Production of Dimethyl Sulphide (DMS) by Scleractinian Coral during Aerial Exposure – Effect of Temperature and Light

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Thesis Summary

In this thesis I investigated how rates of DMS production in three Scleractinian corals were affected by both aerial exposure and different light and temperature regimes. Coral specimens were acclimated over a 1 month period prior to data collection. Gas samples were collected from corals both prior to and during emersion in a hermetically sealed vial system and DMS was content measured via gas chromatography. Major differences in DMS production during emersion were observed between species; *Acropora inermis* production increased significantly upon exposure (from ~20 to ~600 nmol/h/cm^2) with *Turbinaria reniformis* also increasing but to a lesser degree. No significant increase was observed in *Porites cylindrica*. Prolonged acclimation to low light (~20 µmol photons/m²/s) resulted in a general decrease in DMS production in *A. inermis* and *Turbinaria reniformis* compared to control (~200 µmol photons/m²/s). The most dramatic effect was observed in *T. reniformis* where production was very low and in some cases not detected. The effect of temperature on DMS production was dependent on species and light, with either an increase, decrease or no measurable effect being observed. However the magnitude of this effect was smaller compared to other factors. Although interspecific differences in symbiont density, chlorophyll content and total DMSP were observed, no measurable effect of acclimation to light and temperature was recorded, suggesting that intraspecific differences in DMS production were not driven by changes in *Symbiodinium* physiology. The results of this study show that coral reefs exposed regularly at low tide can potentially act as significant contributors to the local DMS-flux. However, interspecific differences in response, as well as the effects of environmental factors, make predicting habitat-wide DMS production challenging. Further investigation into the mechanisms behind these responses is warranted to support potential reef-wide shifts in DMS production.

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I guess DMS isn't all that bad after all...

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Abbreviations

BVOC – Biogenic volatile organic compound

CCN – Cloud condensation nuclei

DMS – Dimethyl sulphide

DMSP – Dimethylsuphoniopropionate

DMSPt – Total DMSP

DMSPp – Particulate DMSP

DMSO – Dimethyl sulphoxide

GSS – Global surface seawater database

CTCL – Control temperature, control light

CTLL – Control temperature, low light

HTCL – High temperature, control light

HTLL – High temperature, low light

Introduction

Recent research has recognised that scleractinian corals generally contain high concentrations of dimethylsuphoniopropionate (DMSP) (Broadbent and Jones, 2004). DMSP is a sulphur compound that has multiple biological and physiological roles as well as being the precursor to dimethyl sulphide (DMS), a climatically active biogenic volatile organic compound (BVOC). With the threat of climate change on the horizon, and previous studies highlighting the susceptibility of corals to environmental disturbance (Donner et al., 2005, Hoegh-Guldberg, 1999) , it is important to understand how these organisms may respond to future environmental change in order to better understand the consequences for reef DMS production as a whole.

The role of DMS and DMSP

DMS is a volatile organosulphur compound formed through the cleavage of DMSP via cellular metabolism in bacteria (Malmstrom et al., 2004), algae (Steinke et al., 1998) and through higher trophic levels via predator-prey interactions with DMSP producing organisms (Lee et al., 2012). With the exception of a recent study on coral juveniles (Raina et al., 2013) and a study on a heterotrophic dinoflagellate *Crypthecodinium cohnii* (Uchida et al., 1996), the majority of known DMSP synthesis occurs in selected photosynthetic taxa including marine phytoplankton, seaweeds and a few intertidal higher plants such as *Spartina sp* (Stefels, 2000).

Even with nearly 30 years of research, the primary role of DMSP in these organisms is yet to be fully determined. One suspected role is that it helps support osmoregulation in maintaining cell solute concentrations (Dickson and Kirst, 1986), however other studies downplay the overall role of DMSP in this capacity, stating that intracellular DMSP levels

increase relatively slowly in comparison to other osmoregulatory compounds (Edwards et al., 1988). The overall role of DMSP in this instance may be to facilitate slower adaptation to long term salinity fluctuations, rather than a metabolic response to osmotic shock.

DMSP, DMS and other cleavage products such as acrylate have also been shown to scavenge potentially toxic reactive oxygen species (ROS) (Sunda et al., 2002). In this study marine phytoplankton *Thalassiosira psuedonana* and *Emiliania huxleyi* were exposed to a range of oxidative stressors including UV radiation and CO_2 limitation. The results showed substantially increased cellular DMSP concentrations and/or DMS production rates *in vitro*. However, the actual measures of oxidation scavenging potential were conducted outside of cell metabolism and may not translate directly *in vivo*.

Another role of DMSP that may seem counterintuitive is that DMSP produced by planktonic algae may actually help their predators locate them, with a recent study showing that the heterotrophic dinoflagellate *Oxyrrhis marina* exhibited a positive chemosensory response to DMSP (Breckels et al., 2010). The importance of DMSP as a signalling molecule has been observed in other motile phytoplankton, zooxplankton and heterotrophic bacteria species (Seymour et al., 2010). However, DMS may reduce the palatability of prey species to their predators in high concentrations (Alstyne et al., 2001, Wolfe et al., 1997). In the Alstyne et al study, measurements of increased DMS production during cellular damage and changes to predator feeding behaviour were observed in parallel rather than simultaneously.

In scleractinian corals in particular, DMSP has been implicated as an infochemical to attract beneficial microbial communities to coral surface membranes (Raina et al., 2010). Here this role is inferred via the overlap in the associated microbial communities' ability to metabolise DMSP as well as being strongly associated to healthy coral epifauna. While not conclusive that DMSP production is the sole driver of coral associated bacteria and viruses, it is likely a key component, providing DMSP production is conserved throughout the Scleractinia.

However a more recent study has shown that when corals are under thermal stress, DMSP may actually act as a biomarker for microbial pathogens that cause disease (Garren et al., 2013).

One of the key roles of organosulphur compounds such as DMS is the enrichment of terrestrial habitats with biologically available sulphur (Sievert et al., 2007). Sulphur is limited inland yet relatively concentrated in the sea, with the average sulphate concentration being 28mM in seawater. Microbial metabolism of DMSP into volatilized forms increases its availability for organisms without direct access to reduced biologically available sulphur. The contribution of DMS (via SO_2 release) to global sulphur output ranges between 17.6 and 34.4 Tg sulphur per year (Lana et al., 2011). In fact, DMS has been described as the most significant source of gaseous sulphur to the marine atmosphere (Bates et al., 1992).

The most publicised role of DMS and its derivative DMSP is its potential in the maintenance of climate at a near-equilibrium point. The basic idea outlined in the original CLAW-hypothesis (Charlson et al., 1987) is that as light irradiance on oceanic surface waters increases, the production of DMS would also increase as a function of higher photosynthetic microorganism activity (Fig 1). Once in the atmosphere, DMS oxidises to SO_2 and methane sulphonic acid. These compounds then combine with other gaseous atmospheric compounds to form particulates, which in turn act as cloud condensation nuclei (CCN) and contribute to cloud formation and also directly reflect UV radiation away from the earth's surface. With increased cloud cover, the percentage of incoming light that is reflected away from the earth is increased (albedo effect). With less incoming light, oceanic DMS productivity would also decrease. The result is constantly fluctuating levels of DMS, cloud cover and incoming solar irradiance around an equilibrium point, with the ability to compensate for additional incoming light with increased DMS output.

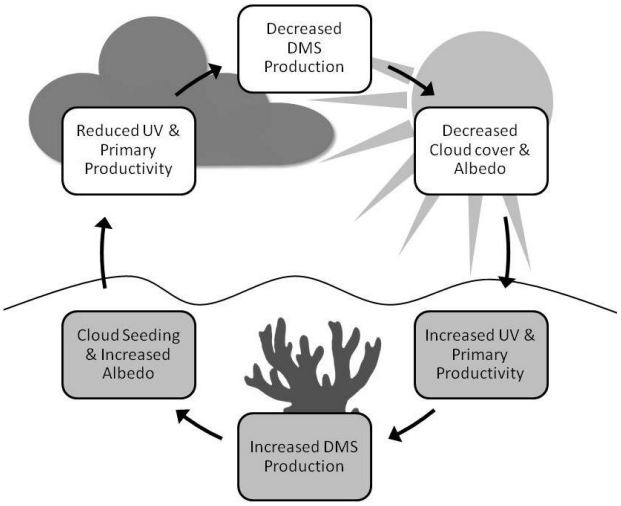

Fig.1. A schematic outlining the key concepts behind the CLAW hypothesis, outlining a potential negative feedback mechanism between DMS, primary productivity and cloud albedo

While elegant in its proposition, the CLAW hypothesis has been criticised for oversimplifying a complex and multi-faceted system (Quinn and Bates, 2011). One criticism is that the CLAW hypothesis overstates the magnitude of DMS and its overall contribution to global CCN levels. One simulation model suggested that for a 1% increase in DMS, only around 0.1% increase in CCN follows (Woodhouse et al., 2010). Other sources state that sea salt aerosols are more important to the contribution of marine CCN, with up to 50% of the particles in the 50-150nm size range originating from sea salt (Murphy et al., 1998). It is important to note at this point that whether DMS contributes to indirect atmospheric cooling is not in question, but whether or not the proposed mechanism of a self-regulatory system for maintaining a stable climate is likely to be true. Regardless of surrounding controversy, the CLAW-hypothesis helped garner interest and funding for investigating the role of DMS in global sulphur biogeochemistry, and has helped to bring attention to earth systems science.

A large scale meta-analysis between DMS and solar radiation data from the global surface seawater database (GSS) analysing over 26,000 individual DMS data points with satellite solar radiation data found a strong positive correlation across the globe (Vallina and Simo, 2007). However, while the pelagic solar irradiation data are complete, the DMS data does not cover all longitudes and latitudes. Additionally, data in the GSS comes from a wide range of investigators and may not be directly comparable, with the likely variability between DMS

data around 25% (Bell et al., 2011). Regardless, the high number of data points included in this analysis as well as the strength of the correlation provides solid evidence for the link between increased light and DMS production in the CLAW hypothesis. Previous studies have also highlighted a non-linear positive relationship between CCN and methanesulphonate, a DMS oxidation product (Ayers and Gras, 1991).

DMS production in the ocean is increased by grazing pressure of zooplankton on phytoplankton blooms (Dacey and Wakeham, 1986, Wolfe and Steinke, 1996). This provides a biotic link between higher light intensity and increased DMS production rates, where grazing pressure increases in response to higher food availability. Consequently, this adds another confounding variable in terms of DMS modelling for both short and long term ecosystem disturbance; even if phytoplankton blooms increase with warming temperatures and increased light, if zooplankton abundance is negatively impacted then this pathway for increased DMS-flux may begin to decouple.

Coral reefs and DMS

Hermatypic corals are well known as the key ecosystem architects of coral reefs, which subsequently support high biodiversity and biomass of other taxa such as fishes. Coral reefs provide a major source of income, ecosystem services and food to many human populations worldwide (Cesar et al., 2003). There are thought to be over 800 species of scleractinian corals, and while not all species are found in a single community, there is a remarkable level of local species co-existence within the most biodiverse regions. Many species of Scleractinia are defined by characteristics such as growth form, with massive slow growing species like *Porites spp.* being more adapted to longevity and survivorship. Other, fast growing species such as the branching *Acropora spp.* are more susceptible to environmental

fluctuations and storm damage (Marshall and Baird, 2000). These differences in life history strategy are linked to ecosystem functionality, with spatially complex species being associated with increased secondary biodiversity (Gratwicke and Speight, 2005). As such, there has been considerable research effort put into attempting to predict which species are more likely to survive in various long and short term patterns of disturbance and the subsequent consequences for reef-wide biodiversity.

One approach to investigating these questions is through eco-physiology experiments, where coral specimens are held in a mesocosm that simulates different environmental conditions. Changes to coral physiology are then measured, and the results used to infer potential changes in coral ecology. However, such studies are confounded by the presence of symbiotic algae and microbial communities associated with the coral host. Together

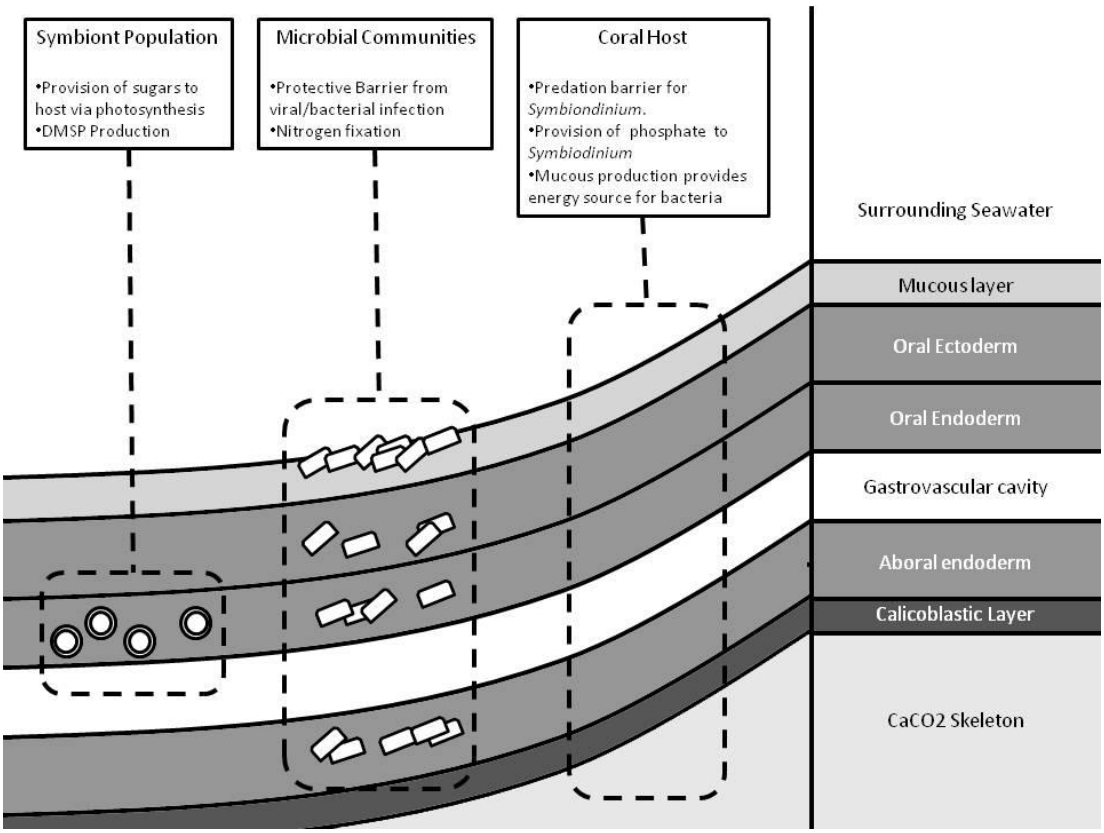

Fig 2. A simplified diagram of a typical coral holobiont showing the coral host, *Symbiodinium* population and microbial communities with descriptions of the role each organism plays in the symbiosis.

these three separate components form the coral holobiont (Fig 2.), with each component having important roles that benefit each other.

The dinoflagellate *Symbiodinium* sp. is one of the primary symbiotic algae in reef environments, with a range of host species including clams, sea slugs and perhaps most importantly scleractinian coral (Baker, 2003). Corals and their symbiotic algae are large reservoirs of DMSP and by extension potentially DMS. However, there are large differences in concentrations of DMSP between species (Broadbent et al., 2002). This is most likely due to high functional, genetic and phenotypic diversity not just within the coral tissues, but their symbionts as well. Genetic diversity within *Symbiodinium* is very high and phylogenetically separating all species would be a resource-intensive task (Baker, 2003). Therefore, strains of *Symbiodinium* are commonly separated into clades, with each clade representing an ecologically and/or functionally distinct strain. However, research into functional differentiation of *Symbiodinium* clades reveals that even closely related taxa can be widely different in their physiological function (Iglesias-Prieto and Trench, 1994). Examples of functional heterogeneities between strains include differences in thermal tolerance and/or growth rates. It is likely that these differences are based on an evolutionary trade-off for either host, symbiont or both depending on biogeography and growth environment (Jones and Berkelmans, 2011), where higher rates of primary productivity in the symbiont may translate to faster skeletal deposition, however this trait may be detrimental when the holobiont is under stress, increasing photo-oxidative stress and damage to the organism.

Concentrations of DMSP within *Symbiodinium* vary greatly between coral hosts, with *in hospite Symbiodinium* samples ranging in concentration from 36 (*Favites* sp.) to 7,590 (*Acropora palifera*) mmol L^{-1} Cell Volume (Broadbent et al., 2002). It should also be noted that DMSP concentrations here were measured from homogenised coral tissue, normalised

to *Symbiodinium* density. In this study, differences were observed between communities rather than explicit clades or genotypes. It is likely that the coral tissues harbour multiple strains simultaneously (Carlos et al., 2000), albeit at reduced relative abundances with typically one or two major clades dominating at around 90% of total abundance.

More conclusive analysis of strain-specific *Symbiodinium* DMSP/DMS levels has been determined via single strain cultures (Steinke et al., 2011). By investigating *Symbiodinium* outside of the host, cross contamination of DMSP/DMS from other sources is accounted for. However this also removes the interaction between the symbiont and coral host. One investigation found that across four tested clades (2 thermally tolerant; A1 & A2, and 2 sensitive; A13 & B1) DMSP and DMS concentrations were independent of their thermal tolerance characteristics under constant growth conditions. In this study both high- and low-tolerance species were found to have high DMSP concentrations and DMS-production profiles, which may call into question the hypothesis of DMS acting as a reactive oxygen scavenger. High temperatures are associated with increases in reactive oxygen species (ROS) production, and so it could be expected that concentrations of antioxidant DMSP and DMS would be higher in thermally tolerant strains, providing that DMSP/DMS have a key role in increasing thermal tolerance. However, this study did not actually subject the cultures to thermal stress, which is likely a key factor in DMSP/DMS stress physiology and may provide supporting evidence for the DMS ROS scavenger hypothesis.

Another study investigated the activity of DMSP-lyases in five strains of *Symbiodinium microadriaticum* (Yost and Mitchelmore, 2009*)*. Again, the level of activity varied drastically between clades, with one of the five strains exhibiting no capacity for DMSP lysis. Given the potential roles of DMSP breakdown products as ROS scavengers, the ability of a particular *Symbiodinium* clade to increase concentrations of these compounds through enzymatic cleavage may correlate with resistance to stress/bleaching.

The high genetic and functional variability of *Symbiodinium*, as well as differences in DMSP production, has major implications for reef derived DMS output as a whole. It is important to understand the dynamics of symbiont community diversity both within host-species and on biogeographical scales, especially as there can be pronounced spatial intracladal genetic diversity within the same host species (Santos et al., 2003). Without clear understanding of these aspects of *Symbiodinium* any large scale inferences of DMS productivity in this genus may prove difficult.

Returning to the role of the coral host, a recent study has upturned the commonly accepted paradigm that DMSP production is purely associated with photosynthetic organisms, showing asymbiotic coral juveniles synthesising DMSP (Raina et al., 2013). Although *Crypthecodinium cohnii* is a non-photosynthetic organism capable of producing DMSP (Caruana and Malin, 2014) , its phylogeny is derived from a photosynthetic algal ancestor and actually retains a vestigial and non-functional chloroplast. In this instance it is possible that DMSP is being synthesised via enzymatic machinery encoded in its chloroplast genome from its evolutionary history as an autotroph. In contrast, the study by Raina et al (2013) showed the first animal biosynthesis of DMSP as corals lack this legacy of once being photosynthetic, and the enzymatic machinery used in DMSP synthesis is likely to have evolved separately than those associated with photosynthetic organisms. Here, the absence of photosynthetic organisms was confirmed via PCR amplification that targeted a range of DNA markers from *Symbiodinium* specific to universal algal plastids, in addition to other methods including visual confirmation via microscopy.

The Raina et al study found that intracellular DMSP concentrations in aposymbiotic juveniles increased over time even when maintained in the dark. While it has been shown that DMSP synthesis can occur in the absence of light providing uptake rates of exogenous sulphate is high enough (Stefels, 2000) further highlighting that the coral host possesses an inbuilt

ability for this to occur. The work also suggested that host-derived DMSP synthesis continues into the adult stage, quoting increased DMSP even when algal symbiont populations were severely depleted through thermal stress, which may suggest a host mediated response to increased ROS. However, expression of a key DMSP producing enzyme, methyltransferase, is relatively low in adult colonies compared to initial juvenile levels, suggesting that host mediated DMSP synthesis may begin to slow as the coral reaches maturity.

How DMSP levels were maintained in both the absence of symbionts and reduced expression of methyltransferase may be explained if the mechanism of symbiont down-regulation in response to thermal stress was via apoptosis and digestion of the *Symbiodinium* cells as opposed to expulsion. In this scenario increased DMSP levels in coral specimens with reduced endosymbiont populations could be due to the liberation of DMSP previously locked up in algal cells into surrounding tissue. Although not investigated in the study, expression of methyltransferase may be up-regulated in the face of thermal stress. This study highlights the difficulties in disentangling the mechanisms and drivers of organosulphur dynamics within the coral holobiont, and should be considered when attempting to interpret such datasets.

The results published in the Raina et al (2013) paper offer a new way of interpreting previous coral-algae DMSP/DMS experiments. Referring back to the Broadbent (2002) paper discussing *Symbiodinium* DMSP levels, the high levels found within *Acropora palifera* may be explained by host-derived DMSP synthesis as the reported concentrations assumed that all DMSP production was via *Symbiodinium* production. An alternative hypothesis here may be that differences between the coral hosts, rather than the symbiote population, are driving the large differences in DMSP concentration. However in other cnidarian species, for example anemones, DMSP synthesis appears to be completely symbiont derived (Van

Alstyne et al., 2008). Why some symbiotic cnidarians seem to possess the enzymatic machinery to produce DMSP in the absence of endosymbionts and others do not is a key area of further research to supplement larger, habitat wide predictions/estimations of reef derived DMS output in the future.

Adding yet another layer of experimental difficulty to the study of organosulphur compound production in scleractinian and other cnidarian species, corals have a secondary symbiosis with ectodermic microbial communities. As mentioned previously, coral derived DMSP may be important for attracting and maintaining beneficial microorganisms (Raina et al., 2010). In this relationship, the bacteria utilise DMSP as an energy source, and are responsible for the cleavage of DMSP to DMS. In the absence of these micro-organisms, it is likely that the contribution to atmospheric CCN of DMSP-producing organisms would be severely diminished, along with the viability and survivorship of the colony itself. However, very high DMS levels as a function of thermal stress may attract harmful pathological bacteria such as *Vibrio sp.* (Garren et al., 2013). The potential of DMS acting as a chemical cue for negative species interactions is also supported by previous research on predator foraging being influenced by DMS release (Breckels et al., 2010).

DMS, Coral reefs and Environmental Change

It is now widely accepted that many ecosystems worldwide are already or will be affected by anthropogenic climate change in the coming decades and centuries. However, how local climate will change, and to what degree and on what timescale is still being debated. As climate change is a global issue, research has been diverse in scope and approach. The complexities of predicting how biogeochemical and ecological systems may or may not respond to long term changes make modelling efforts challenging.

When attempting to make predictions on how DMSP-synthesising organisms may respond to current and future environmental stressors it is important to understand how the biochemistry and physiology of how these organisms react to perturbations in their environment. Understanding the physiological effects of climate change on these organisms can better inform ecological predictions and potential effects on organosulphur output within a particular community.

Increased temperatures have been linked to a change in phytoplankton resource allocation, particularly with regards to protein synthesis (Toseland et al., 2013). The net impact is a shift in organism nitrogen and phosphorous ratios, resulting in a higher cellular demand for nitrogen. This exacerbates nitrogen limitation and could reduce the size of plankton blooms, and by extension increase the concentration of DMSP per cell, since DMSP is a preferred osmolyte under nitrogen limitation (Stefels, 2000). If increased temperatures also increase DMSP/DMS output per cell to combat oxidative stress, this may offset losses caused through reduced overall phytoplankton biomass.

In corals, increased heat and light can cause a phenomenon known as bleaching. In this case, high temperatures (2.3°C or higher above ambient) and high light act synergistically to increase ROS production (fig 3); with temperature increases higher than 2.3°C causing denaturing of the D1 protein of Photosystem II (Warner et al., 1999). The subsequent photoinhibition of PSII then contributes to the production of ROS, causing damage to the holobiont (Lesser, 1997). One proposed mechanism for how this progresses is that photoinhibition causes a build up of electrons in the thylakoid membrane of *Symbiodinium* chloroplasts which are unable to diffuse this excess energy through reemission (fluorescence) or heat dissipation, causing the formation of hydrogen peroxide and other ROS (Suggett et al., 2008). Under long term and/or extreme increases in light and temperature, the extent of down-regulation of photosynthesis in the symbiont increases

and bleaching occurs. One proposed theory of combating oxidative stress as suggested by Sunda et al. (2002) is that if DMSP and related compounds are active scavengers of ROS, and the production of these compounds is up regulated during stress, then DMSP production may help offset tissue damage through nullification of ROS.

Bleaching itself can be separated into a sub-lethal (type-II) or lethal (type-I) response (Suggett and Smith, 2011). In Type-II bleaching the symbiont cell counts and/or photo pigment concentrations are decreased by the expulsion/digestion by the host or potentially via host-mediated down regulation of symbiont photo activity. In this scenario, the coral itself may be able to recover and repopulate its symbiont assemblage once the environmental stress is alleviated. However, in the case of type-I bleaching, the animal tissue decouples from the skeleton, and dies off permanently. Interestingly, studies have shown that the symbiotic algal cells are still viable in the coral tissues that have sloughed away from the skeleton (Gates et al., 1992), suggesting that in these cases the host's physiology is more susceptible than the symbiont's. This may have implications for DMSP production where symbiont-produced DMSP is not increased even when the holobiont as a whole is undergoing a bleaching event.

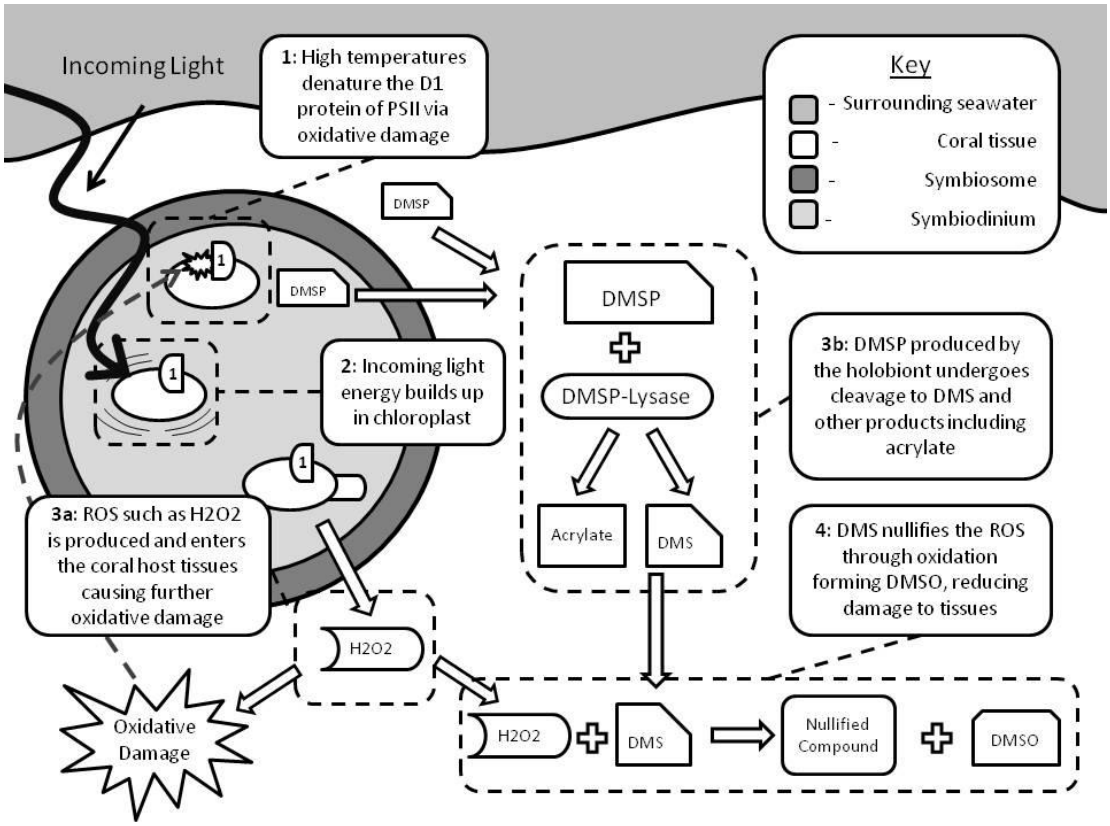

Fig 3. The process of photo-oxidative stress in coral, and the potential role of DMS as a scavenger of reactive oxygen species.

Temporally, the total and relative abundances of the *in hospite* symbiont population can change quite significantly as a function of seasonal fluctuations. A six year study on *Acropora formosa* symbiont populations found a seasonally driven pattern of density regulation, with population abundance peaking during the winter months between June to July each year (Fagoonee, 1999). This is likely a host-mediated response to the environment to avoid further oxidative stress during the high light/high temperature months of summer (Baird et al., 2009).

A recent investigation into how environmental stress can affect DMSP dynamics within corals revealed a more cohesive link between DMSP production, symbiote dynamics and coral bleaching (Jones et al., 2014). After a bleaching event, DMSP levels in *P. damicornis*

were increased post-bleaching and were strongly correlated with chl-a concentration. Interestingly, the high post-bleaching DMSP levels were independent of *Symbiodinium* density. However, considering the recent work outlining host-derived DMSP synthesis, understanding whether this response was primarily due to host or symbiont up regulation of DMSP is difficult. Regardless, this study adds further weight to the ROS scavenging hypothesis.

There is also a caveat with regards to tidal sea level changes and emersion of corals as a large yet inconsistent source of DMS production. When exposed to the air at low tide, large plumes of DMS are given off (Andreae et al., 1983), with values in this study up to 25 µg S(DMS) m^3. While typically exposure to the air is considered a form of stress for corals, in this case it may be a significant contributor to local sulphur cycling and cloud seeding. However, this study took samples from the marine air downwind of the reef and did not investigate further the coral community assemblage or actual rates of DMS production. Additionally, there may be a wide range of other taxa that may contribute to increased DMS production at low tide e.g. algae. Regardless, tidally driven release of DMS from reef habitats is significant, and yet little work to date has investigated the relative importance of different species nor the role light and temperature play in this context.

Aims and Objectives

From the literature it is clear that there are strong links between corals, DMS production and sensitivity to environmental changes. However there remain areas that have yet to be investigated fully, specifically the mechanisms and rates of production of DMS during low tide exposure to the air. While previous research has found evidence for strong up-regulation of reef DMS production at low tide, little is known about interspecific differences or how DMS production rates change temporally during the course of exposure. With links

between corals, climate change and the importance of DMS as a climatically active compound, beginning to understand the dynamics of this source of irregular, yet significant DMS in reef environments is important for future predictions of reefs under climate change scenarios.

This study investigates species specific differences in DMS production rates before and after exposure to the air, overlaying different light and temperature to investigate what effect these environmental variables may have. By investigating a range of taxa representative of different coral life histories and obtaining DMS production rates in a sealed system we can begin to tease out the contributions of different species within a coral community. From this we may be able to infer potential shifts in reef DMS production based on likely community shifts as driven by climate change and anthropogenic disturbances.

The specific objectives of this study are:

1. Construct a suitable system to quantify DMS production from air measurements.
2. Measure DMS production in a variety of scleractinian corals and investigate the effect of emersion, light and temperature.
3. Collect auxiliary data from coral specimens (e.g. chlorophyll content, *Symbiodinium* density, etc) to better inform the main data.
4. If any effects or differences are observed in DMS production, attempt to explain possible physiological mechanisms that drive them

Together, this approach will form a good base from which to begin to inform larger questions such as; differences in coral physiology and organosulphur production in environmentally stressed conditions, reef wide production of DMS and how coral community composition may affect it and potential consequences for organic sulphur transport in reef systems.

Materials & Methods

Coral Specimens

Three species of coral (order: Scleractinia) were used throughout the study; *Acropora inermis*, *Porites cylindrica* and *Turbinaria reniformis* (fig 4. a-c). These species were selected as they represent different life history strategies; *A. inermis* are a fast growing yet less resilient species, *P. cylindrica* is a slow growing species that can survive and persist in environments that other species may not. Finally, *T. reniformis* are an intermediate between the other two species, with larger polyps that suggest a higher instance of heterotrophic feeding. Colonies were imported via the Tropical Marine Centre directly prior to beginning the set-up for the experiments from wild stock from Fiji Island and maintained in the Coral Reef Research Unit at the University of Essex. For each species, a single colony was fragmented into smaller nubbins in order to reduce any effect of genetic variability. After an initial acclimation period of 2 weeks, the coral colonies were fragmented into 20 nubbins, tagged with an ID number

and affixed to a plug with epoxy.

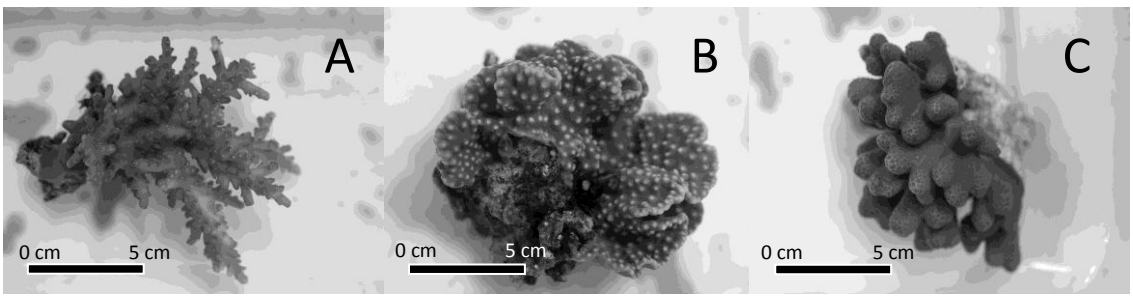

Fig 4 a-c. The three original coral colonies used in this study prior to fragmentation to smaller nubbins. From left to right: A - *Acropora inermis*, a branching acroporid coral usually associated with lower tolerance of stress and high growth rates, B – *Turbinaria reniformis*, a foliose/plate coral characterised by large and dispersed polyps that commonly suggest higher levels of heterotrophy, C – *Porites cylindrica*, a sub-massive species comprised of small densely packed polyps. Member of this genus are characteristically hardier and slower growing.

The nubbins were then allowed to recover and stabilise under normal aquaria conditions for 2 weeks of ~28°C water temperature and 0 to 200 µmol photons/m²/s light conditions on a 12 hr on/off cycle. The aquarium water used throughout the experiment was a mix of tropical aquaria salt with RO water that was in circulation through both a live rock sump and live reef aquarium set-up to help maintain healthy conditions for coral growth and survivorship.

Growth Environment and Maintenance of Specimens

After the recovery period, frags from each species were split into 4 experimental groups; Control Temperature Control Light (CTCL) (Light: ~200 µmol photons/m²/s, Temperature: 28°C), High Temperature Control Light (HTCL) (Light: ~200 µmol photons/m²/s, Temperature: 31°C), Control Temperature Low Light (CTLL) (Light: ~20 µmol photons/m²/s, Temperature: 28°C) and High Temperature Low Light (HTLL) (Light: ~20 µmol photons/m²/s, Temperature: 31°C). The aquaria light operated on a 12hr on 12hr off cycle throughout the experiment. For each group, 5 fragments were placed into the new growth environments in mesocosm aquaria all running on same water inflow system to avoid issues with variations in water chemistry across aquaria. All aquaria started at control conditions, with temperature and light changes being introduced slowly over the course of two weeks (1°C increase and 60 µmol photons/m²/s decrease per 4.5 days) until the desired conditions were met. This was to reduce the chance of shocking and causing mortality in the fragments as well as attempting to match a “real-world” scenario such as an ENSO event. After reaching the desired growth environment conditions, coral fragments were left for a period of 1 month to acclimate before collecting experimental data.

Sample Collection

After acclimation, coral frags were placed in 800ml Duran culture flasks fitted with inflow and outflow supply and sample lines that were purpose-built from OD 1.8 inch (3.2 mm) PTFE tubing and PTFE bulk-head fittings connected to the screw top lid. Flasks containing specimens were held in a water bath/lighting set-up matching the specimen's respective acclimation growth conditions. Flasks were filled with 400ml of pre-purged filtered aquarium water taken directly from the aquaria associated with the specimen. The vials also had an inlet and outlet tube that attached to a compressed air flow system consisting of two interchangeable modes; default with continuous out flow of air bubbled through the water column with the option to attach a tedlar bag for sample collection (fig 5a), or drain mode that pumped the water out, exposing the coral to air (fig 5b). In gas collection mode all vials received 60ml/min flow controlled through the use of stainless steel needle valves. Air flow was constant throughout the experiment; the air entered the vials through the water column at the bottom of the vial with an outflow tube at the top of the vial. As flow was constant, the system was hermetically sealed and checked for any leaks via submersion of the apparatus and through the use of leak detecting liquid, therefore excluding any influence of the surrounding environment on the experiments. The outflow tube could have a tedlar gas bag attached to it for collecting air samples for analysis. In drain mode, flow was reversed, with air entering from the top of the vial. The resulting build-up of pressure forced water through the tube at the bottom and to a drainage vessel. Modes could be switched without opening the vial and/or exposing the coral to the surrounding environment.

The system consisted of four vials; three for holding replicate coral frags and one for a control containing seawater media only for background DMS levels from the water sample. Specimens were held and acclimated overnight for 12 hours prior to sample collection to

settle the specimen after being transferred to the flask, as well as allowing the DMS production rate of the coral to reach equilibrium. For each experimental run, each specimen was analysed in sequence; with all data points (from immersion to 60 minutes of emersion) for a single specimen recorded prior to moving onto the next. To control for any effect of circadian cycling, samples were collected at similar times of day between sampling days starting at 10:00 and finishing at 16:00. Additionally, any residual DMS from the seawater-only flask was deducted from the final DMS concentrations for each respective specimen.

At the beginning of the sampling period a sample of the control vial was taken to check for residual DMS in the water column that would be additional to that produced by the coral. Then, outflow gas was collected from a coral specimen into a tedlar bag whilst the coral was still immersed in water for later processing. The system was then switched to drain the water from the vial and expose the coral to the air. Immediately following exposure, the system was switched and another gas sample collected. Further samples were collected in 20 minute intervals to give a time series of DMS production. Gas samples were processed during the interlude between sampling time points. Gas samples were collected over a period of 5 minutes at 60ml/min flow rate, giving a total bag volume of 300ml. In total five bag samples per specimen were collected. After the final sample collection, the vial was opened and the coral specimen was snap frozen in liquid nitrogen and stored at -80°C for later processing.

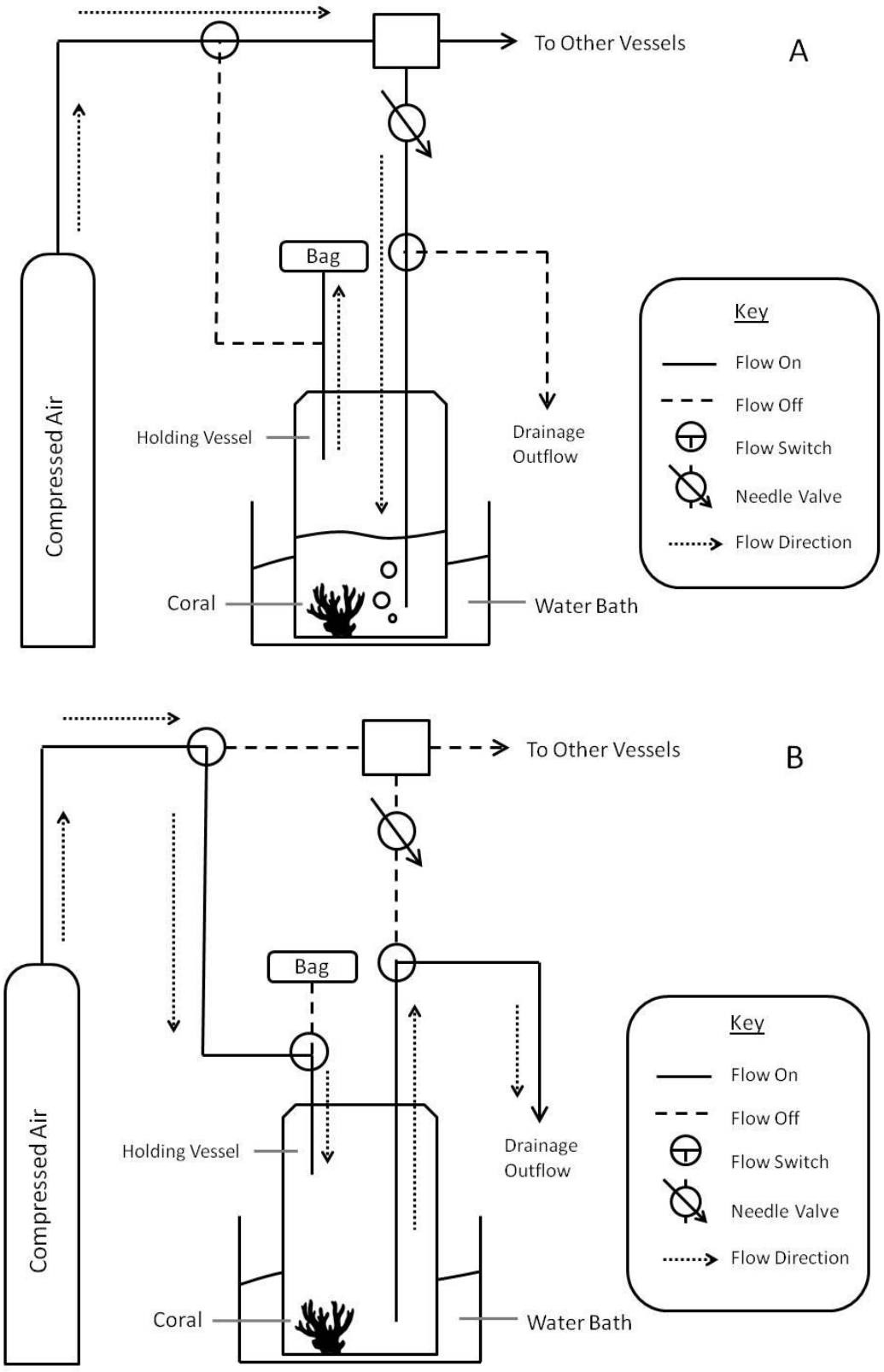

Fig 5 a-b. A schematic of the sealed flow air system for sampling DMS production in coral. A – Gas collection/normal mode: Here a constant flow of air enters the vessel which either exits the vessel into the surrounding air or can be collected in a tedlar bag for processing. B – Drainage mode: Here the system switches to allow the water in the vial to be drained without exposing the system to surrounding atmosphere, simulating a low tide event and exposing the coral to air.

Quantification of DMS production

Each 300ml gas sample was drawn through a purge and trap system to concentrate volatiles and increase signal. In “Trap” mode (fig 6a) the tedlar bag was attached to an inlet valve and the entire sample was pulled through at 60ml/min over a cryo-loop maintained at -160°C, freezing the DMS and other compounds whilst the rest of the sample was purged to the surrounding atmosphere. A bubble flow meter was used in conjunction with a control valve to keep track of and regulate flow. Pulling the sample through too fast would potentially stop some of the compounds from condensing on the loop in time. Once the sample was processed, the system was switched to “Purge” mode (fig 6b) and the cryo-loop was plunged into boiling water to liberate any volatiles including DMS. The sample was then flushed to a gas chromatograph for quantification.

For analysis of the pre-concentrated samples, a GC (GC-2010 Shimadzu Corporation) with flame photometric detector unit was used (FPD 2010-Plus). The GC was equipped with a 23m x 0.53mm x 5µm HP-1 capillary column (Agilent, Wokingham, UK). Instrument settings were 40°C column temperature, detector 175°C, purge flow off. Nitrogen gas was used to carry the sample into the column at 60ml/min. For the flame gases a mix of air and hydrogen were used at 60ml/min and 50ml/min respectively. Output data were analysed using Shimadzu GCSolution Workstation V2.0. Initial runs used a higher column temperature of 120°C, however a contaminant peak originating from the vial incubation system overlapped slightly, and so 40°C was chosen to increase the resolution between these two peaks. The contaminant was likely a sulphur compound leaking from the small amount of silicone tubing used as connectors in the vial set-up.

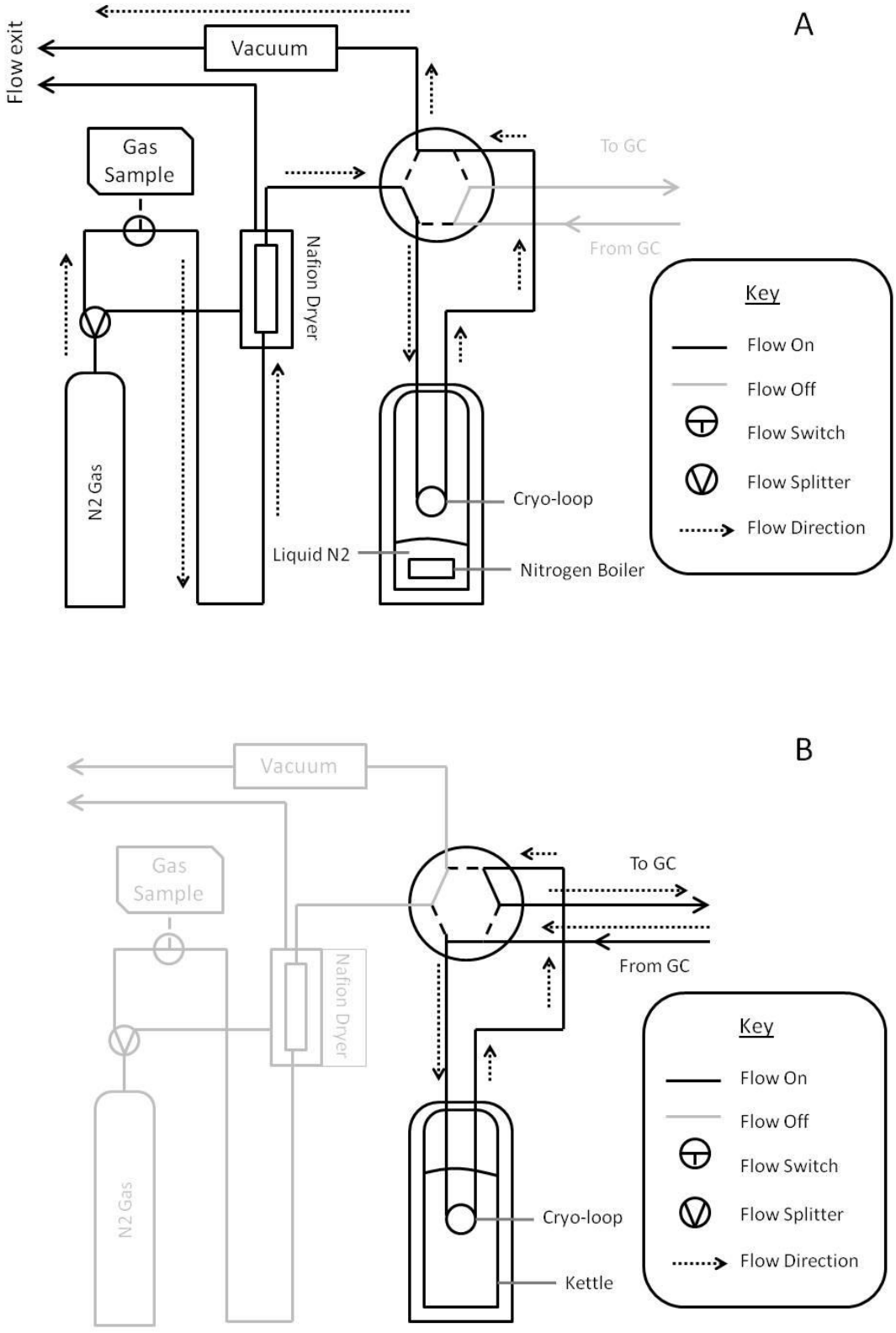

Fig 6 a -b. A schematic showing the purge and trap system used to process the gas bag samples. A – In "trap" mode, the gas sample is pulled through the cryo-loop, frozen and concentrated. When no sample was being pulled through, the system received constant nitrogen flow to keep the system clear. B – In "purge" mode, the cryo-loop is placed into boiling water, vaporising the frozen sample which is carried to the gas chromatographer (GC) for analysis.

To determine the concentration of DMS from the GC data, a series of calibrations were used to match the square root of the peak area with a known concentration of DMS as outlined in (Steinke et al., 2000). Calibrations were run by setting up a series of stock solutions of DMSP at a known concentration. DMSP and DMS are equimolar, allowing easy conversions for concentrations in a sample completely hydrolysed by sodium hydroxide to be calculated. Concentrations were increased in sequential as opposed to exponential increments as data point influence on the derived correlation co-efficient is higher with increasing distance from the rest of the data. As such, any errors/variation in such data would have a larger overall effect on the co-efficient, and therefore any concentrations derived from it. However, as previous calibrations were not inclusive of some rudimentary data early on, extra calibrations with higher stock concentrations were also run. For each calibration, a series of 4ml headspace vials were prepared; with two vials prepared from each of the 7 stock solutions including a MilliQ water blank. Each vial contained 2850µl of stock, with 150µl of 10M NaOH being added immediately before the vials were sealed. The vials were then incubated for 24 hours at 30°C to allow the NaOH to convert all of the DMSP to DMS and to equilibrate between the aqueous and gaseous DMS phases.

Following incubation each vial was analysed using the same settings as described above, with the headspace of each vial being flushed at 60ml/min for 90 seconds to ensure that the entire gaseous phase was collected on the cryo-loop prior to quantification. Using Henry's Law constants, it was possible to calculate the concentration of DMS that was processed, allowing a direct correlation between DMS concentration and peak area to be made. For simplicity, peak areas were square rooted as the response of the FPD is exponential in relation to increasing concentrations. By working with square root areas, a simple linear co-efficient can be used.

Coral Specimen Processing

Once all coral specimens had been processed, samples were removed from cold storage for processing. Frozen frags were blasted with high pressure nitrogen gas whilst submerged in 100ml of filtered artificial seawater as opposed to using a waterpik technique. This method was chosen to avoid wide differences in sample dilution based on species (*P. cylindrica* typically takes longer to strip away the tissue as the polyps are immersed in the skeleton more), and similar pressured gas methods have been used in recent studies. (Szmant and Gassman, 1990). The vessel chosen was high sided with a small opening so as to reduce homogenate loss to the environment. The resulting slurry produced little froth and so a homogeniser was not used. The homogenate was then used in a variety of extra experiments to gather information on DMSPt and DMSPp, DMSO, Chl-a, Chl-c1+2, *Symbiodinium* cell counts and cell volume. Additionally, the remaining coral skeleton was dried for later surface area estimations via wax dipping methods.

DMSPt, DMSPp and DMSO Measurements

Total and particulate DMSP was quantified from the homogenate along with DMSO. For DMSPt, 2ml of the homogenate was extracted in 1 ml of 100% methanol for 24h without filtration as this technique has previously found to yield values up to 2.8 times higher (Hill et al., 1995). Next 2850µl of the extract was transferred into a 4ml headspace vial. Just before the vials were sealed, 150µl of 10M NaOH was added to the vial to facilitate the cleavage of DMSP to DMS. Vials were incubated at 30°C for 24h in the dark. After incubation 60µl of headspace volume was directly injected into the GC column (GC 2010, see above for GC settings), and the DMS quantified. From the calculated DMS from the injection, the total

DMSP was calculated. After injection, the samples were placed back into incubation storage for later DMSO analysis.

DMSPp samples were prepared by filtering 10ml of homogenate onto GF/F filter paper at a low pressure (<25mm Hg). The filter was then placed in a falcon tube containing 5 ml of 100% methanol and refrigerated at 4°C for 24 hours in the dark to prevent photo-oxidation of chlorophyll. After the extraction period, samples were centrifuged at 400rpm for 15 minutes. This sample was then split for DMSPp and Chlorophyll analysis. For DMSPp, a 1 ml aliquot was transferred into a 4ml headspace vial. Just before sealing the vial, 1ml of 0.5M NaOH was added. Samples were incubated for 24h at 30°C and initially analysed using the same protocol for the DMSPt samples (See above).

Unfortunately, the DMS concentrations were so low in the samples that headspace injections failed to yield discernible peaks. An alternative protocol utilising headspace flushing was attempted, however further issues arose from the methanol solvent; where large a volume of the solvent would be trapped on the cryo-loop during the flushing period. This would occasionally cause blockages and when the sample was purged to the GC instrument, large solvent fronts would occur that eclipsed the DMS peak. Due to these complications, no DMSPp data was able to be recorded.

DMSO measurements were taken using the same samples after DMSPt analysis. Here, the headspace vials were purged for 10 minutes to clear out any DMS remaining in the vial. Next a 1g pellet of sodium borohydride was added to the vial and attached to the purge and trap system to convert DMSO to DMS, which was cryogenically trapped. The reaction was facilitated via gentle bubbling until the pellet was fully dissolved. Next, the sample was acidified by injecting 200µl of 20% HCl through the septa drop-wise to force the remaining aqueous DMS out of the sample. This technique quantified the total DMSO in the sample; however similar issues arose with the DMSPp measurements with the methanol solvent

front in the GC. Attempts to mitigate this issue with the DMSO measurements were made by attempting to drive off the methanol by leaving the samples under the fume hood for 24h. However, even after driving off the solvent, the issues persisted. Consequently no DMSO data were recorded.

Normalisation Indices: Chlorophyll, Algal Cell Counts & Volume and Surface Area

Additional data from the coral specimens were recorded for the purpose of normalising DMS production, as well as giving insights into the effect of growth environment on the symbiont population. Chlorophyll a and c1+2 were recorded using a 2 ml aliquot from the filter extraction used for the DMSPp analysis. For each sample a 2ml aliquot was placed in a quartz curvette and placed in a spectrophotometer (GENESYS 10S UV-Vis). Absorbance values from 190-1000nm with a 1nm interval were recorded. Absorbance values at 630 and 664nm were taken and, using the coefficients and formulae taken from (Ritchie, 2006), values for chlorophylls a and c1+2 were calculated. Chl-a values were used to normalise DMSP/DMS values based on the photosynthetic component for comparison to coral biomass estimations from surface area. Chl-c1+2 values were also taken to support potential effects of low-light.

Symbiodinium cell counts and cell volume measurements were taken using microscopy. Cell counts were conducted in a haemocytometer using material from the coral homogenate immediately after the coral was processed. This removed the need for cell preservation which has been shown to cause changes in cell volume. For each coral specimen, three cell counts were taken and averaged. Additionally, cell volume estimations were taken for the first 30 cells of each count. This was measured using a measuring eyepiece in the microscope calibrated to a graticule. For each cell, the diameter was recorded. From this, cell volume could be calculated based on the assumption that *Symbiodinium sp.* are

spherical in shape. Using average cell volume and multiplying the cell counts in the haemocytometer volume to the volume of the homogenate, values for total cell counts and algal biomass could be calculated and use for normalisation of other data.

Surface area estimations were made using the coral wax dipping method outlined in (Stimson and Kinzie, 1991). Dried coral skeletons were first weighed, and then dipped in melted paraffin wax maintained at 65°C for 2 seconds to match with the previous methodology. Deviations from 65°C would result in changes in the density of the wax and therefore the weight of the wax attached to the coral. Fragments were rotated during and after dipping to ensure even coverage of wax and to aid dripping of excess. After 15 minutes of drying time, fragments were inspected to ensure wax only covered to coral skeleton and not the holding plug. Any wax on non coral surfaces, i.e. the plug, was carefully removed with a scalpel. The skeletons were then weighed again and the difference in mass recorded. The dipping was then repeated for all corals and the mass difference between the first and second dip calculated. Surface area was then estimated using the formula and co-efficient from (Veal et al., 2010):

$$\text{Surface area (cm2)} = 34.32(\text{cm}^2/\text{g}) \times \text{mass difference between } 1^{st} \text{ and } 2^{nd} \text{ dip (g)}$$

The mass difference was the mass value from the second dip minus the first dip. This method has been shown to be very close in precision to x-ray CT scanning (Veal et al., 2010), without the associated cost and time constraints. The downside is that internal corralite meso-architecture details are lost and not accounted for with this method; however, this method has been shown to have an approximate spatial resolution of 2mm². This dataset was then used as the primary method of normalising DMS production, as well as giving values for symbiont density and algal biomass per cm².

Surface area was chosen as the primary normalisation index due to the fact that surface area roughly translates to coral tissue biomass, as well as the fact that DMS production is likely to increase along with surface area. Additionally chlorophyll-a was used as another normalisation metric as it is a direct indicator of primary productivity, and as such (and assuming DMSP production is primarily through photosynthesis) DMSP synthesis.

Data Processing and Statistical Analysis

All raw data were processed and arranged in a suitable format before values were imported into SPSS 20 (IBM SPSS Statistics V.20) for statistical analysis and preparation of figures.

Prior to analysis, data were checked for normality and transformed as required. As the homoskedasticity assumption was violated for between group variance, data were analysed primarily through a univariate type-III SS generalised linear model. Species, light and temperature treatment as well as sample timepoint were used as fixed factors, quoting likelihood ratio chi-squared values for significant differences. Initial models were full factorial with non-significant interactions and effects removed prior to running a final GLM to investigate significant interaction effects. Estimated means were compared using post-hoc contrasts with Bonferroni adjustment and 95% confidence intervals.

Results

1 – Overall DMS production.

Overall DMS production was calculated as the mean DMS production across all exposure stages. The range of DMS overall production across the three species was significantly different (SA: $X^2_{(2, 170)}$ = 143.427, P <0.01, Chl-a: $X^2_{(2, 170)}$ = 137.373, P <0.01), with *A. inermis* producing the highest mean DMS values in the CTCL group (fig 7) when normalised to surface area (519 ± 123 nmol/h/cm^2) and chlorophyll-a (77.54 ± 21.55 nmol/h/µg chl-a). The lowest mean values were observed in *T. reniformis* in the CTLL group, 0.71 ± 0.43 nmol/h/cm^2 when normalised to surface area and 0.03 ± 0.02 nmol/h/µg chl-a when normalised to chlorophyll-a. The range between the highest and lowest mean DMS production between treatment groups was also different depending on species. The range in *A. inermis* was greatest at 440 nmol/h/cm^2 or 73.33 nmol/h/µg chl-a. *P. cylindrica* had the smallest range at 14.5 nmol/h/cm^2 or 1.91 nmol/h/µg chl-a. *T reniformis* data ranges were between the other species, but closer to *P.* cylindrica than *A. inermis* at 56.9 nmol/h/cm^2 or 3.2 nmol/h/µg chl-a.

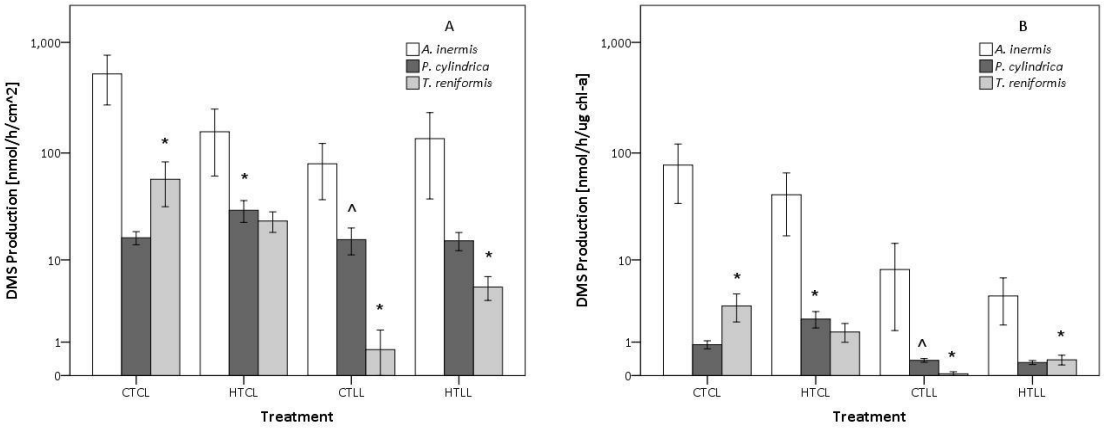

Fig. 7 – Mean overall DMS production of three species of coral; *A. inermis*, *Porites cylindrica* and *Turbinaria reniformis* normalised to surface area (Panel A) and chlorophyll-a (Panel B). Please note that the *y*-axis is on a logarithmic scale. Species were acclimated for 1 month to treatment conditions prior to data collection: CTCL – control temperature control light, HTCL – high temperature control light, CTLL – control temperature low light, HTLL – high temperature low light. N =15 except when marked by '*' where n = 14 or '^' where n = 9. Error bars show 1SE.

1.1 – Between species comparisons

A. inermis produced the highest concentrations of DMS of the three species independent of treatment or normalisation index (fig 7). Species were significantly different independent of treatment (SA: $X^2_{(2, 170)}$ = 143.427, P <0.01, Chl-a: $X^2_{(2, 170)}$ = 137.373, P <0.01), with additional significant interaction effects between species and light (SA: $X^2_{(2, 170)}$ = 24.830, P <0.01, Chl-a: $X^2_{(2, 170)}$ = 8.986, P <0.01), species and temperature (SA: $X^2_{(2, 170)}$ = 4.078, P <0.05, Chl-a: $X^2_{(2, 170)}$ = 1.806, P <0.01) and species, light and temperature combined (SA: $X^2_{(2, 170)}$ = 10.595, P <0.01, Chl-a: $X^2_{(2, 170)}$ = 8.046, P <0.01). In the control treatment, *T. reniformis* mean DMS production was higher than *P. cylindrica* regardless of normalisation index (p<0.01), with a mean difference of 11.2 nmol/h/cm^2 when normalised to surface area and 2.33 nmol/h/µg chl-a when normalised to chlorophyll-a. In the HTCL treatment, there was no significant difference in mean DMS production between *T. reniformis* and *P. cylindrica* regardless of normalisation index.

In the CTLL treatment however, *T. reniformis* DMS production was much lower than *P. cylindrica* (p<0.01), with *T. reniformis* producing the lowest recorded gross DMS values in the study at 0.7 nmol/h/cm^2 or 0.03 nmol/h/µg chl-a. Importantly, and more apparent in later figures (fig 8), in some cases a recorded zero concentration was measured, where no detectable DMS was in the sample. These cases are included in the analysis as they are deemed valid zeros.

For the HTLL treatment group choice of normalisation index had an effect on the analysis results; specifically, no significant difference was observed between *P. cylindrica* and *T. reniformis* when normalised to chlorophyll-a. However, when normalised to surface area, *P. cylindrica* was higher (15.7 ± 1.5 nmol/h/µg chl-a compared to 5.26 ± 0.76 5 nmol/h/µg chl-a, p<0.01). This is the only treatment group where a difference between normalisation indices has an observable impact on the outcome of the data when comparing between species.

Additionally, the *A. inermis* data in this group seems much lower when normalised to chlorophyll-a when considering all data.

1.2 – Within species comparisons

1.2.1 – A. inermis

A. inermis had the largest difference in mean DMS of the three species between treatment groups. However which groups the difference was between was dependent on normalisation index, with the largest difference between the CTCL and CTLL groups when normalised to surface area (440 nmol/h/cm^2 change) and the CTCL and HTLL groups when normalised to chlorophyll-a (73.33 nmol/h/µg chl-a change).

When analysed independent of temperature conditions, low light DMS production is significantly lower than control light ($p < 0.01$). However, further analysis revealed that this is only the case in the control temperature group (from 519.6 ± 123.7 nmol/h/cm^2 to 79.6 ± 21.17 nmol/h/cm^2, $p < 0.01$). When combining the effect of temperature and light treatment is taken into account, high temperatures resulted in reduced DMS production compared to control when in control light conditions (from 519.6 ± 123.7 nmol/h/cm^2 to 155.8 ± 47.1 nmol/h/cm^2, $p < 0.01$). No effect of temperature was observed in the low light group. Comparisons between control and HTLL groups revealed DMS production to be significantly higher in the control group ($p < 0.01$) although the combined effect of low light and high temperatures did not result in an accentuated effect of reduced DMS production when normalised to surface area but did when normalised to chlorophyll-a content.

In summary, for *A. inermis*, both high temperatures and low light result in reduced gross DMS production. However normalisation index appears to have an effect on whether this difference is synergistic when combined. This species also saw the largest difference in DMS production in response to light and temperature treatment.

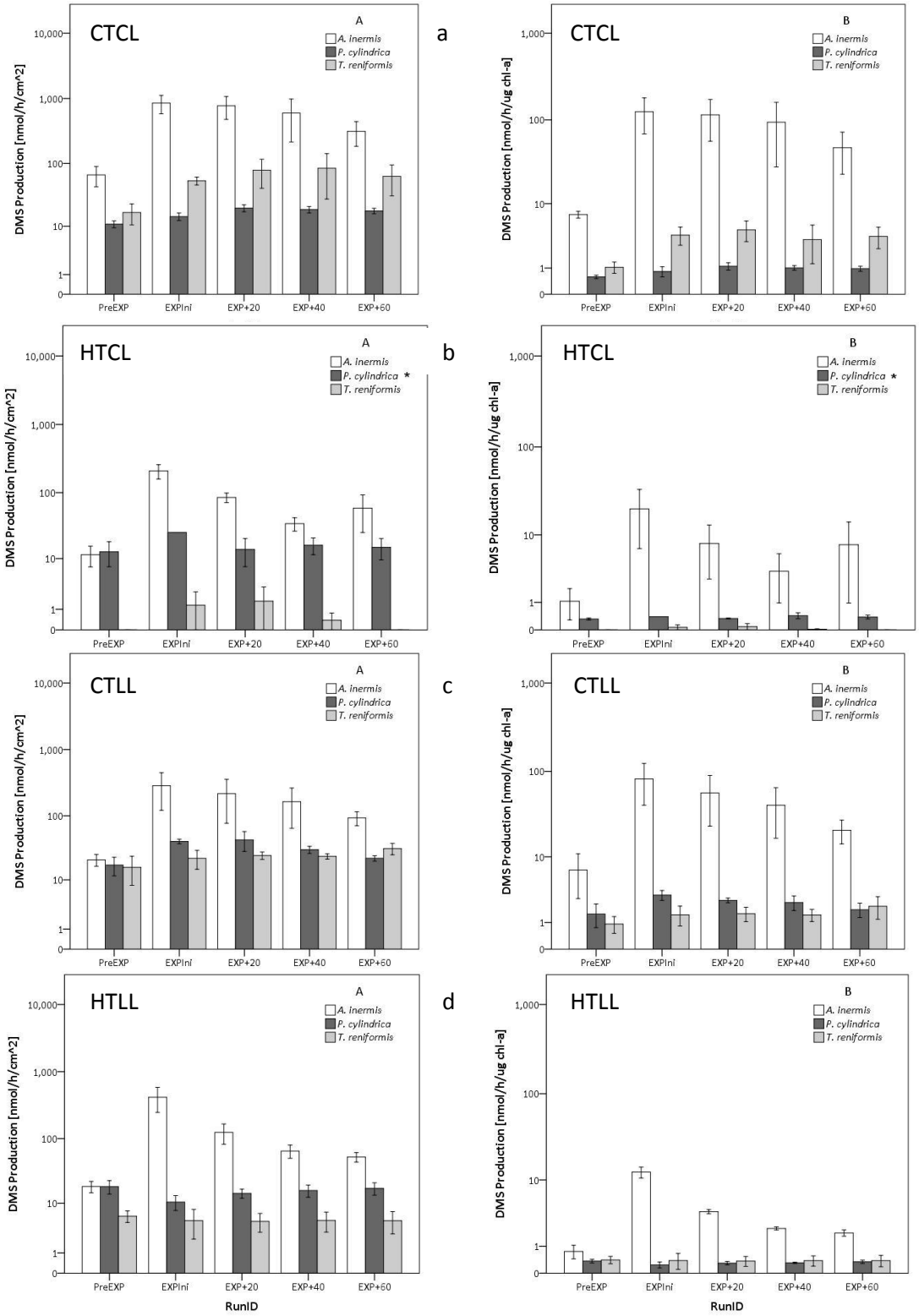

Fig. 8a-d – Mean DMS production of three species of coral; *A. inermis*, *Porites cylindrica* and *Turbinaria reniformis* over a period of aerial exposure normalised to surface area (left panels) and chlorophyll-a (right panels) across multiple acclimation treatments. a – CTCL, b – HTCL, c – CTLL, d - HTLL . Please note that the *y*-axis is on a logarithmic scale. X-axis labels are as follows: PreEXP – Prior to exposure, EXPIni – Immediately following exposure, EXP+x – Exposure plus time in minutes. n =3. Error bars show 1SE.

1.2.2 - Porites cylindrica

For *P. cylindrica* no independent effect of either light or temperature treatment was observed (fig 7), regardless of normalisation metric. However, when analysed together, low light resulted in lower mean DMS values than in the control in the high temperature group ($p < 0.05$), however the magnitude of this difference was rather small compared to other effects observed within the dataset. Additionally, normalisation index choice resulted in slightly different outcomes; when normalised to chlorophyll-a, high temperatures resulted in higher mean DMS values compared to control, than when in control light conditions (from 0.89 ± 0.78 nmol/h/µg chl-a to 2.22.8 ± 0.27 nmol/h/µg chl-a, $p < 0.05$). While this effect was not statistically apparent when surface area was used for normalisation, it is somewhat apparent from visual inspection of the data. The highest and lowest treatment groups for DMS production was the same in *P. cylindrica* regardless of normalisation index, with the biggest difference between the HTCL and HTLL groups (SA = 14.5 nmol/h/cm^2, Chl-a = 1.91 nmol/h/µg chl-a). In general, *P. cylindrica* gross DMS production was not affected by light or temperature treatments, with production rates remaining unchanged during the course of exposure

1.2.3 – Turbinaria reniformis

No major differences between normalisation index for *T. reniformis* was observed between datasets, with similar patterns and statistical outputs for all treatment combinations (fig 7). When independent of temperature treatment, low light DMS production was significantly lower than control ($p < 0.01$). In control light conditions, high temperature DMS production was lower than control ($p < 0.05$), although both of these treatment groups were higher than their low light counterparts. In low light, the effect of increased temperature was reversed, resulting in increased DMS production compared to control (from 0.71 ± 0.42 nmol/h/cm^2 to 5.26 ± 0.76 nmol/h/cm^2 $p < 0.01$). The largest difference in DMS production in *T. reniformis*

was the same regardless of normalisation index, with control and CTLL groups being the most different (SA = 56.9 nmol/h/cm^2, Chl-a = 3.2 nmol/h/µg chl-a). In general, *T. reniformis* lies in the middle of the three study species in terms of overall DMS production and stability in terms of response to treatment variables.

2 – Time series of DMS production during air exposure

2.1 – Between species effects

In general, DMS production rates in *A. inermis* were significantly higher than the other species (fig8 a-d) at most time points across all treatments (SA: $X^2_{(8, 170)} = 7.320$, P <0.01, Chl-a: $X^2_{(8, 170)} = 6.535$, P <0.01). It also appeared to be the species with DMS production most effected by exposure to the air, with significant increases in mean DMS between pre and initial exposure time points in all treatments (all p < 0.05), although the magnitude of this difference was dependant on treatment conditions. *P. cylindrica* DMS production did not increase significantly throughout the exposure period regardless of treatment. *T. reniformis* was similar to *P. cylindrica* with the exception of control conditions where exposure did have an effect on DMS production rates.

2.1.1 – Control conditions

A. inermis DMS production was significantly higher than both *P. cylindrica* and *T. reniformis* at all exposure time points in control conditions (fig 8a) when normalised to surface area (p <0.05) and chlorophyll-a (p <0.05) with the exception of *A. inermis* and *T. reniformis* prior to exposure when normalised to chlorophyll-a. Comparing *P. cylindrica* and *T. reniformis*, *T. reniformis* produced more DMS across all aerial exposure time points (p <0.05). However, pre-exposure mean production values between the two were similar. This was the same response regardless of normalisation index. How DMS production changes over time was also different between species, with *A. inermis* increasing rapidly at initial exposure before

maintaining similar production rates over the course of exposure (SA = from ~90 to ~900 nmol/h/cm^2, Chl-a = from ~9 to ~110 nmol/h/µg chl-a. *T. reniformis* also increased somewhat rapidly on exposure, albeit less pronounced than *A. inermis*. However, DMS production in this species did not begin to decline after 60 minutes of exposure. *P. cylindrica* was largely stable, with little detectable increase in DMS production compared to pre-exposure levels

2.1.2 – High temperature, control light conditions

With the exception of the pre-exposure time point when normalised to surface area, *A. inermis* DMS production was significantly higher than the other species (fig 8b) in all other time points, regardless of normalisation metric ($p < 0.05$). However, the magnitude of this difference was affected by normalisation, with surface area closing the difference between the *A. inermis* data and the other species. When normalised to chlorophyll-a however, the differences were more accentuated. Comparing *T. reniformis* and *P. cylindrica* had shown no discernible difference between the two at any time point, regardless of normalisation. In terms of response over time, *A. inermis* was similar to control conditions, as was *P. cylindrica*. However, *T. reniformis* didn't significantly increase DMS production upon aerial exposure compared to pre-exposure levels as previously noted.

2.1.3 – Control temperature, low light conditions

In the CTLL group more noticeable differences in the data based on normalisation index were apparent (fig 8c), specifically, *P. cylindrica* DMS production values were closer to *T reniformis* when normalised to chlorophyll-a, and closer to *A. inermis* when normalised to

surface area. The other two species did not appear to be effected by normalisation index choice to the same degree, with the overarching trends remaining similar throughout.

When normalised to surface area, *A. inermis* was significantly higher than *T. reniformis* at all time points ($p < 0.01$). This was effectively the same when normalised to chlorophyll-a, with the exception of the pre-exposure group. *A. inermis* was only significantly different from *P. cylindrica* at initial exposure and after 20 minutes of exposure ($p < 0.01$) when normalised to surface area. The same differences were observed when chlorophyll-a was used for normalisation.

The most dramatic difference in this treatment group based on normalisation index was between *P. cylindrica* and *T. reniformis*. When normalised to surface area, *P. cylindrica* DMS production was consistently higher at all time points ($p < 0.01$). However, when normalised to chlorophyll-a, although visual inspection of the data suggests a similar trend, no statistically significant differences between the two were observed. *T. reniformis* DMS production in this treatment group was close to zero in a lot of cases, with some "legitimate zero values" recorded for the initial, plus 20 and plus 40 minutes of exposure. In general, DMS production trends over the course of exposure for each species were similar to control conditions, with the major difference being in terms of decreased DMS production at each time point.

2.1.4 – High temperature, low light conditions

DMS production prior to exposure was not significantly higher for any species regardless of normalisation index in the HTLL group (fig 8d). In all following exposure time points, *A. inermis* was significantly higher than both *P. cylindrica* and *T. reniformis* regardless of normalisation index ($p < 0.05$). When normalised to chlorophyll-a, *P. cylindrica* and *T.*

reniformis data were very similar, with both producing low levels of DMS around 0.5 nmol/h/µg chl-a. This trend was statistically the same when normalised to surface area, although mean production values for *P. cylindrica* were consistently higher at all time points, albeit not drastically. DMS production trends over time in this treatment for each species was similar to the HTCL group, with the primary difference being *T. reniformis* not increasing production over the course of exposure.

2.2 – Within species effects

2.2.1 – A. inermis

The biggest amplitude of DMS production in this species was the control group (fig 9a), where the initial increase during exposure was 117.4 nmol/h/cm^2 followed by a consistent drop over the exposure period of 77.2 nmol/h/cm^2 the same response was observed when normalised to chlorophyll-a. A similar trend was observed for both HTCL and CTLL groups, regardless of normalisation metric, albeit reduced in amplitude. The HTLL group responded differently depending on normalisation metric. When normalised to surface area, the HTLL group had the second largest single increase in DMS production between pre and initial exposure, and also saw the fasted post-peak decrease in DMS between initial exposure and 20 minutes exposure time points. However, when normalised to chlorophyll-a, the HTLL group became the lowest average producer of DMS, as well as having the lowest amplitude of production difference over time.

In general, this species responded in a similar trend in all treatment groups, with the major differences occurring in the range of production values. Specifically, low light resulted in reduced production both initially and during the exposure period when normalised to chlorophyll-a, although this difference is not as strong when normalised to surface area.

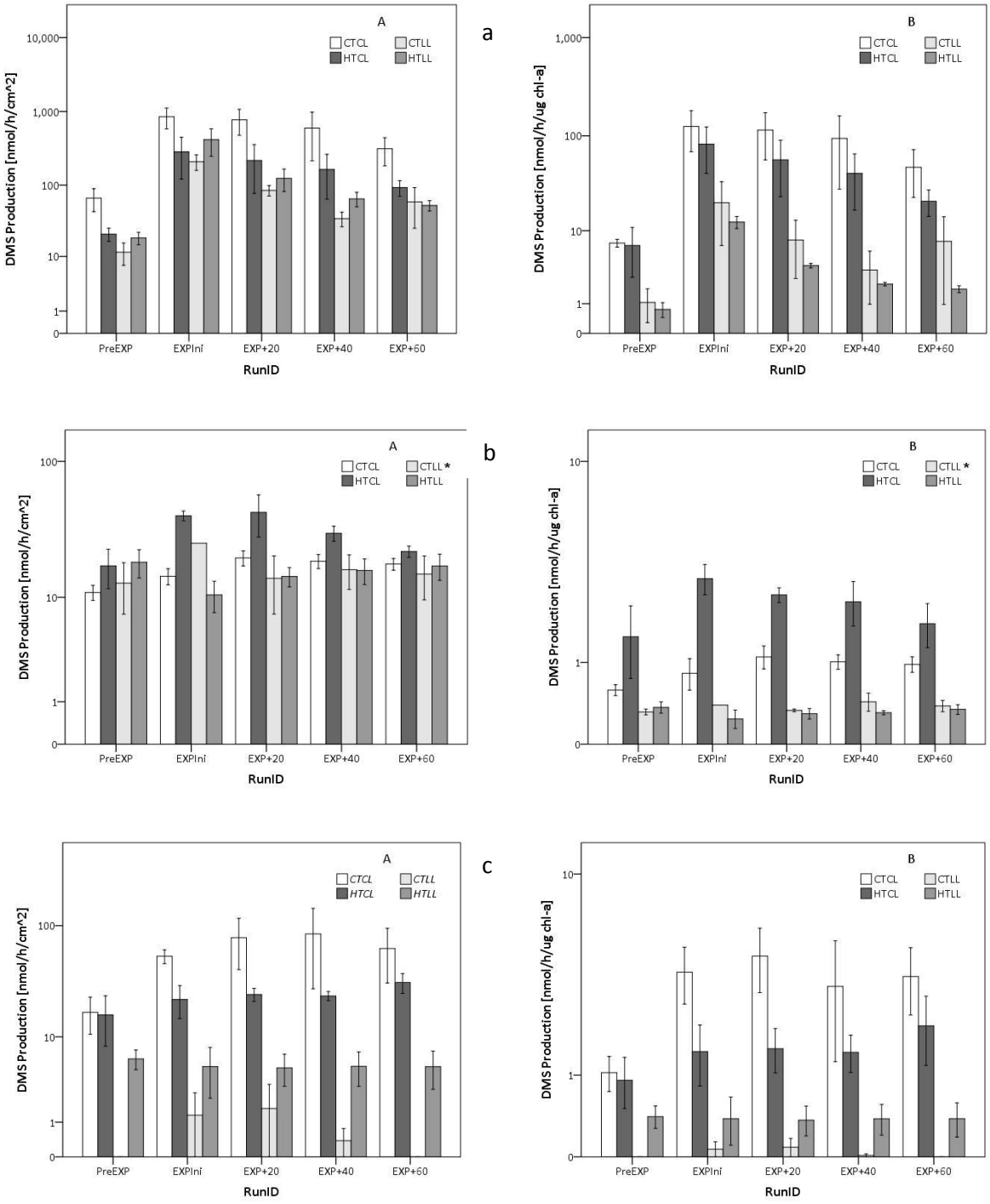

Fig. 9 a-c – Mean DMS production for three species of scleractinian coral over a period of aerial exposure normalised to surface area (left side) and chlorophyll-a (right side). a – *A. inermis,* b – *Porites cylindrica*, c – *Turbinaria reniformis*. Please note that the *y*-axis is on a logarithmic scale. Species were acclimated for 1 month prior to data collection as follows: CTCL – control temperature control light, HTCL – high temperature control light, CTLL – control temperature low light, HTLL – high temperature low light X-axis labels are as follows: PreEXP – Prior to exposure, EXPIni – Immediately following exposure, EXP+x – Exposure plus time in minutes. n =3. Error bars show 1SE

2.2.2 – Porites cylindrica

Regardless of normalisation index, no difference between any exposure time points within treatment group was observed in this species (fig 9b). Additionally, comparisons between treatment groups at each time point yielded no discernible differences. Essentially, there was no effect of treatment or exposure time on *P. cylindrica*.

2.2.3 – Turbinaria reniformis

Aerial exposure only significantly increased DMS production in the CTCL group ($p < 0.05$), regardless of normalisation index (fig 9c). In all other groups, DMS production was essentially the same across the entire exposure period. However, the HTCL group suggests that production may continue to increase gradually over time. Both low light treatments are essentially the same independent of temperature. Pre exposure production data was significantly lower in the CTLL group compared to the control group ($p <0.01$).

3 – Total DMSP content

3.1 – Between species effects

The highest DMSPt values were in the *A. inermis* HTCL group at around 3 µmol/cm^2 (fig 10), which matched to the values obtained by Broadbent et al (2002) for *Acropora palifera.* *P. cylindrica* and *T. reniformis* DMSPt concentrations were the same in each treatment group, regardless of normalisation index at 0.8 µmol/cm^2 when normalised to surface area. For *A. inermis*, the only notable difference when normalised to chlorophyll-a was in the HTCL group ($X^2_{(2, 23)} = 6.150$, $p <0.05$), with DMSPt being much higher compared to the other species. In all other groups however no observable difference between species was

apparent. However, visual inspection suggests that *A. inermis* may be higher if standard errors were reduced. When normalised to surface area however, *A. inermis* DMSPt was consistently higher than the other two species ($X^2_{(2, 25)}$ = 51.510, p <0.01), across all treatment groups, being roughly double the concentration (average 2 µmol/cm^2)

3.2 – Within species effects

In *A. inermis,* when normalised to surface area, DMSPt concentrations were only significantly different between the control and high temperature groups in control light ($X^2_{(1, 23)}$ = 6.202, p <0.05). High temperatures increased mean DMSPt values by 1.11 µmol/cm^2. When normalised to chlorophyll-a, the HTCL treatment group was significantly higher than both the CTCL and CTLL groups (p <0.01). No significant differences in DMSPt within either *P. cylindrica* or *T. reniformis* were observed regardless of treatment or normalisation index.

4 – Auxiliary data: Symbiodinium *population dynamics and chlorophyll content*

4.1 – Average Symbiodinium *cell volume*

In control and HTCL conditions, *P. cylindrica* average cell volume (fig 11 a) was significantly lower than the other two species (p <0.01). No significant differences were observed between *A. inermis* and *T. reniformis*. In the CTLL group, *T. reniformis* cell volume was

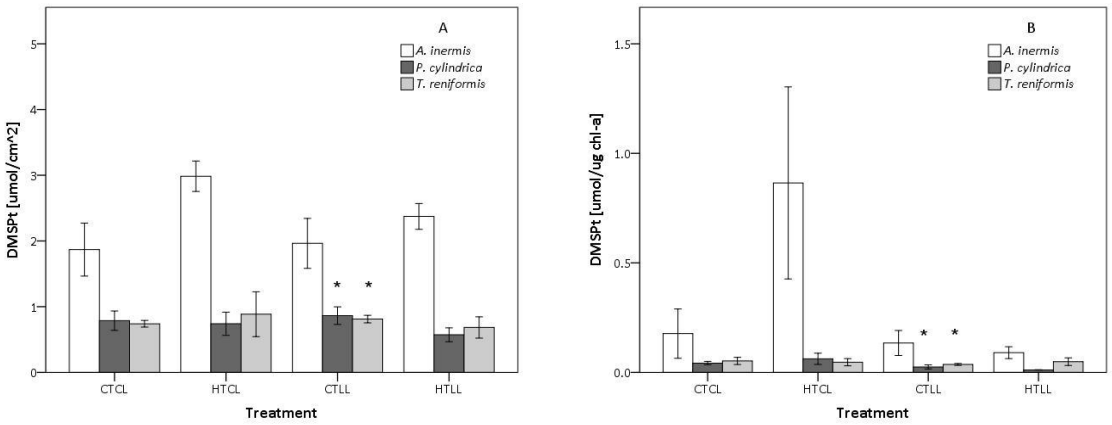

Fig. 10 – Mean DMSPt for three species of coral; *A. inermis*, *Porites cylindrica* and *Turbinaria reniformis* normalised to surface area (left panel) and chlorophyll-a (right panel). Species were acclimated for 1 month prior to data collection to treatment conditions: CTCL – control temperature control light, HTCL – high temperature control light, CTLL – control temperature low light, HTLL – high temperature low light. n= 3 except when marked by '*' where n= 2. Error bars are 1SE.

significantly lower than the other two species ($p < 0.01$), with no observable difference between the other species. No differences were observed between any species combinations in the HTLL group.

In *A. inermis*, low light had a slight, yet significant effect on cell volume, with reductions in mean values of 52 μm^3 between control temperature control light and control temperature low light ($p < 0.01$). High temperatures resulted in increased cell volumes, but only in the low light groups ($p < 0.05$) *T. reniformis* cell volume changes were similar to *A. inermis,* albeit slightly more greater in magnitude. *P. cylindrica* cell volumes were not affected by temperature or light treatment in any case.

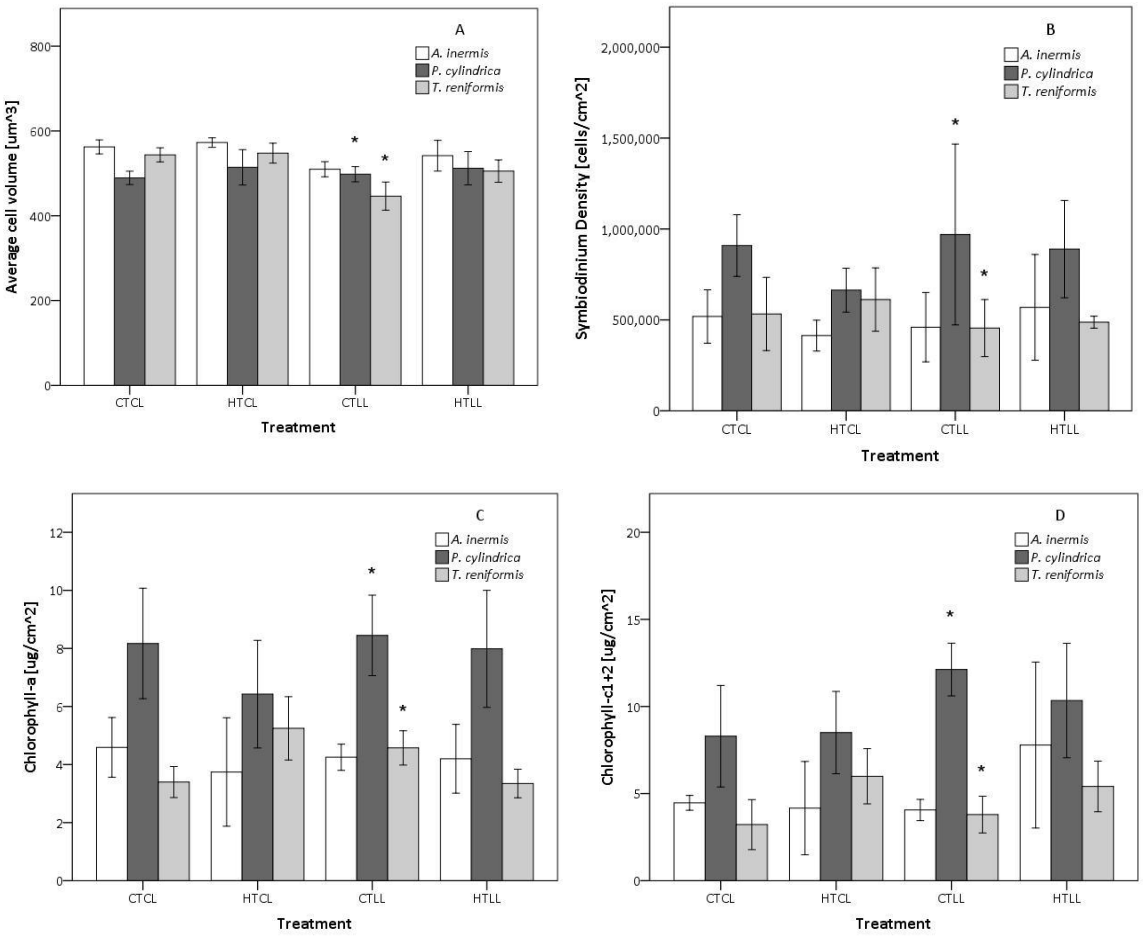

Fig. 11 a-d – Mean data for multiple auxiliary datasets for three species of coral; *A. inermis*, *Porites cylindrica* and *Turbinaria reniformis*: a – Average cell volume of *Symbiodinium*, b – *Symbiodinium* density, c – Chlorophyll-a, d – Chlorophyll-c1+2. All data normalised to total surface area with the exception of cell volumes. Species were split into treatment groups for 1 month prior to data collection to treatment conditions: CTCL – control temperature control light, HTCL – high temperature control light, CTLL – control temperature low light, HTLL – high temperature low light. N=3 except when marked by '*' where n=2. Error bars are 1SE.

4.2 – Symbiodinium Densities and Chlorophyll Content

No significant differences in *Symbiodinium* density or chlorophyll content were observed between species in any treatment group (fig 11b-d). Additionally, no differences were observed between treatment groups within species. However, *P. cylindrica* chlorophyll content appears to be slightly higher on average than the other two species, despite not being significantly different statistically.

Discussion

DMS Production under control conditions

The high levels of DMS production observed in *A. inermis* in comparison to the other species during emersion suggests key intraspecific differences in physiology and response to aerial exposure. While baseline production prior to exposure was marginally higher in some cases for *A. inermis*, on the whole it seemed consistent with the other species under control conditions. The mechanism behind this significant increase in production relative to the other species should be explored further in order to improve our understanding of coral DMS production under fluctuating environmental conditions.

Coral mucus as a source of DMS?

An increase in net DMS production could be attributed to either of the holobiont components (symbiont, coral, bacteria) in isolation or may be the result of more complex changes in the interplay between each component. Since much of the bacterial DMS production will be associated with coral mucus, it is possible that the effect of emersion on mucus production may stimulate DMS output. Mucus production in corals is estimated to account for up to half of the carbon assimilated through primary production (Crossland et al., 1980). It has also been documented in other species of *Acropora* that DMSP concentrations in mucus is extremely high, compared to other coral taxa (Broadbent and Jones, 2004). One hypothesis for the post exposure peak in DMS could be that the microbial communities associated with *A. inermis* metabolise the DMSP, rapidly converting it to DMS. A previous study investigating coral mucus membranes as a secondary source of carbon showed that O_2 consumption rates in the mucosal films are upwards of 10-times faster compared to the

surrounding seawater, as a function of the 100-fold higher bacterial abundance in the mucus (Wild et al., 2004).

However, this fails to explain the observed increase in DMS production upon exposure. The water samples for each specimen were purged free of DMS prior to bag sample collection, and so any DMS collected when submerged should be representative of the production rate for the coral. Mucus production has been observed to rapidly increase during low tides (Krupp, 1984), therefore it may be plausible that the spike in DMS is through its rapid volatilisation to the environment from the extra mucus produced. In normal conditions, the free DMS in the coral tissues first has to go through the mucus barrier and then the surrounding environment; however, if mucus production is rapidly increased during exposure then this new mucus may shuttle DMS from the coral to the surface, where it can volatilise to the air.

The other species, however, reacted differently compared to *A. inermis*. For *P. cylindrica* DMSP production remained unchanged throughout the exposure period in most cases, with only marginal increases in some treatment groups. Whilst this may be somewhat explained by lower DMSPt per unit area in *P. cylindrica*, the fact that no biologically significant change in production occurred at all suggests mechanistic differences in physiology between the two.

P. cylindrica is typically considered a longer lived and hardy species capable of resisting oxidative damage. It could be hypothesised, given the apparent role of DMSP and its breakdown products as antioxidant scavengers, that DMSP concentrations would be higher in this species. However, *P. cylindrica* may combat oxidative stress by down regulation of its symbiont population (Smith et al., 2008) or by harbouring *Symbiodinium* clades that are inherently less productive, yet resistant to oxidative photosystem damage (Fitt et al., 2009).

The density of *Symbiodinium* in *P. cylindrica* appears to be higher than for other species, going against the hypothesis that the lower DMS production is due to down regulation of symbiont populations. And while it may be plausible that the *Symbiodinium* in *P. cylindrica* simply produces less DMSP, which is somewhat supported by the data, the extreme difference in response between species suggests another mechanism driving these differences.

While the spike in *A. inermis* may be explained by increased mucus production shuttling extra DMS to the surface, why is this effect not observed in *P. cylindrica*? The mucus barrier has many useful functions in protecting the coral, especially when exposed to the air as it prevents desiccation. In this case either the mucus barrier in *P. cylindrica* is already present in enough quantity by default so as to not warrant increased production, or *P. cylindrica* simply does not increase mucus production when exposed. While the latter hypothesis would explain the data clearly, it is not likely as the mucus serves an important protective function and is likely conserved in hardier species. Instead, *P. cylindrica* most likely invests in a thicker, longer lived mucosal membrane by default, and so no excess shuttling of DMS occurs upon exposure. Alternatively the composition of the mucus may be different between species. One study has previously found that coral mucus lacks any common structure (Meikle et al., 1988) which is likely a function of different types and histological locations of mucus producing cells (mucocytes) (Brown and Bythell, 2005). In this scenario, *P. cylindrica* still produces extra mucus when exposed, but the mucus produced actually contains little to no DMS/DMSP, instead the DMS produced originates in the coral tissues, and slowly diffuses into the surrounding atmosphere.

DMS from bacterial associations?

It is likely that microbial communities associated with the coral use DMSP as an important source of energy and carbon. Previous work on bacterial community associations have shown strong evidence for host specificity of associated bacterial taxa with coral mucus of different species (McKew et al., 2012). If DMSP is a strong attractant for microbial communities and these communities are different between the study species, then this may explain the interspecific differences observed during this study. While there is a lack of empirical support at this stage, a plausible explanation for the differences between species may be through widely different metabolic rates of DMSP-lyase activity between them. Or alternatively through increased concentrations of bacterial inhibitors in the mucus itself, as suggested by one study that observed that bacterial rates of mucosal consumption was higher in more dilute samples compared to more concentrated (Vacelet and Thomassin, 1991). There may be some component of the mucus that inhibits consumption, which makes sense as constantly replacing mucus sheets may become costly to the organism.

T. reniformis appears to be an intermediate species between the other two, and while its rates of DMS production are much closer to *P. cylindrica* it is closer to *A. inermis* in terms of showing a measureable response to aerial exposure. These properties, combined with the similarities between how *A. inermis* and *T. reniformis* responds to the acclimation variables suggests that these two species are functionally similar in the context of response to aerial exposure.

These species-specific responses suggest taxonomic variation in the potential for tidally driven plumes of DMS release. As such any changes in coral community composition from High to low DMS producing/releasing species would correspond to a dramatic change in DMS production of reefs, given the data presented in this study. Considering the previous research into coral mortality responses to environmental stressors, it may be likely that the

highly productive *Acorpora* species may be lost and replaced with hardier, less productive species. Such a dramatic loss of DMS production may have major implications for local organic sulphur biogeochemistry as well as local cloud seeding capacity.

Light as a modulating variable for DMS production

Light was shown to have a major role in the modulation of DMS production in *T. reniformis* and *A. inermis*, with *P. cylindrica* only showing this effect when normalised to chl-a. One possible explanation as to why DMS production was lower in very low light conditions may be through reduced lipid production. One study investigated the role of light and mucus production and found that the rates of DOC-lipid release during the night were 55% of those during daylight hours (Crossland, 1987), this is likely through lower/zero photosynthesis due to *Symbiodinium* being the primary site for lipid synthesis in coral (Crossland et al., 1980). Also to note is that extended acclimation to low light environments had no effect on total DMSP in any of the species, which suggests that the lower DMS production rates were not due to reduced DMSP production.

A possible explanation for the observed effect of low light is through reduced mucus production and, therefore, lower degradation rates by bacterial communities to DMS. While this effect is pronounced in *A. inermis,* in *T. reniformis* the result (at least in control temperatures) was an almost complete cessation in production in some incubations. Again, total DMSP in this species was similar across all treatments so why *T. reniformis* responded in this way is not likely through a halt in DMSP production through photosynthesis. One explanation as to why DMSP levels were still high even in the supposed absence of photosynthesis could be through host-derived synthesis of DMSP. If both species have the

enzymatic machinery necessary for DMSP production, and that these are still expressed well into the adult stage, then it may be possible that DMSP is still produced.

Marrying this theory with the possibility of reduced mucosal production (and by extension, DMSP degradation to DMS), helps to suggest a possible physiological mechanism that would explain the results. In this case, DMSP levels are maintained through animal biosynthesis, however mucosal production (driven by *Symbiodinium* primary production of lipids) is severely inhibited, and so the produced DMSP is not metabolised to any detectable level (*T. reniformis*) or the capacity to produce DMS is decreased (*A. inermis*).

Why DMS production did not differ in the low light groups in *P. cylindirca* is difficult to explain. One possibility is that the mucosal secretion rate of *P. cylindrica* is constrained to a constant regardless of light or temperature, possibly due to the fact that the mucocytes in this species are located deep within the coral tissues rather than near the surface like in many other species (Brown and Bythell, 2005). This makes sense to a certain degree from a survival perspective as this arrangement avoids excess loss of assimilated carbon to the environment, and the mucus produced may be more structurally attuned to preventing desiccation and therefore does not need to be produced excessively.

Regardless, further investigation into the likely mechanisms behind the responses in these species is needed before more solid conclusions can be drawn. However, what is important is that light seems to be a determining factor for modulating DMS production in those species which are “leaky” to DMS when exposed. When attempting to calculate the production rates of reef DMS at low tide, it is clearly important to consider light irradiance and time of day when exposed. Depending on the dominant mode (if indeed one exists), reef DMS production at low tide at night could be very low (in the case of *T. reniformis*) or decreased (*A. inermis*). Also important to note is that the effect of light on the respective species also applied to the pre exposure DMS production rates in some cases. Regardless of

whether the reef is exposed to the air or not, it is likely that estimations of reef DMS production will need to account for this fluctuation in daily production.

The effect of temperature

Temperature was of secondary importance for the strength of the corals' response. Again, *P. cylindrica* was not affected by prolonged increased temperatures. It is important to note that generally temperatures of 31°C are short of the temperature where physiological damage and bleaching usually occurs, and so is considered sub-lethal. Also to note is that coral specimens were brought up to this temperature gradually, and acclimated for one month prior to data collection. It may be likely, at least with *P. cylindrica*, that no effect was observed due to acclimation and/or the treatment temperature not being high enough to elicit an effect. This is further supported by the fact that there is little to no difference in the auxiliary data where it would be expected that symbiont populations and chlorophyll content would be different in the high temperature groups compared to control, specifically demonstrating lower *Symbiodinium* densities and decreased chlorophyll-*a* concentrations (Suggett and Smith, 2011).

In *A. inermis* and *T.* reniformis the effect of temperature appears to be dependent on the light environment; with high temperature in control light (HTCL) causing a slight decrease in DMS production yet increasing DMS production in low light. The simplest explanation for the former effect is that DMSP and its constituents are being utilised as ROS scavengers. Here the classic, temperature-stress-plus-light-scenario that causes damage to PSII and causes photo-oxidative radical formation holds true and as such DMSP production is increased (as seen in our data as increased DMS). The reason that we observe decreased DMS release in spite of increased DMSP is likely due to the conversion of DMS to DMSO.

Unfortunately we were unable to obtain the DMSO data in this study to back up our hypothesis, however the underlying theory has been explored in previous studies (Jakob and Heber, 1996) (Sunda et al., 2002).

Somewhat contradictory to our data (Deschaseaux et al., 2014b) observed that temperatures of 31°C dramatically increased DMS within the coral holobiont after 5 days of acclimation to these temperatures in *A. aspera*, and also observed increases in DMSP and DMSO. This study also observed that DMS was below the detection limit after 5 hours of air exposure, and also observed no detectable DMS after three days of low light. As this study used the entire coral nubbin and therefore also incorporated the mucosal DMS/DMSP, then it is likely that after a 1 month acclamatory period, the coral specimens in our study may have altered their physiology such that DMS production is regulated. This is also further supported by comparing our auxiliary data to this study; no significant differences in Chl-a or symbiont populations were observed as an effect of treatment, whereas the opposite was true in the Deschaseaux study, with high temperatures being associated with lower *Symbiodinium* counts and reduced chl-a.

Another study that specifically investigated DMS production in *Symbiodinium* clades under thermal stress, observed that clade C1 produced more DMSP than D1, yet C1 up-regulated DMSP consumption when exposed to thermal stress (Deschaseaux et al., 2014a). It may be possible that the *Symbiodinium* population of *P. cylindrica* was that of a thermally tolerant clade that also produced lower concentrations of DMS. In the other species, DMS production was higher in the control as a function of *Symbiodinium* clade, yet this production was negatively impacted by thermal stress. This appears to hold true for *A. inermis* to some degree as total DMSP was higher in this species. However, DMSP also appears to increase when exposed to a 3°C increase in temperature, the opposite of what would be expected from this *Symbiodinium* study.

Returning to our hypothesis that the resulting drop in DMS production is through consistent oxidation to DMSO, the study by Deschaseaux on coral holobiont organosulphur concentrations does offer support in that DMSO levels were higher in the thermally stressed group in that study. It may be possible that our coral specimens exhibited an ability to adapt to their environment. Whether this is through symbiont population reshuffling and/or the production of other heat stress molecules such as heat shock proteins is another area of further study to be explored.

An alternative hypothesis could be that the microbial community associated with the coral surface has shifted due to thermal stress. A previous study found that bacterial communities associated with corals are flexible and can be altered when exposed to high temperatures (Ainsworth and Hoegh-Guldberg, 2009). In this scenario, reduced DMS production may be due to reduced populations of DMSP metabolising bacteria. This has some weight given the fact that DMSP levels in thermally stressed corals (in control light) were actually higher than in the control. It may be possible that this DMSP build-up is due to reduction in bacterial metabolism activity.

The mechanism behind the increased DMS production in *T. reniformis* when in low light is more difficult to explain. Returning to the Deschaseaux et al (2014b) paper, light depletion resulted in no detectable DMS (which also corroborates with the previous section on light modulation). However, this study did not look at the combined effect of both. One explanation for the increased DMS production in *T. reniformis* may be that, faced with thermal stress, the host produces DMSP in the absence of photosynthesis. However, whether or not thermal stress would result in ROS production in extreme low light is important to decipher for this hypothesis to hold up. Likewise, it may also be possible that this observed effect is simply a false positive, which may be likely given the difficulty in reconciling the literature with the data.

Although the observed effect was slight in our data, if SSTs in reef ecosystems approach or exceed 31°C, reef wide DMS production rates may drop more drastically. Even if the drop remains slight however, this evidence does not bode well for the proposed positive feedback mechanism through increased CCN as outlined in the CLAW hypothesis. However, the implication that our specimens' may have in fact adapted over time also offers some hope. In order to come to more solid conclusions however, studies that push not just temperature, but also light irradiance should be undertaken to better understand this dynamic.

Implications of Study

This study highlights the differences in DMS production both between coral species and their response to long term acclimation to different environments. Of particular note is the difference in response to aerial exposure between species. Depending on which species in a coral community release DMS when exposed, and which do not, the estimations for overall reef DMS output are likely to be highly variable. This has implications for local sulphur cycling as well as cloud seeding capacity. Therefore any shifts in coral community composition are also likely to shift these values. More in depth studies into which species exhibit which response should be pursued in order to be better able to predict such changes in future reef communities. If slower growing, hardier species such as *Porites spp.* are all similar in response, and we see a general shift towards the dominance of these species at the expense of other species then offshore terrestrial habitats may also be affected through changes to the carbon and nitrogen cycling of plants (Oulehle et al., 2011). The huge pulses of DMS seen within the first 5 minutes of exposure also suggest that even short time emersion can have a dramatic effect on the DMS loading of the local air.

The second largest finding of this study is that light modulates the DMS production response, as previously mentioned this has implications for temporal estimates of reef organosulphur production. If this effect is conserved across taxa then time of day will have to be taken into account when assessing DMS release from exposed corals. This is especially important for corals that may respond similarly to *T. reniformis*, where the difference between night and day emersion would be dramatic. This applies to DMS production when immersed as well, and so may also affect dissolved organosulphur loading that may also decrease with depth/water clarity. Of course, further studies into how dramatic the effect of light is across the majority of coral taxa is also important, and highlights the difficulties with deciphering the issue of estimating how reef DMS production may change in the future.

The effect of temperature within the control light groups, while not necessarily novel, adds a further dimension to the potential future of reef organosulphur production. In terms of the coral holobiont, more research is needed to work out the mechanisms behind what aspect of the coral is being affected by thermal stress and why species respond differently. Also of importance is to determine the effects of short vs long term stress as well as pushing the degree of stress higher i.e. 32°C and above in order to support future predictions in organosulphur production shifts.

As well repeating these experiments across a wider range of taxa and environmental conditions, it may also be worthwhile in future studies to quantify a whole range of biogenic volatile organic compounds (BVOCs) during coral air exposure. Other examples of important BVOCs include; isoprene, another important compound in atmospheric chemistry that reacts with hydrogen oxide (Lelieveld et al., 2008), simple alcohols and terpenes that act as biochemical signal molecules (Pichersky and Gang, 2000), and whole range of other secondary metabolites. By quantifying these other volatile compounds, especially during low tide exposure, it is wholly possible that a whole range of other compounds are being

released into the local atmosphere by corals each low tide. This has so far not been examined thoroughly, even for intracellular metabolite quantification the literature is more geared towards gorgonian and soft coral species.

In conclusion, this study highlights the complexities associated not just with interspecific differences in organosulphur production in Scleractinian coral, but also the response of these organisms to changing environment. As such, future work is necessary if any realistic and dependable estimations of organosulphur production of reef systems as an ecosystem are to be made. By understanding not only the changes in terms of DMS production, but also the underlying mechanistic cause of these changes, will allow for more comprehensive analysis from the organism to the community level. As such, DMS quantification could prove to be a useful tool for future reef biologists.

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