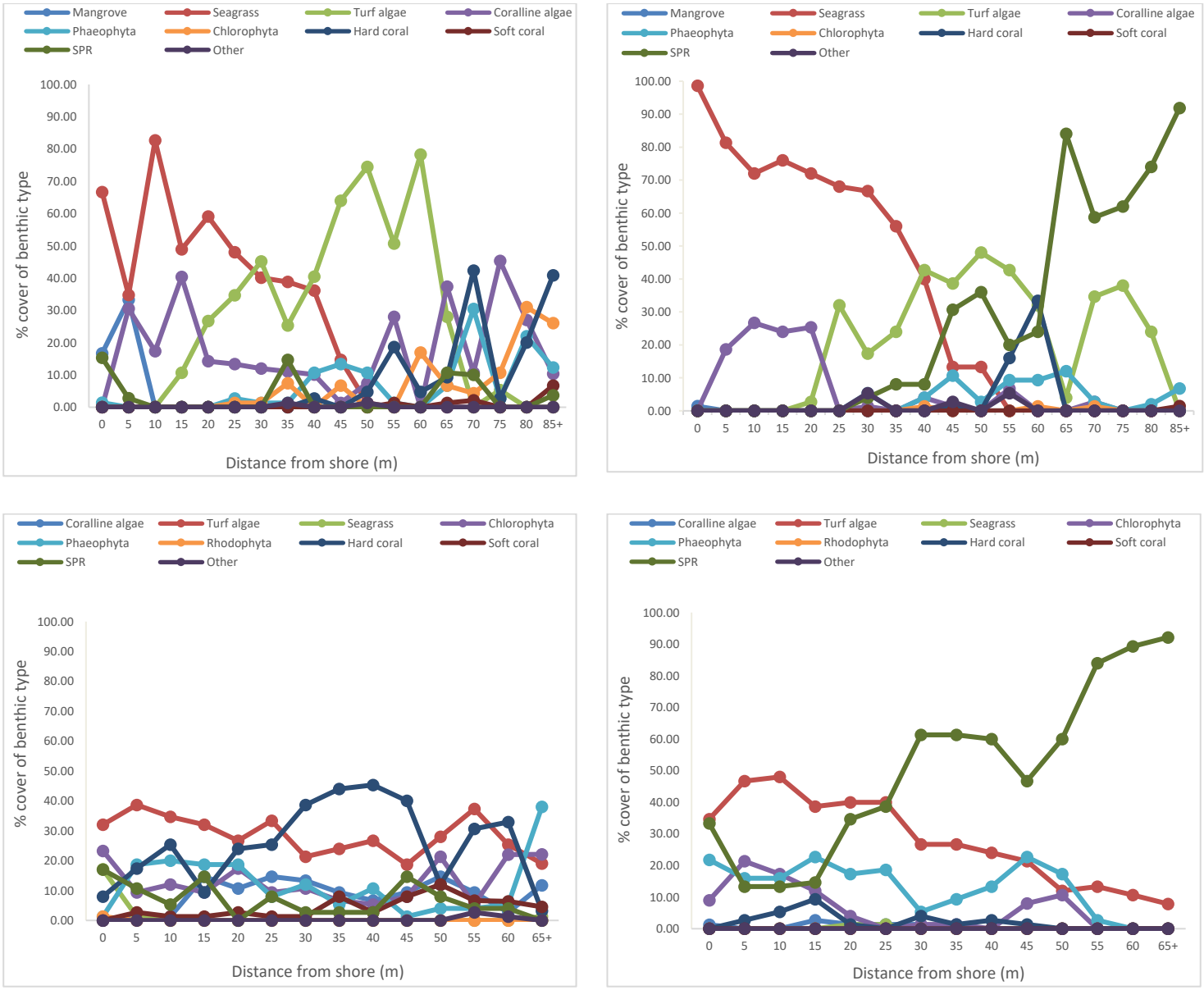


Figure 4.13 The mean percentage covers of major benthic types in the spurs (left hand graphs) and grooves (right hand graphs) at Coral View (top graphs) and Little Bight (bottom graphs) during July 2015. % covers are shown on the y - axes and distance from shore on the x - axes.

The spurs at Coral View were characterised by a back reef (0 - 30 m) dominated by seagrass, which gradually decreases with increasing distance from shore to give way to a mid - reef (30 - 60 m) environment that is primarily composed of macroalgae and coralline algae. Hard and soft coral begins to appear at 55 m and were most evident from 70 m onwards. By contrast, the grooves at Coral View were dominated by fleshy autotrophic assemblages, composed mainly of seagrass in the back reef and turf algae in the mid - and fore - reef

environments. The fore - reef environment was principally composed of SPR and turf algae - corals were almost entirely absent except at 45 - 60 m.

Conversely, the back - reef environment (0 - 20 m) of the spurs at Little Bight was not dominated by seagrass, rather it was composed of a mixture of turf algae, Chlorophyta, Phaeophyta and hard corals, which appear as far back as 5 m. Moreover, there were no mangroves at Little Bight. Coral cover increased noticeably from 20 m onwards and this characterised the start of the mid - reef environment, which was composed mainly of turf algae and hard corals. Soft coral cover increased markedly from 40 m onwards, giving way to a reef - front that was composed mainly of hard and soft corals, macroalgae and coralline algae. The grooves at Little Bight were dominated almost entirely down their length by turf algae, Phaeophyta and SPR, which steadily increased from 33 % to almost 100 % from the back of the reef to the reef front.

To assess intra - site variability, t - tests (Welch's, unequal variances) were conducted on mean benthic cover (Fig 4.14) between spurs and grooves at each reef site. At Coral View, coralline algae ($p = 0.01$), Chlorophyta ($p = 0.01$) and SPR ($p = 0.005$) all exhibit significant differences between spurs and grooves. At Little Bight mean percentage cover of coralline algae ($p < 0.001$), Chlorophyta ($p = 0.01$), hard coral ($p < 0.001$), soft coral ($p < 0.001$) and SPR ($p < 0.001$) all exhibit significant differences between spurs and grooves.

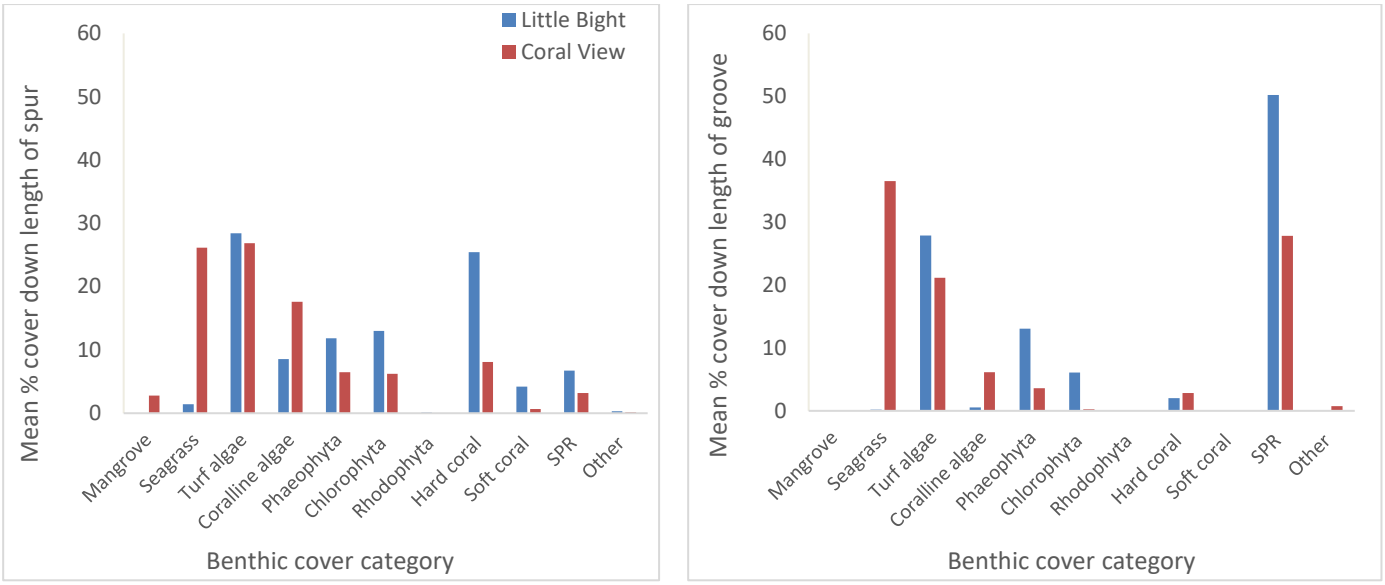


Figure 4.14 Mean % cover of each benthic category for all spurs (left hand graph) and grooves (right hand graph) at Coral View (red bars) and Little Bight (blue bars).

Inter - site variability between spurs and grooves (Fig 4.14) was also evident; in the spurs, coralline algae ($p = 0.02$), seagrass ($p = 0.001$) Chlorophyta ($p = 0.03$), hard coral ($p = 0.001$), soft coral ($p = 0.002$) and SPR ($p = 0.04$) all exhibit significant differences between sites. In the grooves, coralline algae ($p = 0.03$), seagrass ($p < 0.001$), Chlorophyta ($p = 0.009$), Phaeophyta ($p < 0.001$) and SPR ($p < 0.001$) demonstrated significant differences in mean percentage cover between sites.

4.5.6 Reef ecology at Coral View and Little Bight 2016

Percentage cover of the major benthic groups for each distance bin were plotted with increasing distance from shore for both reef sites (Fig 4.15).

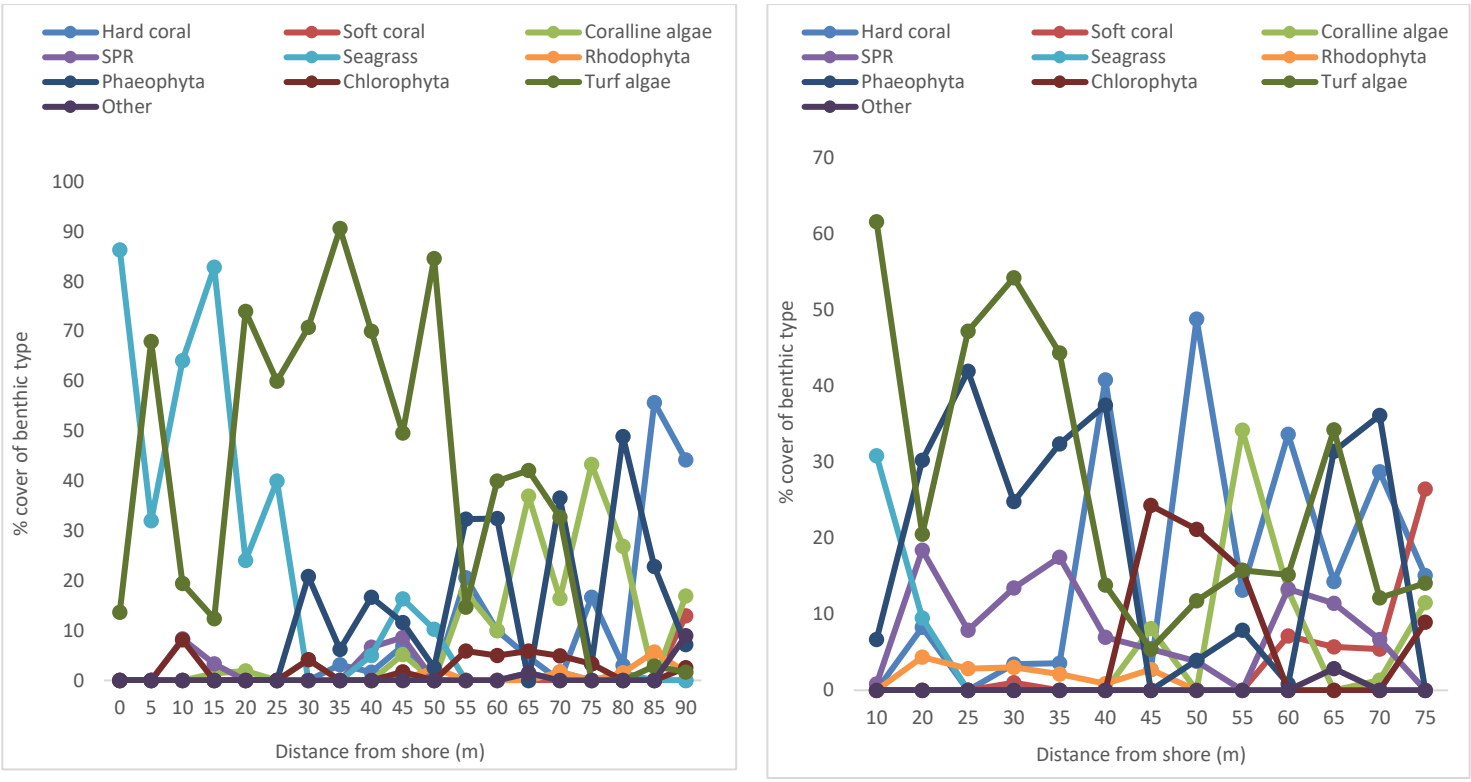


Figure 4.15 Percentage cover of key benthic organisms down the length of a spur at Coral View (left hand graph) and Little Bight (right hand graph) of key benthic community organisms.

The reef at Coral View was characterised by a back reef (0 - 25 m) dominated by seagrass (average cover 0 - 25 m 54 %) and turf algae (average cover 0 - 25 m 41 %). Seagrass decreases markedly after 25 m, leading into the mid - reef, which is primarily composed of turf algae, macroalgae and coralline algae. The macroalgal assemblage was composed mainly of Phaeophyta, of which *Dictyota* spp. appeared to be the dominant class. Hard and soft coral begin to appear at 35 m and were most evident from 75 m onwards, which marks the beginning of the reef - front. Whilst Phaeophyta were still prevalent here, all other algal classes were either absent or rare, conversely coralline algae became more dominant from 55 m and were a major component of the benthic community in the reef - front.

The back - reef environment (0 - 10 m) of the spur at Little Bight was characterised by the presence of seagrass (~ 30 %) and turf algae (~ 60 %). Seagrass coverage reduced abruptly and was entirely absent 25 m from shore, after which the mid - reef environment began. The mid - reef (25 - 60 m) was composed of a mixture of turf algae, Chlorophyta,

Phaeophyta and hard corals, which appeared as far back as 20 m. Soft coral cover was evident from 60 m onwards and coralline algal cover increases markedly from 55 m, which characterised the start of the reef - front environment. Macroalgae were still present on the reef front and although turf algal coverage steadily declined with increasing distance from shore, there was still considerable coverage of the reef - front. Phaeophyta were evident here. SPR distribution appeared homogenous for the entire length of the spur at Little Bight.

To assess which benthic categories exhibited significant differences in % cover between reef sites, t - tests (Welch's, unequal variances) were conducted. With the exceptions of seagrass ($p = 0.03$) and SPR ($p = 0.003$) there were no significant differences in % cover by any benthic category between reef sites.

4.5.7 Reef ecology and seawater DMSP in 2016

The output from mixed model analysis is presented in Table 4.7. The only benthic category to affect seawater DMSP at both sites was seagrass cover. At Coral View in 2016, Chlorophyta and Phaeophyta both significantly affected seawater DMSP, however, when all macroalgal percentage covers were aggregated there was no significant effect of macroalgae on seawater DMSP. At Little Bight Rhodophyta, seagrass, SPR and turf algae all significantly affected seawater DMSP.

Table 4.7 Summary of p - values from ANOVA output of linear models investigating the effect of benthic group on seawater DMSP at Coral View and Little Bight in 2016. Significant values are highlighted in bold.

Benthic category	*Coral View*	*Little Bight*
CCA	0.31	0.24
Chlorophyta	**0.01**	0.32
Hard coral	0.28	0.12
Macroalgae	0.36	0.18
Phaeophyta	**0.03**	0.48
Rhodophyta	0.31	**0.03**
Seagrass	**0.008**	**0.03**
Soft coral	0.32	0.19
SPR	0.16	**0.05**
Turf algae	0.45	**0.04**

4.5.8 Comparison: 2015 and 2016

T - tests (Welch's, unequal variances) were conducted on the data from 2015 and 2016 to establish whether there were significant differences in seawater DMSP, DIC, TA, mean daily temperature and benthic ecology.

Because temperature was only recorded at two points on the spur at Coral View in 2015, corresponding temperature data from 2016 was used to assess whether there were significant differences in temperature between years. Temperature data in 2015 (Table 4.8) was taken from the closest point to the corresponding time point in 2016; where the time point from 2016 occurred between time points for 2015, both data points were included in the statistical analysis.

There were significant increases in temperature at Coral View between 2015 (average daily temperature 28.57 °C) and 2016 (average daily temperature (29.81 °C) for both the far shore ($p = 0.0005$) and near shore ($p = 0.02$).

Because DMSP, DIC and TA from the back, middle and front locations on the reef were all sampled in both years and at both sites, these data were analysed for significant differences between years at each site. Significant differences between years were noted for DMSP at Coral View ($p = 0.001$) and TA at Little Bight ($p = 0.0006$), but no other variables exhibited significant differences at either site between years.

Since no significant differences were noted between spurs at the same site in 2015, the mean percentage covers for each benthic category of all three spurs at each site were used in the comparison (Fig 4.16).

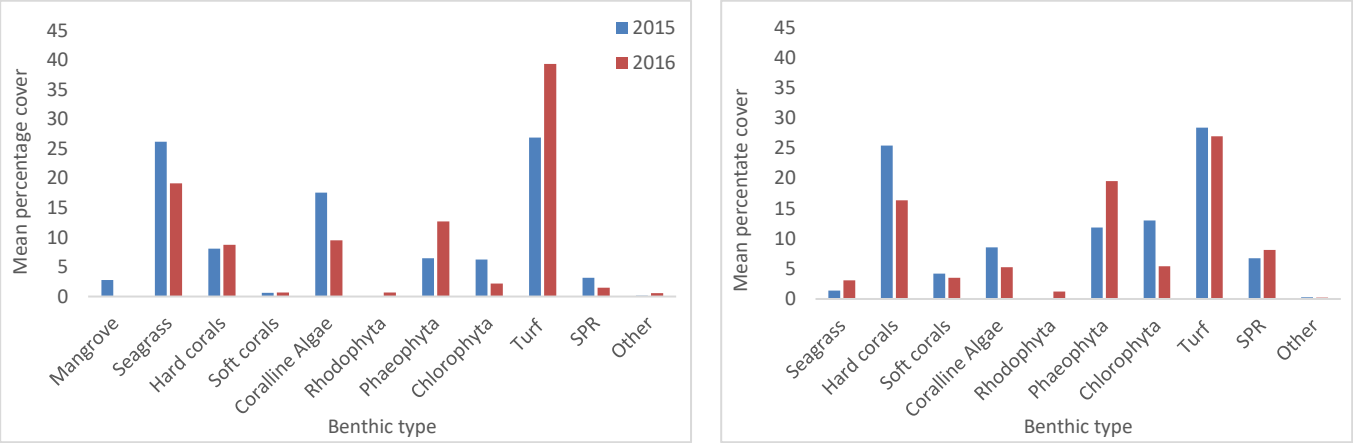


Figure 4.16 Mean percentage cover by benthic type for Coral View (left hand graph) and Little Bight (right hand graph) for 2015 (blue bars) and 2016 (red bars).

No significant differences between years were observed for in percentage cover of any benthic category at Coral View, however at $p < 0.10$, coralline algae ($p = 0.07$) and Chlorophyta ($p = 0.09$) exhibited significant declines in percentage cover between years. A significant increase in Rhodophyta ($p = 0.02$) and significant decline in Chlorophyta ($p = 0.02$) was observed at Little Bight, however no other benthic type demonstrates a significant difference in percentage cover between years.

4.6 Discussion

4.6.1 Concentrations and comparison with values reported in the literature

Mean concentrations of DMSP compounds reported for Little Bight in 2016 (11.3 – 13.2 ± 0.9 nmol DMSP) were lower than those at Coral View (17.7 - 53.7 ± 15.8 nmol DMSP), however 2015 concentrations were higher at Little Bight (8.9 - 47.2 ± 33.6 nmol DMSP) than at Coral View (6.7 - 20.9 ± 8.6 nmol DMSP). It is unlikely that this is driven by changes in benthic cover, since no significant differences were observed between years, which indicates the driving factor(s) is abiotic in nature. This study has reported that temperature is a significant driver of seawater DMSP concentrations, however temperature was not recorded at Little Bight in 2015. It is possible, therefore, that sea surface temperatures at Little Bight in 2015 were higher than at Coral View, which would explain the higher seawater DMSP concentrations. It is also possible that changes in the sediment load in the water column at each site result in subtle changes to the light regime between sites in 2015. For example, Coral View is typified by an extensive seagrass bed (with necessarily higher amounts of benthic sediment) shallower topography and more restricted circulation (Fig 4.16). It is possible that increased sediment load in the water column restricts light penetration, resulting in less DMSP production by the benthos and by extension, less DMSP release to the water column. Differences in wind regime may also lead to the higher seawater DMSP concentrations observed at Little Bight in 2015; the greater depth at Little Bight is likely to enhance greater water column mixing, which could result in a greater flux of DMS to the atmosphere leading to lower total DMSP concentrations being recorded. Equally likely is the restricted seawater mixing and/or flushing in the back - reef at Coral View caused by the extremely shallow depths (often ≤ 20 cm) observed during this study. This topography may serve to allow accumulation of DMSP in seawater, which would otherwise leave the system and result in lower concentrations being measured. Other studies into seawater DMSP in shallow water tropical reef systems report DMSP concentrations ranging from 0.66 – 134 nmol DMSP in the Great Barrier Reef (Broadbent and Jones 2006; Jones et al. 2007) and 14.7 – 23.9 nmol DMSP in the Red Sea (Burdett et al. 2013). Thus, the range of values reported here for the Caribbean are within the range of concentrations reported for other reef sites.

4.6.2 Spatial variability of seawater DMSP

Distance from shore (i.e. longitudinal), benthic composition and reef site are all important factors in determining seawater DMSP concentrations in this study, however lateral spatial variability was only evident (i.e. between identical distances on different spurs) at Coral View. Seawater DMSP concentrations at Coral View were found to be highest in the seagrass dominated back - reef environment in both sampling years. The finding that seawater DMSP concentrations are higher in areas dominated by seagrasses echoes the findings of Burdett et al. (2013), who also reported higher seawater DMSP concentrations in a seagrass dominated back - reef environment, with seawater DMSP concentrations decreasing with increasing distance from shore. Higher concentrations of seawater DMSP were also evident at Little Bight in both years, however rather than a seagrass dominated benthic ecology, the back - reef at Little Bight was a mixed autotrophic assemblage with the most abundant component being turf algae. This study found that turf algae does affect seawater DMSP distributions at Little Bight, as does the coverage of SPR but the same was not observed at Coral View. These results suggest that the composition of the benthos does exert an effect on seawater DMSP distributions, however each site is characterised by a different physical environment (Fig 4.17) and this is likely to play a role. As previously mentioned, both sites are characterised by a shallower back - reef environment which can serve to trap DMSP and result in the higher concentrations measured. However, this is more pronounced at Coral View, where depths in the back - reef often do not exceed ~ 20 cm. At this site, DMSP production in the back - reef is driven by seagrasses, but the shallow topography restricts seawater circulation and results in DMSP being accumulated in seawater. At Little Bight, however, DMSP production in the back - reef is driven not only by seagrass, but also by turf algae and the absence of any DMSP producing organisms (SPR). There is still a significant effect of distance from shore on seawater DMSP concentrations. Thus, the fact that distance from shore is a significant driver on seawater DMSP concentrations at both sites, despite different benthic compositions, and that this is coupled with decreasing concentrations with increasing distance from shore is suggestive of increased mixing at the front of the reef than the back (Fig 4.17). Thus, DMSP distributions probably do not follow a strictly linear

relationship driven by the underlying benthic ecology but are subject to an array of biotic and abiotic factors.

4.6.3 Diel variability of seawater DMSP

Seawater DMSP was significantly affected by time of day at both Coral View and Little Bight reef sites in 2016, however diel trends varied between sites. Seawater DMSP in the back - reef environment at Coral View was lowest in the morning, with highest values recorded at 1.30 pm after which concentrations began to decrease again. The back - reef environment at Coral View was dominated almost exclusively by seagrass, turf algae and Chlorophyta. As a photosynthetically derived product, it is likely that DMSP production is driven, at least in part, by photosynthetic rates. However, this study did observe higher concentrations of seawater DMSP first thing in the morning (Fig 4.6) at Coral View, when photosynthetic activity would have been lower and based on the DO_2 measurements made, does appear to be the case. It has been proposed that DMSP can act as a grazing deterrent (Alstyne et al. 2001; Van Alstyne and Houser 2003; Erickson et al. 2006), and with grazing pressure typically highest at night on tropical reefs (Lewis and Wainwright 1985), it had been anticipated that seawater DMSP levels would be highest in the morning and at the end of the day in the algal dominated back - reef. Whilst this was not the case for the back - reef, DMSP concentrations were higher in the morning and evening in the mid - and front - reef at Coral View, which is in line with findings from Burdett et al. (2013) and appears to support the macroalgal defence hypothesis. The disparity in diel trends in seawater DMSP between the back - mid - and front - reef sites is possibly driven by the physical environments (Fig 4.17). Whilst measurements were not made of water depth, visual observations suggest the mean depth of the back reef at Coral View was ~ 20 cm and often much shallower. Thus, this environment is characterised by a high light regime and extreme changes in salinity, temperature and restricted water circulation. The presence of significantly greater coverage by seagrasses at Coral View, coupled with the physical environment, as previously discussed, may allow accumulation of DMS/P in seawater, resulting in the greater concentrations observed here relative to Little Bight. Little Bight, conversely, had a deeper back - reef that allowed greater mixing and potential efflux of DMS from seawater.

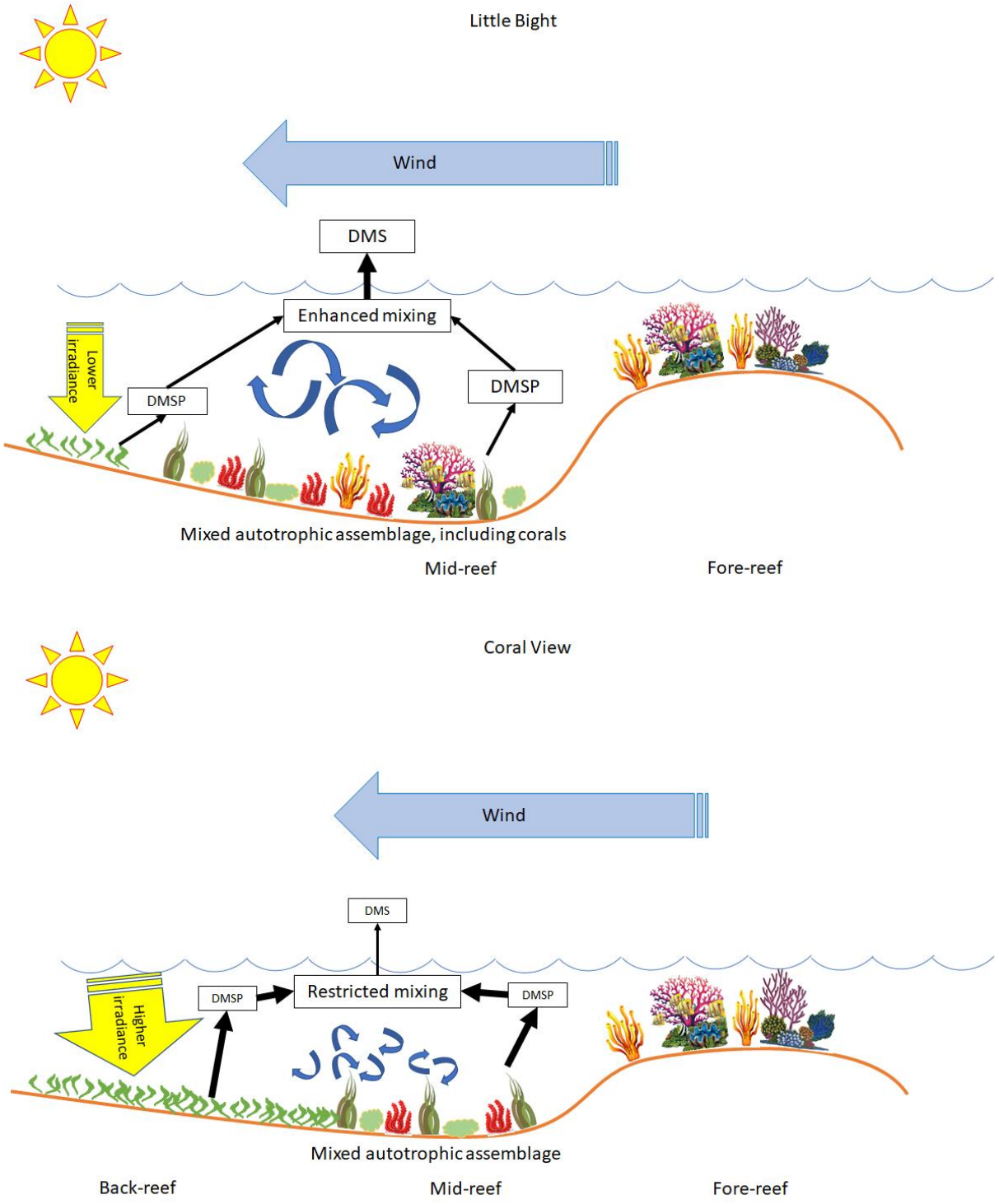


Figure 4.17 Conceptual diagram illustrating the physical and biological processes at Coral View (top) and Little Bight (bottom) reef sites. The strength of individual processes relative is indicated by the arrow thickness.

Amongst the other functions proposed for DMSP in marine algae is that of an osmolyte or antioxidant (Reed 1983; Sunda et al. 2002), enabling organisms to deal with changes to salinity, temperature and light. Both salinity and temperature were observed to increase throughout the day in the back reef at Coral View, with highest values at 1.30 pm and this would have been coupled with increases in photosynthetically active radiation (Fig 4.17); it is possible that seawater DMSP in this portion of the reef is driven by diel changes in salinity, light and temperature leading to increased production of DMSP by the macroalgal and seagrass community. Other studies have reported significant relationships between DMSP production and temperature, salinity and light exposure in seagrasses (Borges and Champenois 2015), corals (Deschaseaux et al. 2014b), *Symbiodinium* (McLenon and DiTullio 2012; Deschaseaux et al. 2014a) and macroalgal (Kirst 1996) species. Thus, the research presented here supports previous findings and suggests that the effects of abiotic variables on DMSP production by all reef organisms warrants further research.

4.6.4 Controls on carbonate chemistry variability

Differences in DIC concentrations between sites were significant in both years, however differences in seawater TA between sites was only significant in 2015 at $p < 0.10$. The values reported here are within the range of values reported elsewhere in the literature (Kleypas et al. 2006). There was no observed difference in carbonate chemistry between spurs and grooves in 2015, however DIC and TA at both sites is significantly affected by distance from shore in both years. Whilst TA increased with increasing distance from shore at both sites, DIC increased between the back - and mid - reef environments and decreased between the mid - and front - reef. This was likely to be driven by the shift from a seagrass/macroalgal dominated benthic ecology in the back - and mid - reef to coral/mixed assemblage in the front - reef environments. Furthermore, the back - and mid - reef environments at both Coral View and Little Bight were relatively shallow (visual observations suggest 0.1 - 0.5 m deep), and diel variability in seawater carbonate chemistry was more pronounced here. Biological processes, namely calcification/dissolution and photosynthesis/respiration probably exerted a strong influence on the carbonate chemistry in this portion of the reef. This has also been observed in other reef environments (Kleypas et al. 2006, 2011; Gagliano

et al. 2010), and is most pronounced in shallow waters due to the reduced surface area to volume ratio (Kleypas et al. 2006).

4.6.5 Seawater DMSP between years

This study demonstrates that temperature had a significant effect on seawater DMSP at Coral View in both years and on DMSP at Little Bight in 2016. Also noted was the significant increase in both temperature and seawater DMSP at Coral View between years. Whilst coverage of some benthic categories appeared to change between years at both sites, there were no consistent patterns between sites that could account for the increase between years. Furthermore, no abiotic variable (TA, DIC etc) indicates any change in biological activity (i.e. growth, photosynthesis, calcification etc) between years. It appears likely that the biological community exhibited no change in composition or biological activity between years and suggests that the increase in seawater DMSP observed between years was driven by an increase in temperature. It is well reported in the literature that temperature plays a crucial role in driving DMSP production in a wide range of algal classes and corals (Stefels 2000; Jones et al. 2007; Raina et al. 2013), with the antioxidant function of DMSP (Sunda et al. 2002) making this compound of particular interest with respect to corals and coral reefs. The global mass - bleaching event that was reported for a range of reef systems in 2016 was not visually confirmed during this study, however reefs in the Caribbean were known to be affected. The global mass - bleaching event was driven by El Niño and its associated increases in sea surface temperature; this event is likely to have driven increased production of DMSP by a range of coral reef taxa at these reef sites, which resulted in the elevated concentrations reported here. It is interesting to note that whilst seawater DMSP increased, no visible signs of bleaching were evident, perhaps suggestive of increased thermal tolerance conferred to organisms through production of DMSP.

4.6.6 Summary and conclusions

This study highlights that, as well as corals, other prolific DMSP producers, i.e. seagrasses & turf algae, are crucial organisms in determining seawater DMSP concentrations and distributions in coral reef systems. However, biological activity and community composition within a reef setting are not the sole factors determining DMSP cycling; temperature, light and the physical regime of a reef are all important in determining how DMSP is cycled/accumulated. Whilst of coral reef DMSP research has tended to focus on production of DMSP by corals and coral associated algae/bacteria, the results reported here indicate that future research should consider other producers and abiotic factors if a more complete understanding of coral reef DMSP cycling is to be gained.

The previous study highlighted the importance of increases in pCO_2 and temperature on coral production of DMSP and DMSO, which resulted in increased concentrations of DMSP in the surrounding water. However, the conditions to which corals were subjected were, necessarily, tightly controlled. Wild corals in a typical reef setting, however, are subject to an array of variable conditions and despite the name, coral reefs are not solely comprised of corals, but a wide range of different taxa including coralline algae, soft corals and macroalgae. A study was designed to investigate what factors drive the spatial variability of coral reef DMSP in a Caribbean reef system, which have hitherto been unstudied with respect to DSC. Abiotic and biotic variables are considered over two summer seasons, to ascertain what the current drivers of seawater DMSP are. Only when we understand contemporary reef sulphur cycling, can we being to forecast the likely impacts of climate change on DSC in reef systems.

5 Impacts of climate change on production of dimethylated sulphur compounds by a range of coral reef taxa

5.1 Introduction

Oceanic pH has begun to decrease from pre - industrial levels as more carbon dioxide (CO_2) dissolves into the oceans and this is projected to continue to decrease in the future. The pH of the oceans is regulated by a carbonate equilibrium that is driven by the dissolution of atmospheric CO_2 into the oceans. Between 30 – 50 % of the CO_2 released from the burning of fossil fuels, cement production and deforestation have been absorbed by the oceans (Sabine et al. 2004). Once dissolved in the oceans, CO_2 forms carbonic acid (H_2CO_3), which can dissociate into (bi)carbonate (HCO_3^- and CO_3^{2-}) and hydrogen ions (H^+). An increase in atmospheric CO_2 causes a shift in the oceanic carbonate equilibrium, favouring the formation of H^+, which controls oceanic pH by the relationship:

$$pH = - \log_{10}(H^+)$$

Consistently increasing levels of atmospheric carbon dioxide have driven an increase in H^+ formation, resulting in a decrease in the saturation state of seawater and decreasing oceanic pH. It is projected that continued anthropogenic emissions of CO_2 will cause the pH of the oceans to drop by 0.3 – 0.5 units by 2100 (Caldeira and Wickett 2003). Although this drop sounds negligible, the logarithmic nature of the pH scale means a drop of 0.3 units will have potentially devastating effects on calcifying marine organisms. Furthermore, rising atmospheric CO_2 levels have also driven increases in sea surface temperature (SST), with the Atlantic Ocean warming by an average of 0.41°C between 1950 – 2009 (IPCC 2013). Conversely, macroalgae and seagrasses are expected to fare better under these conditions, leading to the suggestion that 'coral' reefs will become 'algal' reefs (Dubinsky and Stambler 2011)

Coral reefs in tropical and subtropical regions contribute to the ocean carbon cycle through the processes of photosynthesis, respiration, $CaCO_3$ production and dissolution (Barnes 1983; Barnes and Devereux 1984). Coral reef ecosystems are vulnerable to OA and climate

change induced ocean warming (Hoegh-Guldberg et al. 2007a) with a range of effects on the ecosystem (Fabricius et al. 2011). Specifically, increases in oceanic CO_2 will reduce the aragonite saturation state, which decreases the ability of many coral species to produce their carbonate skeletons (Anthony et al. 2008a), potentially resulting in stress of the coral and/or its symbiotic algae. Additionally, increases in sea surface temperatures are known to cause cellular stress in coral associated algae (Lesser 2011), rendering these organisms highly susceptible to the effects of climate change.

Dimethylsulphoniopropionate (DMSP) is a sulphur compound produced by many marine algae and is the major precursor to dimethylsulphide (DMS), a gas that may be linked to local climate regulation through aerosol production and cloud formation (Charlson et al. 1987). A number of cellular functions have been described for DMSP and DMSO in marine algae, (Karsten et al. 1996b; Kirst 1996; Sunda et al. 2002; Van Alstyne and Puglisi 2007; Seymour et al. 2010a), but in tropical reef environments, these compounds may be particularly important as antioxidants, grazing deterrents and/or compatible solutes. Further research has also suggested that DMSP may play a role in improving tolerance to variable carbonate chemistry conditions (Burdett et al. 2012), thus its production is persuasively linked to cellular stress.

5.2 Aims of this study

Whilst there are several published investigations into the effects of rising SSTs on cellular concentrations of DMSP (Broadbent et al. 2002; Yost and Mitchelmore 2010; Yost et al. 2012; Tapiolas et al. 2013), there are relatively few that have also considered DMSO (Deschaseaux et al. 2014b) and no studies have considered the impacts of reefal carbonate chemistry on seawater DMSP production or intracellular concentrations of DMSO. Furthermore, whilst there has been evidence to suggest that reef sediments are potential sources of water column DMSP (Broadbent and Jones 2004), no further work has yet been conducted to quantify how much DMSP may be released from sediments to the overlying water column, nor how much DMSO they may contain. This study aims to assess the contribution of key reef organisms to the overall DSC biogeochemistry. Incubations of various non-coral species were conducted to quantify rates of seawater DMSP production,

as well as DMSP/DMSO content. Coral production of seawater DMSP was also assessed using incubations with corals *in situ*. Using transplant techniques (King & Jones, 2015), the potential impacts of climate change on these rates of production were investigated by transplanting corals to warmer, more acidic seawater. It was hoped that using this method would enable a field - based experiment similar to that of Chapter 3 to be conducted to see if wild corals behaved differently than laboratory specimens.

5.3 Hypotheses

1. The major sources of seawater DMSP in a reef setting are seagrasses → macroalgae → corals → sediments in descending order of magnitude
2. Sediments are an overlooked source of seawater DMSP in coral reefs and also act as a source and sink for DMSP/O
3. Coral production of seawater DMSP will increase when transplanted to a warmer, more acidic location on the reef
4. Non – coral production of DMSP will vary according to time of day, with production being lowest in the morning and highest in the afternoon and this will be driven by changes to temperature/light/pH conditions

5.4 Methods

5.4.1 Research and transplant sites

Transplants were conducted at one reef site in July 2016, Coral View, during this study (Fig 5.1). Coral View is located on the island of Utila, which is located within the Honduran Bay Islands on the southern end of the Meso - American Barrier Reef (See Chapter 4 for a detailed map showing the location of Coral View and Utila).

Figure 5.1 Map showing the aerial view of Coral View and locations of *in situ* corals and the transplant site

Coral samples were found growing naturally at the *in situ* site shown in Fig 5.1 and, once removed from the reef, this was the site of the *in situ* incubations. After the *in situ* incubations, corals were moved to a warmer, more acidic portion of the reef, hereafter referred to as the transplant site, which is also shown in Fig 5.1.

Measurements made the previous year and during the experimental year indicated that the transplant site was characterised by significantly greater sea surface temperatures (SSTs, see section 4.5.8) than the *in situ* location of the corals. This natural (i.e. not manipulated artificially) temperature increase could therefore help assess the response of wild corals to increases in SSTs.

Coral selection was based on ecological surveys during 2015 that identified the main species of coral at the reef site; these were identified as *Porites astreoides* and *Undaria tenuifolia* (Fig 5.2). It is worth noting that *Undaria tenuifolia* was recently reclassified and was formerly *Agaracia tenuifolia*; *Undaria* is also a genus of macroalgae and should not be confused with the *Undaria* coral genus referred to in this study.

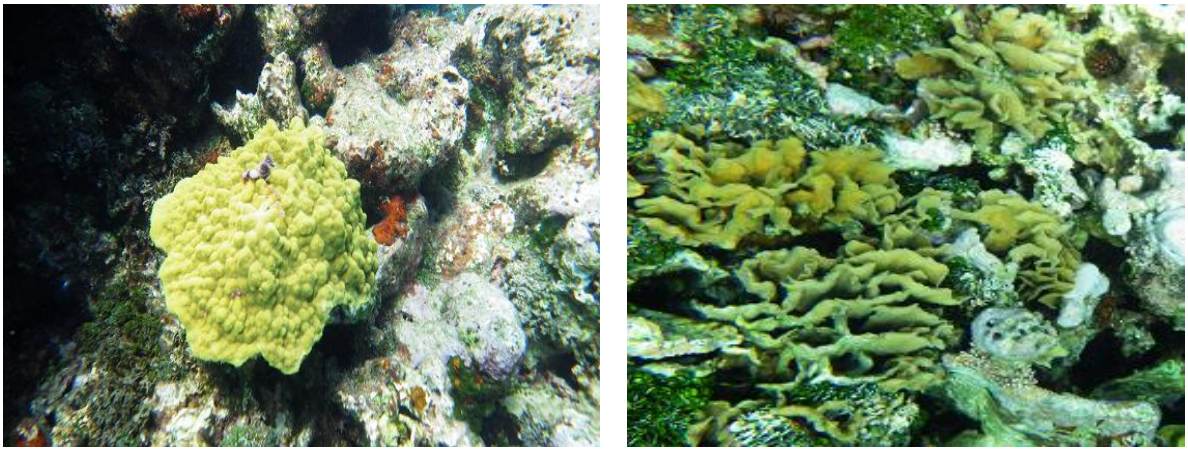


Figure 5.2 Pictures taken in the field in 2016 of transplant coral species. P.astreoides (left) and *U.tenuifolia* (right) are two key reef building coral species at the research site used in this study in the Caribbean.

Certain non-coral species (Fig 5.3) were selected for incubation *in situ*; seagrass (believed to be *Thalassia testudinum*), *Dictyota* spp, *Halimeda* spp (believed to be *Halimeda opuntia*) and reef sediment were all selected based on their dominant coverage at Coral View reef site.

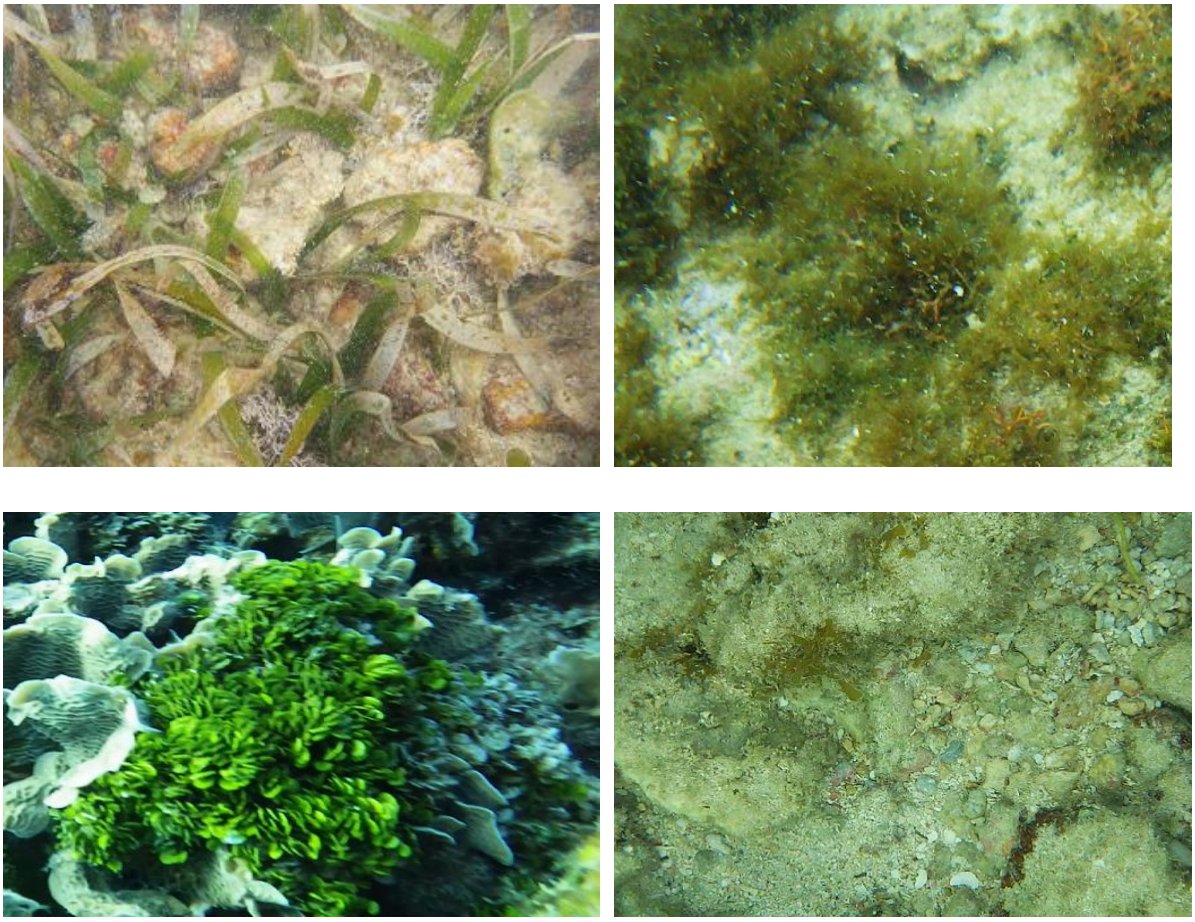


Figure 5.3 Pictures taken in the field in 2016 of key non - coral benthic cover specimens selected for incubation. Clockwise from top left: Seagrass, Dictyota spp, sediment and Halimeda spp.

All non - coral species were incubated *in situ* (Fig 5.4) according to where each specimen occurred in the most dominant amounts. Incubations were conducted in the morning and afternoon (except sediment) and the same locations were used for both times of day.

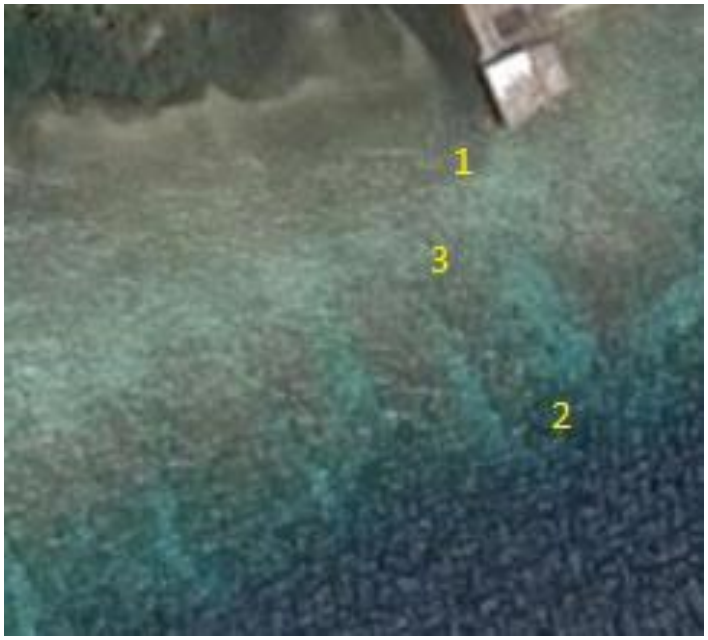


Figure 5.4 Locations of non - coral incubations at the Coral View study site in 2016. 1 = seagrass incubations, 2 = Halimeda incubations, 3 = Dictyota spp. & sediment incubations.

5.4.2 Incubations

Incubations involved placing each specimen sample in a modified chamber (see section 5.4.3 for detailed methodology) for a specified period of time. Variables being measured were:

Seawater DMSP

Temperature

Dissolved oxygen

Salinity

Carbonate chemistry (DIC & TA)

A sample was taken for each variable at the beginning and end of each incubation and the rate of change calculated as the difference between the two divided by the length of the incubation.

5.4.3 Coral incubations – experimental design

P.astreoides and *U.tenuifolia.* Fragments (n = 3 per species) were either broken off manually or using a hammer and chisel, set in epoxy putty (Milliput®), anchored to the reef and left to recover *in situ* for 48 h prior to the first incubation.

Incubations (Fig 5.5) were conducted in clear Tupperware™ containers that had been modified to enable sampling to take place and further tested for water tightness by conducting a control 4 h control incubation (no coral); no discernible change was observed in either dissolved oxygen or salinity, whilst the internal chamber temperature remained consistent with the external seawater temperature.

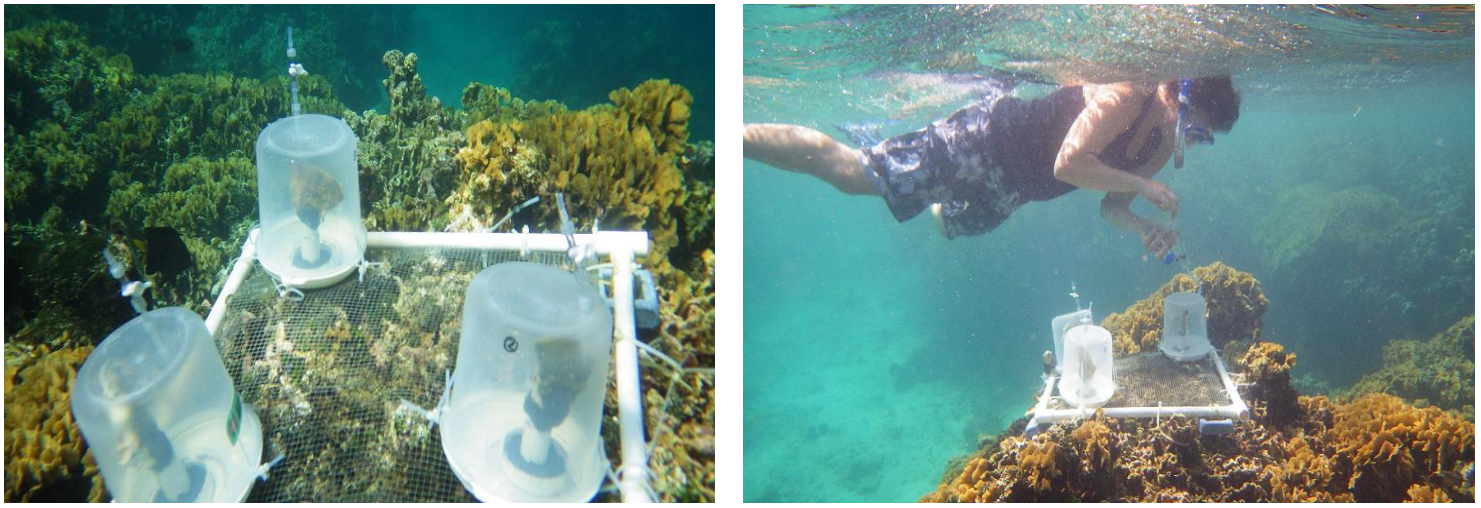


Figure 5.5 Pictures showing the experimental set up *in situ* for coral incubations (left) and sampling from each chamber (right).

To enable sampling to occur, holes had been made in the top of each chamber through which a Luer valve was inserted and glue applied to ensure there were no leaks; a small (~ 5 cm) length of silicon tubing was placed on the Luer valve on the inside of each chamber so that water would be sampled from the middle of the chamber whilst ensuring the tubing did not touch the coral fragment. Corals were incubated for 4 h from 10 am to 2 pm so that the hottest part of the day coincided with the halfway point in the incubation. After an individual coral fragment was placed in the incubation chamber, samples were taken

immediately, returned to shore and analysed in the order of for DO_2/salinity/temperature, DIC/TA and DMSP.

5.4.4 Coral incubation seawater analyses

Water samples were returned to shore and processed as described in section 4.4.5 and when returned to the University of St Andrews, were analysed per the methods outlined in sections 2.5.2 (TA), 2.5.3 (DIC) and 2.3 (DMSP).

5.4.5 Seagrass, sediment and macroalgal incubations – experimental design

The key macroalgal species on the reef had previously been identified as *Dictyota* spp. and *Halimeda* spp., whilst the back part of the reef at Coral View was dominated by seagrass which was present, but not as abundantly, at Little Bight. It had also been noted that sediment accounted for a significant proportion of the reef benthic environment, with previous research reporting that sediment pore waters contain higher concentrations of DMSP than are typically found in seawater (Broadbent and Jones 2004). The high concentrations of DMSP in sediment pore waters is likely to be the result of free-living *Symbiodinium* living within reef sediment; because of their lack of theca, when not associated with a host, studies have reported that the highest abundances of *Symbiodinium* are to be found in reef sediments, rather than in the water column (Littman et al. 2008; Takabayashi et al. 2012).

Incubations were conducted on four key species/substrate types to assess their relative contributions to the overall reef water DMSP budget. Tests to assess the change in dissolved oxygen were conducted on *Halimeda* and *Dictyota* and 2 h was determined as an acceptable incubation time, which would pick up detectable changes in seawater DMSP. Seagrasses are prolific producers of DMSP so 1 h incubations were used for this species, whilst sediment samples were incubated for 4 h. Apart from sediment, all species were incubated twice daily; once in the morning (11 am - 1 pm) and once in the afternoon (2 – 4 pm). Sediment samples were incubated from 10 am - 2 pm.

Because *Dictyota* and *Halimeda* are not vascular plants and have a holdfast system rather than true roots, they can attach to varied substrata and do not have to be incubated precisely *in situ*. Samples (n = 5) of each species were taken from a 2 m^2 patch of reef (including the holdfasts) and placed in small incubation chambers, which were anchored to the reef in the same 2 m^2 area of reef. Seawater samples were taken at the start and end of each incubation for temperature, dissolved oxygen, salinity and DMSP (as previously described). After each incubation, the whole specimen was weighed and subsamples (~ 0.1 g) were taken and weighed for tissue DMSP and DMSO analysis in the lab at the University of St Andrews (see section 2.3).

As angiosperms, seagrasses have extensive and complex below - ground root systems, which are believed to contain significant amounts of both DMSP and DMSO (Husband and Kiene 2007; Borges and Champenois 2015). Seagrass samples (n = 5) were therefore incubated *in situ* by placing a small plastic bag around two fronds and sealing it at the base of the plant. At the end of the incubation the plant was cut at the base and immediately returned to shore where water samples were taken for dissolved oxygen, salinity, temperature and DMSP (see section 4.4.6).

5.4.6 Control incubations

Five control chambers comprising seawater only were conducted for 4 h in the back - reef environment (10 am – 2 pm) to investigate whether DMSP produced in any of the incubations could be derived from other processes, however no significant production of seawater DMSP was observed (Welch's t - test, p = 0.371).

5.4.7 Species tissue sample extraction and preparation

Incubation chambers containing water and the species sample were returned to shore so that samples could be documented and subsampled. Coral species were photographed (Fig 5.6) with a scale marker to enable surface area analysis to be completed using ImageJ™, after which the tissue was removed using a WaterPik™ (Fig 5.6) containing DI water (transported from St Andrews). The tissue slurry was homogenised using a small blender and 1 ml of the

slurry was added to each of 5 replicate vials, after which the vials were fixed with 10 M NaOH and immediately crimp sealed. The volume of water used for each tissue extraction was also measured so the total DMSP for each coral fragment could be quantified.

Figure 5.6 Example picture of a coral specimen post - transplant (left) and post - Waterpiking (right) with a scale added to enable surface area measurements to be made.

Five replicates of approximately 0.1 g of *Halimeda*, *Dictyota* and seagrass were placed into vials, 1 ml of 10 M NaOH was added and the vials were immediately crimp sealed. Samples were returned to St Andrews where they were analysed for DMSP/O by headspace injection as described below. Sediment chambers were returned to shore where the chamber and contents were weighed; the water was collected after passing through a 0.6 µM mesh filter and its volume noted. The sediment weight was then recorded and replicate vials (n = 5) prepared for DMSP/O analysis as previously described.

5.4.8 Tissue DMSP and DMSO analysis

Samples were stored in the dark until analysis back at St Andrews could be completed. DMSP samples were analysed as per the method in section 2.3.3, whilst DMSO samples were analysed as per the method in section 2.3.4

5.4.9 Statistical analyses

In terms of DMSP production between days, coral species demonstrated different behaviour and so statistical analyses were run on each species separately. Mixed models were run using the 'nlme' package in R, with the mean temperature and pH during each incubation as well as the sampling day inputted as fixed effects. The location of the transplant was included as a random effect, since the purpose of location was to subject each coral fragment to decreased pH and increased temperature. The dependent variable was the rate of production of DMSP, normalised to coral surface area and per hour. The generic model input was thus:

```
speciesmodel <- lme(fixed = ΔDMSP ~ MeanTemp + MeanpH ~ Day, random = ~
1|Location, data = data.table, na.action = na.omit)
```

Mean macroalgal/seagrass production of DMSP between times of day was similar, with increased production in the afternoon compared with the morning for all species. Welch's two sample t - tests were used to assess differences in seawater DMSP production between species. Mixed models were conducted on all non-coral species to assess which factors significantly affected production of seawater DMSP. The mean temperature during each incubation, background pH and carbonate saturation state for each reef location, and time of day were all included as fixed effects.

The location of each incubation on the reef was inputted as a random effect, with the generic model input thus:

```
speciesmodel <- lme(fixed = ΔDMSP ~ MeanTemp + BackgroundpH +
BackgroundSatCa + TimeofDay, random = ~ 1|Location, data = data.table,
na.action = na.omit)
```

Two - way ANOVA was used to assess which environmental variables affected tissue DMSO in all samples; the generic model input was:

```
model <- Anova(lm(dmso ~ variable1+ variable2…)
```

For the transplant experiment, paired t - tests were conducted on tissue DMSO concentrations and all environmental variables to establish significant differences in the pre - and post - transplant conditions.

5.5 Results

5.5.1 Coral - derived seawater DMSP production

Background levels of seawater DMSP in the *in situ* incubations ranged from 9.28 – 24.97 nmol DMSP and 12.5 – 118.3 nmol DMSP at the transplant site. On the morning of the *P.astreoides in situ* incubation, seawater DMSP was measured at 9.6 nmol DMSP whilst for the *U.tenuifolia in situ* incubations it was 7.2 nmol DMSP. At the transplant site, background levels of seawater DMSP ranged from 12.5 – 118.3 nmol DMSP.

Measured concentrations of seawater DMSP at the beginning and end were used in a one-tailed paired t-test model to assess whether there were significant increases of DMSP in the seawater in the chamber for each day of the experiment (Table 5.1). There was significant seawater DMSP production in the *in situ* incubations for both *P.astreoides* and *U.tenuifolia* (Table 5.1, in situ rows). However, on day 1 of the transplant (Table 5.1, Transplant T1 rows), there was no significant production of seawater DMSP by *U.tenuifolia* and production

of seawater DMSP by *P.astreoides* was only significant at $\alpha < 0.10$. By day 7 (Transplant T2) of the transplant (Table 5.1, Transplant T2 rows) significant seawater DMSP production by *P.astreoides* was apparent, however this was only significant for *U.tenuifolia* at $\alpha < 0.10$.

Table 5.1 Summary of p - values for seawater DMSP production at the start and end of each incubation

Species	*In situ*	*Transplant T1*	*Transplant T2*
U.tenuifolia	0.05	0.13	0.08
P.astreoides	0.05	0.07	0.03

Mean (n = 3) seawater DMSP production (Fig 5.7) between coral species incubated *in situ* was not significantly different (Welch's t - test, $p = 0.10$), however seawater DMSP production between species was significant at days 1 ($p < 0.001$) and 7 ($p = 0.02$). Production of seawater DMSP by *P.astreoides* increased in response to the transplant (Fig 5.7) increased between days 0 and 1, but decreased between days 1 and 7. Conversely, *U.tenuifolia* exhibited decreased seawater DMSP production on day 1 of the transplant, which increased between days 1 and 7.

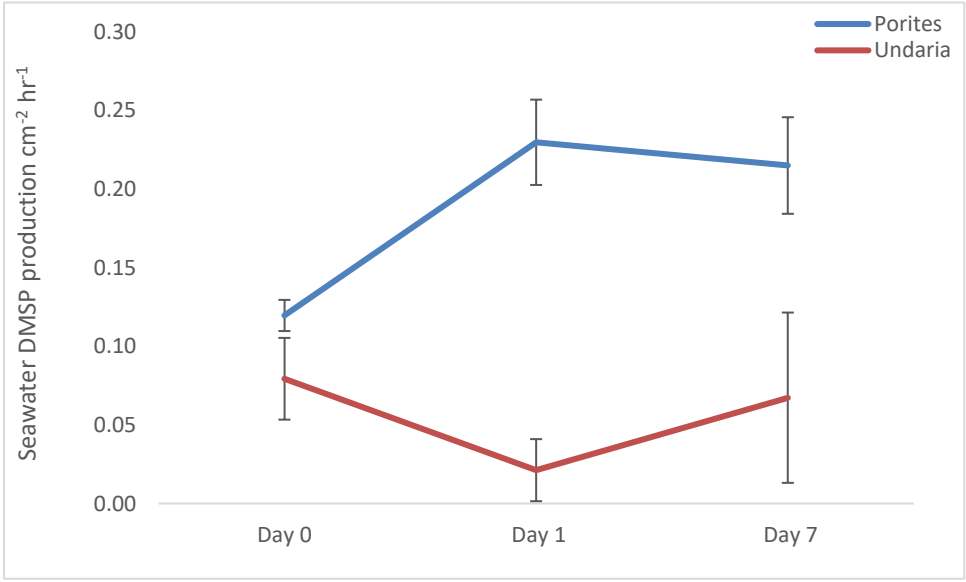


Figure 5.7 Mean (n = 3) coral production of seawater DMSP by *P.astreoides* and *U.tenuifolia*, both *in situ* (Day 0) and post - transplant (Days 1 & 7). Values are normalised to coral surface area per hour. Error bars are standard deviations (n = 3) of hourly DMSP production for each species.

Mixed models were used to assess the effect of the transplant on DMSP production by each species of coral and there are notable differences between species. Production of seawater DMSP by *P.astreoides* was significantly affected by temperature ($p = 0.04$) and pH ($p = 0.04$), and DMSP production between days was also significantly different ($p = 0.03$). However, neither temperature ($p = 0.49$) nor pH ($p = 0.99$) significantly affected seawater DMSP production by *U.tenuifolia*, nor were there significant differences in seawater DMSP produced between days ($p = 0.14$).

5.5.2 Non - coral derived seawater DMSP production

Background levels of seawater DMSP for the *Halimeda* incubations on the reef front ranged from 7.3 – 9.6 nmol DMSP, however the background level of seawater DMSP on the day of the incubation was 45.5 (morning) to 28.1 nmol DMSP (afternoon). *Dictyota* and sediment incubations were conducted in the mid - reef where background concentrations of seawater DMSP ranged from 7.2 – 33.5 nmol DMSP; concentrations on the day of the *Dictyota* incubations, were within this range and measured from 24.4 (morning) to 17.8 nmol DMSP (afternoon) whilst seawater DMSP at the start of the sediment incubations was measured at 28.8 nmol DMSP. Seagrass incubations were conducted in the back - reef environment where the range of seawater DMSP concentrations ranged from 12.5 – 118.3 nmol DMSP. On the day of the incubations, seawater DMSP in the back - reef ranged from 59.9 (morning) to 46.2 nmol DMSP (afternoon).

Measured concentrations of seawater DMSP at the beginning and end were used in a one-tailed paired t-test model to assess whether or not there were significant increases of DMSP in the seawater in the chamber during each incubation (Table 5.2)

Table 5.2 Summary of p - values for seawater DMSP production at the start and end of each non - coral incubation.

Species	*Morning incubation*	*Afternoon Incubation*	*Morning & afternoon*
Halimeda	0.04	0.02	
Dictyota	0.03	0.01	
Seagrass	0.008	0.005	
Sediment	n/a	n/a	0.01

Production of seawater DMSP in the *Halimeda* and *Dictyota* incubations was significant in the morning, but only at $p < 0.10$ however production is significant at $p < 0.05$ in the afternoon incubations. Significant levels of seawater DMSP production by seagrass was evident in both the morning and afternoon incubations. Sediment was incubated for four hours (10 am to2 pm) and production of seawater DMSP normalised to sediment weight and time (h^{-1}) was significant at $p = 0.01$. Control incubations ($n = 5$) comprising seawater only were conducted for 4 h (10 am –2 pm) to investigate whether DMSP produced in any of the incubations could be derived from other processes, however no significant production of seawater DMSP was observed in the control chambers (Welch's t - test, $p = 0.371$).

Rates of seawater DMSP flux by *Halimeda, Dictyota* and seagrass (Fig 5.8) appeared to be consistently greater in the morning than in the afternoon. However, t - tests (Welch's two sample with unequal variances) showed that the differences between seawater DMSP produced in the morning and afternoon were not significant for *Dictyota* ($p = 0.32$), *Halimeda* ($p = 0.69$) or seagrass ($p = 0.80$).

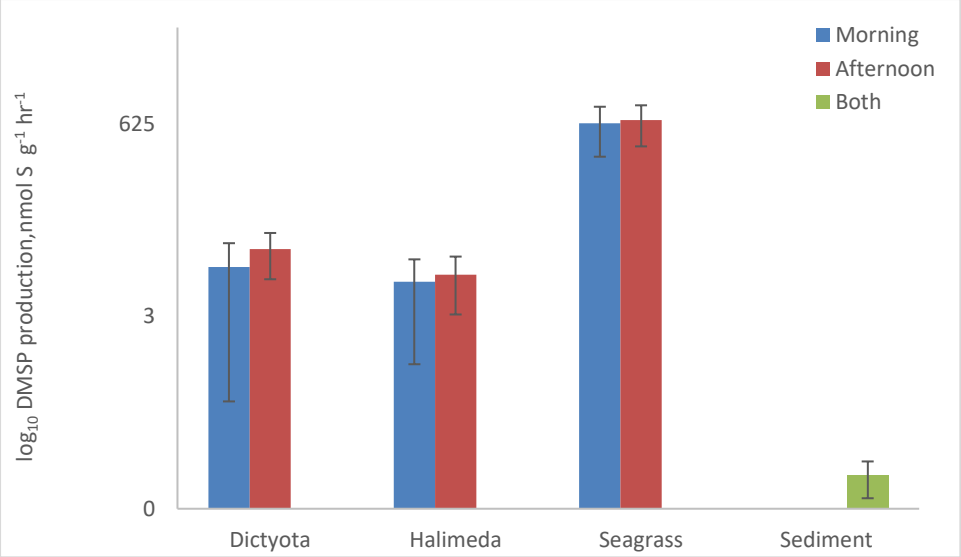


Figure 5.8 Mean (n = 5) seawater DMSP production in the morning and afternoon for non - coral species at Coral View reef site on a log scale (base 10). Values are normalised to the mass of each species incubated per hour. Error bars are standard deviations (n = 5) of hourly DMSP production for each species.

It is evident that seagrass produced the greatest amount of seawater DMSP of all the incubated species and this is the case for both the morning and afternoon incubations. Conversely, sediment produced the least amount of seawater DMSP. Furthermore, t - tests of seawater DMSP production in the morning and afternoon confirmed that there were significant differences between all species ($p < 0.005$) except between *Halimeda* and *Dictyota* ($p = 0.12$).

Mixed model output was used to assess which abiotic factors might influence seawater DMSP production by non-coral species. Whilst the carbonate saturation state ($p = 0.87$) and time of day ($p = 0.32$) were not significant factors, pH ($p < 0.001$) and temperature ($p < 0.001$) both significantly affected seawater DMSP production.

5.5.3 Tissue DMSO

Unfortunately owing to the delay in tissue samples being returned to the UK, all samples analysed for DMSP returned a blank result. Samples were therefore analysed for DMSO in the absence of DMSP. Tissue DMSO concentrations for non-coral species (Fig 5.9), except

sediment (which was incubated for one 4 h period), were consistently higher in the afternoon incubations than in the morning.

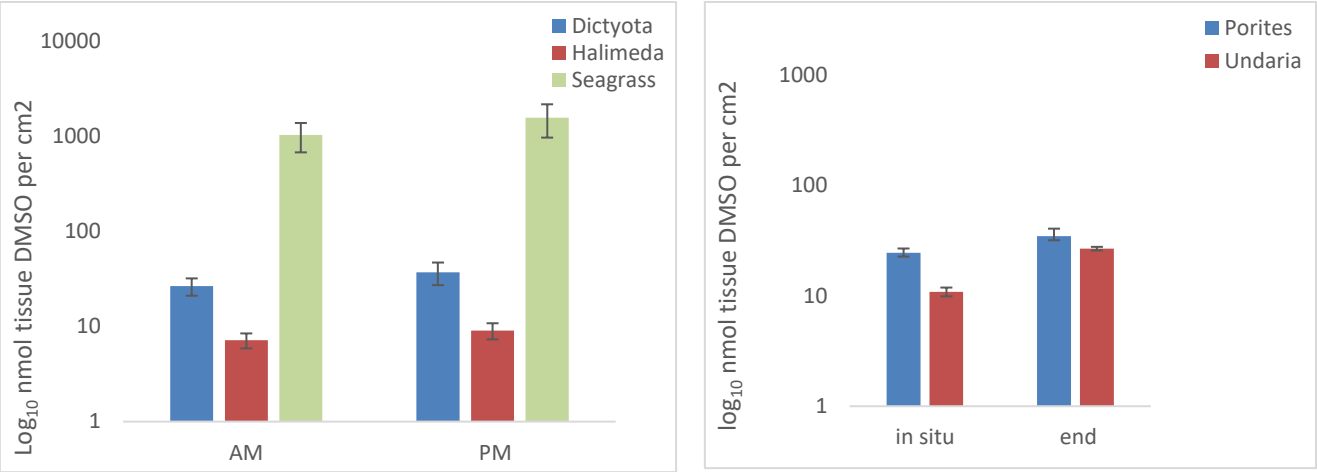


Figure 5.9 Tissue DMSO concentrations on a log scale (base 10) normalised per gram tissue (fresh weight) for non - coral (left hand graph) or per cm^2 surface area for coral (right hand graph) species. Mean values are shown for non - coral species (n = 5) and coral species (n = 3) and error bars represent standard deviations of replicates. Note the different log y-axes between graphs. Sediment DMSO content could not be displayed in these graphs owing to the extremely low concentrations but is explained in the main text.

DMSO concentrations in *Dictyota* in the morning ranged from 21 – 35.3 ± 5.1 nmol DMSO g^{-1} fw (mean 26.6 nmol g^{-1} fw), increasing to 27.9 – 50.2 ± 9.2 nmol DMSO g^{-1} fw (mean 37.7 nmol g^{-1} fw) in the afternoon. Tissue concentrations of DMSO in *Halimeda* samples in the morning incubations ranged from 7.6 - 11.2 ± 1.2 nmol DMSO g^{-1} fw (mean 9.1 nmol g^{-1} fw) and decreased to 5.5 – 10.5 ± 1.6 nmol g^{-1} fw (mean 7.3 nmol g^{-1} fw) in the afternoon. The highest tissue DMSO concentrations were observed in seagrass, which exhibited DMSO concentrations of 392.3 – 1728 ± 557.4 nmol g^{-1} fw (mean 1067.2 nmol g^{-1} fw) in the morning, increasing to 757.6 – 2438.6 ± 324.7 nmol g^{-1} fw (mean 1567.4 nmol g^{-1} fw). Sediment concentrations of DMSO ranged from 0.01 – 0.04 ± 0.01 nmol g^{-1} fw (mean 0.03 nmol g^{-1} fw). Paired t - tests were conducted to assess differences in tissue DMSO between the morning and afternoon incubations; seagrass ($p = 0.003$), *Halimeda* ($p = 0.002$) and *Dictyota* ($p = 0.002$) were all found to have significantly higher tissue DMSO concentrations in the afternoon than in the morning.

ANOVA was conducted to see which factors affect non - coral tissue DMSO concentrations. There were significant differences in tissue DMSO between species (ANOVA, $p < 0.001$), with temperature ($p = 0.001$) and pH ($p < 0.001$) significantly affecting tissue concentrations of DMSO. There was also a significant effect of time of day on tissue DMSO ($p = 0.009$).

Concentrations of tissue DMSO, normalised to coral surface area, were higher at the end of the transplant experiment (Fig 5.9) for both species. *P.astreoides* samples exhibited significantly higher concentrations than *U.tenuifolia* samples for tissue DMSO both before and after the transplant (One - way ANOVA, $p < 0.001$). Tissue DMSO concentrations *in situ* ranged from 22.2 – 27.6 nmol cm^{-1} (mean 24.5 nmol cm^{-1}) in *P.astreoides* samples, whilst DMSO in *U.tenuifolia* samples ranged from 8.8 – 13.2 nmol cm^{-1}. At the end of the transplant, concentrations of tissue DMSO in *P.astreoides* samples had increased to 28.2 – 40.9 nmol cm^{-1} (mean 34.9 nmol cm^{-1}) and 23.6 – 32.4 nmol cm^{-1} (mean 26.8 nmol cm^{-1}) in *U.tenuifolia*.

Paired t - tests confirmed that increases in tissue DMSO concentrations were significant for both species ($p < 0.001$). The same test was conducted on the environmental data recorded at the time the samples were collected before and after the transplant experiment. There were significant ($p < 0.001$) differences in temperature, pH, salinity and pCO_2 between the beginning and end of the transplant experiment. To assess which of these factors could drive increases in tissue DMSO in coral samples, a two - way ANOVA was conducted and confirmed that pH ($p < 0.001$) and temperature ($p < 0.001$) significantly affected tissue concentrations of DMSO. Moreover, significant differences were observed in tissue concentrations of tissue DMSO between species (ANOVA, $p < 0.001$).

Overall differences in DMSO content between coral and non - coral species were evident (Fig 5.10); seagrass concentrations of DMSO are considerably higher than all other species, whilst sediment concentrations were lower than all other species.

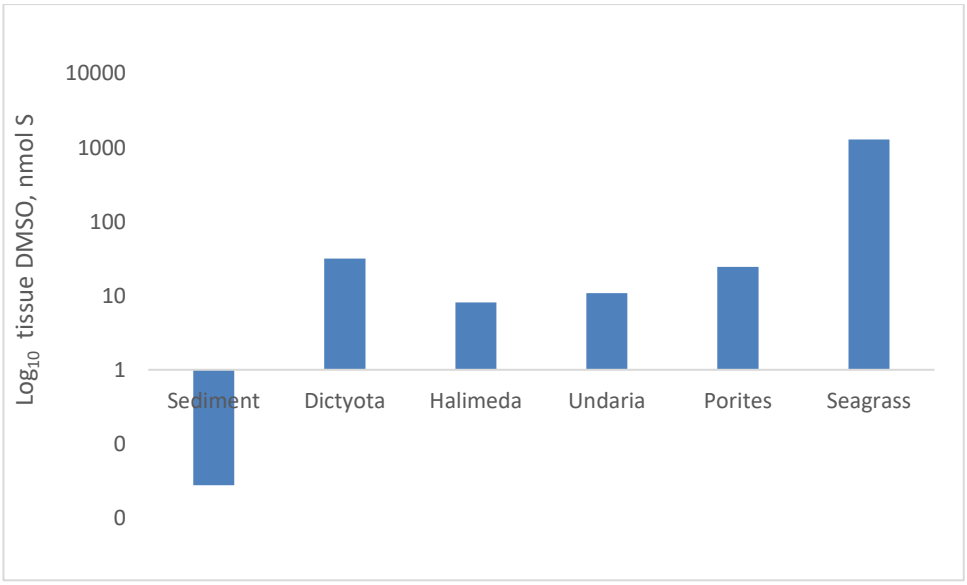


Figure 5.10 Mean tissue DMSO concentrations by species on a log scale (base 10). Coral species values use *in situ* values (n = 9). Sediment (n = 5), Dictyota (n = 10), seagrass (n = 10) and Halimeda (n = 10) use mean values across morning and afternoon incubations. Normalisation indices for coral is surface area and for non - corals is per gram fresh weight. Note that sediment values are extremely low and appear negative, but are not.

ANOVA and *post-hoc* testing (Tukey analysis) was conducted to assess differences between species. Seagrass contained significantly more DMSO than all other species and sediment ($p < 0.001$), whilst sediment contained significantly less DMSO than all other species ($p < 0.001$). *Dictyota* concentrations of DMSO were significantly higher than *Halimeda* ($p < 0.001$).

5.6 Discussion

5.6.1 Contribution of different species to the seawater DMSP burden

Coral derived production of seawater DMSP in this study varied between species. Notably, less seawater DMSP was produced by *P.astreoides* than *U.tenuifolia* at all points in the experiment. Furthermore, production by *U.tenuifolia* did not significantly change over the course of the experiment, whilst production by *P.astreoides* significantly increased by 180 % which was driven by increased temperature and decreased pH. Whilst there are no studies to have reported either decreases (or no change) in seawater DMSP production by a coral

species in response to a change in environmental conditions (as was the case in this study for *U.tenuifolia*), decreases in production of DMSP in response to increased light have been observed (Jones et al. 2007; Fischer and Jones 2012). The range of values for seawater DMSP production by each species reported here (0.38 – 0.48 nmol S cm^{-2} h^{-1}) are lower than reported in other studies (Fischer and Jones 2012; Jones et al. 2014). Species - specific variability is likely to account for the differences in reported values; DMSP biosynthesis is intimately linked with the coral microbial assemblage. Different symbiont species produce variable amounts of DMSP (Steinke et al. 2011; Borell et al. 2016) and this can further vary according to host - symbiont interactions and associated bacteria (Frade et al. 2016).

Incubation experiments to determine production of DMSP in seawater by tropical non - coral species had not, to date, been conducted and studies have instead focussed on tissue concentrations (Broadbent et al. 2002; Burdett et al. 2013). However, studies by Burdett et al. (2013) and Broadbent et al. (2002) have indicated that macroalgae and seagrass might be greater producers of seawater DMSP than corals. This study demonstrates that seagrass is a prolific producer of seawater DMSP (236.6 – 1213.6 ± 361.2 nmol S g^{-1} h^{-1}), whilst *Dictyota* and *Halimeda* are intermediate producers (0.55 – 30.9 ± 8.8 nmol S g^{-1} h^{-1} and sediment is a minor source of DMSP to seawater (0.01 – 0.04 nmol S g^{-1} h^{-1}). Similar findings were suggested by Burdett et al. (2013) in the Red Sea, where the highest seawater DMSP measurements were observed from water sampled over a seagrass bed. However, Broadbent et al. (2002) suggested, based on a simplified model using tissue concentrations, that corals might contribute a more significant amount of DMSP to the water column. The results presented here remove the challenges faced by Broadbent et al. (2002) in constructing their model, by assessing seawater DMSP production during experimental incubations. Contrary to the suggestion that corals might contribute a greater amount of DMSP to the water column, these results show that, except for sediment, all non-coral species are greater producers of seawater DMSP than corals.

The results presented here also offer an insight into the contribution of coral reef sediment to the water column DMSP burden, which has previously been suggested by Broadbent & Jones (2004). Analyses of sediment pore waters at several reef sites in the Great Barrier Reef revealed concentrations of DMSP that were considerably higher than reef waters (53 -

1840 nmol). This finding is, perhaps, not unsurprising; it has been previously shown that free - living *Symbiodinium* (dinoflagellates) are more abundant in sediments than in the water column (Littman et al. 2008; Takabayashi et al. 2012). This study is, to date, the first to demonstrate flux, albeit extremely low, of DMSP from reef sediments into the overlying water column. The mechanism by which this happens (lysis, apoptosis, vertical migration etc.) requires further research.

5.6.2 The effect of temperature and pH on coral and non - coral seawater DMSP production

Production of seawater DMSP was not significantly different between the morning and afternoon incubations in the seagrass and macroalgal incubations. However, temperature and pH did affect seawater DMSP production in all non-coral species, suggesting that production of DMSP in seawater is more likely to be driven by changes to environmental conditions, rather than through any natural diurnal rhythm. Furthermore, there was significantly more seawater DMSP produced by *P.astreoides* at the end of the transplant experiment, which was driven by increased temperature and reduced pH.

It has previously been suggested that marine macroalgae and seagrasses produce DMSP for a number of reasons; DMSP can act as an anti - grazing agent (Alstyne et al. 2001; Van Alstyne and Houser 2003; Burdett et al. 2013), as an osmolyte (Kirst 1996) or as an antioxidant (Sunda et al. 2002). The results presented here suggest that coral and non - coral production of seawater DMSP could be related to increased cellular stress, caused by changes to pH (decrease) and temperature (increase). However, since no additional stress biomarkers were measured during this study, this remains speculative and further research would be required.

To date, studies using experimental incubations to assess seawater production of DMSP in corals have been limited to *Acropora* species (Broadbent and Jones 2006; Jones et al. 2007; Fischer and Jones 2012), in which production of seawater DMSP has been shown to exhibit diurnal variability (Broadbent and Jones 2006), and increases in response to increased temperature (Jones et al. 2007; Fischer and Jones 2012). Fischer & Jones (2012) reported ~

6000 % increases in seawater DMSP flux, whilst Jones et al. (2014) reported more modest 45 % increases under elevated temperature conditions. Previous studies have estimated that coral expel ~ 10 % of their algae each day (Broadbent and Jones 2006), with Fischer & Jones (2012) suggesting that zooxanthellae expulsion could account for increased seawater DMSP flux. It is also worth considering the role of coral mucus; previous studies have shown that coral mucus production increased at higher temperatures (Sawall et al. 2015) as well as being higher during the day than during the night (Crossland et al. 1980; Crossland 1987). Since coral mucus is known to contain considerable quantities of DMSP (Broadbent and Jones 2004), it is possible that the increased levels of DMSP flux to seawater were driven, at least in part, by increased mucus production and its subsequent bacterial breakdown.

Temperature and pH are also likely to induce cellular stress in macroalgal cells, leading to upregulation and resulting in increased seawater production, possibly following lysis (Stefels 2000). It is also likely that changes to temperature and pH enhance photosynthetic and growth processes; since DMSP is a tertiary metabolite, its production is linked with photosynthesis (Stefels 2000). Indeed, there is the potential for DMSP to serve multiple functions within a species (Stefels 2000). Burdett et al. (2013) suggested that intracellular DMSP may play a role in maintaining macroalgal cellular function whilst under low carbonate saturation conditions. Furthermore, they reported that seawater DMSP was well described by pH and the results described here support those findings.

5.6.3 Tissue DMSO significantly affected by species

Whilst DMSP analysis was inconclusive in this study, previous studies have shown that DMSO and DMSP co-occur in algal samples (Hatton and Wilson 2007; Deschaseaux et al. 2014b). Previous studies have also shown that where DMSP concentrations do not significantly increase/decrease in response to changing environmental conditions, the same is not true of DMSO, which significantly increased with each experimental condition (Deschaseaux et al. 2014 b). Based on this, Deschaseaux et al. (2014) suggested that DMSO was a more effective stress biomarker because of its propensity to change under all experimental treatments.

Differences in tissue DMSO concentrations reported here were found to be significant between most species. The lowest tissue DMSO concentrations, normalised to weight, were found in sediment, whilst the highest occur in seagrass. Consistent with the intra- and interspecies variability in tissue DMSP for algal samples in the Great Barrier Reef (Broadbent et al. 2002; Deschaseaux et al. 2014 b) and Red Sea (Burdett et al. 2013), algal samples analysed in this study exhibited DMSO concentrations that were variable both between and within species. Intraspecific variability is to be expected, with factors such as the presence/abundance of epiphytes (Dacey et al. 1994), light intensity (Hatton 2002) and general physiological condition potentially contributing to variable DMSO concentrations.

Tissue DMSO concentrations in both coral species were similarly variable, with significantly less DMSO occurring in *U.tenuifolia* than in *P.astreoides*. Mean DMSO tissue concentrations for *U.tenuifolia* (10.9 ± 1.8 nmol DMSO cm^{-2}), pre - transplant, reported in this study were notably lower than DMSO concentrations (28.4 ± 6.5 nmol DMSO cm^{-2}) reported for Acroporid coral species (Deschaseaux et al. 2014b), whilst *P.astreoides* concentrations were more comparable (24.5 ± 2.4 nmol DMSO cm^{-2}). Intraspecific variability was evident in incubated coral samples and this variability increased post - transplant, however the variability reported here was lower than reported for tissue DMSP & DMSO in other studies (Broadbent et al. 2002; Deschaseaux et al. 2014b). This could be attributed to the sampling regime, which in this study sampled corals from adjacent colonies whereas Broadbent et al. (2002) often collected samples from different colonies, including those with different morphologies and from differing environmental conditions.

5.6.4 Tissue DMSO significantly affected by time of day, temperature and pH

Whilst time of day did not affect non-coral production of seawater DMSP, there were significant differences in tissue concentrations of DMSO between the morning and afternoon incubations for all non-coral species. A significant increase in tissue concentrations of DMSO was also observed in both coral species at the end of the transplant, despite there being no apparent effect of the transplant on seawater production of DMSP by *U.tenuifolia*.

The temperature measured in the morning and afternoon incubations of macroalgal /seagrass samples was significantly higher than the temperature measured in the mornings. Temperature has been shown to be a key factor driving increases in tissue DMSO production by all species. Similarly, the temperature measured at the transplant site for coral incubations was significantly higher than the temperature measured at the *in situ* reef location, which led to increases in coral tissue DMSO concentrations.

Much of the research into DMSP/DMSO reveals broad similarities in the physiological function of these compounds (Hatton et al. 1996; Karsten et al. 1996 b; Stefels 2000; Sunda et al. 2002). Previous studies have shown cellular DMSP concentrations were highest at night (Burdett et al. 2013), acting to combat grazing pressure which is typically highest at night (Alstyne et al. 2001; Van Alstyne and Houser 2003). However, the pattern evident in the data presented here suggests that DMSO concentrations were higher in the afternoon, when temperatures are highest. The role of DMSO as an antioxidant in algal cells is widely reported in the literature (Sunda et al. 2002; Deschaseaux et al. 2014 b), with changes in light and temperature regime being two crucial factors known to drive production of this compound in algal cells (Hatton et al. 1996; Sunda et al. 2002). An 'antioxidant cascade' mechanism has been proposed (Sunda et al. 2002) in which DMSO is produced as a by - product of DMSP, acting to 'mop up' reactive oxygen species produced during photosynthesis or elevated temperature.

Whilst light was not measured at either the *in situ* or transplant locations in this study, previous data obtained (Chapter 4) and observations regarding the depth, show that the transplant site was significantly shallower and received higher light levels than the *in situ* location (158 – 205 % higher) throughout the day. Water temperature was a significant factor contributing to the observed DMSO concentrations in all species, suggesting that DMSP production was up - regulated in response to increasing temperature, acting in concert with increased light levels to generate increased cellular DMSO levels.

Increases to light and temperature levels have been previously shown to lead to increases in tissue concentrations of DMSO (Deschaseaux et al. 2014 b), and these results support the

emerging view that DMSO production may be involved in the maintenance of the antioxidant system of the algal cell when subjected to increased temperature/light.

No previous research has linked decreased pH with increased concentrations of DMSO. Studies to-date have either focussed on the effects of increases in temperature/light on cellular DMSP/DMSO (Fischer and Jones 2012; Deschaseaux et al. 2014 b, 2014 a; Jones et al. 2014; Jones and King 2015), or where pH/carbonate chemistry has been investigated (Burdett et al. 2013), it is with respect to water column/cellular concentrations of DMSP. However, Burdett et al. (2013) reported DMSP concentrations that were driven, at least in part, by the carbonate chemistry of reef seawater. The relationship between pH and cellular DMSO concentrations (as well as the relationship between pH and seawater DMSP production) reported in this study supports this finding. Furthermore, it seems likely that as well as a key antioxidant for algal species under times of cellular stress (i.e. increased temperature, light), an osmolyte and potential anti-grazing deterrent, this research suggests DMSO may also be used maintain metabolic function during periods of low carbonate saturation state and indicates that further research is warranted.

5.6.5 Implications for coral reefs

Previous studies have suggested that coral reefs could, under climate change projections, switch to a fleshy macroalgal/seagrass dominated assemblage (Pandolfi et al. 2011). Indeed, rising sea surface temperatures are already causing population shifts in temperate and tropical macroalgal species across various biogeographic regions (Wernberg et al. 2011). It has also been suggested that seagrasses and macroalgal species subjected to increased CO_2 levels are likely to experience increases in photosynthetic and growth rates (Koch et al. 2013). The temperature optima for photosynthesis in tropical seagrasses range from 27 to 33 °C and for temperate species from 21 to 32 °C, while their growing temperatures average ~ 3 and 8 °C lower respectively (Lee et al. 2007). Thus, tropical seagrasses are growing closer to their photosynthetic and physiological optimum in comparison to their temperate counterparts. As a tertiary metabolite, produced in response to increased cellular stress (such as that caused by lower pH or increased temperature), it is possible that reefs composed primarily of seagrasses or fleshy macroalgae will experience higher water column

concentrations of this compound. As seawater becomes progressively more acidic and warmer, the results presented here indicate that species-specific changes in cellular and seawater concentrations of DMSP/DMSO will also change, as organisms adapt to changing conditions. This study suggests that intracellular DMSO concentrations may be regulated in response to variability in reef water pH, helping to maintain cellular function. A shift toward macroalgal dominance, and/or increased seawater DMSP/O production (possibly via increased cellular DMSO production) will impact sulphur biogeochemistry, affecting ecosystem function and potentially increasing atmospheric dimethyl sulphide emissions (the CLAW hypothesis, see section 1.4), with subsequent changes to climate regulation on local scales (Ayers and Gillett 2000; Jones and Trevena 2005). The direction this impact will take, i.e. increasing or decreasing the impact of global warming, and its magnitude is uncertain since the CLAW hypothesis is still disputed (Green & Hatton 2014, Quinn & Bates 2011), but it is unlikely this will be the main ecosystem effect. Whilst studies have shown that increased production of DMSP can lead to increases in atmospheric DMS (Fischer and Jones 2012), which would reduce the impact of global warming by increasing cloud cover, investigations into production of DMSO and the resultant effect on atmospheric DMS emissions do not currently exist. Since the biogeochemistry of DMSO is affected by both biotic (e.g. bacteria) and abiotic (e.g. photochemistry) processes, the relationship between DMSO production and DMS emissions, and by extension local cloud cover, may not be as intimately related as that of DMSP and DMS. It is more likely that the main effect of increases in DMSO production by reef organisms will be to increase seawater concentrations of DMS, since DMSO is photolyzed to DMS and reef environments receive high levels of solar radiation. DMS is an important compound for marine microbes, acting as a substrate for growth (Kiene and Linn 2000; Raina et al. 2010) and helping structure bacterial communities (Frade et al. 2016). DMS is also known to serve as a cue for foraging fish and birds (Nevitt 2000; DeBose et al. 2008; Paul et al. 2008) and is implicated in grazing/predation of marine algae by a range of organisms (Dacey et al. 1994; Wolfe et al. 1997; Seymour et al. 2010a). It seems more likely, therefore, that upregulation of tissue DMSO production will result in increased seawater DMS concentrations. This could increase the microbial biomass with implications for biogeochemical cycling of all major nutrients, as well as potentially opening reef organisms up to elevated levels of predation and grazing.

6 Contribution of mesophotic reef systems to the spatial variability of seawater dimethylated sulphur budget

6.1 Introduction

This study has reported the spatial variability of DMSP and that a general decrease in seawater DMSP concentrations occurs with increasing distance from shore (see Chapter 4). Also noted was the lack of data for Caribbean and mesophotic reef systems. Mesophotic reefs differ from cold water deep reefs in that they harbour zooxanthellate Scleractinian corals, which are often the same species as those found in shallower (< 30 m) environments. This study investigates seawater DMSP down a depth gradient at five mesophotic reef sites in the Caribbean, providing the first ever data for DMSP in a mesophotic reef setting. DMSP and DMSO concentrations in a key mesophotic coral species was also included in the study, to better understand the contribution of corals to the seawater DMSP budget at greater depths than previously reported.

The oceans absorb approximately one third of the anthropogenic CO_2 emitted to the atmosphere (Barker and Ridgwell 2012). While this CO_2 uptake helps ameliorate human caused greenhouse warming, the amount of absorbed CO_2 is so great that it is significantly changing the chemistry of the oceans (Feely et al. 2004). Once anthropogenic CO_2 enters the oceans there is no practical way to remove it and the oceans will require thousands of years to naturally return to a higher pH state (Raven et al. 2005b). Increasing dissolved CO_2, described as the partial pressure of CO_2 (pCO_2), and decreasing pH will likely affect many marine organisms and alter ecosystem community structure (Kleypas et al. 2005b; Raven et al. 2005b; Pandolfi et al. 2011). Corals and other calcifying organisms are particularly at risk due to their dependence on CO_3^{2-} concentration and $CaCO_3$ saturation states (Gledhill et al. 2008; Andersson and Gledhill 2011).

Mesophotic coral ecosystems (MCEs) are deep fore - reef communities that occur at intermediate depths (30–200 m) of the photic zone in which light - dependent (zooxanthellate) corals are present that form a symbiosis with dinoflagellate protists from the genus, *Symbiodinium*. This symbiotic relationship allows a coral's intracellular symbionts

to photosynthesize and provide the host coral with a rich source of sugars, glycerol, lipids, and other organic compounds (Hoegh-Guldberg et al. 2017). In shallow waters, this relationship enables corals to grow and calcify at high rates in the clear, warm, water conditions along tropical coastlines (Hoegh-Guldberg et al. 2017). However, the abundance of Scleractinian corals hosting *Symbiodinium* decreases with depth beyond 20–40 m, depending on the clarity of the water column. Light in the ocean decreases exponentially with depth and it is one of the most important factors shaping coral communities (Ziegler et al. 2015). As light levels decrease with depth, decalcification dominates and the overall carbonate balance of reef ecosystems shifts to negative and net dissolution of the reef occurs (Barnes and Chalker 1990; Bongaerts et al. 2010). The deepest Scleractinian corals that associate with *Symbiodinium* are found 100 m or more below the surface of tropical waters (Englebert et al. 2015). The productivity of this symbiosis is complemented by the ability of corals to capture and feed on waterborne particles and plankton (i.e., polytrophy, mixotrophy). Studies so far suggest that MCE areal coverage may equal or surpass that of shallow reefs in many of the world's coral reef systems, including the Great Barrier Reef (Harris et al. 2013) and the Caribbean (Locker et al. 2010).

Dimethylsulphoniopropionate (DMSP) represents a major fraction of organic sulphur within marine systems (Stefels 2000; Yoch 2002) and is produced by many macroalgae and microalgal species, including dinoflagellates from the genus *Symbiodinium* (e.g Steinke et al. 2011; Yost et al. 2012). *Symbiodinium* are known to be prolific producers of DMSP but there is considerable variability, according to species and/or host dynamics (Steinke et al. 2011; Borell et al. 2016). A range of biological functions have been proposed for DMSP, but it is generally thought to act as a compatible solute, aiding in osmoprotection (Welsh 2000) and cryoprotection (Karsten et al. 1996a). Dimethylsulphoxide is also produced *de novo* in algal cells (Simo et al. 1998b; Lee et al. 1999), where it is thought to perform the same range of functions as DMSP. In tropical reef environments, intracellular DMSP may be important as an antioxidant, grazing deterrent and/or compatible solute. Recent studies also suggest that intracellular DMSP may play a role in improving tolerance to variable carbonate chemistry conditions (Burdett et al. 2012, 2013). However, research into coral-derived DMSO production is sparse (Deschaseaux et al. 2014b) and there are no reports into DMSO production by mesophotic corals.

Both DMSP and DMSO enter seawater either in particulate (i.e. contained within an algal cell) or dissolved (i.e. following cell lysis) form (Stefels 2000), where they can be further degraded to DMS, a gas that may be linked to local climate regulation through aerosol production and cloud formation (Charlson et al. 1987). More recently, studies have demonstrated that DMSP can act as a chemo-attractant for certain virus species (Garren et al. 2014), indicating that seawater concentrations of this compound are likely to exert an effect at the ecosystem level.

Spatial variability of seawater concentrations of DMSP have been extensively studied throughout the world's oceans (Belviso et al. 1993; Kiene and Linn 2000; Merzouk et al. 2004; Yang et al. 2011; Borges and Champenois 2015; Espinosa et al. 2016) and have been shown to be strongly influenced by changes to the micro - and phytoplankton community assemblage. Spatial variability of seawater DMSP in tropical reef systems is somewhat limited, but studies have shown that the underlying benthic ecology (Broadbent and Jones 2006; Burdett et al. 2013) and water chemistry (i.e. temperature, Jones et al. 2007; Burdett et al. 2013) play crucial roles in seawater DMSP distributions. However, to date no studies have investigated the spatial distribution of seawater DMSP in mesophotic reefs, nor the likely controls on this compound at depth. MCEs are considered to be crucial environments for corals in a changing climate and their role as potential refugia has already covered (see section 1.3. Shallow coral reefs are subject to inherent natural variability whilst MCEs are characterised by more steady conditions, thus in order to accurately project the impact of climate change on MCEs and their role as refugia for shallow reefs, we must first understand their natural variability.

6.2 Aims of this study

Whilst studies into DMSP/O are reported in the literature, they are geographically and spatially limited to a) Indo-Pacific reefs (notable exceptions are the Red Sea (Burdett et al. 2013) and Bermuda (Yost and Mitchelmore 2010, Borell et al. 2016)), and b) to the upper ~24m of a reef system. The potential importance of MCes as refugia for shallow reef organisms (including corals) has recently gained momentum owing to the improvement in diving technology that has facilitated more studies. The deep reef refugia hypothesis, as it has become known (Bongaerts et al. 2010), suggests that because MCEs are more protected (by their depth) from the same threats facing their shallow water counterparts, they may be able to harbour organisms that would otherwise struggle in a changing climate. Implicit in this is the notion that MCE corals are less "stressed" than their shallow water cousins. This study investigated coral tissue concentrations of DMSP and DMSO by a key mesophotic coral species at two different sites in the Caribbean, *Agaracia lamarcki*, to assess possible stress levels as determined by the DMSO:DMSP ratio. Seawater DMSP concentrations were also investigated at five different sites, along with benthic ecology and water chemistry parameters, to determine the likely controls on the depth distribution of seawater DMSP concentrations.

6.3 Hypotheses

1. Mesophotic corals will not exhibit signs of cellular stress would be evidenced by a higher DMSO:DMSP ratio
2. Seawater concentrations of DMSP will decrease with increasing depth owing to lower abundance of prolific DMSP producers (i.e. seagrasses, macroalgae)
3. Seawater DMSP concentrations will be driven primarily by the composition of the benthic community, rather than by extreme variations in abiotic factors which are not present at greater depths
4. There will be a significant effect of depth on tissue concentrations of DMSP/O owing to more stable conditions
5. Tissue concentrations of DMSP/O will be lower than those of shallow water corals owing to lower temperatures and solar irradiance

6.4 Methods

6.4.1 Research sites

Utila is located within the Honduran Bay Islands on the southern end of the Meso - American Barrier Reef (Fig 6.1).

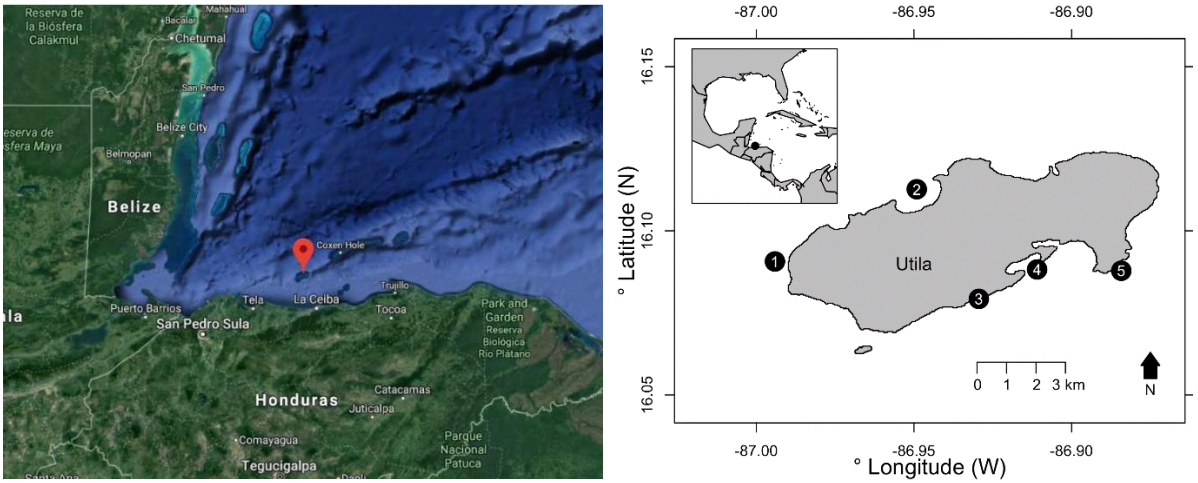


Figure 6.1 Google Map showing the location of Utila Island (Honduras) on the southern end of the Meso - American Barrier Reef (left), with the location of all mesophotic dive sites (right). Dive sites are listed with GPS coordinates and abbreviations starting left in WGS84 format: (1) Raggedy Cay (RC: N 16.09065964, W - 86.9941015), (2) The Maze (TMA: N 16.11266214, W - 86.94911793), (3) Little Bight (LB: N 16.07926302, W - 86.92942222), (4) Coral View (CV: N 16.08823274, W - 86.91094506), (5) Rocky Point (RP: N 16.08784039, W - 86.88423403). The dive site map was sourced with permission from Laverick et al. (2017).

A quantitative benthic description exists to a maximum depth of 85 m (Laverick et al. 2017) and five sites were sampled in this study:

- The Maze (TMA): 0 - 85 m
- Raggedy Cay (RC): 0 - 55 m
- Rocky Point (RP): 0 - 55 m
- Coral View (CV): 0 - 40 m
- Little Bight (LB): 0 - 40 m

These sites are known to have differing maximum depth ranges for *A. lamarcki* (Laverick et al. 2017).

6.4.2 Water sampling

Samples were collected on a series of morning dives (8 a.m. – 10 a.m.) during July and September 2015 at the 5 sites listed above. Sampling dates varied between sites and are shown in Table 6.1.

Table 6.1 Dates on which water sampling occurred at all mesophotic dives sites included in this study

Site	***Sampling Date***
The Maze	01.10.15
The Maze	29.09.15
The Maze	28.09.15
The Maze	27.09.15
Raggedy Cay	25.09.15
Raggedy Cay	22.09.15
Rocky Point	21.09.15
Coral View	23.09.15
Little Bight	26.09.15
Little Bight	14.7.15

Sampling was started at the deepest depth at each site to minimise mixing of the water by the divers, potentially confounding the results. Logistics prevented replicate collections at all sites, consequently The Maze (n = 4), Raggedy Cay (n = 2) and Little Bight (n = 2) represent the only sites with samples taken on more than one day. Sampling effort was distributed as equidistantly down the depth gradient as possible at each site and replicate samples over two or more days were taken from the same locations on each day. Samples were retrieved in 1 Litre Flexi Water Bottles (Mountain Warehouse™) and returned to shore for subsampling of dissolved inorganic carbon (DIC), total alkalinity (TA) and DMSP. DIC and TA samples were decanted using Tygon tubing from the bottom of the bag into 12 ml borosilicate glass vials and were immediately fixed with 10 µL of HgCl and sealed with no headspace. Samples for DMSP were pipetted into 10 ml crimp top vials, to which 1 ml 10 M NaOH was added and immediately sealed with PTFE lined gas tight crimp top caps. Samples were stored in the dark until analysis could be conducted at the University of St Andrews.

6.4.3 Ecological sampling

50m video transects were collected by Jack Laverick (University of Oxford) using mixed-gas closed-circuit rebreathers (Hollis Prism 2, Hollis, San Leandro, California, USA). A Veho K2 action camera (Veho, Southampton, UK) in a 100m depth-rated housing was aimed down at the sea floor 20 cm from the bottom with a dive torch for illumination (Laverick et al. 2017). Sample depths were 5, 15, 25, 40, 55, 70 and 85m, with four replicate transects collected at each depth following the respective depth contour at each site (Laverick et al. 2017). Two transects were collected reef-on-left, and two reef-on-right starting 10m from the GPS location (Fig 6.1) with 10m between adjacent transects (Laverick et al. 2017). Videos were analysed as per J. Laverick (2017) to determine percentage cover of broad benthic categories; Scleractinia, soft coral, macroalgae, coralline crustose algae, sponge and sand/pavement/rubble (SPR). The bottom cover under the transect tape was identified every 25cm. Scleractinian corals, as well as Millepora observed 10cm either side of the tape were recorded in preference to other benthic cover types. The justification by Laverick et al (2017) was that the primary concern was Scleractinian community composition, and this approach maximised collected information. However, it does produce an over-estimate in

Scleractinian percentage cover, especially when in low abundance. Counts were used instead of percentages to prevent elevating the importance of rare species in the analysis.

6.4.4 Mesophotic coral fragment collection

Coral fragments were collected by Jack Laverick (University of Oxford, Permit number: ICF - 261 - 16) using SCUBA equipment comprising mixed gas closed circuit rebreathers (Hollis Prism 2, Hollis, San Leandro, California, USA) during July 2015. Sampled depths were from 10 m to 45 m at Little Bight and 16 m to 60 m at The Maze. These depths reflect the shallowest and deepest observed colonies of *A. lamarcki* at each site. Sampled coral colonies were at least 40 cm in diameter to minimise sampling damage to newly recruited colonies, and 5 m from their nearest neighbour to minimise the effect of sampling of clones. All colonies were sampled as they were found, so long as they satisfied these selection criteria, and up to 12 colonies per 10 m vertical depth band.

When a suitable colony of *A. lamarcki* was identified, a thumb sized fragment was excised from the plate margin using a chisel. This was placed in a labelled zip - lock bag stored within an opaque PVC tube attached to the diver. Fragments were kept in the dark prior to analysis to mitigate light associated stress during the divers' ascent. Once stowed, the fractured margin of the colony was lined with pre - mixed Milliput modelling putty to prevent infection or fouling (Downs 2011).

The samples were returned to a temperature - controlled field laboratory and placed within an opaque plastic aquarium filled with water from the fore-reef. The aquaria were heated to 28°C (= ambient *in situ* temperature), aerated, and covered in four layers of opaque plastic to allow dark acclimation of fragments. Samples were acclimated in the dark for 12 h prior to analyses.

Surface coral tissue was removed using a Waterpik filled with sea water (Johannes & Wiebe 1970) and the resulting slurry frozen at - 20°C. The mass of the air - dried skeleton was later recorded to allow the mass of coral tissue to be determined (= original mass – mass of bare skeleton).

6.4.5 Sample analyses

6.4.5.1 Carbonate chemistry

Water samples were analysed per the methods outlined in sections 2.5.2 (TA) and 2.5.3 (DIC). During analysis of TA procedural issues limited the number of titration points for each sample, resulting in reduced precision.

6.4.5.2 Seawater DMSP and tissue DMSP/O

Samples for seawater DMSP were analysed per the method outlined in section 2.3, samples for tissue DMSP were analysed as described in section 2.3.3 and tissue DMSO as described in section 2.3.4.

6.4.6 Statistical analyses

To assess the spatial variability of seawater DMSP down a depth gradient, a linear mixed model was run on the full 2015 dataset to identify which benthic components (macroalgae, crustose coralline algae (CCA), hard corals and soft corals) significantly affected seawater DMSP. Reef site was included as a fixed effect but the model was run controlling for sampling day, which was inputted as a random effect. The full model was run as:

model <- lme(fixed = dmsp ~ depth + site + macroalgae + cca + hardcoral + softcoral, random = list(~ 1|day), na.action = na.omit, data = dataset)

ANOVA was run on the model output using the “anova.lme” function.

For the mesophotic coral tissue samples, linear models were run on each sulphur component (DMSP, DMSO and the DMSO:DMSP ratio) to assess whether site and/or depth significantly affected each component and whether there was a significant interaction between depth and site. The model input was:

model <- lm(sulphurcomponent ~ depth*site)

Anova was conducted on the model output using the Anova function in the 'car' package.

6.5 Results

6.5.1 Seawater DMSP concentrations

Seawater DMSP exhibited a general decrease with increasing depth at all sites sampled (Fig 6.2), although there was variability between sites. Seawater DMSP values at The Maze ranged from 3.5– 19.1 ± 9.1 nmol, Raggedy Cay 5.5 – 22.2 ± 6.0 nmol, Rocky Point 11.3 – 17.9 ± 2.8 nmol, Coral View 11.4 – 20.7 ± 3.8 nmol and Little Bight 7.4 – 20.9 ± 4.8 nmol.

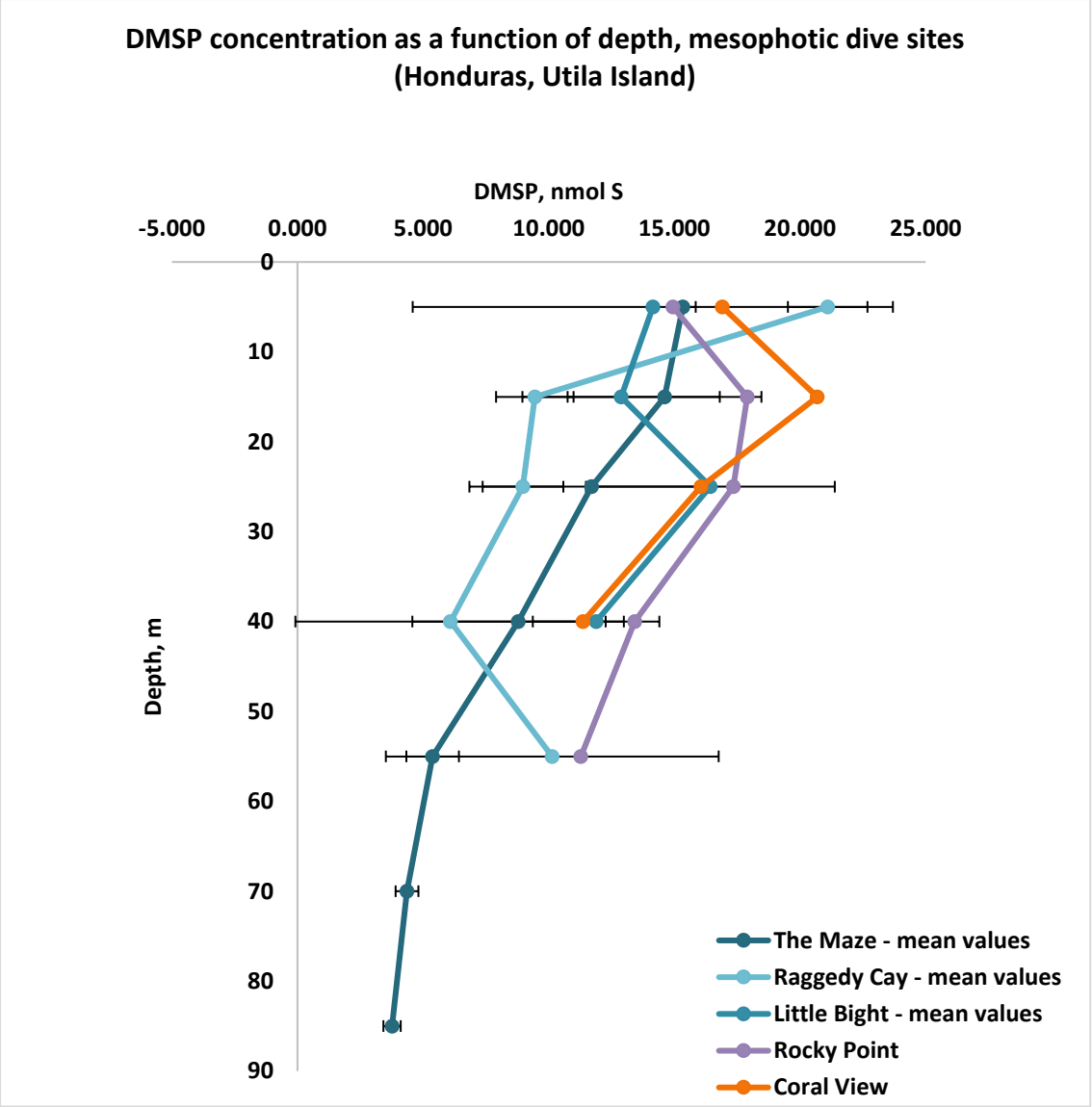


Figure 6.2 Seawater DMSP as a function of depth for The Maze, Raggedy Cay, Rocky Point, Coral View and Little Bight reef sites. Dates on which sampling occurred for all mean values are included in Table 6.1. Mean values are shown for The Maze (n = 4), Raggedy Cay (n = 2) and Little Bight (n = 2). Error bars represent the standard deviation of values taken across all sampling days per site.

Whilst the general trend was for decreasing seawater DMSP with depth, there were instances where seawater DMSP concentrations increase; notably between 5 m and 15 m at Coral View and Rocky Point, between 15 m and 25 m at Little Bight and between 40 m and 55 m at Raggedy Cay.

Mixed models, controlling for sampling day, were conducted to determine which factors (underlying benthic ecology, depth and site) affected seawater DMSP at depth. There was

no significant effect on seawater DMSP by any biotic variable, nor was there an effect of site, however depth (ANOVA, $p < 0.001$) significantly affected seawater DMSP.

6.5.2 Tissue DMSP & DMSO

DMSP and DMSO for all tissue samples were normalised to weight (per gram tissue) and to surface area (cm^2). Tissue DMSP values ranged from 4544.6 – 151448.0 ± nmol g^{-1} at Little Bight and 8198.1 – 155580.4 ± 38174.7 nmol g^{-1} at The Maze (Fig 6.3). When normalised to surface area, tissue DMSP values at Little Bight ranged from 27.3 – 127.9 nmol DMSP cm^{-2} (mean 54.7 nmol cm^{-2}). At The Maze, tissue DMSP per cm^2 ranged from 17.6 – 91.655 nmol DMSO cm^{-2} (mean 46.3 nmol DMSO cm^{-2}). Whilst differences in tissue DMSP between sites were not significant when normalised to gram of tissue (Paired t - test, $p = 0.11$), DMSP normalised to surface area between sites was significantly different (Paired t - test, $p = 0.02$).

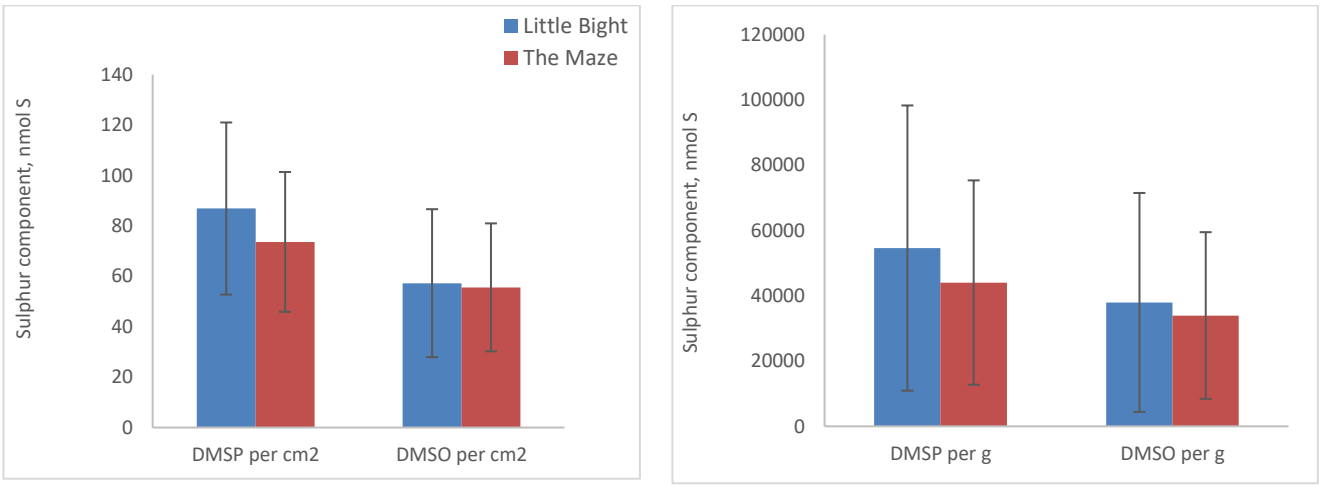


Figure 6.3 Mean tissue DMSP and DMSO concentrations in *A.lamarcki* at The Maze (n = 45) and Little Bight (n = 45) reef sites normalised to surface area (left hand graph) and tissue weight (right hand graph). Error bars are standard deviation of the normalised sulphur component at each site.

Tissue DMSO concentrations at Little Bight ranged from 2.48 – 13.92 µmol g^{-1} and The Maze values ranged from 5.76 – 10.93 µmol g^{-1}, although differences between sites were not significant (Paired t - test, $p = 0.44$). Tissue concentrations of DMSO normalised to

surface area ranged from 0.012 – 0.12 µmol cm $^{-2}$ at Little Bight and 0.009 – 0.088 µmol cm $^{-2}$ at The Maze. There was no significant difference in tissue DMSO normalised to surface area between sites (Paired t - test, $p = 0.76$). Tissue concentrations of DMSP normalised to either parameter was significantly higher than tissue DMSO concentrations at both sites (Paired t - test, $p < 0.001$ for all sites/combinations).

To assess whether site and/or depth had any effect on tissue concentrations of DMSP/O and whether this was the same or different for each normalisation index, linear models were run (Fig 6.4) and a type II ANOVA was conducted on the output. Significant interactions were observed when tissue DMSP and DMSO concentrations were normalised to tissue weight, so type III ANOVA were used. No significant interactions were observed for the DMSO:DMSP ratio ($p = 0.98$) and so type II ANOVA was used in the final analysis. When normalised per gram of coral tissue, tissue DMSP was significantly affected by depth ($p = 0.04$) whilst tissue DMSO was not ($p = 0.11$). There was no effect of site on either compound when normalised to tissue weight, however there was still an interactive effect of depth and site for both DMSP ($p = 0.04$) and DMSO ($p = 0.05$). There was, however, a significant effect of site on the DMSO:DMSP ratio ($p = 0.02$). When normalised to surface area, depth significantly affects tissue DMSP ($p < 0.001$) and DMSO ($p = 0.03$). Whilst there was a significant interactive effect between site and depth for tissue DMSP ($p < 0.001$), the interactive effect of site and depth on tissue DMSO was only significant at $p < 0.10$ ($p = 0.09$).

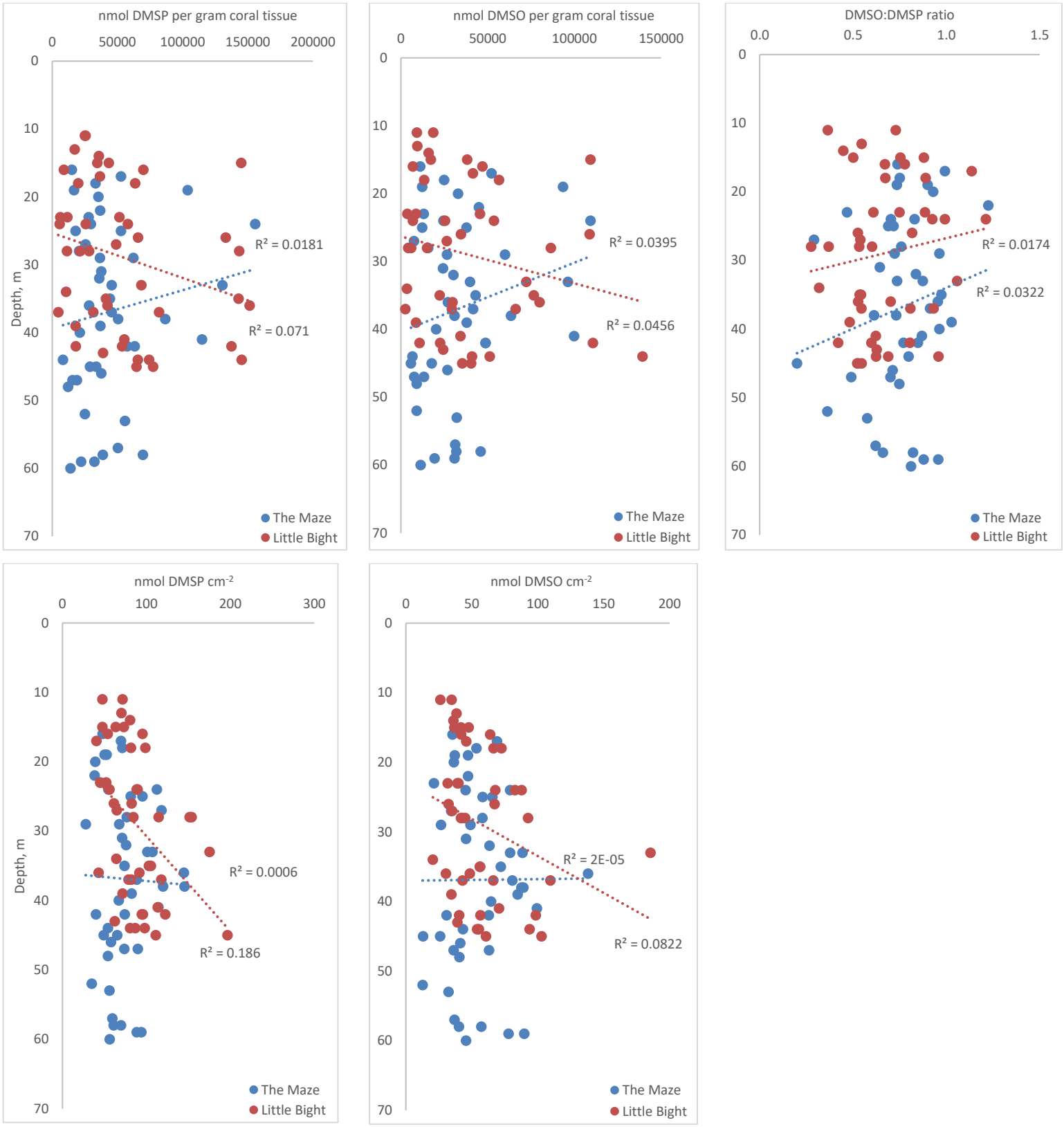


Figure 6.4 The effect of depth on tissue concentrations normalised to tissue weight (top graphs) and surface area (bottom graphs) for DMSP (left hand graph), DMSO (middle graph) and the DMSO:DMSP ratio (right hand graph, top graph only) in *A. lamarcki* at The Maze (TMA) and Little Bight (LB) reef sites. Depth is shown on the y – axis for all graphs.

6.5.3 Carbonate chemistry

There was variability between sites for both carbonate chemistry parameters and within the same site down a depth gradient (Fig 6.5). Dissolved inorganic carbon (DIC) values at The Maze across all four sampling days ranged from 2008.1 – 2103.2 µmol kg $^{-1}$ with TA measurements ranging from 2190 – 2524 µeq. Raggedy Cay values across two sampling days for DIC ranged from 2044.4 – 2089.5 µmol kg $^{-1}$ and 2362 – 2409 µeq for TA. DIC concentrations at Rocky Point ranged from 2105.5 – 2139.4 µmol kg $^{-1}$, at Coral View from 2063.4 – 2089.6 µmol kg $^{-1}$ and at Little Bight from 2056.4 – 2093.4 µmol kg $^{-1}$. TA values at Rocky Point ranged from 2359 - 2399 µmol kg $^{-1}$, at Coral View from 2382 - 2400 µmol kg $^{-1}$and at Little Bight from 2350 - 2401 µmol kg $^{-1}$.

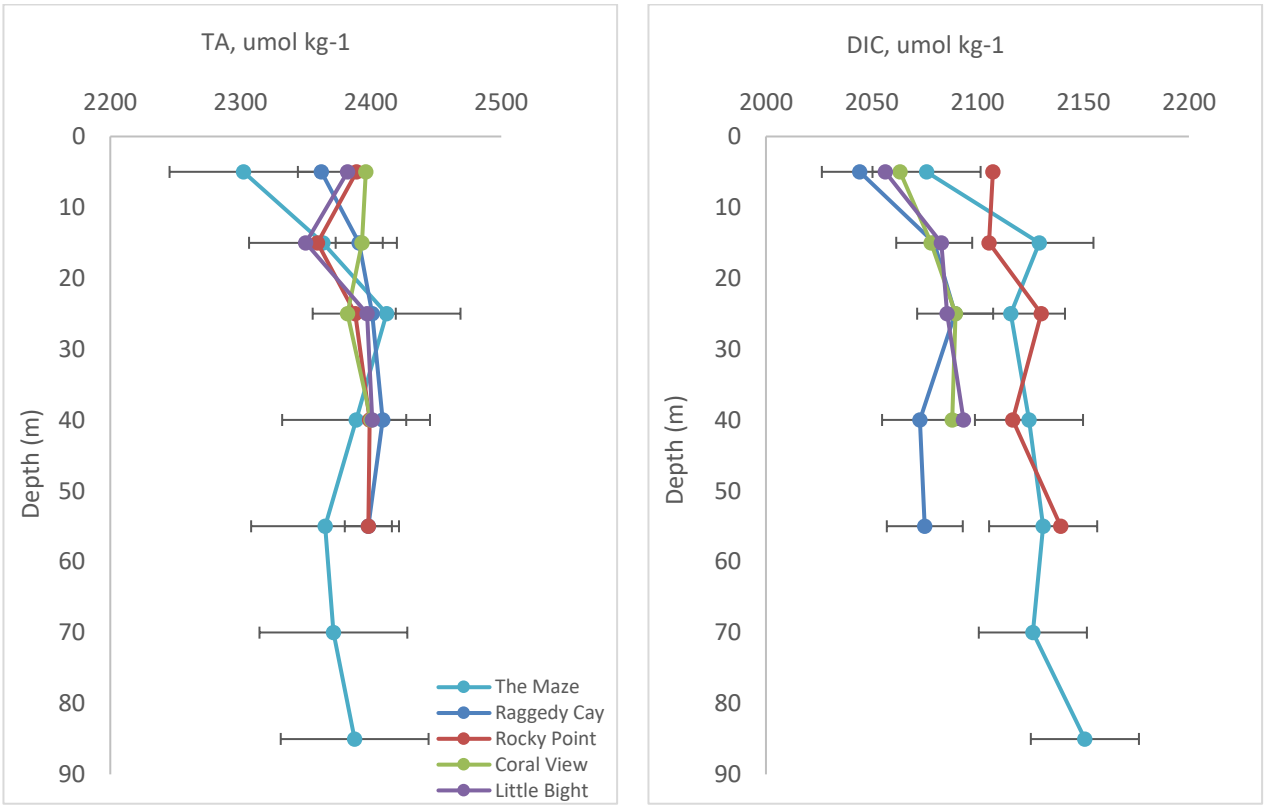


Figure 6.5 TA, (left hand graph) and DIC (right hand graph) concentrations as a function of depth (y - axis) at all mesophotic sites. Mean values are shown for The Maze (n = 4) and Raggedy Cay (n = 2), but all other sites represent n = 1. Error bars represent the standard deviation of values taken across all sampling days per site. Note the different x – axes between graphs.

Both TA and DIC generally exhibit lowest concentrations in shallower water and increase with depth. However, there are exceptions, for example seawater DIC concentrations increase between 15 m and 25 m at The Maze and Little Bight, and TA concentrations increase between 5 m and 15 m at The Maze, Coral View, Little Bight and Raggedy Cay. Below 55 m and 85 m there is also a minor increase in DIC and a more noticeable increase in TA at The Maze.

Mixed models were conducted to assess which factors control carbonate chemistry down a depth gradient; models were identical to the model run for seawater DMSP. There was a significant effect of both depth (ANOVA, $p > 0.001$) and site (ANOVA, $p < 0.001$) on seawater DIC, however this was not true of TA, which was only affected by the presence of macroalgae (ANOVA, $p = 0.001$).

The calcium and aragonite saturation states (Table 6.2) were also derived from TA and DIC measurements using CO2SYS (see section 2.5.4).

Table 6.2 Carbonate and aragonite saturation states for each depth sampled at all sites used in this study. Mean values are shown for The Maze (n = 4), Raggedy Cay (n = 2) and Little Bight (n=2), but all other sites represent n = 1.

Depth	*The Maze*	*Raggedy Cay*	*Rocky Point*	*Coral View*	*Little Bight*
5	4.621	4.836	4.806	5.599	4.496
15	4.972	4.532	4.346	5.313	4.026
25	6.010	4.458	4.427	4.947	4.378
40	5.418	5.633	4.796	5.251	4.340
55	4.803	5.404	4.424		3.501
70	5.173				
85	4.983				

The values for both saturation states reported here are within the range of normal values for tropical reef systems and not below minimum thresholds required for reef precipitation to occur (Hoegh-Guldberg et al. 2007b).

Mixed models were run to establish whether carbonate chemistry affected seawater DMSP down a depth gradient; models were run accounting for variability between sites and sampling day. Whilst TA did not significantly affect seawater DMSP (ANOVA, $p = 0.08$), DIC

did exert a significant effect (ANOVA, $p < 0.001$). Linear models were run on each site separately (Fig 6.6) to establish individual relationships between seawater carbonate chemistry and DMSP.

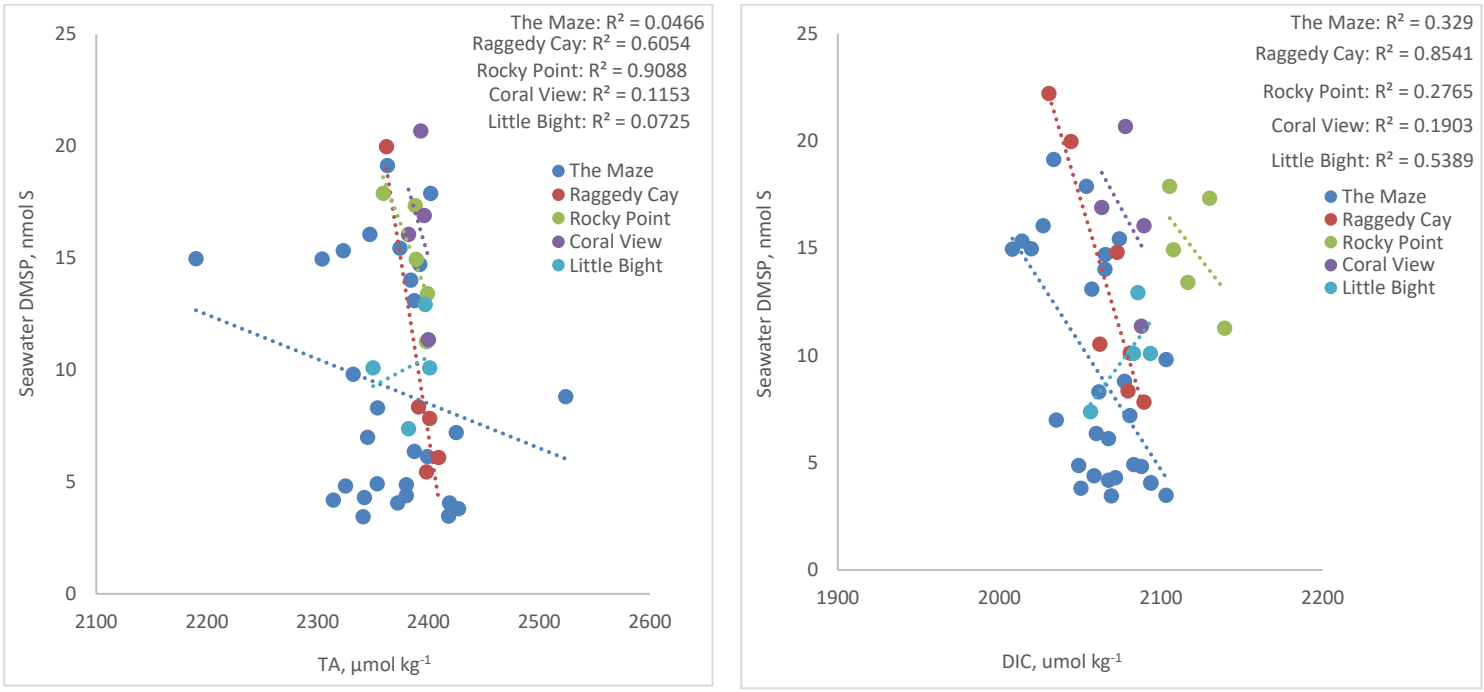


Figure 6.6 Seawater DMSP as a function of TA (left hand graph) and DIC (right hand graph) at each of the five mesophotic sites sampled. Mean values are shown for The Maze (n = 4) and Raggedy Cay (n = 2), but all other sites represent n = 1. Note the different x – axes for both graphs. Linear lines of best fit are indicated by the dotted lines for eac site (denoted by colour), with R^2 values for each trendline given in the legend.

There were evident linear trends between seawater DIC and DMSP at The Maze ($R^2 = 0.329$), Raggedy Cay ($R^2 = 0.854$) and Little Bight ($R^2 = 0.538$), although this was only significant for The Maze ($p < 0.001$) and Raggedy Cay ($p < 0.001$). To assess the overall impact of carbonate chemistry on seawater DMSP concentrations, depth, DIC and TA measurements were inputted into CO2SYS (see section 2.5.4), using default values for salinity and temperature and the carbonate chemistry was calculated. Site affected the aragonite ($p = 0.01$) and calcium carbonate ($p = 0.01$) saturation states, as did the presence of sand/pavement/rubble (both $p = 0.04$). There was an indication that macroalgae (both $p = 0.06$) and hard coral (both $p = 0.07$) affected the carbonate saturation state of the surrounding seawater. To assess whether either carbonate saturation state affected

seawater DMSP, mixed models were run controlling for sampling day and site, but no significant effect was found for either (both p = 0.41).

6.6 Discussion

6.6.1 Concentrations and comparison with values reported in the literature

Studies into dimethylated sulphur compounds in mesophotic reef systems have not yet been conducted, thus the data presented here stand alone. However, the range of values for seawater DMSP reported for all depths at all sites (3.452 – 22.222 ± 5.697 nmol DMSP) in this study are within the range of values reported for shallow water tropical reef systems (Broadbent and Jones 2006; Jones et al. 2007; Fischer and Jones 2012; Burdett et al. 2013)

Measurements made for tissue DMSP in tropical corals at mesophotic (> 30 m) depths are limited in the literature, and to our knowledge there have been no measurements made for tissue DMSO in mesophotic corals and only limited measurements made for shallow water coral DMSO concentrations (Deschaseaux et al. 2014b). DMSP and DMSO values are usually normalised to polyp numbers, surface area, chlorophyll - a content, protein content or symbiont cell numbers/volume. Owing to logistical constraints, it was only possible to normalise tissue DMSP/O to coral tissue weight and surface area. The lower depth range of corals sampled in this study (11 – 60 m) is deeper than other studies, and no studies have previously published tissue concentrations of either DMSP or DMSO for *A.lamarcki*. The DMSP values presented here (17.586 - 127.901 nmol DMSP cm^{-2}, mean 50.876 nmol DMSP cm^{-2}) are comparable to values previously reported for shallow water (< 30 m) tropical corals, with concentrations ranging from 17 – 2200 nmol cm^{-}(Broadbent et al. 2002; Yost et al. 2012; Tapiolas et al. 2013; Deschaseaux et al. 2014b; Jones and King 2015; Borell et al. 2016). Tissue DMSO concentrations reported here (8.47 – 117.59 nmol DMSO cm^{-2}, mean 35.94 nmol DMSO cm^{-2}) are similarly comparable with previous reports of tissue DMSO in shallow water corals (Deschaseaux et al. 2014) for both the control (28.4 ± 6.48 nmol cm^{-2}) and light depleted (30.1 ± 4.40 nmol cm^{-2}) corals, although no significant difference between these treatments was reported. It was expected that, because of reduced solar irradiance and lower/more stable temperatures, tissue DMSP concentrations in mesophotic

corals would be lower than shallow water corals. However, this was not the case and suggests that these are not the driving production of these compounds in the coral holobiont. The possibility that DMSP/O were being produced in response to reduced carbonate saturation levels was considered, however the values reported here do not support this. It is more likely that species – specific changes in DMSP/O production in both the host and symbiont are responsible for the concentrations we see here. However, the fact that mesophotic corals produce comparable amounts of DMSP/O at depth may impact shallow water sulphur biogeochemistry via vertical mixing, suggesting these reefs are capable of not only acting as refugia for shallow water species, but also are biogeochemically linked.

6.6.2 Spatial variability and drivers of seawater DMSP concentrations

This investigation of seawater DMSP at 5 mesophotic reef sites demonstrated that this compound is significantly affected by depth, whilst site and benthic ecology both appear to exert no effect on the spatial variability. This is in contrast to previous studies that have reported relationships between the presence of macroalgae/seagrass and higher concentrations of seawater DMSP (Burdett et al. 2013). However, we noted that macroalgal percentage cover ranged from 0 – 52% across all sites and did not extend deeper than 55 m, whilst Burdett et al. (2013) reported seawater DMSP concentrations where the reef was dominated by fleshy and coralline algae. It is likely that reduced coverage by macroalgae coupled with greater potential for water mixing at depth act in concert, reducing the effect of specific benthic types on seawater DMSP concentrations. Furthermore, since seawater DMSP concentrations are affected by a suite of variables (i.e. bacterial action, temperature, light), it is perhaps not unexpected that no significant relationship exists between benthic ecology and seawater DMSP. This study previously reported results which indicated that water mixing, and the physical reef environment, exert an effect on seawater DMSP by restricting or enhancing atmospheric degassing of DMS. Whilst no data exists for the hydrodynamic nature of, or physical environment of the reef sites included in this study, it seems unlikely that all 5 reef sites were subject to the same physical processes given their locations around the island (Fig 6.1) and the relative effects of the prevailing wind, as well as the variable site depths and topographies. Further investigations into biogeochemical

cycling of DSC should seek to include measurements of physical and hydrodynamic properties, to establish how seawater DMSP is cycled at depth. This work is crucial in developing our understanding of how mesophotic reefs may impact the sulphur biogeochemistry of shallow tropical reef systems. Upwelling of DMSP and DMS from deeper reefs could lead to increases in microbial biomass since both are important compounds for marine microbes, acting as a substrate for growth (Kiene and Linn 2000; Raina et al. 2010) and helping structure bacterial communities (Frade et al. 2016). DMS/P are also known to serve as a cue for foraging fish and birds (Nevitt 2000; DeBose et al. 2008; Paul et al. 2008) and is implicated in grazing/predation of marine algae by a range of organisms (Dacey et al. 1994; Wolfe et al. 1997; Seymour et al. 2010a). Understanding how deeper reefs are biogeochemically linked to their shallow water counterparts is crucial in assessing the impacts of climate change on shallow water reef systems.

The results presented here show evidence of an inverse relationship between seawater DIC and seawater DMSP, although the same is not true of TA. A general increase in saturation states, DIC and TA with increasing depth was also reported, although temperature and salinity measurements were not made, potentially confounding any accurate calculation of either carbonate saturation state. Burdett et al. (2013) noted a significant relationship between seawater carbonate chemistry and tissue DMSP concentrations, reporting that maximum intracellular DMSP and water column DMS/P concentrations were observed at night, coinciding with the time of lowest carbonate saturation state. It was suggested that CO_2 uptake for photosynthesis during the day reduced DIC, and release by respiration at night (which increases DIC) were the probable drivers of the observed diel pattern in DIC concentrations. Increases in cellular concentrations of DMSP may therefore be responsible for elevated seawater concentrations, and the same could also apply to DMSO.

This study reports increasing DIC with depth, indicating increasing respiration rates at depth and may be related to reduced light conditions and/or changing feeding strategies. In the mesophotic zone the attenuation of light is the most significant change in the abiotic environment affecting the physiology of corals (Lesser et al. 2009b, 2010). It follows then that the decreased ability of a coral to photosynthesize in the mesophotic zone will lead to increases in seawater DIC as corals rely on other trophic strategies, such as heterotrophy, to

meet their overall metabolic costs for growth (Muscatine et al. 1989). Indeed, *A. lamarcki* has been previously identified as a heterotrophic coral (Crandall et al. 2016). As a downstream product of photosynthesis (DMSP is a tertiary metabolite), it is logical that intracellular concentrations of DMSP would decrease in response to decreased photosynthesis. However, this was not observed in this study; tissue concentrations of DMSP and DMSO in *A.lamarcki* exhibited contrasting weak but significant relationships with depth at both sites (Fig 6.4), with tissue concentrations of DMSP decreasing with depth at The Maze, but increasing at Little Bight when normalised to weight. When normalised to surface area, however, tissue concentrations of both compounds exhibited increases with depth at both sites, albeit weakly.

Numerous studies have demonstrated that catabolic action by bacteria (Simó et al. 2000; Raina et al. 2009; Hatton et al. 2012) and viruses (Bratbak et al. 1995; Evans et al. 2007) serve to release DMSP held in algal cells into surrounding seawater. Degradation of DMSP by marine microorganisms takes place by at least two major pathways; the cleavage pathway involves degradation of DMSP to DMS by phytoplankton or bacteria using enzymes known as DMSP lyases, whilst the alternative demethylation pathway, carried out by bacteria alone, involves an initial demethylation of DMSP to methylmercaptopropionate (MMPA) (Frade et al. 2016). The relative incidence of these competing microbial pathways has important consequences for the biogeochemical cycling of dimethylated sulphur compounds and is driven by coral - microbial associations. *A.lamarcki* has previously been identified as exhibiting symbiont zonation with depth (Bongaerts et al. 2013), so it is possible that whilst intracellular DMSP concentrations increase with increasing depth, changing bacterial - coral associations alters the DMSP - degradation pathway in favour of demethylation and resulting in lower seawater concentrations of DMSP.

It is also possible that whilst seawater DMSP decreases with depth, the majority fraction of dimethylated sulphur in the water column exists as DMSO. Studies have shown that whereas DMS and DMSP are usually restricted to the euphotic zone, DMSO has been reported at concentrations greater than 1.5 nmol dm^{-3} at depths up to 1500 and 4000 m in the equatorial Pacific Ocean and Arabian Sea, respectively (Hatton et al. 1998, 1999). Consequently, when the whole water column is considered, depth-integrated DMSO levels

are significantly higher than those for DMSP. Hence DMSO can be the dominant DMS-related sulphur species throughout the water column, especially in eutrophic regions (Hatton et al. 1998). Concentrations of tissue DMSP were significantly higher than DMSO; since cell membranes are more permeable to DMSO, it can more easily leave algal cells where photobiological mechanisms can act to reduce it to DMS (Hatton 2002; Hatton et al. 2004). This study measured seawater DMSP as total DMSP, which includes DMS; previous studies have reported that DMS can account for a significant portion of total seawater DMSP (up to 22 nmol) (Curran et al. 1998), although the ratio of these two compounds reported here is unknown. However, in a reduced light environment, it is conceivable that any algal derived DMSO in seawater is not photochemically converted to DMS, making DMSO, not total DMSP, the dominant sulphur compound in seawater at depth. Further research would be needed to ascertain the partitioning of the various dimethylated sulphur species at depth. Further research should include separate measurements of DMSP and DMSO in their dissolved and particulate fractions, which would help improve our understanding of how these compounds are cycled at depth. Measurements of DMS should also be included so that the photochemical pathways involved in DMSO cycling can be elucidated.

6.6.3 Tissue DMSP and DMSO

Depth was found to affect tissue concentrations of both DMSP and DMSO when normalised to surface area, but only affected tissue DMSP when normalised to weight. These results suggest that the accumulation of DMSP in host tissues may not simply be a function of tissue depth or coral morphology and that both factors potentially influence the interpretation of DMSP concentrations within a coral species. Nonetheless, both coral surface area and biomass indices may be informative for various DMSP investigations relating to, for example, mass-transfer limited processes or coral biomass, respectively (Edmunds and Gates 2002; Yost et al. 2012). An effect of depth on tissue concentrations of DMSP has previously been reported, although not for all species studied, nor for all normalisation indices and the depths used did not exceed 30 m (Yost et al. 2012). Of the depth-related factors most likely to affect tissue DMSP/O concentrations are changes to temperature and light (Sunda et al. 2002; Deschaseaux et al. 2014b). Whilst neither factor

was measured in this study, both temperature and light are known to decrease with increasing depth; light in the ocean decreases exponentially with depth and it is one of the most important factors shaping coral communities (Falkowski and Dubinsky 1981; Dubinsky et al. 1984). Increases in temperature and irradiance are known to increase algal production of DMSP/O (Hatton 2002; van Rijssel and Gieskes 2002; McLenon and DiTullio 2012; Deschaseaux et al. 2014b), as part of an antioxidant cascade response (Sunda et al. 2002), however the opposite was observed here. Increased tissue DMSP and DMSO concentrations with depth were also noted in *Diploria labyrinthiformis* (1 - 30 m) and were attributed to the host-symbiont association and its impact on photosynthetic response (Yost et al. 2012). Whilst we cannot rule out changes to symbiont structure with depth here, no genetic analyses were conducted but the results indicate a direction for future research efforts. Most studies have concentrated on DMSP/O production in response to increased light rather than decreases in irradiance. However, Deschaseaux et al. (2014) noted no significant difference in tissue DMSP concentrations in response to light depletion for any normalisation index, however tissue DMSO did significantly increase when normalised to protein content. The authors reported that light depletion led to an increase in the overall antioxidant capacity (AOC) of the coral holobiont, resulting in increased tissue DMSO concentrations. They further suggest that DMSO production in coral tissue is intimately linked to the up-regulation of the coral AOC and the results presented here support that hypothesis.

Although there was no effect of site on each compound individually, there was a significant effect of site on the DMSP:DMSO ratio. Moreover, a stronger linear relationship was observed in tissue DMSP/O at Little Bight than at The Maze. In comparison to Little Bight, very few physiological changes with depth have been observed at The Maze (Laverick et al. 2018), despite colonies being collected across a 44 m depth range, and the mean values and spread of data being comparable between both sites. One potential explanation is that the topography of The Maze allows for more light-equivalent microhabitats for colonies to exploit (Brakel 1979). Photosynthetic capacity in Scleractinia has been shown to correlate with the light environment of microhabitats (Anthony and Hoegh-Guldberg 2003), so we would expect greater variation in the light levels of potential microhabitats in the shallows than at depth. Since DMSO production has been shown to be intimately linked with DMSP

biosynthesis (Sunda et al. 2002), with irradiance exerting considerable influence over production of both compounds, it is likely that the effect of site observed here is related to subtle variations in irradiance levels of potential microhabitats.

6.6.4 Implications and future focus for research

The results presented here demonstrate that there is a clear need to understand the role of mesophotic reefs in biogeochemical cycling of dimethylated sulphur compounds, to comprehend how they are biogeochemically linked to shallow water reefs through vertical mixing of seawater. It seems unlikely that no exchange of seawater between both shallow and deep reefs occurs - were that the case then it would be unlikely that mesophotic reefs could act as refugia for shallow water reefs. The role that DMSP plays in structuring ecosystems from the microbial to the macrofaunal is of vital importance, but how mesophotic reefs will impact DMSP dynamics in shallow water systems is hitherto speculative. Whilst considerable attention has been given to shallow water reefs and the likely impacts of climate change, mesophotic reefs are understudied, despite being a comparable source of biogenic sulphur to marine waters. As well as withstanding numerous environmental stressors (i.e. temperature fluctuations), production and cycling of biogenic sulphur compounds are further complicated by depth - related factors and the presence of micro - habitats, which are site specific. If we are to accurately project the impacts of climate change on biogeochemical cycling of biogenic sulphur compounds in these ecosystems, we must first understand contemporary natural variability.

7 Discussion and conclusions

Research presented in this thesis significantly improves current understanding of sulphur cycling in coral reef ecosystems, specifically by determining drivers of seawater and tissue concentrations of DMSP/O in a range of coral reef taxa across spatial and temporal scales. In this concluding chapter, the results presented in this thesis are synthesised, to develop a broader view of how contemporary sulphur cycling is likely to be altered in response to climate change. Future research directions are then highlighted that would further advance our understanding of coral reef sulphur biogeochemistry.

The production and release of dimethylsulphoniopropionate (DMSP) by algae is a major component of the marine sulphur cycle, accounting for ~ 50 x 10^{12} moles of sulphur per year (Sievert et al., 2007). The subsequent formation of dimethylsulphide (DMS) may help to regulate local climate by promoting the formation of clouds (Charlson et al. 1987). Dimethylated sulphur compounds (DSC) are also important in maintaining marine ecosystem function, acting as a cryoprotectant (Karsten et al. 1996a), antioxidant (Sunda et al. 2002), compatible solute (Stefels 2000), grazing deterrent (Dacey and Wakeham 1986) and attractant (Seymour et al. 2010b; Garren et al. 2014) and microbial energy source (Green et al. 2011; Hatton et al. 2012). More recently, DMSP has been shown to act as a chemoattractant to marine *Vibrio* bacteria (Garren et al. 2014), which use this compound as a cue to target heat - stressed corals. Thus, this suggests that DMSP may act not only as an indicator of stress, which has been the focus of research to date, but could also exert a top - down control on coral populations. Thus, rates of production by corals are likely to have significant and potentially detrimental effects on coral ecosystems in a manner that has, so far, been largely overlooked. Amongst the many studies on DMSP (i.e. Green and Hatton 2014 and references therein), most papers report concentrations of seawater DMSP, often characterising the form P (i.e. particulate or dissolved), however information on the rates of DMSP production by any marine taxa are lacking. For dimethylsulphoxide (DMSO) there are even fewer studies, despite its significant role in the marine sulphur biogeochemical cycle (Hatton et al. 2004). For example, whilst DMSP can act as a source for DMS, DMSO can act as a source and sink for DMS, owing to the biotic and abiotic processes involved in its synthesis and degradation. Crucially, DMSO reduction by bacteria or light is a potentially

significant source of DMS (Hatton 2002; Spiese et al. 2009) to seawater, whilst bacterial oxidation of DMS can ultimately remove this compound from seawater (Shooter and Brimblecombe 1989; del Valle et al. 2009; Green et al. 2011).

There have also been suggestions that the DMSO:DMSP ratio may be a proxy for cellular stress (Husband and Kiene 2007; McFarlin and Alber 2013; Deschaseaux et al. 2014b). However, two of these studies refer to *Spartina* grasses that are unlikely to act as comparable analogues for symbiotic dinoflagellates, with only one study having considered this ratio with respect to corals (Deschaseaux et al. 2014b). This ratio is further complicated by the variety of indices by which DSC concentrations can be normalised (i.e. per cell, per cell volume, surface area, chlorophyll - a, protein content, weight). The question of which normalisation index to use in coral holobiont research has proven to be problematic for some time (Edmunds and Gates 2002) and there is no general consensus on which index is "better". This is largely driven by the numerous and varied coral research foci that span habitats from whole ecosystems down to microhabitats occupied by their smallest inhabitants (bacteria, viruses, algae). Biogeochemical cycling of DSC is driven chiefly by the latter, i.e. algae & bacteria, but photochemistry plays a crucial role in cycling DMS and DMSO (Hatton 2002; Deschaseaux et al. 2014b). Thus, the question of which index might be the most appropriate to use should be driven by the research question being addressed; for example, it would be more appropriate to use cellular concentrations when considering the role of DSC as stress biomarkers, since stress occurs at cellular level. However, when considering the role of DSC in ecosystem function, surface area measurements in which the overall contribution of coral production of DSC can be quantified would be more useful.

The effects of increased (or decreased for that matter) carbon dioxide are similarly under-reported in the literature with respect to corals/coral reefs, with the studies that do exist being focussed on temperate/polar regions and with variable or conflicting results (Lee et al. 2009; Avgoustidi et al. 2012; Archer et al. 2013; Hopkins and Archer 2014). The spatial and temporal range of studies to-date have also been somewhat limited, with most occurring in shallow coral reef systems in the Red Sea (Burdett et al. 2013) or the Great Barrier Reef (Broadbent et al. 2002; Broadbent and Jones 2004, 2006; Jones and Trevena 2005; Swan et al. 2012b, 2016, Deschaseaux et al. 2012, 2014a; Fischer and Jones 2012; Suzanne and

Deschaseaux 2013; Jones et al. 2014; Jones and King 2015), whilst the Atlantic reefs are under-represented save for studies in Bermuda (Yost and Mitchelmore 2010; Yost et al. 2012; Borell et al. 2016) and no studies exist for mesophotic coral ecosystems at all.

Using a variety of techniques in the laboratory and the field, this research was designed to provide a better understanding of:

1. How intracellular concentrations of DMSP and DMSO vary between coral taxa (Chapters 3, 5 & 6) and how this compares with other coral taxa
2. How climate change is likely to affect coral production of DMSP and DMSO intracellularly and in seawater (Chapters 3 & 5)
3. The natural spatial and temporal drivers of reef seawater DMSP (Chapters 4 & 6)
4. The role of non-coral taxa in natural variability of coral reef sulphur biogeochemistry (Chapter 5)

7.1 Variability of intracellular concentrations of DMSP and DMSO between coral taxa

DMSP and DMSO concentrations in marine phytoplankton are known to be highly variable between different taxa and species (Karsten et al. 1996a; Hatton and Wilson 2007; Steinke et al. 2011; Caruana and Malin 2014). On a global scale, the main producers of DMSP are phytoplankton species confined to the classes Dinophyceae (dinoflagellates) and Prymnesiophyceae (including the coccolithophorids) (Keller et al. 1989). Some members of the Chrysophyceae and Bacillariophyceae (diatoms) can also produce significant amounts of DMSP, but generally intracellular concentrations are low (Hatton and Wilson 2007). Within dinoflagellates, concentrations are highly variable and this extends to coral-associated *Symbiodninium*, where concentrations can vary according to not only strain (Steinke et al. 2011) but also according to the host-symbiont relationship (Borell et al. 2016). Symbiodinium species have been broadly categorised into different groups (often called clades), labelled A – G, with groups (clades) A-D occurring most commonly in corals in varying proportions (LaJeunesse et al. 2004a; Silverstein et al. 2012). There is also a suggestion that *Symbiodinium* distributions vary considerably between the Caribbean and Indo-Pacific (Baker 2003; LaJeunesse et al. 2003). Generally, clades A & B are more common

in the Caribbean and tropical Atlantic (LaJeunesse 2002; Baker 2003; Garren et al. 2006), whilst tropical Indo-Pacific corals are dominated by clades C and D (LaJeunesse 2001; Fabricius et al. 2004; LaJeunesse et al. 2004a; Chen et al. 2005a). Physiological differences have also been ascribed in a very general sense to each clade; clade C *Symbiodinium* have been reported to be more thermally sensitive than clade D, which have been shown to be more thermally tolerant (Tchernov et al. 2004; Berkelmans and van Oppen 2006; Jones et al. 2008; Stat and Gates 2011; Kemp et al. 2014). The corals studied during this research are from both the Indo - Pacific (*Porites* spp., Chapter 3) and the Caribbean (*A.lamarcki*, *U.tenuifolia*, *P.astreoides*, Chapters 5 & 6). Thus, it is reasonable to expect that tissue concentrations of DMSP/O in the coral samples used in Chapter 4 & 6 (Caribbean samples) would be lower than samples collected from the field in Chapter 3 (Indo - Pacific). However, this was not observed and DSC concentrations from field samples were two orders of magnitude higher than those found in laboratory conditions. This is not unexpected due, largely, to the conditions experienced by laboratory specimens compared with those sampled from the field – notably, laboratory experimental conditions did not include UVA/B light, which is known to influence DMSP/O production and degradation (Hatton 2002; Sunda et al. 2002). Whilst tissue DMSP results for incubated field corals came back blank due to sample transport issues , the research presented in this thesis has consistently observed higher tissue DMSP concentrations compared with DMSO, which is in line with other studies in the literature (Hatton and Wilson 2007; Deschaseaux et al. 2014b). It would therefore be expected that tissue DMSP concentrations would be higher than DMSO in field specimens and to be higher than tissue DMSP in laboratory specimens. The differences between laboratory and field conditions might, at first glance, appear to confound any results or their interpretation, however importantly laboratory studies go a long way to illustrating a key control on coral production of DMSP/O. Laboratory corals exhibited considerably lower tissue DMSP/O than field samples, but were not subject to UV radiation. Field samples, however, were subject to UV radiation and exhibited considerably higher DMSP/O concentrations – even mesophotic corals from a low light environment, where tissue DSC concentrations increased with increasing depth. Whilst light was not measured at mesophotic sites, it is reasonable to suggest that depth is a proxy for light and that the increases in tissue DSC observed are in response to decreases in light. It might also be inferred that increasing depth is accompanied by decreasing temperature, but this would

likely be accompanied by decreases in tissue DSC concentrations, as has been reported widely in the literature (Sunda et al. 2002; Fischer and Jones 2012; McLenon and DiTullio 2012; Deschaseaux et al. 2014b) and in this study (Chapters 3 & 5). The results from Chapter 6 are in line with previous reports that light depletion acts as an oxidative stressor, resulting in increased tissue DSC (Deschaseaux et al. 2014b). Whilst the lower concentrations of DSC observed between laboratory and field corals can be attributed to the absence of UV radiation, and increases in DSC in mesophotic corals with increasing depth can be attributed to light depletion, transplanted field specimens (Chapter 5) exhibited significantly higher tissue DMSO concentrations after the transplant than before. This was determined to be related to temperature, although it is likely that corals were also exposed to higher levels of light and that this contributed to increased tissue DSC. This inability to single out single factors is a weakness of field studies that can be further addressed in laboratory work once data from filed work points the way.

7.2 The role of increased seawater surface temperature on production of DSC in coral reef ecosystems

The importance of temperature as a control on coral production of DSC is a consistent trend in this research; transplanted corals (Chapter 5), laboratory corals (Chapter 3) and the spatio-temporal variability of shallow reef systems (Chapter 4) have all demonstrated that temperature exerts considerable influence in determining production and distribution of DSC in tropical reef systems. Chapter 3 demonstrated that corals increase production of DSC in response to increases in temperature regardless of normalisation index, whilst the same does not hold true of pCO_2/pH which affects DMSO but only when normalised to cellular concentrations. Similarly, transplanted corals (Chapter 5) exhibited significantly higher tissue DMSO at the end of the transplant, which was driven by increases in temperature and decreases in pH. Seawater production of DMSP was also affected by pH and temperature in both experiments and the same pattern was evident in the spatio-temporal distribution of seawater DMSP (Chapter 4) in 2016. The importance of temperature in cellular upregulation of DMSP has been widely reported in the literature for a wide range of marine algal taxa (van Rijssel and Gieskes 2002; Lee et al. 2009; Spielmeyer and Pohnert 2012b), including coral associated dinoflagellates (McLenon and DiTullio 2012; Deschaseaux et al. 2014b;

Jones and King 2015) and the coral animal itself (Raina et al. 2013). This research observed increased seawater production of DMSP alongside increased tissue concentrations of DSC in one coral and all non-coral species (Chapters 3, 4 & 5), suggesting that production by the organism is a significant driver of seawater DMSP levels and distribution. Additionally, the highest tissue concentrations of DSC were seen in seagrasses (Chapter 5), which had a significant effect on seawater DMSP (Chapter 4). Additionally, temperature affected seawater DMSP at both reef sites, where DMSP concentrations were highest later in the day and were driven by increases in temperature. Coral tissue concentrations of DSC were significantly higher in both species after the transplant, however in the case of *U.tenuifolia*, this was not accompanied by increased seawater DMSP production, suggesting that species specific contributions are likely to exert a significant influence over the seawater DMSP budget. Furthermore, coral production of DSC, both in tissues and seawater, was not the dominant source of DMSP to reef seawater. In fact, production of seawater DMSP by *U.tenuifolia* was, at points, comparable with production by reef sediments.

This could have serious ramifications for coral reef sulphur biogeochemistry under a changing climate depending on who the "winners" and "losers" are, or as reef ecosystems switch to a state of net dissolution thereby increasing sediment coverage. For example, increases in temperature known to increase incidences and severity of coral bleaching, and are likely to lead to increases in reef macroalgal coverage (Hoegh-Guldberg et al. 2007a; Anthony et al. 2008b; Carpenter et al. 2008). Under these conditions, the data presented in this study indicate that seawater concentrations of DMSP are likely to increase as macroalgal dominated assemblages increase and coral cover decreases. Aside from the loss of habitat for a diverse range of coral reef taxa that will occur following the loss of this vital three-dimensional habitat, concomitant increases in DSC are likely to have serious and potentially deleterious consequences for reef ecosystem function (Bourne et al. 2009; Garren et al. 2014) and for atmospheric DMS concentrations and local cloud cover (Jones and Trevena 2005; Fischer and Jones 2012).

Increased production of DMSP at the cellular level is a tactic employed by the majority of marine algal taxa to help them deal with temperature induced stress (Sunda et al. 2002; Deschaseaux et al. 2014b). However, this same tactic could open already susceptible and

stressed coral to infection by pathogenic bacteria (Garren et al. 2014), which would likely increase mortality rates of corals and potentially accelerate rates of coral loss.

It has also been suggested that increases in seawater DMSP may drive increases in atmospheric DMS, thereby increasing local cloud cover in a negative feedback loop known as the CLAW hypothesis (Charlson et al. 1987). However, subsequent research has revealed that the CLAW hypothesis is a somewhat simplified explanation for a highly complex biogeochemical system (Quinn and Bates 2011; Green and Hatton 2014). Indeed, recent research from the Great Barrier Reef has shown that whilst DMSP production may increase in response to elevated sea surface temperatures, this does not necessarily lead to increases in atmospheric DMS, which could equally result from *in situ* production of atmospheric DMS by corals (Fischer and Jones 2012; Hopkins et al. 2016).

The lack of a direct link between atmospheric DMS and DMSP production by coral reef taxa is likely due to the numerous and varied mechanisms by which DMSP and DMSO are produced and cycled in seawater (Hatton et al. 2004; Stefels et al. 2007). These mechanisms are predominantly driven by microbial processes and, in the case of DMS/O, additionally by the presence of UV radiation. Whilst the role of marine bacteria in the biogeochemistry of DSC has been relatively well studied (Kiene et al. 2000; Reisch et al. 2011; Hatton et al. 2012; Moran et al. 2012; Vila-Costa et al. 2014), the role of coral-associated bacteria in DMSP cycling is a relatively recent focus (Raina et al. 2009; Frade et al. 2016). As sea surface temperatures increase, the research presented in this thesis suggests that coral and non-coral taxa alike will increase cellular production of DMSP/O and that this results in increased seawater DMSP concentrations. What is not clear, however, is how seawater concentrations of DMSO are likely to vary in response to climate change. The research presented here has shown that DMSO production is affected by pCO_2 and temperature in all settings, suggesting it is a sensitive indicator of the coral response to changing environmental conditions. Given the significance of this compound as a sink for DMS and the role of UV radiation in governing how it is cycled in seawater, understanding contemporary DMSO cycling in reef waters will aid understanding as to why atmospheric DMS emissions are not directly linked to marine DMSP production. This will aid in future efforts to understand how coral reefs will

respond to climate change, as well as improving the accuracy of forecast models that aim to predict global DMS emissions and cloud cover.

7.3 The role of ocean acidification on production of DSC in coral reef ecosystems

Whilst this present research has observed consistent trends regarding the role of increased temperature on DSC production, studies into the effects of lower pH or increased pCO_2 on production of DMSP have often reported inconsistent results. For example, some investigations report increased DMSP production in response to decreases in pH/increased pCO_2 (Lee et al. 2009; Archer et al. 2013), whilst others have shown that decreases in pCO_2 is accompanied by reduced DMSP concentrations (Hopkins et al. 2010; Avgoustidi et al. 2012; Hopkins and Archer 2014).

The inconsistencies in published results has been attributed variously to phytoplankton community structure changes, alterations to the growth rates of various algal taxa or differences in physiological responses (i.e. variable cell boundary layer thickness) of different algal taxa to increases in CO_2 concentrations. Given that coral reef seawater is oligotrophic, it is reasonable to assume that the same changes to phytoplanktonic community structure referred to above are unlikely to apply in a reef setting. As such, the effects of lower pH on DSC production reported here are likely to result from either an alteration in zooxanthellae growth rates, or from changes in physiological response. Although not found in this study, it is known that DMSP can be synthesized in the dark. A coupling between photosynthesis (and by extension, growth) and DMSP production has also been reported for dinoflagellates (Simo et al. 2002). It is possible, therefore, that increases in seawater pCO_2 that drive ocean acidification are responsible for the increases in DSC observed in this study, by driving growth rates of zooxanthellae and with subsequent increases in tissue and seawater DSC concentrations. Additionally, since CO_2 limitation is a known oxidative stressor, capable of inducing DMSP/O upregulation, it is possible (although thus far unreported in the literature) that decreases in pH may serve to upregulate DMSP/O production in the same way that light depletion and excess light exposure are known to drive DMSP/O upregulation.

However, as with temperature, changes to pH/pCO_2 are also likely to affect coral-associated bacterial communities and it may be this that regulates production and cycling of DSC in corals and coral reef waters. In coral reef environments, bacteria are extremely dependent on organic compounds produced by *Symbiodinium*; photosynthetic products are released into coral tissues and mucus and have been shown to determine the microbial community structure present in the holobiont and surrounding seawater (Ritchie and Smith 2004; Ritchie 2006; Frade et al. 2016). However, coral mucus is also known to have a lower pH (around 7.7) compared with the surrounding seawater (~ 8.3), which indicates the presence of acidic compounds in mucus, most likely acrylate, carboxylic acid and sulphate (Raina et al. 2009), and this is an effective anti-microbial defence. It is possible that ocean acidification will alter the community composition of bacteria associated with corals and in seawater, resulting in reduced DMSP/O degradation and elevated seawater concentrations of both compounds.

7.4 Future work and research direction:

7.4.1 Laboratory based trials

Research conducted during this PhD has identified several key areas for focus and consideration, the most notable of which is the similar trends between results from field and laboratory-based experiments, although the concentrations measured were often orders of magnitude apart. Whilst tissue concentrations of DMSP were considerably lower in laboratory coral specimens, the overall trends and response of corals to experimental conditions between field and laboratory-based studies was consistently the same. This suggests that laboratory-based manipulation experiments are well placed to act as analogues for field studies, potentially increasing the research effort and aiding reef management strategies. Furthermore, field - based studies could complement laboratory - based studies by providing a broader focus for research, which could be investigated more precisely in controlled trials.

7.4.2 DMSO

DMSO is a significant compound in the marine sulphur biogeochemical cycle (Hatton et al. 2004), but there is a paucity of data surrounding its production and cycling in reef environments. One study has reported intracellular concentrations (Deschaseaux et al. 2014b) and one has reported values for coral reef seawater (Broadbent and Jones 2006). This is clearly a gap in our knowledge. Given the significant role that light has in structure reef environments, both in shallow and mesophotic reefs, there is the potential for DMSO to act as a significant removal pathway for DMS, with consequences for local climate control. In the same manner as DMSP, which is arguably more studied, DMSO can act as a source of DMS via microbial pathways that are still not fully understood. Thus, future work should focus on cellular production and ecophysiology in a range of coral reef taxa, as well as the biotic and abiotic factors that govern how it is cycled.

7.4.3 Mesophotic reefs

To-date, no research has considered sulphur biogeochemistry in mesophotic reefs, thus the research presented in this PhD stands alone. Mesophotic reefs are receiving renewed interest from coral reef scientists and managers because they are linked physically and biologically to their shallow water counterparts, have the potential to be refugia for shallow coral reef taxa such as coral and sponges, and can be a source of larvae that could contribute to the resilience of shallow water reefs (Lesser et al. 2009a). In a future in which shallow water reefs seem destined to drastically diminished, these deeper reefs may play an important role in the global marine sulphur cycle. However, very little is presently understood with regards the production of DSC in tissues and seawater by mesophotic reef taxa, nor how climate change is likely to affect this dynamic. Laboratory-based studies, with strict controls on light levels, could be employed to assess contemporary DSC production and cycling in a range of mesophotic taxa, with further studies designed to manipulate pCO_2/temperature.

7.5 Summary and concluding remarks

In the past, coral reefs have been neglected within dimethylated sulphur research under the assumption that their relatively small area (0.5 % of the total ocean surface area) implies they are not an important component of the global marine sulphur cycle. The data presents here, however, suggests that coral reef sulphur cycling has the potential to restructure entire ecosystems from deep to shallow reefs and microbes to macrofaunal communities. Given the reliance of coral reefs for food, medicine, tourism and biodiversity, it could be argued that changes to the sulphur biogeochemistry of coral reefs could have far – reaching consequences. However, our continued exploitation of coral reefs necessitates a requirement to better understand the ecosystem processes involved. This research focussed on the likely effects of climate change (ocean acidification, elevated sea surface temperature) on production of DMSP/O in a range of coral reef taxa to better understand the possible impacts facing these vulnerable ecosystems under a changing climate. Furthermore, DSC research in coral reefs has been somewhat limited both by geographic location and species selected - this study aimed to provide more balance, by focussing efforts on Caribbean reefs and a variety of coral and non-coral taxa. This research has shown that production of DMSP by Caribbean reefs is comparable with other reef environments, i.e. the Great Barrier Reef or Red Sea. However, the high biodiversity and highly variable physicochemical environments found in coral reefs result in variability both temporally and spatially, even on local scales. Thus, DSC production and cycling in these habitats is mediated by biological (i.e. community composition, grazing activity) and abiotic (i.e. depth, light intensity, temperature) factors that are more complex than the relatively stable open ocean environment. In the future, increases in sea surface temperatures and decreases in seawater pH are likely to result in a loss of coral reefs and the transition to a fleshy/macroalgal dominated reef system. The consequences of this include reduced habitat for juvenile fishes and the loss of resources (i.e. medicines, food, money) for millions of people worldwide. However, the impact this will have on production of DSC has been, thus far, unclear. The research presented here suggests that reef waters will contain much higher concentrations of DSC, which is likely to be derived predominantly from seagrass/macroalgae but also from corals. The impacts of this extend beyond the marine environment; increased DMS in the water column is likely to drive net degassing of this

compound to the atmosphere, where it can seed new cloud growth and promote net cooling over reef areas. Other research has also pointed to the role of DSC as olfactory cues for birds (Nevitt 2000), fish (Dacey et al. 1994) and squid (Paul et al. 2008). Thus, increases in seawater DSC could increase numbers of these organisms in reef systems, subjecting the reef to increased grazing pressure or increasing predation of reef inhabitants by predators. Furthermore, increased production of DSC by reef organisms is likely to increase the abundance of pathogenic bacteria that could infect already stressed and vulnerable corals, thereby exacerbating the effects of climate change and potentially accelerating their decline. The impact of the loss of corals and increased coverage by fleshy/macroalgal species on sulphur cycling is one of the ecosystem services that must be considered.

This research ultimately provides a stepping stone to gain a broader understanding of the effects of climate change on DSC production and cycling, which may be used to inform further investigations into the possible effects of climate change on reef environments in the future.

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