Gas Powered Reefs: Exploring the Nature and Variability of the Coral Volatilome

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Certificate of original authorship

I, Caitlin Alinya Lawson declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctorate of Philosophy, in the Faculty of Science at the University of Technology Sydney.

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Figure S5.2 Schematic of volatile sampling set up. Coral nubbins were placed in gas tight chambers and maintained under growth conditions while undergoing a 30 minute purge of instrument grade air (BOC Gases, Linde Group, Australia). The outlet of this purge was passed over Markes thermal desorption tubes (Tenax TA).

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Thesis Abstract

Biogenic volatile organic compounds (BVOCs) are a diverse class of chemicals well studied in terrestrial ecosystems, where they play important ecological and physiological roles, while influencing atmospheric processes and climate. As the health of tropical coral reefs rapidly deteriorates, BVOCs can potentially mitigate stress events directly by functioning as antioxidants and indirectly through atmospheric chemistry. Yet, the composition of the coral volatilome (total BVOCs) and its potential importance for coral reef functioning is understudied.

I investigated the BVOC capacity of corals systematically – from microbial symbiont to coral holobiont. I examined Symbiodiniaceae, the photosynthetic microbes that provide nutrients to corals. Across six species, I detected 82 BVOCs and using a targeted thermal stress experiment on two key species, *Cladocopium goreaui* and *Durusdinium trenchii*, I identified significant changes in specific BVOCs, with the majority of these significantly increasing following stress.

Given that Symbiodiniaceae are associated with complex and abundant bacterial communities, I described the microbiome of 18 species of Symbiodiniaceae. Three bacterial genera were consistently present across all Symbiodiniaceae species: *Labrenzia*, *Marinobacter* and an unclassified Chromatiaceae. I then characterised the volatilome of *Labrenzia* sp. 21p and *Marinobacter adhaerens* HP15 and detected 35 BVOCs between them. The composition of the *Labrenzia* sp. volatilome significantly changed following incubation in Symbiodiniaceae exudate and additional changes were detected in the quantities of individual BVOCs in both bacterial species. This indicates the ability of Symbiodiniaceae-associated bacteria to alter their BVOC production in the presence of Symbiodiniaceae-derived chemicals.

I finally characterised the volatilomes of two common, heat-sensitive, reef-building corals (*Acropora intermedia* and *Pocillopora damicornis*) during a simulated heat stress event. I detected 88 BVOCs from these holobionts however, the BVOC richness of both

species decreased following stress (by 41% in *A. intermedia* and 62% in *P. damicornis*) and the abundance of multiple BVOCs significantly decreased. As such, this study revealed that thermal stress influences the coral holobiont by decreasing the richness and abundance of the volatilome.

From microbe to holobiont, I consistently detected a wide range of BVOCs, many of which are implicated in stress response, signalling, antimicrobial defence or potentially impacting local climate. I demonstrated that corals and therefore potentially coral reefs, are significant sources of numerous BVOCs that should receive increased attention to determine their biological functions. This work highlights the diversity of BVOCs produced by corals and their constituents, and provides important new knowledge for the successful management and conservation of these threatened ecosystems.

Thesis Structure

This thesis is comprised of four data chapters (Chapters 2 to 5), each constructed around an independent experiment, in the form of a journal manuscript for peer-review. At the time of thesis submission, all chapters have been either published, are under peer-review, or in final draft for submission. Because the introduction of each data chapter is exhaustive, the general introduction (Chapter 1) is written as a brief but focussed overview to develop and outline the over-arching aims of this thesis.

Chapter 2: Lawson, C.A., Possell, M., Seymour, J.R., Raina, J.B. and Suggett, D.J., 2019. Coral endosymbionts (Symbiodiniaceae) emit species-specific volatilomes that shift when exposed to thermal stress. *Scientific Reports*, 9(1), pp.1-11.

Chapter 3: Lawson, C.A., Raina, J.B., Kahlke, T., Seymour, J.R. and Suggett, D.J., 2018. Defining the core microbiome of the symbiotic dinoflagellate, *Symbiodinium*. Environmental Microbiology Reports, 10(1), pp.7-11.

Chapter 4: Lawson, C.A., Seymour, J.R., Possell, M., Suggett, D.J. and Raina, J.B., In Review. The volatilomes of Symbiodiniaceae-associated bacteria are influenced by chemicals derived from their algal partners. Frontiers in Microbiology, submitted on 8th October 2019.

Chapter 5: *Lawson, C.A., Seymour, J.R., Deschaseaux, E., Hreiben, V., Possell, M., Raina, J.B. and Suggett, D.J., Final Draft. Volatile trade-offs amongst reef-building corals: thermal stress negatively affects the diversity of the coral volatilome. Intended Journal, Global Change Biology.–*Author list to be finalised.

Chapter 1

General Introduction & Thesis Outline

Coral Reefs

Coral reefs comprise only 0.1% of the ocean floor, yet they make up some of the most productive biomes and diverse ecological communities. This diversity might at first seem surprising given that coral reefs inhabit nutrient-depleted waters, but this ecosystem achieves high levels of primary productivity through numerous symbiotic associations which enable tight nutrient cycling (Ceh et al., 2013; Rädecker et al., 2015). The best recognised of these symbioses occurs between reef-building cnidarians and photosynthetic microalgal dinoflagellates (family: Symbiodiniaceae). Coral-Symbiodiniaceae relationships are of great importance to coral health since functional characteristics of the microalgae govern the environmental stress tolerance and recovery of their coral hosts (e.g. Baker et al., 2008; Suggett et al., 2017) and in turn, of the entire coral reef ecosystem (Bernasconi et al., 2019; Kemp et al., 2011; Wegley Kelly et al., 2018).

Symbiodiniaceae cells are harboured within coral tissues at high densities (0.5 to 5 million cells per cm^2 of coral tissue), and provide the bulk of their photosynthetically-derived carbon to the coral in exchange for limiting nutrients (Muscatine et al., 1984). Other symbiotic associations also occur in coral tissues, for example numerous prokaryotes contribute to overall coral health and resilience (Blackall et al., 2015). Corals are thus considered “holobionts”, complex meta-organisms comprising the cnidarian host along with its complex assortment of microorganisms, Symbiodiniaceae, bacteria, fungi, archaea, and viruses that inhabit coral tissues and the external mucus layer (Bernasconi et al., 2019; Rohwer et al., 2002; Thompson et al., 2015). Slight changes in abiotic factors, such as light or temperature, can cause complex responses at the holobiont level as each of its constituents reacts to external stressors (Gardner et al., 2019; Leggat et al., 2019; Thompson et al., 2015; Wegley et al., 2007; Ziegler et al., 2019). One of the most studied perturbations affecting the entire holobiont is thermal stress-induced coral bleaching, which is characterised by the expulsion of Symbiodiniaceae from the coral tissue and the concomitant shift in the associated bacterial community (Gardner et al., 2019; Ziegler et al.,

2019). Furthermore, following coral bleaching, corals can be quickly encased in microbial biofilms that accelerate skeletal dissolution (Leggat et al., 2019). However, a mechanistic understanding of how each holobiont component contributes to coral health is yet to be achieved – a key goal to achieve if we are to understand how corals can rapidly acclimatise/adapt to a changing climate (e.g. van Oppen et al., 2017).

Functional Diversity of Symbiodiniaceae

The family Symbiodiniaceae includes multiple distinct genera (*Symbiodinium* – formally clade A*, Breviolum* (B)*, Cladocopium* (C)*, Durusdinium* (D)*, Effrenium* (E)*, Fugacium* (F)*, Gerakladium* (G); LaJeunesse et al., 2018). Coral reefs throughout the Indo-Pacific are almost exclusively dominated by *Cladocopium* and *Durusdinium* (LaJeunesse et al., 2003, 2004), with 50-100 possible "species" currently identified around Australia alone (LaJeunesse et al., 2003; Silverstein et al., 2011); yet this number is almost certainly an underestimate. Within the remarkable genetic diversity of Symbiodiniaceae lies an equally astounding functional diversity (Suggett et al., 2017); however, most studies attempting to reconcile phylogenetic diversity against physiological diversity have, to date, largely focussed on photosynthetic differences (Abrego et al., 2008; Goyen et al., 2017; Hennige et al., 2009; Robison and Warner, 2006; Suggett et al., 2008, 2015). For example, corals harbouring *Cladocopium* C1 types incorporate twice as much Symbiodiniaceae photosynthate as corals associated with *Durusdinium* (Cantin et al., 2009). However, this functional diversity extends to far more than just Symbiodiniaceae photosynthetic capabilities; for example, nitrogen acquisition efficiency varies between species (Baker et al., 2013), as does inorganic carbon uptake (Brading et al., 2011, 2013; Oakley et al., 2014). Such large functional diversity results in certain species being far more resilient to stress, which can ultimately be a key determinant of host fitness (Silverstein et al., 2015). Indeed, corals preferentially associate with Symbiodiniaceae species that best provide nutrients to them (Berkelmans and van Oppen, 2006). This selectivity is not specific to the

photosynthetic symbionts, the bacterial symbionts can also aid the coral host through provision of essential resources (Ceh et al., 2013; Lema et al., 2016; Robbins et al., 2019).

Bacteria in the Coral Holobiont

Prokaryotes represent the majority of the genetic diversity on the planet (Thompson et al., 2015) and the most diverse part of coral reef ecosystems (Robbins et al., 2019; Rohwer et al., 2002). Thousands of bacterial species are typically associated with an individual coral host, making the coral holobiont an exceptionally diverse meta-organism (Bayer et al., 2013; Robbins et al., 2019). Although the functional roles played by the vast majority of coral-associated bacteria remain unknown, it is established that: (1) nitrogen-fixing bacteria can provide inorganic nitrogen to Symbiodiniaceae for photosynthesis (Krediet et al., 2013; Lema et al., 2014; Lesser et al., 2004); (2) specific bacteria can degrade complex polysaccharides, thus providing the holobiont with additional nutrients (Rosenberg and Zilber-Rosenberg, 2011); (3) others can produce antimicrobial compounds, protecting the holobiont against pathogens (Nissimov et al., 2009; Raina et al., 2016; Rosenberg and Zilber-Rosenberg, 2011; Shnit-Orland and Kushmaro, 2009); (4) while other coral-associated bacteria play an important role in the cycling of sulfur (Raina et al., 2009). As both Symbiodiniaceae and bacteria provide the corals with energy and influence their ecological success, understanding how these two components interact is vital for understanding the holobiont as a whole. Although numerous studies have investigated the associations between corals and Symbiodiniaceae, or corals and bacteria in isolation, very little is known about the bacteria associated with Symbiodiniaceae and how they might affect Symbiodiniaceae phenotypes. Moreover, coral-associated microbes are suspected to further aid the coral holobiont by detoxifying reactive oxygen species during thermal stress (Diaz et al., 2016), a particularly beneficial trait given the recent extreme heat waves damaging the tropics.

Coral Holobionts Produce Volatile Metabolites

Tropical corals are major producers of dimethyl sulfide (DMS) (Broadbent and Jones, 2004), a volatile sulfur gas known to function as an antioxidant, effectively scavenging reactive oxygen species in microalgae (Sunda et al., 2002). This capacity is also thought to aid Symbiodiniaceae and corals in coping with stress (Deschaseaux et al., 2014a; Hopkins et al., 2016). Furthermore, once emitted to the atmosphere, DMS molecules can form secondary organic aerosols (SOA) and then cloud condensing nuclei (CCN) which can influence local climate (Ayers and Gras, 1991). These key functions have led to a strong focus on DMS which is important – and perhaps logical given the high concentrations emitted (Broadbent et al., 2002; Broadbent and Jones, 2004; Deschaseaux et al., 2014c; Hopkins et al., 2016) – however, this has resulted in a major knowledge gap on whether and why corals produce any other biogenic volatile organic compounds (BVOCs).

BVOCs broadly encompass those gases that are produced as a direct result of biological activity and are characterised by their high vapour pressure and their low boiling point (Kesselmeier and Staudt, 1999). The majority of BVOC emissions originate from photosynthetic organisms, with tropical rainforests recognised as hotspots (Guenther et al., 1995). Coastal ecosystems are known for their high productivity and recent work has identified coastal macroalgae species that produce atmospherically relevant concentrations of BVOCs (Broadgate et al., 2004; Tokarek et al., 2019). Recently, the coral species *Acropora aspera* was shown to also produce the abundant terrestrial BVOC, isoprene (albeit at much lower levels than DMS) and other reduced sulfur compounds including dimethyl disulfide, methyl mercaptan, carbon disulfide, thiirane, 2,4-dithiapentane, dimethyl trisulfide and 1,2,4-trithiolane (Swan et al., 2016). However, it remains unknown whether there are any other BVOCs that may play important functional roles in coral reefs.

Functional Diversity of Volatile Metabolites

Often overlooked, BVOCs are responsible for global biogeochemical cycling and can influence everything from the grazing behaviour of caterpillars (Laothawornkitkul et al., 2008)

to atmospheric processes (Kulmala et al., 2013). Understanding the production, emission and interactions of these chemicals is crucial for our comprehension of the world around us. Natural emissions of BVOCs are often an order of magnitude higher than those resulting from anthropogenic VOCs (Guenther et al., 1995) and hundreds of different compounds are continually being emitted from the biosphere to the atmosphere (Guenther et al., 2012). In terrestrial systems, isoprene and monoterpenes are the most abundantly emitted BVOCs followed by alcohols and carbonyls, yet there are still large knowledge gaps regarding the biological sources and functions of the remaining groups (e.g. alkanes, alkenes, esters, ethers and acids) (Kesselmeier & Staudt 1999).

Some of the most common functions of BVOCs include signalling and response to stress (Himanen et al., 2010; Peñuelas and Llusià, 2003; Yuan et al., 2009). Furthermore, BVOCs can also influence climatic processes. Following sub-optimal abiotic conditions, plants can alter their BVOC production to increase their chance of survival. For example, isoprene production is strongly influenced by abiotic stressors (Loreto et al., 2001; Sharkey et al., 2008; Sharkey and Monson, 2014; Vickers et al., 2009a). Isoprene has thermo-protective properties and is hypothesised to have the potential to modify the structure of plant membranes in order to improve thermotolerance (Sharkey and Singsaas, 1995). In the development of transgenic tobacco plants, isoprene-emitting variants were far more resistant to oxidative damage – clearly indicating that isoprene production offers protection against oxidative stress (Vickers et al., 2009b). Other isoprenoid BVOCs (limonene, myrcene, sabinene, α-pinene, β-pinene) produced in higher plants have also been shown to respond to thermal stress – increasing in concentration with higher temperatures (Loreto et al., 1998). While there have been fewer targeted studies on other BVOCs, classes such as aromatic hydrocarbons (Misztal et al., 2015) and halogenated hydrocarbons (Paul and Pohnert, 2011) have also recently been suggested to respond to thermal stress.

Multiple BVOCs have been shown to function as infochemicals. Certain bacterial BVOCs act as antifungals and protect both the bacterium and the host plant (Gamliel and Stapleton, 1993; Yuan et al., 2012). Bacteria can also utilise their BVOCs in trophic

interactions – in some cases these volatiles can act against competitive microbes (Garbeva et al., 2014). Additional signalling roles of BVOCs are multifaceted, whereby emitted BVOCs can attract pollinators (Schiestl, 2015), affect trophic interactions (Loivamäki et al., 2008; Rasmann et al., 2005) and prime plant defence responses to herbivory (Laothawornkitkul et al., 2009). Other BVOCs are produced for protection from pathogens by utilising their antifungal or antimicrobial abilities (Croft et al., 1993; Shiojiri et al., 2006), or by influencing the behaviour and physiology of herbivores to protect the emitting plant (De Moraes et al., 2001; Laothawornkitkul et al., 2008).

Once released into the atmosphere, BVOCs can play further crucial roles in atmospheric processes, notably the formation of SOA that can scatter sunlight directly and indirectly by forming CCN (Kulmala et al., 2004). Once in the atmosphere, BVOCs can also react and ultimately oxidise to carbon dioxide unless intermediate molecules are formed (Atkinson and Arey, 2003a). Furthermore, these intermediate molecules can have direct impacts on climate; for example, BVOCs can readily react with and form tropospheric ozone (Atkinson and Arey, 2003b; Peñuelas and Staudt, 2010). Additionally, as the climate continues to change, BVOC formation of ozone is expected to increase (Peñuelas and Staudt, 2010).

A Volatilomic Approach

Volatilomics, a subset of metabolomics, is the study of all volatile compounds produced by an organism and it has been gaining recognition as a valuable non-invasive tool for monitoring the health of organisms and/or ecosystems. The use of volatilomics was recently proposed as a diagnostic tool for determining functional diversity and for monitoring and management in aquatic systems (Steinke et al., 2018). Understanding how volatilomes will respond to climate change and affect climate regulation will likely prove crucial for successful management of marine ecosystems.

Thesis Roadmap, Aims & Objectives

Tropical biomes are highly productive, diverse ecosystems that produce significant levels of BVOCs. Coral reefs are particularly highly diverse, biogeochemically complex marine ecosystems, as illustrated by the nature of the reef-forming coral holobiont – how corals contribute to BVOC emissions in tropical reefs remains almost entirely unexplored. Therefore, the overall aim of this thesis is to identify coral BVOCs and how they vary between (i) the different components of the holobiont and in response to (ii) anomalous thermal stress, a key environmental perturbation known to affect coral functioning. To address this aim, I applied volatilomics for the first time to Symbiodiniaceae, their associated bacteria, and the coral holobiont itself.

The specific objectives of this project are to:

Aim 1: To describe the volatilome for a range of Symbiodiniaceae under steady state growth conditions and in response to heat stress. Photosynthetic organisms (Symbiodiniaceae) are thought to be the main producers of BVOCs in corals, but the identity of the compounds produced, as well as their consistency and species-specificity were unknown. Here, I assayed for the first time the volatilomes of five species of Symbiodiniaceae under steady state growth and two Symbiodiniaceae species during a thermal stress event (Chapter 2).

Aim 2: To characterise the bacterial communities that associate with Symbiodiniaceae. Whilst bacteria are important constituents of coral holobionts, they are also inherent members of Symbiodiniaceae cultures. Therefore, to address whether and how BVOC emissions within Symbiodiniaceae cultures (Chapter 2) may be impacted by phylogenetically diverse and numerically abundant bacterial communities, I initially used next generation sequencing to characterise the Symbiodiniaceae microbiome and identify the core bacteria within diverse Symbiodiniaceae cultures (Chapter 3).

Aim 3: To identify the bacterial volatilomes of the Symbiodiniaceae core microbiome. I subsequently isolated core Symbiodiniaceae-associated bacteria identified in Chapter 3. I then identified the BVOCs these bacteria produce in order to assess their putative emissions and contribution to the Symbiodiniaceae volatilome (Chapter 4).

Aim 4: To describe the volatilome of the coral holobiont and how it varies with anomalous temperature exposure. Whilst understanding the contribution of each microbial component of the coral holobiont (Chapters 2, 4) is central to understanding their putative role in coral BVOC emissions, the coral holobiont volatilome is likely more than the sum of its parts as complex metabolic interactions within this meta-organism are likely to influence BVOC emissions. Therefore, I next applied the same volatilomic assay to coral holobionts, while also applying a targeted approach to quantify the trace production of isoprene. This approach was conducted within the framework of a heat-stress experiment mimicking a thermal anomaly typical of recent heat waves on the Great Barrier Reef (Chapter 5).

The knowledge gathered by addressing these four aims is then considered in Chapter 6 where I synthesise my findings and identify possible future directions for BVOC research in corals.

Chapter 2

Coral endosymbionts (Symbiodiniaceae) emit species-specific volatilomes that shift when exposed to thermal stress

Author contributions: CA Lawson, JB Raina, JR Seymour and DJ Suggett conceived and designed the project, CA Lawson performed the experiments, analysed the data and produced figures. M Possell provided technical and data analysis assistance. DJ Suggett provided financial support. CA Lawson wrote the paper. All authors edited the manuscript.

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Abstract

Biogenic volatile organic compounds (BVOCs) influence organism fitness by promoting stress resistance and regulating trophic interactions. Studies examining BVOC emissions have predominantly focussed on terrestrial ecosystems and atmospheric chemistry – surprisingly, highly productive marine ecosystems remain largely overlooked. Here I examined the volatilome (total BVOCs) of the microalgal endosymbionts of reef invertebrates, Symbiodiniaceae. I used GC-MS to characterise five species (*Symbiodinium linucheae, Breviolum psygmophilum, Durusdinium trenchii, Effrenium voratum, Fugacium kawagutii*) under steady-state growth. A diverse range of 32 BVOCs were detected (from 12 in *D. trenchii* to 27 in *S. linucheae*) with halogenated hydrocarbons, alkanes and esters the most common chemical functional groups. A thermal stress experiment on thermally-sensitive *Cladocopium goreaui* and thermally-tolerant *D. trenchii* significantly affected the volatilomes of both species. More BVOCs were detected in *D. trenchii* following thermal stress (32°C), while fewer BVOCs were recorded in stressed *C. goreaui*. The onset of stress caused dramatic increases of dimethyl-disulfide (98.52%) in *C. goreaui* and nonanoic acid (99.85%) in *D. trenchii*. This first volatilome analysis of Symbiodiniaceae reveals that both species-specificity and environmental factors govern the composition of BVOC emissions among the Symbiodiniaceae, which potentially have, as yet unexplored, physiological and ecological importance in shaping coral reef community functioning.

Introduction

Photosynthetic organisms, ranging from complex vascular plants to single-celled microalgae, are major producers of biogenic volatile organic compounds (BVOCs), which can represent up to 10% of fixed carbon and play numerous physiological and ecological roles (Peñuelas and Llusià, 2003; Shaw et al., 2010). BVOCs are exceptionally diverse and highly reactive, with chemical lifetimes ranging from minutes (e.g. β-caryophyllene) to months (e.g. acetone) (Kesselmeier and Staudt, 1999), and can be synthesised as by-

products of metabolic pathways (Loreto and Schnitzler, 2010) or be produced to maintain metabolic homeostasis (Kesselmeier and Staudt, 1999). The roles played by these compounds are multifaceted, from protection against abiotic stress (Loreto et al., 2006; Sunda et al., 2002) and pathogens (Croft et al., 1993; Shiojiri et al., 2006), to chemical signalling (De Moraes et al., 2001; Laothawornkitkul et al., 2008; Loivamäki et al., 2008; Schiestl, 2015).

Terrestrial tropical ecosystems are well recognised “hotspots” of global BVOC emissions (Guenther et al., 2006), but growing evidence also highlights the role of tropical marine ecosystems, such as coral reefs (Exton et al., 2015; Jackson et al., 2018) as major sources of BVOC emissions. Reef-building corals emit the highest recorded concentrations of the sulfur gas dimethyl sulfide (DMS; up to 18.7 μM reported in coral mucus; Broadbent and Jones, 2004; Hopkins et al., 2016), a compound potentially influencing climate (Ayers and Gras, 1991), the coral stress response (Deschaseaux et al., 2014a; Sunda et al., 2002) and putatively functioning as an infochemical (Nevitt et al., 1995; Seymour et al., 2010). Furthermore, the presence of acetone and dichloromethane was recently identified in reef seawater samples, indicating BVOCs produced on coral reefs are likely to be a complex mixture (Swan et al., 2016). In addition, cultures of the dinoflagellate endosymbionts of reef-building corals (Family: Symbiodiniaceae) have revealed that these microalgae are capable of producing DMS (Broadbent et al., 2002; Deschaseaux et al., 2014a; Steinke et al., 2011) and isoprene (Exton et al., 2013). However, these observations are derived from targeted quantifications of specific BVOCs and likely represent only a small pool of the compounds they are producing.

Emission of BVOCs from corals are thought to largely originate from Symbiodiniaceae due to the large quantities of carbon and metabolites they translocate (Burriesci et al., 2012; Van Alstyne et al., 2009) however, the host and other associated prokaryotes are likely additional BVOC sources. Metabolic coupling between coral host and algal endosymbionts is critical for viability of coral reef functioning – whereby the Symbiodiniaceae drive coral productivity, but can also govern susceptibility of their host to

stressors by controlling the exchange of nutrients (Suggett et al., 2017). Recent studies have moved towards identifying the functional diversity amongst the Symbiodiniaceae to determine key traits of resistance to stress (Díaz-Almeyda et al., 2017; Goyen et al., 2017), which is critical to understand their susceptibility to thermal stress. Whilst different metabolic traits and hence diagnostic metabolites appear central in governing this functional diversity (Matthews et al., 2017, 2018), the role of BVOCs remains largely unexplored.

The assortment of BVOCs produced by an organism has recently been termed the 'volatilome' (D'Alessandro, 2006; Steinke et al., 2018). Assessments of volatilomes, or volatilomics, gained interest in medical research, where the approach was used to diagnose patient health via bio-markers in exhaled breath (Amann et al., 2014). More recently, volatilomics has been used to quickly distinguish between varieties of plant species and to successfully monitor long-term plant health (Jud et al., 2018), with this approach showing promise for the development of new non-invasive monitoring tools of taxonomic and functional diversity in aquatic ecosystems (Steinke et al., 2018). Indeed, volatilomics may be particularly useful in connecting ecological processes and biogeochemical cycles in marine systems, since emissions of BVOCs from photosynthetic organisms can have a strong influence on atmospheric chemistry by increasing local cloud albedo or the residence time of greenhouse gases (Atkinson and Arey, 2003b, 2003a). Within this context, characterising the volatilome of highly productive, habitat-forming marine organisms should enable more accurate modelling of the impact of tropical BVOCs on climate regulation (Laothawornkitkul et al., 2009; Peñuelas and Llusià, 2003).

Here I provide the first characterisation of the volatilome of different Symbiodiniaceae species (spanning five genera) to determine whether and how volatile metabolite signatures are conserved across divergent taxa, and to identify volatiles that may be involved in previously overlooked physiological processes, ecosystem interactions and atmospheric cycling. In addition to this screening across-strains, I also examined the volatilomes of two Symbiodiniaceae strains with different thermal tolerance thresholds under conditions of heat

stress, to investigate the extent of thermally-induced changes in the volatilome and identify BVOCs potentially involved in Symbiodiniaceae thermal tolerance.

Methods

Across strain screening of Symbiodiniaceae volatilomes

Five isolates, each representing a distinct Symbiodiniaceae genus (*Symbiodinium linucheae, Breviolum psygmophilum, Durusdinium trenchii, Effrenium voratum* and *Fugacium kawagutii*; see Table 2.1), were maintained in exponential growth in a temperature controlled incubator (Labec; Marrickville, Australia) maintained at 25°C ± 1.5°C and under a light intensity of ca. 50 ± 5 µmol photons m^{-2} s^{-1} (HYDRA; Aquaillumination, Ames, Iowa) on a 12:12 hr light:dark cycle. Cultures were grown in triplicate in 0.2 µm filtered artificial seawater (Berges et al., 2001) with IMK medium (Diago, Japan) in sterile 250 mL Schott bottles. Growth and physiological condition of each culture were monitored daily in the five days prior to sampling with direct cell counts and Fast Repetition Rate fluorometry (FRRf; FastOcean, Chelsea Technologies Group, UK). The FRR fluorometer was programmed to deliver single turnover induction of photosystem II (PSII) (i.e. 100 × 1.1 µs flashlets spaced at 2.8 µs intervals) via a blue excitation LED (450 nm). Each acquisition recorded was the mean of 40 consecutive single turnover fluorescent transients, with intervals of 150 ms between acquisitions (Robinson et al., 2014). FRRf measurements were performed on 3 mL live culture, samples were first acclimated to low light (<5 µmol photons m^{-2} s^{-1}) for ~15 minutes to relax non-photochemical quenching while simultaneously avoiding build-up of chlororespiration to ensure that only the maximum quantum yield of PSII (F_v/F_m) was assessed (Suggett et al., 2015). Cell counts were performed on live cultures using a Neubauer haemocytometer and 20x magnification on a Nikon Eclipse Ci-L compound microscope (Nikon Instruments; Melville, New York). In addition to these daily monitoring data, additional samples were taken on the day of BVOC sampling for FRRf, cell counts, and cell size. For cell size analysis, an aliquot of culture was loaded onto a Neubauer

haemocytometer and using a Nikon Upright Fluorescence Microscope (Nikon Instruments; Melville, New York) a series of 48 images were taken for each sample. Using FIJI (Rueden et al., 2017; Schindelin et al., 2012) and White Balance software, these images were processed and the mean cell volume was recorded for each strain (Suggett et al., 2015). Values of F_v/F_m (dimensionless) varied on the day of sampling from 0.412 ± 0.016 (*E. voratum*) to 0.479 ± 0.002 (*D. trenchii*) (mean ± SE, n = 3; Figure S2.2), a range expected across isolates growing in nutrient replete exponential growth (Goyen et al., 2017; Suggett et al., 2015).

Table 2.1 Symbiodiniaceae isolates used in the screening experiment. Isolates were maintained in culture and no corals were directly handled in this study. Strains in bold were used for the thermal stress experiment, * indicates use in the screening experiment.

ID	ITS2 Type	Origin	Host	Species
*RT379	A4	Bahamas	*Plexaura homamalla* (Coral)	*Symbiodinium linucheae*
*RT141	B2	Bermuda	*Oculina diffusa* (Coral)	*Breviolum psygmophilum*
123SCF 058-04	**C1**	**Magnetic Island (Pacific)**	***Acropora millepora* (Coral)**	***Cladocopium goreaui***
***amur-D-MI**	**D1a**	**Magnetic Island (Pacific)**	***A. muricata* (Coral)**	***Durusdinium trenchii***
*CCMP421	E	Wellington, NZ	Free-living	*Effrenium voratum*
*f156	F1	Hawaii (Pacific)	*Montipora veruccosa* (Coral)	*Fugacium kawagutii*

Symbiodiniaceae thermal assay experiment

Two Symbiodiniaceae strains characterised by different levels of thermal sensitivity, including the relatively heat sensitive SCF058-04 (*Cladocopium goreaui*) and heat tolerant amur-D-MI (*Durusdinium trenchii*) (Berkelmans and van Oppen, 2006; Jones et al., 2008; Rowan, 2004; Stat and Gates, 2011), were subjected to a thermal stress experiment. These isolates were selected based on their differing thermal tolerance and their abundance on the Great Barrier Reef (Ulstrup and Van Oppen, 2003; Van Oppen et al., 2001). A control incubator (ARALAB; Sintra, Lisboa) was maintained at 26°C ± 1.5°C with a light intensity of ca. 100 ± 10 µmol photons m^{-2} s^{-1} (LEDs) on a 12:12 hr light:dark cycle. A parallel incubator (ARALAB; Sintra, Lisboa) was used for the heat treatment assay with identical settings to the control, except that temperature was ramped from 26°C to 30°C over 4 days and then

maintained at 30°C for 4 days prior to resampling. The temperature was then ramped from 30°C to 32°C over 2 days and finally maintained at 32°C for a further 4 days (Figure S2.1). Six biological replicates of each culture were grown (n = 3 per each control and treatment). All cultures were monitored daily with FRRf and cell counts following the same procedures as used in the screening experiment. BVOC sampling was performed at the end of the stress period when the treatment cultures had been at 32°C for 4 days. Additional samples were taken for cell imaging as per the screening experiment on these BVOC sampling days.

BVOC sampling and volatilome retrieval

Two aliquots of 50 mL from each sample were each placed into a sterile 100 mL crimp cap vial to yield technical duplicate samples with 50 mL headspace. Vials were capped and placed in a water bath under light intensity (cool white light, HYDRA; Aquaillumination, Iowa, USA) and temperature that matched their growth or treatment conditions. Samples were purged with instrument grade air (BOC Gases, Linde Group, Australia) for 30 minutes, whereby the purge outlet was passed over thermal desorption (TD) tubes (Tenax TA; Markes International Ltd, Llantrisant, UK; see Figure S2.3), which were immediately capped post purge and stored at 4°C until processing. All TD tubes were analysed within two weeks of sampling by desorbing samples with automated thermal desorption (ULTRA 2 & UNITY 2; Markes International Ltd, Llantrisant, UK) for 6 minutes at 300°C and concentrated on a Tenax TA cold trap at -30°C. This cold trap was then flash heated to 300°C and the concentrated sample injected via a heated transfer line (150°C) onto a 7890A GC-MS (Agilent Technologies Pty Ltd, Melbourne) fitted with a BP1 capillary column (60 m x 0.32 mm, 1 µm film thickness; SGE Analytical Science Pty Ltd, Melbourne) at a flow rate of 2.3 mL min^{-1}. Samples were run splitless to allow detection of trace compounds. To allow for complete desorption, the GC oven was heated at 35 °C for 5 minutes then 4°C min^{-1} to 160°C and then 20°C min^{-1} to 300°C for 10 minutes. The GC-MS was coupled to a mass-selective detector (Model 5975C; Agilent Technologies Pty Ltd,

Melbourne) that was set to a scanning range of 35 – 250 amu for the screening experiment and 35 – 300 amu for the stress experiment. The higher scanning range in the stress experiment was chosen as I examined only two species. The increased scanning range combined with the higher light levels likely led to the higher number of compounds detected in the stress experiment.

Peaks were identified by manually comparing mass spectra against a commercial library (NIST08 library in NIST MS Search v.2.2f; NIST, Gaithersburg, MD). Blank media samples were run in conjunction with all analyses; the average values for compounds present in the blanks were subtracted from all samples. Common contaminating ions (73, 84, 147, 149, 207 and 221 m/z) were removed using the Denoising function in OpenChrom (Wenig and Odermatt, 2010). Chromatograms were integrated in ChemStation (Agilent Technologies Pty Ltd, Melbourne) with an initial threshold of 14.5 and an initial peak width of 0.068. Files were processed using the MSeasyTkGUI package (Nicolè et al., 2012) in R version 3.5.3 (R Development Core Team, 2015) and all putative compounds clustered. Any compound that did not occur in at least 4 of the 6 replicates (2 technical replicates per biological replicate) was considered contamination and removed. UC denotes an unclassified compound (the number following UC indicates the retention time of the compound if the functional group could not be determined).

Statistical analyses

A Principal Components Analysis (PCA; Bootstrap N = 1000) on data normalised to total cell volume was completed in the statistical package PAST (Hammer et al., 2001) to contrast the volatilomes between Symbiodiniaceae species (Screening experiment) and between temperatures (Stress experiment). Compounds were considered “core” components of the volatilome if they appeared in at least 2 out of 3 biological replicates in all species. To test for significant differences between species in the screening experiment, data were processed in MetaboAnalyst4.0, undergoing a generalised logarithm transformation and tested with a one-way ANOVA and Tukey’s HSD post hoc (Chong et al.,

2018; Xia et al., 2009). For treatments in the stress experiment, a Kruskal-Wallis test was used (IBM SPSS Statistics, version 25), as data did not meet the assumptions required for parametric tests.

Results

Initial screening experiment

Across *Symbiodinium linucheae, Breviolum psygmophilum, Durusdinium trenchii, Effrenium voratum* and *Fugacium kawagutii*, a total of 32 BVOCs were detected following blank subtraction and quality control (Table S2.1). Of these, 50% were successfully identified and a further 28% could be assigned to functional chemical groups. Six compounds were present in at least two out of three replicates in all species tested and defined as core components of the *Symbiodiniaceae* volatilome. These included DMS, 2,3-dimethyl hexane, 6-methyl octadecane and three compounds that could not be fully identified (including a halogenated hydrocarbon, an organosulfur compound and an unclassified compound (UC; UC40.63) (Figure 2.1a). Excluding unclassified compounds (22.6%), halogenated hydrocarbon compounds constituted the largest functional group (12.9%), followed by alkanes and esters (both 9.7%) (Figure 2.1b).

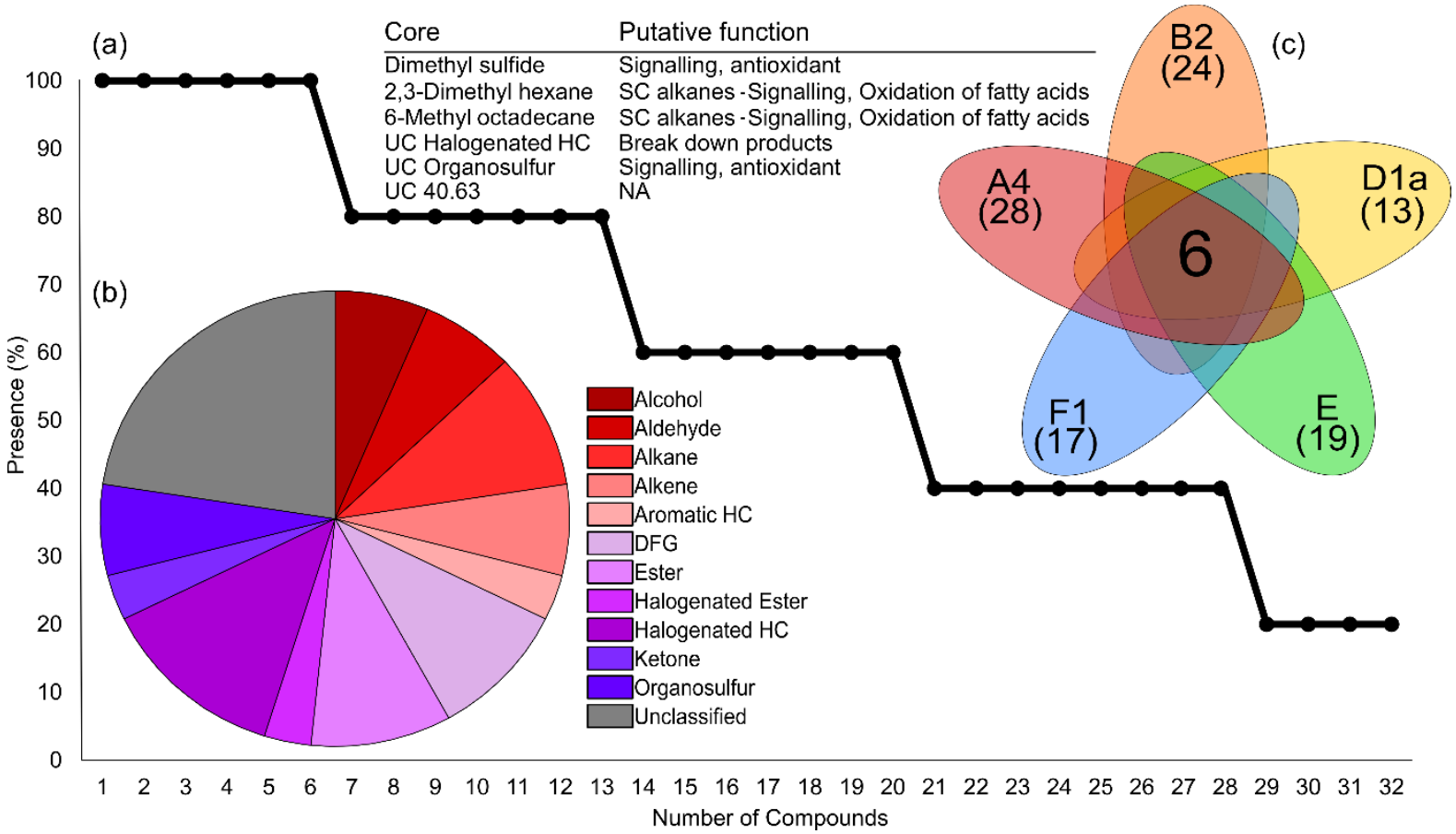

Figure 2.1 (a) Number of compounds detected across all Symbiodiniaceae isolates (present in at least 2 out of the 3 biological replicates). (b) The proportion of chemical functional groups present across the 32 detected compounds from five Symbiodiniaceae species. (c) Venn diagram (Heberle et al., 2015) showing the six core volatiles and their putative functions, as well as the number of compounds detected in each species (*Symbiodinium linucheae* (A4, red), *Breviolum psygmophilum* (B2, orange), *Durusdinium trenchii* (D1a, yellow), *Effrenium voratum* (E, green) and *Fugacium kawagutii* (F1, blue)). HC: hydrocarbon, DFG: diverse functional groups, SC: short chain, UC: unclassified (the number following UC indicates the retention time of the compound if the chemical functional group could not be determined).

Four BVOCs were unique to *S. linucheae*, including 3-trifluoroacetoxypentadecane, squalene, an unknown halogenated hydrocarbon and a compound that could not be identified to functional chemical group (UC18.34). None of the volatilomes associated with any of the other *Symbiodiniaceae* strains comprised any unique BVOCs. The most BVOCs were detected in *S. linucheae* (27), followed by *B. psygmophilum* (23), *E. voratum* (18), *F. kawagutii* (16) and *D. trenchii* (12) (Figure 2.1c). The abundance of five compounds differed significantly between species (one-way ANOVA; $P < 0.01$) – these included DMS, 3-trifluoroacetoxypentadecane, UC18.34, UC39.59 and an unclassified halogenated hydrocarbon (Figure 2.2). Principal component analysis (PCA) revealed tight groupings of *B. psygmophilum, E. voratum* and *F. kawagutii* volatilomes, while the *S. linucheae* and *D. trenchii* volatilomes overlapped, potentially due to higher variation between replicates (Figure 2.2). The principal components were strongly influenced by DMS (Principal Component (PC) 1 loading = 0.72), UC40.45 (PC1 loading = 0.68), methyl jasmonate (PC1 loading = 0.03, PC2 loading = 0.15) and styrene (PC1 loading = -0.09) (Figure 2.2).

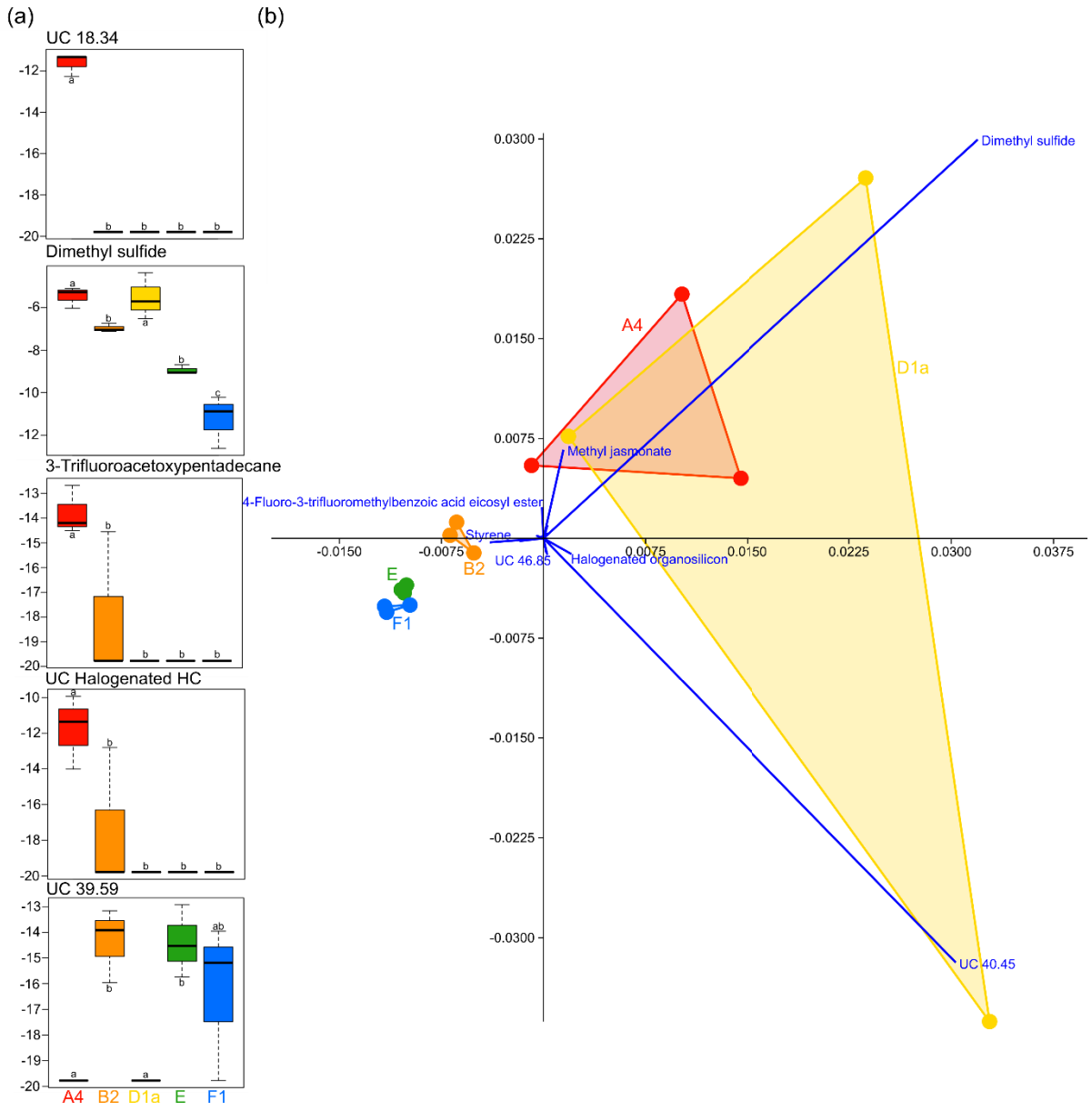

Figure 2.2 (a) Principal Component Analysis (PCA) of the volatilomes of *Symbiodinium linucheae* (A4, red), *Breviolum psygmophilum* (B2, orange), *Durusdinium trenchii* (D1a, yellow), *Effrenium voratum* (E, green) and *Fugacium kawagutii* (F1, blue). PCA component 1 = 42.0% and component 2 = 40.0% of variance. Bootstrap N = 1000. (b) Generalised logarithm of normalised volatile abundance for the five compounds that differed significantly (ANOVA, $P < 0.05$, Metaboanalyst 4.0; Xia et al., 2009) between species, significant differences are annotated on the plots. Error bars are standard deviation. HC = Hydrocarbon, UC = Unclassified (the number following UC indicates the retention time of the compound if the chemical functional group could not be determined).

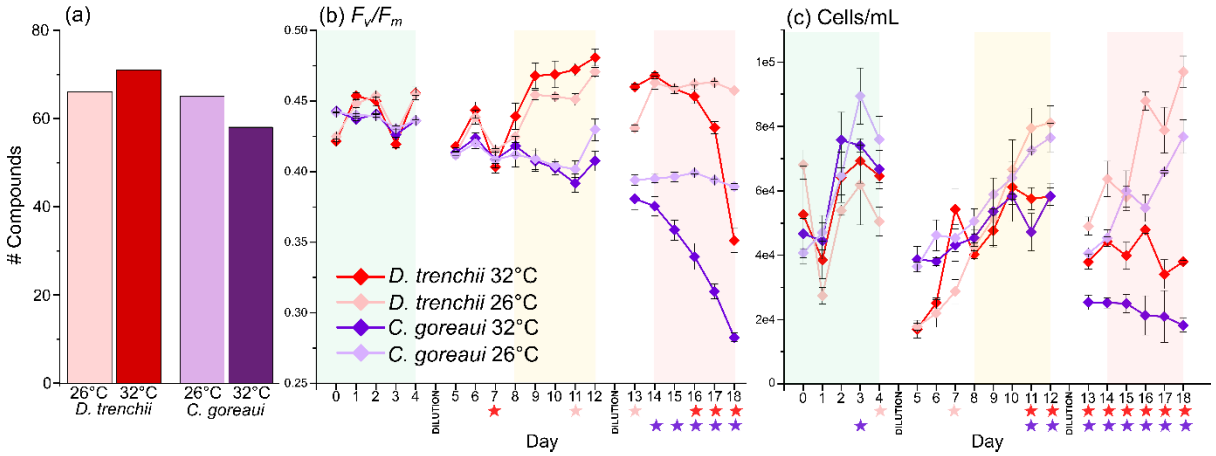

Figure 2.3 (a) Total number of compounds (present in at least two out of three replicates) detected in *Durusdinium trenchii* and *Cladocopium goreaui* control and stress treatments. Photosynthetic health *(F_v/F_m)* (b) and cell density (cell mL^{-1}) (c) of *D. trenchii* and *C. goreaui* throughout the thermal stress experiment. Significant differences ($P < 0.05$; Kruskal Wallis; IBM SPSS version 25) between treatments are indicated with a star, the star colour indicates the significantly decreased treatment. Shading on panels (B) and (C) indicates the time period the treatment cultures were held at 26°C (green), 30°C (yellow) and 32°C (red), controls were held at 26°C throughout, see Figure S2.1 for full temperature profile. All error bars are standard error.

Thermal Stress Experiment

Both the heat-tolerant *D. trenchii* and heat-sensitive *C. goreaui* species exhibited a decrease in photochemical efficiency (F_v/F_m, dimensionless) and cell density during the thermal stress experiment, but the more thermally sensitive strain *C. goreaui* exhibited significant (Kruskal-Wallis, $P < 0.05$) declines in F_v/F_m two days earlier than *D. trenchii* (Figure 2.3B-C). A total of 72 BVOCs were detected in this stress experiment after quality control, following thermal stress there was a significant shift in volatilome composition. The relative abundance of eighteen compounds differed significantly ($P < 0.05$; see Table S2.2 for full list of significant differences) between the treatments in *C. goreaui*. This shift was also reflected in a decrease in the total number of compounds detected during stress (65 to 58; present in at least two out of three replicates; Figure 2.3A). Conversely, more compounds were detected in *D. trenchii* during stress (66 to 71; Figure 2.3A) and 25 BVOCs differed significantly ($P < 0.05$; Table S2.2) between treatments. Only four compounds (2,4-bis(1,1-dimethylethyl)-phenol, two UC ketones and UC40.78) that differed significantly between

treatments were shared by both species during stress (Figure 2.4B-C). DMS was recorded in lower amounts under thermal stress in *D. trenchii* and UC42.37 became undetectable in *C. goreaui* during thermal stress (Figure 2.4B-C). All other significantly different compounds were measured in higher quantities during thermal stress (Figure 2.4B-C). Of the compounds that differed significantly between control and heat stress treatments in *C. goreaui,* 1,3-dimethoxy-2-propanol and dimethyl disulfide (DMDS) showed the largest increase (94.94% and 98.52%, respectively) under stress (Figure 2.4B). In *D. trenchii*, nonanoic acid and UC44.07 exhibited the largest increases (99.85% and 100%, respectively) in the heat stress treatment (Figure 2.4C). Five compounds were only detected under thermal stress conditions: benzoic acid pentadecyl ester, UC44.07, UC33.89 and UC17.25 (Figure 2.4C). PCA visualised the strong differentiation between the control and thermal stress volatilomes of *C. goreaui* (Figure 2.4A), the principal components in this PCA were influenced by DMDS (PC1 loading = 0.66), UC40.78 (PC1 loading = 0.52) and 1,3-dimethoxy-2-propanol (PC1 loading = 0.48), which were all detected in higher levels during thermal stress (Figure 2.4A). PCA also revealed clear distinction between control and stress treatments in *D. trenchii*, whereby the principal components in the *D. trenchii* PCA (Figure 2.4D) were largely influenced by UC40.78 (PC1 loading = 0.46), nonanoic acid (PC1 loading = 0.18) and a stronger negative forcing from DMS (PC1 loading = -0.84).

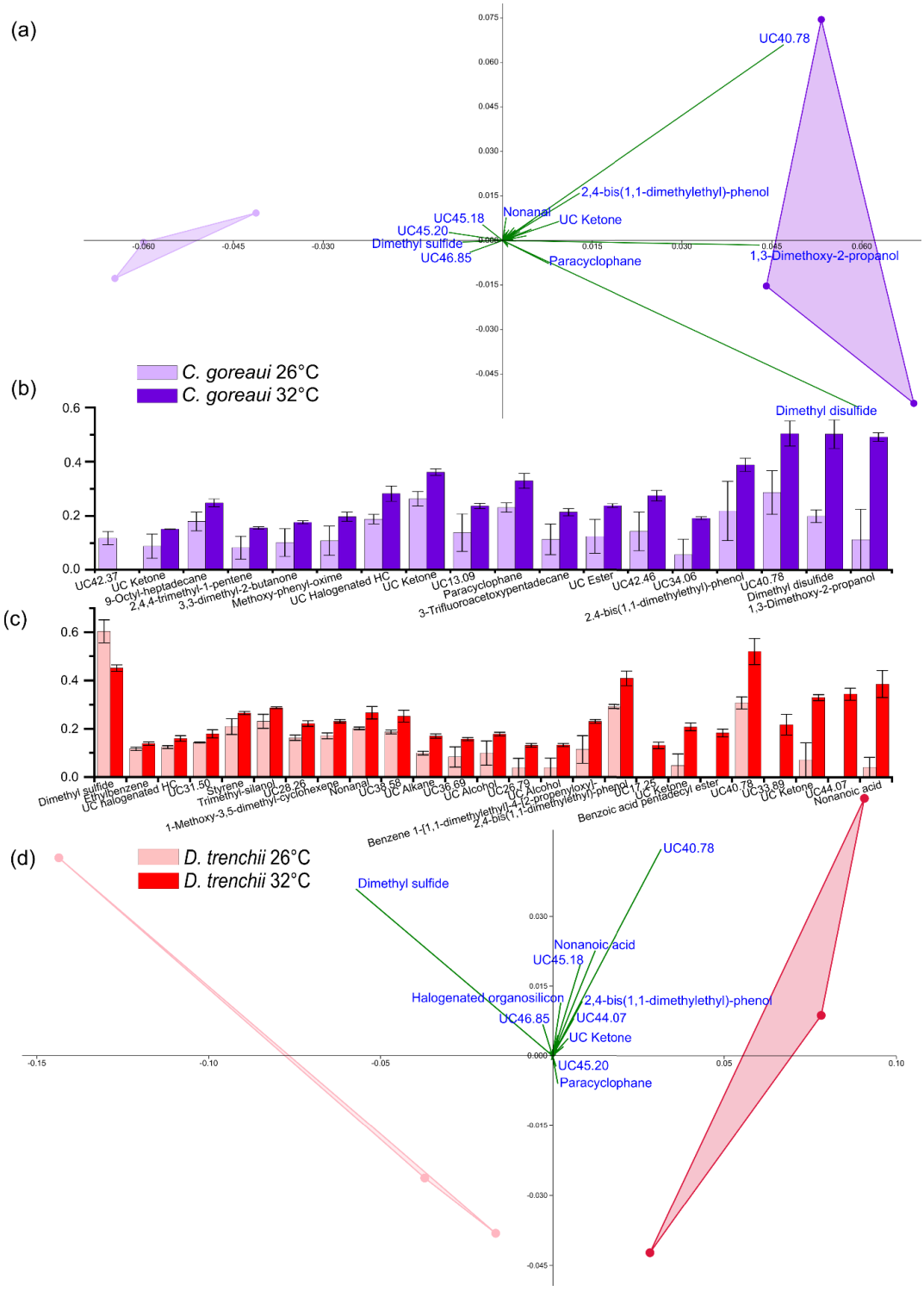

Figure 2.4 Principal Component Analysis (PCA) of *Cladocopium goreaui* (A) (component 1 = 62.1% and component 2 = 29.6% of variance) and *Durusdinium trenchii* (D) (component 1 = 74.5% and component 2 = 17.7% of variance) during the thermal stress assay experiment (PAST; Bootstrap N = 1000). Fourth root normalised abundance of compounds that varied significantly between control (26°C) and treatment (32°C) ($P < 0.05$; Kruskal Wallis; IBM SPSS version 25) are shown for *C. goreaui* (B) and *D. trenchii* (C). All error bars are standard error. HC = Hydrocarbon, UC = Unclassified (the number following UC indicates the retention time of the compound if the functional group could not be determined).

Discussion

The multifaceted biological and ecological functions of BVOCs can influence ecosystem resilience (Peñuelas and Llusià, 2003), and therefore understanding the roles of these compounds in shaping 'healthy' functioning of threatened ecosystems such as coral reefs will be particularly important (Steinke et al., 2018). To contribute to an enhanced understanding of coral reef volatilomics, I performed the first characterisation of the volatilome of the fundamentally important coral algal endosymbionts Symbiodiniaceae, which revealed substantial diversity of BVOC production. Our study screened species spanning a wide range of Symbiodiniaceae genera (LaJeunesse et al., 2018), demonstrating that key species produce far more BVOCs than simply the iconic BVOC dimethyl sulfide. This diverse pool of volatiles included halogenated hydrocarbons, alkanes and esters among the most commonly detected compounds.

By screening five different species (spanning five genera), *S. linucheae, B. psygmophilum, D. trenchii, E. voratum* and *F. kawagutii,* I detected 32 different BVOCs. This diversity of BVOCs is consistent with a recent screening of three species of marine macroalgae (*Ulva prolifera, Ulva linza* and *Monostroma nitidum*), which identified 41 volatile compounds (Yamamoto et al., 2014). Similarly to our study, alkanes, alkenes, ketones, aldehydes, sulfur compounds, alcohols and esters were detected in the macroalgae studied (Yamamoto et al., 2014). Additionally, Symbiodiniaceae also produced halogenated hydrocarbons which were absent in macroalgae, and only benzaldehyde (known to be involved in signalling amongst insects; Yang et al., 2018) was identified in both Symbiodiniaceae and macroalgae.

Of the 32 volatile compounds detected in this study, six were present in all five Symbiodiniaceae species and were therefore defined as core volatiles. Recognising the ubiquitous nature of certain volatiles is a first step in understanding the potential ecological relevance of compounds produced by this important family of algae. I defined DMS, 2,3-dimethyl hexane, 6-methyl octadecane, an UC halogenated hydrocarbon, UC organosulfur

and UC40.63 as core volatiles. Two of these compounds, 2,3-dimethyl hexane and 6-methyl octadecane, are both short chain alkanes, which may have originated from the oxidation of fatty acids. The inclusion of DMS in the core set of volatiles produced by all Symbiodiniaceae adds weight to the importance and ubiquitous nature of this compound in these organisms. However, it is notable that the relative abundance of DMS varied substantially between Symbiodiniaceae species, with significantly more DMS detected in *S. linucheae* and *D. trenchii* than all other species, while DMS quantities detected in *F. kawagutii* were significantly less than all other species. Both *S. linucheae* and *D. trenchii* are known to be more heat resistant species (Díaz-Almeyda et al., 2017; Goyen et al., 2017; Swain et al., 2017), potentially suggesting that the high amounts of DMS detected under steady state conditions could positively influence their resilience, allowing these species to have a larger existing pool of antioxidants (Hopkins et al., 2016; Steinke et al., 2011).

In addition to DMS, differences between Symbiodiniaceae volatilomes were largely driven by the relative abundance of UC40.45, methyl jasmonate, styrene and 4-fluoro-3-trifluoromethylbenzoic acid eicosyl ester. Methyl jasmonate is ubiquitous in higher plants and is an important signalling molecule that regulates plant development and also plays a role in defence against biotic (e.g. herbivory) and abiotic (e.g. heat) stresses (Dar et al., 2015). Methyl jasmonate was detected in all replicates of *S. linucheae,* and given observations in higher plants it follows that this BVOC might be involved in the thermal resistance of this species. Styrene can occur naturally and has been reported in higher plants (Pagot et al., 2007), while 4-fluoro-3-trifluoromethylbenzoic acid eicosyl ester is a halogenated compound. However, the potential function of these two molecules remains unknown.

This study identified multiple halogenated compounds however, only three were fully classified (1,2-dichloropropane, 3-trifluoroacetoxypentadecane & 4-fluoro-3-trifluoromethylbenzoic acid, eicosyl ester). Halogenated compounds are of particular importance as they can degrade atmospheric ozone (Lim et al., 2017). Halogenated compounds are known to be produced naturally (Gribble, 2003) and have previously been reported from marine microalgae (Lim et al., 2018). For example, three tropical microalgae

(*Amphora* sp., *Synechococcus* sp. & *Parachlorella* sp.) have been demonstrated to produce a range of iodinated and brominated compounds (methyl iodide, bromoform, dibromomethane, dibromochloromethane, and chloroform), with production shown to be species-specific and growth-phase dependent (Lim et al., 2018), highlighting the importance of the physiological state of the cell for volatile emissions. Other examinations of a suite of common phytoplankton (*Calcidiscus leptoporus, Emiliania huxleyi, Phaeodactylum tricornutum, Chaetoceros neogracilis* and *Dunaliella tertiolecta*) demonstrated that all tested species emitted chloromethane, bromoform, bromomethane, chlorobenzene and dichlorobenzene (Colomb et al., 2008). Our understanding of the function of halogenated compounds remains in its infancy, with current hypotheses suggesting a tight coupling of halogenated compound production with oxidative processes, with these compounds potentially formed as side products during the breakdown of reactive oxygen species (Pedersen et al., 1996; Zuo, 2019). Halogenated compounds are also thought to sometimes function as 'infochemicals', with studies on macroalgae demonstrating that bromoform supports the alga's defence by functioning as an antimicrobial (Cabrita et al., 2010; Ohsawa et al., 2001; Paul and Pohnert, 2011). I observed that two halogenated compounds, including 3-trifluoroacetoxypentadecane and another unclassified halogenated BVOC, differed significantly between Symbiodiniaceae species and notably seem to co-occur with each other (Correlation: 0.73, P Value = 0.002, Pearson R, Metaboanalyst4.0; Chong et al., 2018), with significantly higher levels of both compounds present in *S. linucheae* compared to all other species tested. 3-trifluoroacetoxypentadecane has previously been shown to have antimicrobial properties (Hussein et al., 2016), potentially aiding the survival of this species however, far more work is needed to accurately define the function of halogenated compounds.

This initial screening of a range of Symbiodiniaceae species has demonstrated the diversity and species-specific nature of the volatilome. Yet despite this diversity, a consistent core emerges, suggesting that some compounds have a conserved role across species. While appreciating these potential roles is important, most natural systems rarely remain in

steady-state conditions for prolonged amounts of time. Currently, marine systems are experiencing an increase in the frequency and severity of harmful thermal stress events. How corals respond to heat-wave induced bleaching and mortality is often influenced by the thermal tolerance of their endosymbiotic algae (Symbiodiniaceae; Suggett et al., 2017). A number of physiological traits appear to differentiate stress-tolerant versus susceptible species (or genetic variants) of Symbiodiniaceae, including maintaining integrity of photosynthetic constituents (Goyen et al., 2017; Tchernov et al., 2004) and upregulation of pathways to 'detoxify' organelles (Levin et al., 2016). However, the effect of thermal stress on the Symbiodiniaceae volatilome had not been explored until now.

With the onset of thermal stress, a larger number of BVOCs were detected in the thermally tolerant *D. trenchii*, while the thermally sensitive *C. goreaui* produced fewer compounds relative to control conditions. The ability to synthesise specific compounds under the onset of stress could be involved in the thermal tolerance of *D. trenchii,* as '*de novo'* BVOC synthesis has been previously observed in higher plants during abiotic stresses (Beauchamp et al., 2005; Loreto et al., 2006; Loreto and Schnitzler, 2010). During thermal stress there was a dramatic increase in nonanoic acid in *D. trenchii*. Nonanoic acid is a fatty acid and potentially results from increased cell membrane lysis (Hillyer et al., 2016; Yusupov et al., 2017). Furthermore, significantly less DMS was detected in *D. trenchii* during stress, which was the only compound to significantly decrease in this species. Previous work targeting DMS production in other *Cladocopium* and *Durusdinium* Symbiodiniaceae strains also observed a decrease in DMS with the onset of heat stress (Deschaseaux et al., 2014a). Lower levels of DMS under thermal stress may either indicate that Symbiodiniaceae decrease their production, or that DMS degradation increases due to reactions with harmful molecules in response to thermal stress. Interestingly, in this study, I detected higher amounts of another sulfur compound, dimethyl disulfide (DMDS) in *C. goreaui* during stress. DMDS can be formed from the photo-oxidation of methanethiol (MeSH) (Bentley and Chasteen, 2004). Current research focuses on DMS however, these results indicate that we need to consider other sulfur compounds to fully elucidate the role of these sulfur chemicals

in stress response and trophic interactions in coral reefs. An additional 17 compounds significantly increased during thermal stress in *C. goreaui*, with 1,3-dimethoxy-2-propanol, UC40.78 and DMDS the main drivers of the differentiation between treatments. Increases in 1,3-dimethoxy-2-propanol may have resulted from lipid peroxidation (Farmer and Mueller, 2013; Sousa et al., 2017). Whether these BVOCs are released prior to other visual (e.g. bleaching) or physiological (Goyen et al., 2017) stress indicators remains unknown and will be important to assess for the potential use of specific BVOCs to diagnose early stress responses.

The sheer quantity of detected and unidentified compounds that differed significantly across species and under heat stress highlights a critical need to robustly quantify and classify these BVOCs. However, lack of a comprehensive marine BVOC database severely limits our interpretation of volatilomic data, an issue that similarly limits progress for other metabolomic approaches (Steinke et al., 2018). Terrestrial BVOC studies have already identified ~30,000 volatile compounds identified to date (Peñuelas and Llusia, 2004). The unidentifiable chemical diversity highlighted here teases at the potential unexplored roles of BVOCs in biological and ecological interactions in marine systems. These unidentified compounds should therefore not be discarded from future analyses as they may play key roles in stress response or could function as useful stress biomarkers that can be measured non-invasively. Volatile databases are continually improving and as such I have made available our mass spectra files (MSV000084436; MassIVE; doi:10.25345/C5RD5C) for future studies as more comprehensive databases become available.

Numerous BVOCs are known to induce the formation of secondary organic aerosols that enhance cloud formation and albedo (eg. DMS, benzaldehyde, toluene & styrene; Bruns et al., 2016; Charlson et al., 1987), while other compounds can result in increased formation and residence time of crucial greenhouse gases such as ozone (Na et al., 2005). Marine BVOC emissions are often overlooked when it comes to global modelling of BVOC emissions, largely due to the lack of data from these ecosystems. However, marine ecosystems can have a large influence on atmospheric chemistry. Tropical areas are known

to have stronger convection forces that lead to greater transport of emitted BVOCs to the troposphere and stratosphere (Randel and Jensen, 2013). It is therefore essential to understand current tropical baseline emissions if we wish to accurately model future climate scenarios. Examining the Symbiodiniaceae volatilome is a key step towards understanding the prevalence and function of tropical marine BVOCs. However, further work is needed to clarify how free living Symbiodiniaceae BVOC production varies from endosymbiotic Symbiodiniaceae. Coral holobionts are one of the most complex symbiotic systems and the multiple microbial partners they harbour are likely to contribute to reefs' BVOC emissions.

Here I demonstrate for the first time that volatile metabolites produced by Symbiodiniaceae are not only composed of a broad spectrum of BVOCs, but that their production can be influenced by stressful – suboptimum – conditions, suggesting that these overlooked BVOCs likely operate as key constituents regulating metabolic competency. I detected six BVOCs with putative signalling and antioxidant functions that were ubiquitous across five Symbiodiniaceae genera and were defined as core. Many of the BVOCs reported here are as yet uncharacterised, highlighting an urgent need to develop marine-specific annotation pipelines and to further identify new and abundant compounds. This is of particular relevance given that some of these compounds might play currently uncharacterised roles in resistance and survival of corals during thermal stress events. This work provides direction for future studies to start unravelling the complex functions of volatile metabolites.

Acknowledgments

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Supplementary Material

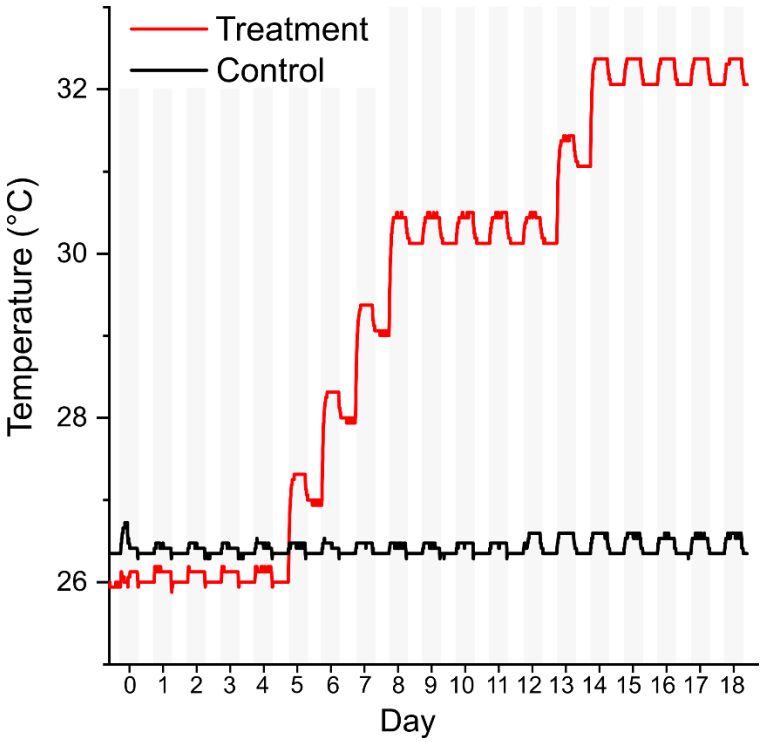

Figure S2.1 Temperature profile for the Symbiodiniaceae stress experiment. Light grey shading indicates the light period. Volatiles were sampled on day 18.

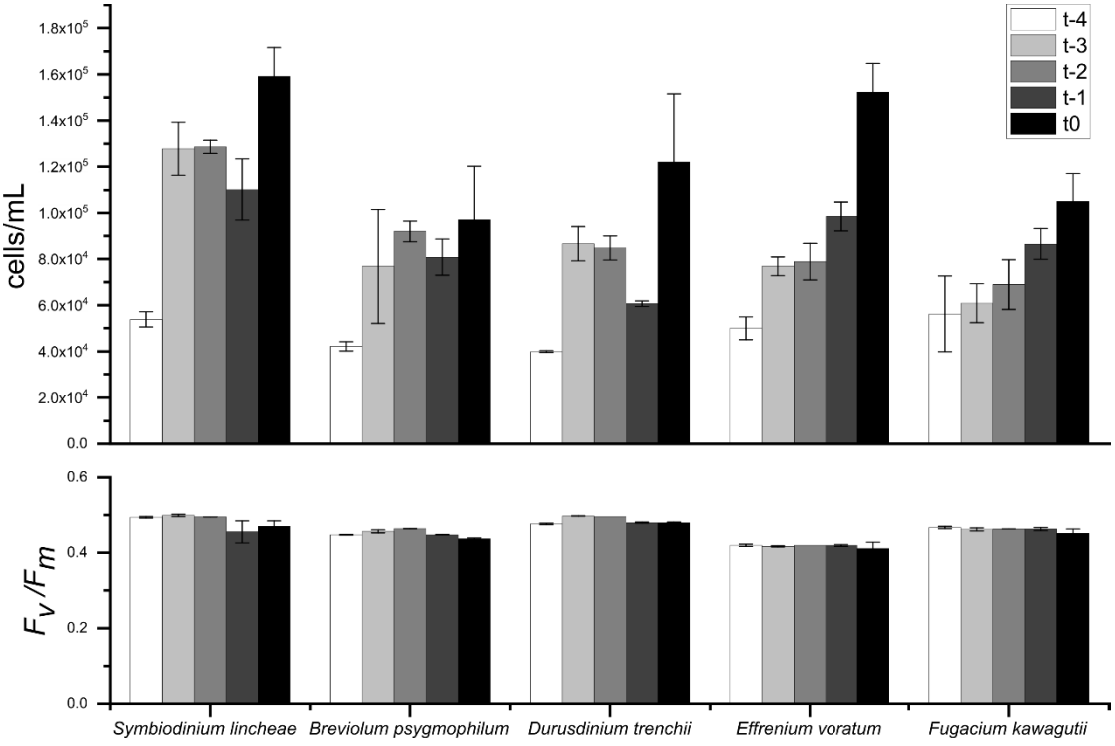

Figure S2.2 Cell density (cells/mL) and F_v/F_m of all Symbiodiniaceae cultures used in the screening experiment in the 4 days prior to BVOC sampling.

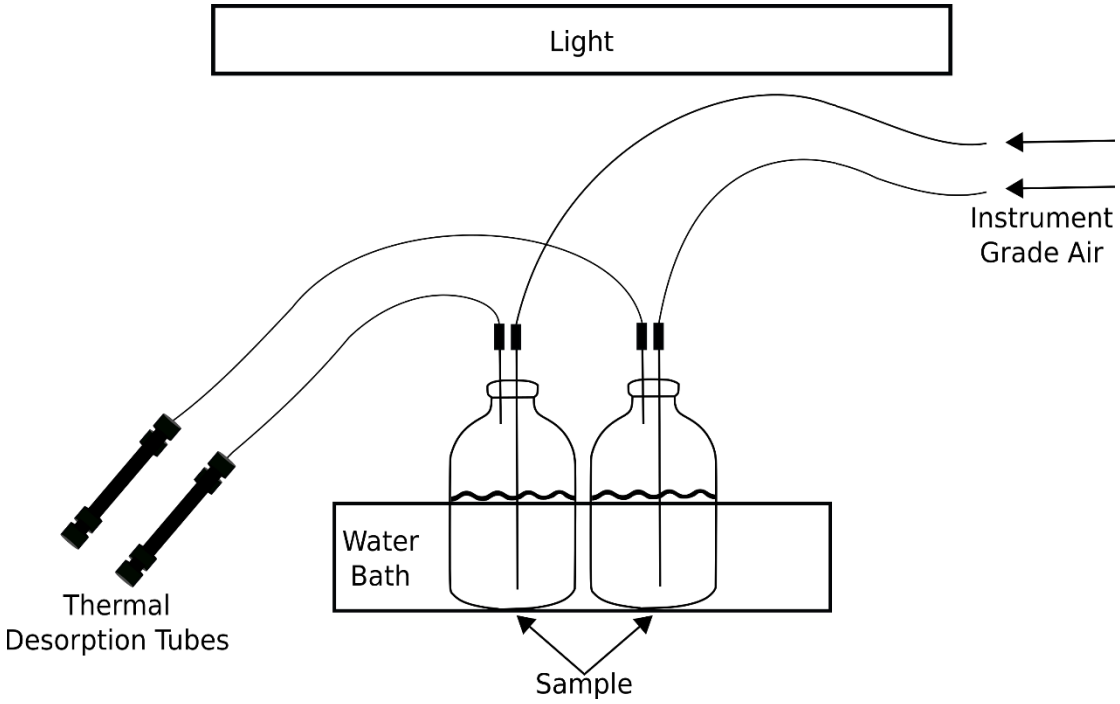

Figure S2.3 Schematic of volatile sampling set up. Culture was placed in gas tight vials and maintained under growth conditions (25°C ± 1.5°C and ca. 50 ± 5 µmol photons m^{-2} s^{-1}) while undergoing a 30 minute purge of instrument grade air. The outlet of this purge passes over Markes thermal desorption tubes (Tenax TA).

Table S2.1 List of all compounds detected in Symbiodiniaceae cultures using the NIST08 library in NIST MS Search v.2.2. DFG = diverse functional groups; UC = Unclassified (the number following UC indicates the retention time of the compound if the functional group could not be determined).

SCREENING EXPERIMENT		
Cluster Number	COMPOUND ID	Functional group
13	UC_18.34	
17	Octadecane, 6-methyl-	Alkane
41	Halogenated organosilicon	Halogenated organosilicon
50	Nonanal	Aldehyde
63	UC 41.89	
93	Benzaldehyde	DFG; aromatic hydrocarbon, aldehyde
94	Cyclooctyl alcohol	Alcohol
104	UC alcohol	Alcohol
109	Squalene	Alkene (Terpenoid)
124	3-Trifluoroacetoxypentadecane	Halogenated ester
127	Styrene	Aromatic hydrocarbon
134	UC 28.26	
139	UC halogenated hydrocarbon	Halogenated hydrocarbon
146	2,4-Dimethyl-1-heptene	Alkene
155	UC halogenated hydrocarbon	halogenated hydrocarbon
156	UC_39.59	
159	UC Aldehyde	Aldehyde
162	UC 40.63	
173	4-Fluoro-3-trifluoromethylbenzoic acid, eicosyl ester	Halogenated hydrocarbon
185	Dimethyl sulfide	Organosulfur
186	Propane, 1,2-dichloro-	Halogenated hydrocarbon
190	Hexadecane	Alkane
195	UC Halogenated hydrocarbon	Halogenated hydrocarbon
196	UC benzoquinone	Ketone
220	1-Propanol, 2,2-dimethyl-, benzoate	Ester
226	UC 40.45	
245	UC 46.85	
265	UC Ester	Ester
269	Hexane, 2,3-dimethyl-	Alkane
271	UC Organosulfur	organosulfur
272	UC ester	Ester
274	Methyl jasmonate	DFG; aromatic hydrocarbon, carboxylic acid, ketone
STRESS EXPERIMENT		
1	l-Alanine ethylamide, (S)-	Amide
11	Silanol, trimethyl-	Organosilicon
17	Octadecane, 6-methyl-	Alkane
20	UC_26.79	
24	3-Trifluoroacetoxypentadecane	Halogenated ester
25	Dimethyl disulfide	Organosulfur
29	2-Propanol, 1,3-dimethoxy-	DFG; ether, alcohol
33	UC 24.96	
41	Halogenated organosilicon	Halogenated organosilicon
47	UC_33.89	
50	Nonanal	Aldehyde
51	UC Ether	Ether

61	UC_38.08	
62	UC Ketone	Ketone
64	Nonanoic acid	Carboxylic acid
71	UC_36.69	
74	Phenol, 2,4-bis(1,1-dimethylethyl)-	Aromatic hydrocarbon
89	Toluene	Aromatic hydrocarbon
91	UC alcohol	Alcohol
95	UC_33.16	
104	UC alcohol	Alcohol
117	1-Pentene, 2,4,4-trimethyl-	Alkene
119	UC Aromatic hydrocarbon	Aromatic hydrocarbon
120	UC ester	Ester
125	Ethylbenzene	Aromatic hydrocarbon
127	Styrene	Aromatic hydrocarbon
133	UC 27.81	
134	UC_28.26	
136	Benzene, 1-ethyl-3-methyl-	Aromatic hydrocarbon
137	UC_30.06	
139	UC halogenated hydrocarbon	Halogenated hydrocarbon
140	UC 31.50	
149	Azulene	Aromatic hydrocarbon (Monoterpene)
153	UC_38.58	
159	UC Aldehyde	Aldehyde
160	1-Hexadecanol, 2-methyl-	Alcohol
161	UC_40.78	
162	UC 40.63	
166	UC 42.02	
168	UC Ketone	Ketone
172	UC 44.07	
177	UC 26.31	
178	UC_39.00	
180	UC 39.36	
181	UC Alkane	Alkane
185	Dimethyl sulfide	Organosulfur
187	2-Butanone, 3,3-dimethyl-	Ketone
193	Benzene, 1-[1,1-dimethylethyl]-4-[2-propenyloxy]-	Ether
194	1-Methoxy-3,5-dimethyl-cyclohexene	Ether
195	UC Halogenated hydrocarbon	Halogenated hydrocarbon
197	UC 45.20	
203	Silanediol, dimethyl-	Organosilicon
210	UC ketone	Ketone
214	Cyclooctyl alcohol	Alcohol
217	UC_45.18	
225	Benzoic acid, pentadecyl ester	Ester
236	Hexane, 2,3-dimethyl-	Alkane
237	UC_42.46	
243	Heptadecane, 9-octyl-	Alkane
245	UC 46.85	
248	Oxime-, methoxy-phenyl-_	DFG, aromatic hydrocarbon, ether, oxime
253	[2.2]Paracyclophane	Aromatic hydrocarbon
256	Pentane	Alkane
257	Naphthalene, 2-methyl-	Aromatic hydrocarbon
264	UC Alkane	Alkane
265	US Ester	Ester
267	UC_42.37	

272	UC ester	Ester
273	UC 17.25	
276	UC_13.09	
278	UC 38.65	
280	UC_34.06	

Table S2.2 P values for all significant statistical differences detected. The screening experiment one-way ANOVA and Tukey's HSD post hoc were performed in MetaboAnalyst4.0 (Chong et al., 2018; Xia et al., 2009). For the stress experiment analysis, a Kruskal-Wallis test was used (IBM SPSS Statistics, version 25), as data did not meet the assumptions required for parametric tests.

Screening experiment		
Compound ID	**P value**	**Tukey's HSD**
UC_18.34	4.34E-12	B2-A4; D1a-A4; E-A4; F1-A4
Dimethyl sulfide	1.54E-05	E-A4; F1-A4; F1-B2; E-D1a; F1-D1a; F1-E
3-Trifluoroacetoxypentadecane	0.001583	B2-A4; D1a-A4; E-A4; F1-A4
UC Halogenated hydrocarbon	0.0025555	B2-A4; D1a-A4; E-A4; F1-A4
UC_39.59	0.003286	B2-A4; E-A4; D1a-B2; E-D1a
Stress experiment		
***Cladocopium goreaui* - testing for differences between control and stress**		
1,3-Dimethoxy-2-propanol	0.046	
2,4,4-trimethyl-1-pentene	0.050	
2,4-bis(1,1-dimethylethyl)-phenol	0.050	
3,3-dimethyl-2-butanone	0.050	
3-Trifluoroacetoxypentadecane	0.050	
9-Octyl-heptadecane	0.050	
Dimethyl disulfide	0.050	
Methoxy-phenyl-oxime	0.050	
Paracyclophane	0.050	
UC Ester	0.050	
UC Halogenated hydrocarbon	0.050	
UC Ketone	0.050	
UC ketone	0.050	
UC_13.09	0.050	
UC_34.06	0.046	
UC_40.78	0.050	
UC_42.37	0.037	
UC_42.46	0.050	
***Durusdinium trenchii* - testing for differences between control and stress**		
1-Methoxy-3.5-dimethyl-cyclohexene	0.050	
2,4-bis(1.1-dimethylethyl)-phenol	0.050	
Benzene 1-[1.1-dimethylethyl]-4-[2-propenyloxy]-	0.050	
Benzoic acid pentadecyl ester	0.037	
Dimethyl sulfide	0.050	
Ethylbenzene	0.050	
Nonanal	0.050	
Nonanoic acid	0.046	
Styrene	0.050	
Trimethyl-silanol	0.050	
UC alcohol	0.046	
UC alcohol	0.050	

UC Alkane	0.050	
UC halogenated hydrocarbon	0.050	
UC Ketone	0.046	
UC Ketone	0.046	
UC_17.25	0.037	
UC_26.79	0.046	
UC_28.26	0.050	
UC_31.50	0.050	
UC_33.89	0.037	
UC_36.69	0.050	
UC_38.58	0.050	
UC_40.78	0.050	
UC_44.07	0.037	

Chapter 3

Defining the Core Microbiome of the Symbiotic Dinoflagellate, Symbiodiniaceae.

Author contributions: CA Lawson, JB Raina, JR Seymour and DJ Suggett conceived and designed the project, CA Lawson performed the experiments, analysed the data and produced figures. T Kahlke provided bioinformatics expertise and assistance. DJ Suggett and JR Seymour provided financial support. CA Lawson wrote the paper. All authors edited the manuscript.

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Lawson, C.A., Raina, J.B., Kahlke, T., Seymour, J.R. and Suggett, D.J., 2018. Defining the core microbiome of the symbiotic dinoflagellate, *Symbiodinium*. Environmental Microbiology Reports, 10(1), pp.7-11.

Please note: At the time of publication, the genus *Symbiodinium* had not yet been redefined as the family: Symbiodiniaceae. As such, in the published version *Symbiodinium* = Symbiodiniaceae, clade A = *Symbiodinium,* clade B = *Breviolum*, clade C = *Cladocopium*, clade D = *Durusdinium*, clade F = *Fugacium.* To maintain consistency for this thesis, the methods section (Appendix S1 in the published version) has been moved to the main text.

Abstract

Dinoflagellates of the family Symbiodiniaceae underpin the survival and ecological success of corals. The use of cultured strains has been particularly important to disentangle the complex life history of Symbiodiniaceae and their contribution to coral host physiology. However, these cultures typically harbour abundant bacterial communities which likely play important, but currently unknown, roles in Symbiodiniaceae biology. I characterised the bacterial communities living in association with a wide phylogenetic diversity of Symbiodiniaceae cultures (18 types spanning 5 genera) to define the core Symbiodiniaceae microbiome. Similar to other systems, bacteria were nearly two orders of magnitude more numerically abundant than Symbiodiniaceae cells and I identified three operational taxonomic units (OTUs) which were present in all cultures. These represented the α-proteobacterium *Labrenzia* and the γ-proteobacteria *Marinobacter* and *Chromatiaceae*. Based on the abundance and functional potential of bacteria harboured in these cultures, their contribution to Symbiodiniaceae physiology can no longer be ignored.

Introduction

Abundant bacterial communities occur in association with reef-building corals, where they play critical roles in recycling nutrients and protection against pathogens (Bourne et al., 2016). Recent research has focussed on identifying the stable and consistent members within coral-associated microbial communities in order to better characterise their functional importance (Astudillo-Garcia et al., 2017), with “core coral microbiomes” recently characterised (Ainsworth et al., 2015). However, despite evidence that microalgae typically also live in close association with bacterial partners (Amin et al., 2015; Seymour et al., 2017), little is known about the bacterial consortia associated with the key symbiotic partner of corals, Symbiodiniaceae.

Dinoflagellates of the family Symbiodiniaceae are globally important primary producers across coastal ecosystems, and also form a key symbiotic partnership with reef-

building corals (Suggett et al., 2017). The genetic and functional diversity of this family is extremely high (Suggett et al., 2015; Thornhill et al., 2017), and the phylogenetic identity of the dominant Symbiodiniaceae symbiont directly influences the environmental stress tolerance and recovery of their coral hosts (Suggett et al., 2017). Consequently, Symbiodiniaceae biology and its impact on the ecological success of reef building corals over space and time, has been a central research focus for decades (Warner and Suggett, 2016). These dinoflagellates can be cultivated *ex-hospite* in monocultures, which remains a primary platform to understand their biology and the complex role they play in coral functioning (Warner and Suggett, 2016). However, these cultures inherently contain abundant bacterial communities co-isolated with Symbiodiniaceae (Frommlet et al., 2015; Ritchie, 2011; Shoguchi et al., 2013). Bacteria are involved in sophisticated interactions with microalgae and their complex metabolic exchanges typically influence the health and physiological performance of both partners (Seymour et al., 2017). To date, the potential roles bacteria may play in Symbiodiniaceae physiology have been almost entirely overlooked. I characterised the bacterial communities associated with 18 Symbiodiniaceae types (spanning 5 genera; Table S3.1), to define the Symbiodiniaceae core microbiome and thus develop a framework for considering the putative functional roles of bacteria in moderating Symbiodiniaceae biology.

Methods

Culturing

All cultures were grown in a Labeq Incubator maintained at 25°C ± 1.5°C and a light intensity of ca. 50 ± 5 µmol photons m^{-2} s^{-1} (Philips TLD 18W/840 Cool White) on a 12:12 hr light:dark cycle. Cultures were grown in sterile 250 mL Schott bottles in 0.2 µm filtered IMK medium and maintained in exponential growth by regularly transferring cultures into new medium (Mcbride et al., 2009). Biological replication was achieved through time (as per Brading et al., 2011); specifically, after each sampling point the culture was diluted and

allowed to re-grow for more than 2 generations prior to the next sampling event. Replication over time was conducted at three consecutive time points, to yield three replicates for each of the 18 types (n=54).

Monitoring

Fast Repetition Rate Fluorometry (FRRf) was utilised to assess the physiological status of the Symbiodiniaceae cultures for the duration of the experiment. FRRf delivers a series of short, but intense, excitation flashlets that stimulates a fluorescence yield from the transient closure of PSII reaction centres within a very short time-frame (<200 µs). Several important photophysiological parameters can be subsequently derived from FRRf, including F_v/F_m which incorporates the maximum (F_m) and initial (F_o) fluorescence yields to gain an understanding of overall 'photosynthetic potential' (F_v/F_m = (F_m –F_o)/F_m). FRRf data were collected non-destructively by agitating the cultures to prevent cells from settling and then directly measuring samples through the clear glass of the Schott bottles. This was achieved by removing the optical head of the fluorometer and standing the Schott bottles upon a custom-made Perspex cover fitted over the instrument lens. This method was tested prior to experimentation and was seen to be equivalent to samples measured in the optical head. Samples were adapted to low light (<5 µmol photons m^{-2} s^{-1}) for a period of 15 minutes to fully relax any nonphotochemical quenching, whilst simultaneously avoiding potential build-up of chlororespiration, thereby ensuring that the maximum quantum yield of PSII was recorded (Suggett et al., 2015). Single turnover protocols (100×1.1 µs flashlets at 2.8 µs intervals) with the excitation delivered via a blue LED (450 nm) excitation source (Robinson et al., 2014) were used with the FastOcean programmed to record the mean of 40 consecutive single turnovers at intervals of 150 ms (Figure S3.2).

An Accuri C6 Flow Cytometer (BD) was used to count Symbiodiniaceae and bacterial cells. For Symbiodiniaceae cells, an aliquot of 1 mL of culture was diluted 10 fold with sterile medium and then directly analysed using the 640 nm Excitation Laser Line and the 670 nm optical filter. For bacterial cells, 80 µL of culture were aliquoted and fixed using 2%

glutaraldehyde, snap frozen in liquid nitrogen and stored at -80°C until processing. Prior to analysis preserved samples were flash-thawed and bacterial DNA was stained using SYBR-I Green (Applied Biosystems) (Marie et al., 1997). Samples were then analysed using 488 nm Excitation Laser Line and using the 533/30 nm optical filter. Calibration beads were run daily.

DNA extraction

Culture samples (30 mL) were filtered using a peristaltic pump (Watson Marlow Sci-Q 323) onto a 0.22 µm GV Durapore® membrane filter (Merck Millipore). Filters were then stored immediately in CryoPure cryotubes (SARSTEDT) in the -80°C freezer until processing. DNA was extracted using the PowerWater® DNA Isolation Kit (MO-BIO Laboratories, Inc.) following the manufacturer's instructions. The raw genomic DNA was stored at -20°C and sent to the Australian Genome Research Facility (AGRF) for sequencing using the Illumina MiSeq platform targeting the 16S region (27F: AGAGTTTGATCMTGGCTCAG – 519R: GWATTACCGC GGCKGCTG).

Bioinformatics & data analysis

Sequencing reads were processed as outlined in https://github.com/timkahlke/ampli-tool. In summary, sequences were joined using FLASh (Magoč and Salzberg, 2011) and subsequently trimmed using mothur (Parameters: maxhomop, maxambig, minlength, maxlength). The resulting fragments were clustered into operational taxonomic units (OTUs) with an identity threshold of 97% and chimeric sequences were identified using vsearch (Rognes et al., 2016) and the Silva v128 database. To assign taxonomy, QIIME (Caporaso et al., 2010) was used with the BLAST algorithm against the Silva v128 database. Data were rarefied to 14000 sequences per sample (Figure S3.3) and the subsequent .biom file was used for all downstream applications. Methylobacterium was present in all samples from 0.02 – 1.37% but was removed from the cores as it is a known contaminant from the DNA extraction process (Salter et al., 2014). The statistical package PRIMER+PERMANOVA (version 6.1; UK) was used to uncover and test the patterns within the data. SIMPER and ANOSIM were used to test the similarities between the bacterial communities; the

communities were visualised using multidimensional scaling (MDS) plots with Bray Curtis similarity (Figure S3.1).

The core was defined as those OTUs that were present in 100% of samples at a minimum relative abundance of 0.0001%. For a conserved core analysis, 21 OTUs were removed based on NCBI quality checks using UCHIME2 (Edgar 2016). The Venn style diagram in Figure 3.2 was created using the online tool, Interactivenn (Heberle et al., 2015).

Results & Discussion

Bacterial cells were on average 65 times more numerically abundant than Symbiodiniaceae in culture ($1.04\times10^7 \pm 1.37\times10^6$ versus $1.59\times10^5 \pm 2.38\times10^4$ cells/mL respectively; Figure 3.1A), which is consistent with bacterial densities commonly reported from other microalgae cultures (Amin et al., 2015). These bacterial communities were also diverse (Table S3.2; Shannon diversity index: 2.72–5.61) and, perhaps notably, their composition and structure differed substantially from that typically observed in other microalgae cultures and coral-associated communities (Ainsworth et al., 2015; Neave et al., 2017). For example, the common coral endosymbiont *Endozoicomonas* (Neave et al., 2017) was not detected in any Symbiodiniaceae strains.

Three different OTUs were present in all Symbiodiniaceae cultures and were identified as core members of the Symbiodiniaceae microbiome (Figure 3.2; Table S3.3). These OTUs belonged to bacterial genera from the α- and γ-proteobacteria (Figure 3.2), and included *Labrenzia*, *Marinobacter* and an unclassified purple sulfur bacterium from the *Chromatiaceae* family. The most abundant core member, *Labrenzia*, represented up to 38.4% of the Symbiodiniaceae-associated communities (Figure 3.1B), has been previously identified in corals and other microalgae cultures (Table S3.7) and is notable for its ability to produce dimethylsulfoniopropionate (DMSP) (Curson et al., 2017). High concentrations of this compound, which likely plays a role in stress tolerance (Sunda et al., 2002), are present in Symbiodiniaceae cultures (Steinke et al., 2011) and have until now been fully attributed to

the microalgae. However, the large proportion of the DMSP-producing *Labrenzia* in Symbiodiniaceae culture illustrates how bacteria might in fact be partially responsible for some traits solely ascribed to Symbiodiniaceae. Another core member, *Marinobacter*, has been demonstrated to produce specific siderophores which can provide enhanced levels of bioavailable iron to phytoplankton (Amin et al., 2009) and could therefore positively impact Symbiodiniaceae growth (Ritchie, 2011), this bacterium has also been previously identified in Symbiodiniaceae cultures (Frommlet et al., 2015). *Marinobacter*, together with the last core member, an unclassified *Chromatiaceae*, have also both been previously reported in corals and other microalgae cultures (Table S3.7).

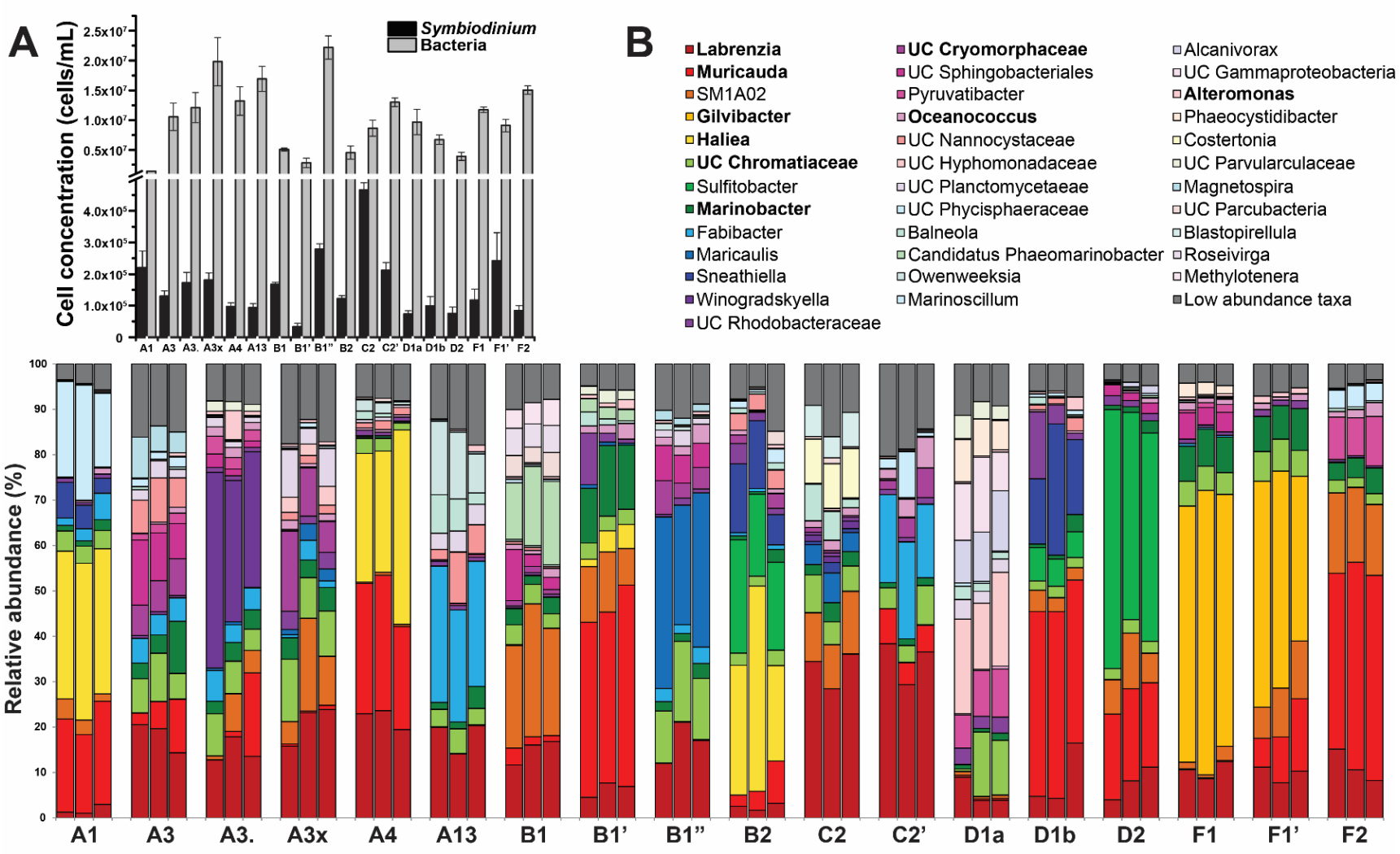

Figure. 3.1 A. Symbiodiniaceae and bacterial cell concentrations (cells mL^{-1}) across 18 cultures of Symbiodiniaceae (spanning 5 genera) maintained in exponential growth in IMK medium (Table S3.1). B. Bacterial community composition (relative abundance %) of each Symbiodiniaceae type at the genus level. The low abundance category contains the sum of all genera that made up < 5% of the community in at least one replicate. Bacteria listed in bold are members of Symbiodiniaceae genera cores. UC: Unclassified.

The small number of OTUs shared across the 18 Symbiodiniaceae strains investigated here is perhaps unsurprising given the large phylogenetic and functional diversity of these dinoflagellates (Figure 3.1B). Therefore, I also defined the core microbiome for each Symbiodiniaceae genera (Figure 3.2). The number of OTUs in these genera-specific cores ranged from 4 in *Symbiodinium* (A) to 35 in *Cladocopium* (C) (which is potentially a consequence of the lower number of *Cladocopium* representatives used here). Four bacterial genera were present and abundant in more than one Symbiodiniaceae genera. These included the Flavobacterium *Muricauda* and the γ-proteobacteria, *Haliea*, *Oceanococcus* and *Algiphilus*. Notably, the latter preferentially uses polycyclic aromatic hydrocarbons that accumulate on phytoplankton cell surfaces as a primary carbon source (Gutierrez et al., 2012). Furthermore, Flavobacteria such as *Muricauda* are known for their association with phytoplankton: they can degrade both high and low molecular weight compounds exuded from these cells and secrete surface proteins that might facilitate their attachment (Buchan et al., 2014).

Notably, *Fugacium* (F) harboured bacterial communities that were significantly different from all other genera (ANOSIM; significance level = 0.1%) and were also the most uniform (similarity: 63.50%) (Table S3.4-S3.5). Furthermore, some unique core members from this genera have potentially been conserved after years of growth in culture (Table S3.1): *Gilvibacter* represented, on average, 51.4 ± 3.65% of the F1 and F1' Symbiodiniaceae type communities, while only having a mean abundance of 0.02±0.003% across all other types. These two F1 strains were isolated from Hawaii and Heron Island (Australia), respectively (Table S3.1). This bacterium has been isolated from the sediment of tropical marine environments, where F1 Symbiodiniaceae is often found in association with Foraminifera (Pochon et al., 2001) and I propose that, given its conserved occurrence across two strains isolated 7000 km apart, it is possible that F1 Symbiodiniaceae might have coevolved in tight association with this nitrate-reducing bacterium (Khan et al., 2007).

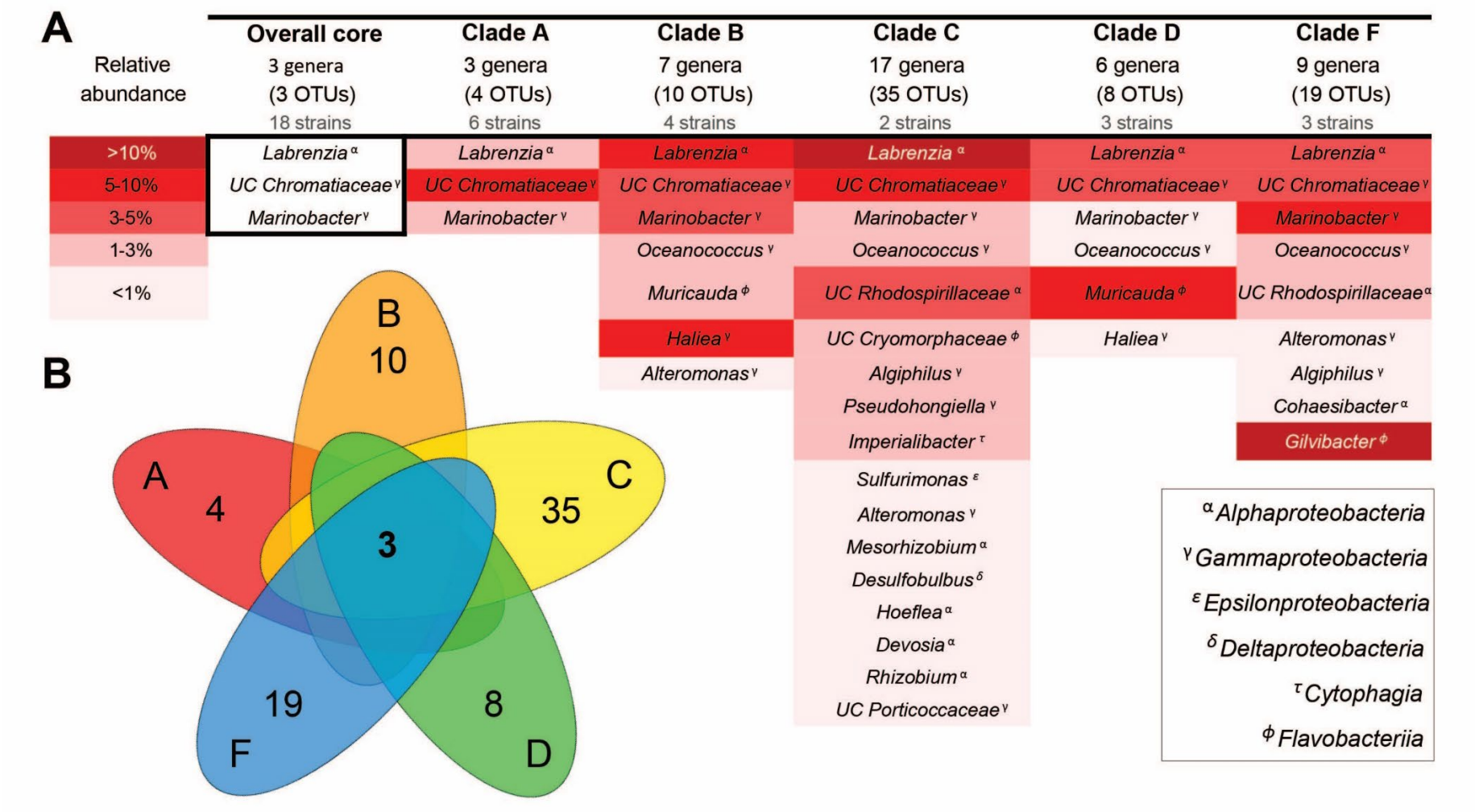

Figure. 3.2 A. The Symbiodiniaceae core microbiome and the individual genera core microbiomes. To be considered core, a single OTU (clustered at 97%) must be present in 100% of samples and have a minimum abundance of 0.0001%, each OTU was then identified to the highest taxonomic resolution possible. B. Diagram showing the number of OTUs in each genera core and the overall core, exclusively. UC: Unclassified.

Less than 5% of the hundreds of studies on Symbiodiniaceae cultures have worked with axenic cultures (Table S3.6). Our results clearly show that bacteria are abundant and diverse within most Symbiodiniaceae cultures and that some bacteria are conserved across the large Symbiodiniaceae phylogenetic diversity, and may be potentially responsible for traits that are presently solely attributed to these dinoflagellates. Consequently, the role played by bacteria in Symbiodiniaceae physiology should no longer be overlooked and future work should precisely target some of the core members identified here and clearly resolve the metabolic interactions that likely occur between core bacterial taxa and this globally important dinoflagellate.

Acknowledgments

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Supporting Information

Table S3.1 Summary of Symbiodiniaceae ITS2 type, strain identification number, location of original isolation, taxa of the cnidarian host species and the time spent in culture. Types are organised alphabetically (determined *via* ITS2 as per LaJeunesse et al., 2012; Lee et al., 2015); species names are given where known (as per Suggett et al., 2015). ' indicates same type but different isolation location.

ITS2 Type	Species	Strain ID	Origin	Host	Years in culture
A1	*S. microadriaticum*	CCMP2464, rt61	Florida (Caribbean Sea)	*Cassiopeia xamachana* (Jellyfish)	30/07/2004▲
A3	*S. tridacnidorum*	CS159	Palau (Pacific)	*Tridacna maxima* (Giant Clam)	01/01/1983●
A3	*S. tridacnidorum*	CCMP2465, rt292	Palau (Pacific)	*Tridacna maxima* (Giant Clam)	30/07/2004▲
A3*x*	*S. tridacnidorum*	CS73	Heron Island (Pacific)	*Tridacna maxima* (Giant Clam)	01/01/1978■
A4	*S. linchuae*	CCMP2456, 379	Sargasso Sea (Caribbean Sea	*Plexaura homomalla* (Gorgonian)	29/07/2004▲
A13	*S. necroappetens*	CCMP2469, JS879, rt80	Jamaica (Caribbean Sea)	*Condylactis gigantean* (Anemone)	30/07/2004▲
B1	*S. minutum*	CCMP3345, CCMP2460, rt2	Florida (Caribbean Sea)	*Aiptasia pallida* (Anemone)	26/02/2013, 30/07/2004▲*
B1'	*S. pseudominutum*	rt12	Puerto Rico (Caribbean Sea)	*Aiptasia tagetes* (Anemone)	Unknown
B1''		UTSB	S. Taiwan (Indo-Pacific)	*Euphyllia glabrescens* (Coral)	Unknown
B2	*S. psygmophilum*	CCMP3364	Florida (Caribbean Sea)	*Orbicella faveolata* (Coral)	26/03/2013▲
C2		HHIB	Unknown	*Hippopus hippopus* (Giant Clam)	Unknown
C2'		rt203	Palau (Pacific)	*Hippopus hippopus* (Giant Clam)	Unknown
D1b		PHMS T54 D1b	Philippines (Pacific)	*Tridacna* sp. (Giant Clam)	Unknown
D1a		UTSD amur-D-MI	Magnetic Island (Pacific)	*Acropora muricata* (Coral)	Unknown
D2		rt401	Gulf of Aqaba (Red Sea)	Unknown foraminifera sp.	Unknown
F1	*S. kawagutii*	CS156	Hawaii (Pacific)	*Montipora veruccosa* (Coral)	17/12/2013●
F1'	*S. kawagutii*	UTSC	Heron Island (Pacific)	*Pocillopora damicornis* (Coral)	Unknown
F2		CCMP2455, rt133	Jamaica (Caribbean Sea)	*Meandrina meandrites* (Coral)	29/07/2004▲

▲Deposit Date at the National Center for Marine Algae and Microbiota.
● Date of Receipt at the Australian National Algae Culture Collection
■ Date of Isolation by E.M Deane, Date of Receipt at the Australian National Algae Culture Collection was 01/01/1979
* Deposit dates for each CCMP3345 and CCMP2460 resepctively.

Table S3.2 Shannon diversity index (H') and number of observed species (O_sp.) calculated using the QIIME alpha_diversity.py command for bacterial communities present 18 ITS2 types (in triplicate). Analysis done on rarefied data (to 14000 reads). Colour scale for H': lowest = white, highest = purple; O_sp.: lowest = white, highest = green.

ITS2	H'	O_sp.	ITS2	H'	O_sp.	ITS2	H'	O_sp.	ITS2	H'	O_sp.	ITS2	H'	O_sp.	ITS2	H'	O_sp.
A1	3.63	184	**A3x**	5.49	350	**B1**	4.57	232	**B2**	4.29	264	**D1a**	4.75	213	**F1**	3.50	225
A1	3.54	196	**A3x**	4.81	316	**B1**	4.22	217	**B2**	3.83	231	**D1a**	4.75	240	**F1**	3.22	196
A1	3.93	220	**A3x**	5.61	322	**B1**	4.35	219	**B2**	4.86	326	**D1a**	5.00	265	**F1**	3.51	216
A3	5.10	346	**A4**	4.43	254	**B1'**	3.85	217	**C2**	4.43	244	**D1b**	3.68	217	**F1'**	3.61	134
A3	5.19	317	**A4**	4.44	251	**B1'**	3.92	230	**C2**	4.89	247	**D1b**	3.37	197	**F1'**	3.63	127
A3	5.34	348	**A4**	4.08	237	**B1'**	3.82	235	**C2**	4.33	231	**D1b**	4.02	241	**F1'**	4.01	159
A3.	4.04	284	**A13**	4.31	224	**B1"**	4.32	255	**C2'**	5.04	320	**D2**	2.72	161	**F2**	4.05	244
A3.	4.54	313	**A13**	4.52	238	**B1"**	4.87	290	**C2'**	5.15	313	**D2**	3.19	176	**F2**	3.80	204
A3.	4.31	262	**A13**	4.88	245	**B1"**	4.42	258	**C2'**	5.17	309	**D2**	3.31	183	**F2**	3.79	203

Table S3.3 Operational taxonomic units (OTUs) defined as core members of the bacterial communities of Symbiodiniaceae cultures and their corresponding GenBank accession numbers.

Overall core OTUs	**Taxonomic ID**	**Genbank**
Symbiodinium core 1	*Labrenzia*	MF598485
Symbiodinium core 2	*Marinobacter*	MF598486
Symbiodinium core 3	UC Chromatiaceae	MF598487
Clade A core OTUs	**Taxonomic ID**	**Genbank**
Symbiodinium clade A core 1	*Labrenzia*	MF598488
Symbiodinium clade A core 2	*Marinobacter*	MF598489
Symbiodinium clade A core 3	UC Chromatiaceae	MF598490
Symbiodinium clade A core 4	UC Chromatiaceae	MF598491
Clade B core OTUs	**Taxonomic ID**	**Genbank**
Symbiodinium clade B core 1	*Labrenzia*	MF598492
Symbiodinium clade B core 2	*Alteromonas*	MF598493
Symbiodinium clade B core 3	*Labrenzia*	MF598494
Symbiodinium clade B core 4	*Marinobacter*	MF598495
Symbiodinium clade B core 5	*Muricauda*	MF598496
Symbiodinium clade B core 6	*Marinobacter*	MF598497
Symbiodinium clade B core 7	*Oceanococcus*	MF598498
Symbiodinium clade B core 8	UC Chromatiaceae	MF598499
Symbiodinium clade B core 9	*Haliea*	MF598500
Symbiodinium clade B core 10	UC Chromatiaceae	MF598501
Clade C core OTUs	**Taxonomic ID**	**Genbank**
Symbiodinium clade C core 1	UC Chromatiaceae	MF598502
Symbiodinium clade C core 2	*Labrenzia*	MF598503
Symbiodinium clade C core 3	UC Chromatiaceae	MF598504
Symbiodinium clade C core 4	*Alteromonas*	MF598505
Symbiodinium clade C core 5	*Labrenzia*	MF598506
Symbiodinium clade C core 6	*Rhizobium*	MF598507
Symbiodinium clade C core 7	*Devosia*	MF598508
Symbiodinium clade C core 8	*Hoeflea*	MF598509
Symbiodinium clade C core 9	*Labrenzia*	MF598510
Symbiodinium clade C core 10	*Labrenzia*	MF598511
Symbiodinium clade C core 11	*Labrenzia*	MF598512
Symbiodinium clade C core 12	*Labrenzia*	MF598513
Symbiodinium clade C core 13	*Desulfobulbus*	MF598514
Symbiodinium clade C core 14	UC Porticoccaceae	MF598515
Symbiodinium clade C core 15	*Pseudohongiella*	MF598516
Symbiodinium clade C core 16	*Marinobacter*	MF598517
Symbiodinium clade C core 17	*Marinobacter*	MF598518
Symbiodinium clade C core 18	*Oceanococcus*	MF598519
Symbiodinium clade C core 19	*Alteromonas*	MF598520
Symbiodinium clade C core 20	UC Cryomorphaceae	MF598521
Symbiodinium clade C core 21	*Algiphilus*	MF598522
Symbiodinium clade C core 22	*Oceanococcus*	MF598523
Symbiodinium clade C core 23	*Oceanococcus*	MF598524
Symbiodinium clade C core 24	*Imperialibacter*	MF598525
Symbiodinium clade C core 25	UC Chromatiaceae	MF598526
Symbiodinium clade C core 26	*Sulfurimonas*	MF598527
Symbiodinium clade C core 27	*Algiphilus*	MF598528
Symbiodinium clade C core 28	UC Chromatiaceae	MF598529
Symbiodinium clade C core 29	UC Chromatiaceae	MF598530
Symbiodinium clade C core 30	UC Chromatiaceae	MF598531
Symbiodinium clade C core 31	UC Chromatiaceae	MF598532
Symbiodinium clade C core 32	UC Rhodospirillaceae	MF598533
Symbiodinium clade C core 33	UC Rhodospirillaceae	MF598534
Symbiodinium clade C core 34	*Mesorhizobium*	MF598535
Symbiodinium clade C core 35	*Labrenzia*	MF598536
Clade D core OTUs	**Taxonomic ID**	**Genbank**
Symbiodinium clade D core 1	*Labrenzia*	MF598537
Symbiodinium clade D core 2	*Muricauda*	MF598538
Symbiodinium clade D core 3	*Muricauda*	MF598539
Symbiodinium clade D core 4	*Marinobacter*	MF598540
Symbiodinium clade D core 5	*Oceanococcus*	MF598541
Symbiodinium clade D core 6	UC Chromatiaceae	MF598542
Symbiodinium clade D core 7	*Haliea*	MF598543
Symbiodinium clade D core 8	UC Chromatiaceae	MF598544
Clade F core OTUs	**Taxonomic ID**	**Genbank**
Symbiodinium clade F core 1	UC Chromatiaceae	MF598545
Symbiodinium clade F core 2	*Labrenzia*	MF598546
Symbiodinium clade F core 3	UC Chromatiaceae	MF598547
Symbiodinium clade F core 4	*Alteromonas*	MF598548
Symbiodinium clade F core 5	*Cohaesibacter*	MF598549
Symbiodinium clade F core 6	*Labrenzia*	MF598550
Symbiodinium clade F core 7	*Labrenzia*	MF598551
Symbiodinium clade F core 8	*Marinobacter*	MF598552
Symbiodinium clade F core 9	*Marinobacter*	MF598553
Symbiodinium clade F core 10	*Marinobacter*	MF598554
Symbiodinium clade F core 11	*Oceanococcus*	MF598555
Symbiodinium clade F core 12	*Alteromonas*	MF598556
Symbiodinium clade F core 13	*Gilvibacter*	MF598557
Symbiodinium clade F core 14	UC Chromatiaceae	MF598558
Symbiodinium clade F core 15	*Algiphilus*	MF598559
Symbiodinium clade F core 16	UC Rhodospirillaceae	MF598560
Symbiodinium clade F core 17	UC Chromatiaceae	MF598561
Symbiodinium clade F core 18	UC Chromatiaceae	MF598562
Symbiodinium clade F core 19	UC Chromatiaceae	MF598563

Table S3.4 ANOSIM results on the bacterial communities associated with five clades (A, B, C, D & F) of Symbiodiniaceae in cultures. Significant values in bold.

Clades	Significance Level %	R Statistic
A, B	3.1	0.117
A, C	11.1	0.133
A, D	**0.1**	0.385
A, F	**0.1**	0.36
B, C	40.7	0.011
B, D	2.4	0.194
B, F	**0.2**	0.288
C, D	1.1	0.374
C, F	**0.1**	0.827
D, F	**0.1**	0.445

Table S3.5 SIMPER analysis of the bacterial communities associated with five genera (A, B, C, D & F) of Symbiodiniaceae in cultures. Showing the main drivers of the cladal similarity and dissimilarity.

Similarity	Mean Abundance	Contribution (%)
A: 50.21%		
Labrenzia	3.81	13.28
UC Chromatiaceae	2.39	8.45
Muricauda	2.50	5.94
B: 47.45%		
Labrenzia	2.99	10.34
UC Chromatiaceae	2.30	8.16
Marinobacter	2.02	6.33
C: 63.11%		
Labrenzia	5.81	18.00
UC Chromatiaceae	2.41	6.95
Oceanococcus	1.67	4.41
D: 50.24%		
Muricauda	3.76	11.70
Labrenzia	2.60	10.29
Sulfitobacter	3.22	7.60
F: 63.50%		
Labrenzia	3.23	12.81
Gilvibacter	4.83	12.73
Marinobacter	2.59	10.12

Dissimilarity	Mean Abundance	Mean Abundance	Contribution (%)
A&B: **55.99%**	**A**	**B**	
Haliea	2.02	1.97	4.96
Muricauda	2.5	2.58	4.78
SM1A02	1.33	2.13	3.95
A&C: **53.80%**	**A**	**C**	
Fabibacter	2.16	2.27	4.34
Muricauda	2.5	1.33	4.18
Haliea	2.02	0.21	4.01
B&C: **55.89%**	**B**	**C**	
Labrenzia	2.99	5.81	5.27
Muricauda	2.58	1.33	4.08
Fabibacter	0.69	2.27	3.91
A&D: **60.85%**	**A**	**D**	
Sulfitobacter	0.19	3.22	6.05
Muricauda	2.5	3.76	4.93
Haliea	2.02	0.26	3.83
B&D: **60.09%**	**B**	**D**	
Sulfitobacter	1.22	3.22	6
Muricauda	2.58	3.76	5.43
SM1A02	2.13	1.86	3.8

	Mean Abundance	Mean Abundance	Contribution (%)
C&D: **63.00%**	**C**	**D**	
Sulfitobacter	0.09	3.22	5.77
Labrenzia	5.81	2.6	5.58
Muricauda	1.33	3.76	5.39
A&F: **56.79%**	**A**	**F**	
Gilvibacter	0.11	4.83	10.04
Muricauda	2.5	3.42	5.7
Haliea	2.02	0.16	4.45
B&F: **57.28%**	**B**	**F**	
Gilvibacter	0.11	4.83	9.9
Muricauda	2.58	3.42	5.83
SM1A02	2.13	2.82	4.48
C&F: **54.81%**	**C**	**F**	
Gilvibacter	0.17	4.83	9.96
Muricauda	1.33	3.42	5.8
Labrenzia	5.81	3.23	5.46
D&F: **58.63%**	**D**	**F**	
Gilvibacter	0.14	4.83	10.23
Sulfitobacter	3.22	0.1	7.17
Muricauda	3.76	3.42	5.99

Table S3.6 Web of Science literature search to identify studies on Symbiodiniaceae using cultures. Search parameters: Search term "*Symbiodinium* culture" and "axenic *Symbiodinium* culture. These two lists were cross checked to ensure they did overlap and axenic studies were manually checked to ensure viability. All studies that used axenic cultures of Symbiodiniaceae are highlighted (12 studies out of 268 use axenic cultures). Search date: 27/06/2017.

Baillie, B. K., et al. (1998)
Darius, H. T., et al. (1998)
Deschaseaux, E. S. M., et al. (2014)
Hambleton, E. A., et al. (2014)
Polne-Fuller, M. (1991)
Santos, S. R., et al. (2001)
Schoenberg, D. A. and R. K. Trench (1980)
Silva-Lima, A. W., et al. (2015)
Trench, R. K. and L. V. Thinh (1995)
Xiang, T. T., et al. (2013)
Xiang, T. T., et al. (2015)
Yost, D. M. and C. L. Mitchelmore (2009)
Aihara, Y., et al. (2016)
Alagely, A., et al. (2011)
Al-Moghrabi, S., et al. (1996)
Andras, J. P., et al. (2009)
Annis, E. R. and C. B. Cook (2002)
Arif, C., et al. (2014)
Baillie, B. K., et al. (2000)
Banaszak, A. T. and R. K. Trench (1995a)
Banaszak, A. T. and R. K. Trench (1995b)
Banaszak, A. T., et al. (2000)
Banaszak, A. T., et al. (2006)
Barnay-Verdier, S., et al. (2013)
Baumgarten, S., et al. (2013)
Beedessee, G., et al. (2015)
Behzad, H., et al. (2016)
Belda, C. A. and D. Yellowlees (1995)
Belda, C. A., et al. (1993)
Belda-Baillie, C. A., et al. (1999)
Belda-Baillie, C. A., et al. (2002)
Benstein, R., et al. (2014)
Bergmann, N., et al. (2011)
Berner, T., et al. (1993)
Blackall, L. L., et al. (2015)
Brading, P., et al. (2013)
Burghardt, I. and H. Wagele (2014)
Buxton, L., et al. (2009)
Buxton, L., et al. (2012)
Carlos, A. A., et al. (1999)
Carlos, A. A., et al. (2000)
Chen, C. S., et al. (2005)
Chen, C. S., et al. (2012)
Chen, H. K., et al. (2015)
Chong, G., et al. (2016)
Cohen, Y., et al. (2013)
Correa, A. M. S., et al. (2013)
Costa, A. P. L., et al. (2016)
Crafts, C. B. and J. R. Tuliszewski (1995)
Cumbo, V. R., et al. (2013)
Davies, S. W., et al. (2013)
Decelle, J., et al. (2012)
Dunn, S. R., et al. (2012)
Farmer, M. A., et al. (2001)
Fitt, W. K. and R. K. Trench (1983)
Fitt, W. K., et al. (1981)
Fitt, W. K., et al. (1995)
Franklin, D. J., et al. (2004)
Frommlet, J., et al. (2015a)
Frommlet, J., et al. (2015b)
Fuchinoue, Y., et al. (2012)
Fujise, L., et al. (2014)
Fukatsu, T., et al. (2007)
Gaither, M. R. and R. Rowan (2010)
Gardner, S. G., et al. (2015)
Gibbin, E. M. and S. K. Davy (2013)
Gierz, S. L., et al. (2017)
Goiran, C., et al. (1997)
Gomez, F. (2014)
Goulet, T. L., et al. (2005)
Graham, M. H. (2015)
Granados-Cifuentes, C. and M. Rodriguez-Lanetty (2011)
Grant, A. J., et al. (2003)
Gustafsson, M. S. M., et al. (2014)
Hagedorn, M. and V. L. Carter (2015)
Hagedorn, M., et al. (2010)
Hagedorn, M., et al. (2015)
Hallegraeff, G., et al. (2014)
Hartle-Mougiou, K., et al. (2012)
Hawkins, T. D., et al. (2016)
Hayashi, A., et al. (2016)
Hill, R. and S. Takahashi (2014)
Hill, R., et al. (2009)
Hill, R., et al. (2011)
Hill, R., et al. (2012)
Hill, R., et al. (2014)
Hirose, M., et al. (2008)
Hoogenboom, M. O., et al. (2012)
Howells, E. J., et al. (2012)
Huang, J., et al. (2012)
Huertas, I. E., et al. (2011)
Hume, B. C. C., et al. (2014)
Hume, B., et al. (2013)
Iglesiasprieto, R. and R. K. Trench (1994)
Iglesias-Prieto, R. and R. K. Trench (1997)
Iglesiasprieto, R., et al. (1992)
Iida, S., et al. (2009)
Ikeda, S., et al. (2016)
Ishikura, M., et al. (2004)
Janouskovec, J., et al. (2013)
Jeong, H. J., et al. (2012)
Jeong, H. J., et al. (2014)
Jiang, J., et al. (2012)
Jiang, J., et al. (2014)
Jiang, J., et al. (2015)
Jimbo, M., et al. (2010)
Jimbo, M., et al. (2013)
Karako-Lampert, S., et al. (2005)
Karim, W., et al. (2015)
Khalesi, M. K. (2008)
Kii, S. I., et al. (2007)
Kita, M., et al. (2006)
Kita, M., et al. (2007)
Klueter, A., et al. (2015)
Kneeland, J., et al. (2013)
Kobayashi, J., et al. (1988)
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Krueger, T. and R. D. Gates (2012)
Krueger, T., et al. (2014)
Kuniya, N., et al. (2015)
Kurihara, T., et al. (2012)
LaJeunesse, T. C. (2001)
LaJeunesse, T. C. (2002)
LaJeunesse, T. C. and D. J. Thornhill (2011)
LaJeunesse, T. C. and R. K. Trench (2000)
LaJeunesse, T. C., et al. (2005)
LaJeunesse, T. C., et al. (2014)
Laurent, K. L. and C. G. Trick (2012)
Lawrence, S. A., et al. (2014)
Lee, J. J., et al. (1997)
Lee, J. J., et al. (2009)
Lee, J. J., et al. (2010)
Lee, S. Y., et al. (2015)
Lesser, M. P. (1996)
Lesser, M. P. and J. M. Shick (1990)
Lewis, L. A. and G. Muller-Parker (2004)
Lilley, R. M., et al. (2010)
Littman, R. A., et al. (2009)
Logan, D. D. K., et al. (2010)
Lohr, J., et al. (2007)
Loram, J. E., et al. (2007)
Markell, D. A. and E. M. Wood-Charlson (2010)
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Mayfield, A. B., et al. (2010)
Mayfield, A. B., et al. (2014)
Mays, M. M. and S. C. Kempf (2014)
Mcauley, P. J. and V. J. Smith (1995)
McBride, B. B., et al. (2009)
McGinley, M. P., et al. (2012)
McGinley, M. P., et al. (2013)
McIlroy, S. E., et al. (2016)
McIlroy, S., et al. (2014)
McLenon, A. L. and G. R. DiTullio (2012)
Mellas, R. E., et al. (2014)
Mies, M., et al. (2017a)
Mies, M., et al. (2017b)
Montresor, M., et al. (1999)
Morera, C. and M. A. Villanueva (2009)
Mydlarz, L. D., et al. (2003)
Mylne, J. S., et al. (1998)
Nakamura, H. (1998)
Nakamura, H., et al. (1998)
Newberger, N. C., et al. (2006)
Nitschke, M. R., et al. (2015)
Oakley, C. A., et al. (2014a)
Oakley, C. A., et al. (2014b)
Obornik, M., et al. (2011)
Onodera, K. I., et al. (2004)
Onodera, K., et al. (2003)
Onodera, K., et al. (2005)
Ortiz-Matamoros, M. F., et al. (2015a)
Ortiz-Matamoros, M. F., et al. (2015b)
Park, J., et al. (2016)
Parkinson, J. E. and M. A. Coffroth (2015)
Parkinson, J. E., et al. (2016)
Pasaribu, B., et al. (2015)
Pasaribu, B., et al. (2016)
Paxton, C. W., et al. (2013)
Pierangelini, M., et al. (2017)
Pontasch, S., et al. (2017)
Porto, I., et al. (2008)
Potvin, E., et al. (2015)
Price, D. C., et al. (2016)
Probert, I., et al. (2014)
Ragni, M., et al. (2010)
Rawlinson, K. A., et al. (2011)
Rehman, A. U., et al. (2016)
Reimer, J. D., et al. (2013)
Roberty, S., et al. (2014)
Robison, J. D. and M. E. Warner (2006)
Rodriguez-Roman, A. and R. Iglesias-Prieto (2005)
Rogers, J. E. and D. Marcovich (2007)
Rogers, J. E. and R. H. Davis (2006)
Rogers, J. E., et al. (2010)
Roopin, M. and N. E. Chadwick (2009)
Roopin, M., et al. (2011)
Roopin, M., et al. (2013)
Rosic, N. N., et al. (2011a)
Rosic, N. N., et al. (2011b)
Rowan, R. (1991)
Rowan, R. and D. A. Powers (1991)
Santiago-Vazquez, L. Z., et al. (2007)
Santos, S. R. and M. A. Coffroth (2003)
Santos, S. R., et al. (2002)
Santos, S. R., et al. (2003)
Santos, S. R., et al. (2004)
Saragosti, E., et al. (2010)
Schlichter, D., et al. (1997)
Schwarz, J. A. and V. M. Weis (2003)
Schwarz, J. A., et al. (2002)
Shick, J. M. (2004)
Shick, J. M., et al. (2005)
Slavov, C., et al. (2016)
Soffer, N., et al. (2014)
Sorek, M. and O. Levy (2012a)
Sorek, M. and O. Levy (2012b)
Sorek, M., et al. (2013)
Starzak, D. E., et al. (2014)
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Stern, R. F., et al. (2010)
Stern, R. F., et al. (2012)
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Takahashi, S., et al. (2008)
Takahashi, S., et al. (2009)
Takahashi, S., et al. (2013)
Takishita, K., et al. (2003)
Thornhill, D. J., et al. (2006)
Thornhill, D. J., et al. (2008)
Thornhill, D. J., et al. (2010)
Tolleter, D., et al. (2013)
Tsunematsu, Y., et al. (2009)
van Dam, J. W., et al. (2015)
Villanueva, M. A., et al. (2012)
Villanueva, M. A., et al. (2014)
Villanueva, M. A., et al. (2015)
Voolstra, C. R. (2013)
Voolstra, C. R., et al. (2009)
Wagner, D., et al. (2012)
Wakefield, T. S., et al. (2000)
Wang, C., et al. (2016)
Wang, J. T. and A. E. Douglas (1999)
Wang, L. H., et al. (2008)
Wang, L. H., et al. (2013)
Wang, L. H., et al. (2015)
Watanabe, T., et al. (2006)
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Weng, L. C., et al. (2014)
Wham, D. C., et al. (2013)
Wietheger, A., et al. (2015)
Wilcox, T. P. (1998)
Yacobovitch, T., et al. (2004)
Yamashita, H. and K. Koike (2013)
Yamashita, H., et al. (2009)
Yamashita, H., et al. (2014)
Yang, I., et al. (2011)
Yost, D. M. and C. L. Mitchelmore (2012)
Yuyama, I. and T. Higuchi (2014)
Yuyama, I., et al. (2005)
Yuyama, I., et al. (2011)
Yuyama, I., et al. (2016)
Zhou, J., et al. (2017)
Zielke, S. and A. Bodnar (2010)

Table S3.7 Summary of studies reporting *Labrenzia*, *Marinobacter* and Chromatiaceae sequenced or isolated from corals (pale blue) and microalgae (pale green). Literature search completed on the 17th of September 2017 using Google Scholar.

Bacteria	Host	Location/Strain ID	Method	Reference
Labrenzia	*Alcyonium digitatum*	Kosterfjorden, Sweden	Isolation	***Alsmark et al. 2013***
Labrenzia	Soft coral	Nanwan Bay, Taiwan	Isolation	***Chen et al. 2012***
Labrenzia	*Platygyra carnosus*	Hong Kong	Isolation	***Chiu et al. 2011***
Labrenzia	*Acropora hemprichii*	Al-Fahal Reef, Red Sea	Amplicon	***Jessen et al. 2013***
Labrenzia	*Acropora millepora*	Orpheus Island, Great Barrier Reef	Amplicon	***Raina et al. 2013***
Labrenzia	*Orbicella faveolata*	Weimberg reef, Caribbean	Amplicon	***Roder et al. 2014***
Labrenzia	*Orbicella franksi*	Weimberg reef, Caribbean	Amplicon	***Roder et al. 2014***
Labrenzia	*Ctenactis echinata*	Al Quad Reef, Red Sea	Amplicon	***Roder et al. 2015***
Labrenzia	*Acropora cervicornis*	Caribbean	Amplicon	***Sunagawa et al. 2010***
Labrenzia	*Alcyonium gracillimum*	Zhaoan Bay, East China Sea	Amplicon	***Yang et al. 2013***
Labrenzia	*Tubastraea coccinea*	Zhaoan Bay, East China Sea	Amplicon	***Yang et al. 2013***
Labrenzia	*Pocillopora verrucosa*	Jeddah, Red Sea	Amplicon	***Ziegler et al. 2016***
Labrenzia	*Acropora hemprichii*	Jeddah, Red Sea	Amplicon	***Ziegler et al. 2016***
Labrenzia	*Aureococcus anophagefferens*	CCMP1708	Amplicon	***Baker et al. 2009***
Labrenzia	*Nannochloropsis gaditana*	Ocean University of China	Isolation	***Han et al. 2016***
Labrenzia	*Nannochloropsis oculata*	Ocean University of China	Isolation	***Han et al. 2016***
Labrenzia	*Cylindrotheca fusiformis*	Ocean University of China	Isolation	***Han et al. 2016***
Labrenzia	*Karenia mikimotoi*	Ocean University of China	Isolation	***Han et al. 2016***
Labrenzia	*Nannochloropsis gaditana*	N/A	Amplicon	***Nam et al. 2015***
Labrenzia	*Scrippsiella trochoidea*	CCAP 1134/1	Isolation & Amplicon	***Hatton et al. 2010***
Labrenzia	*Picochlorum* sp.	Strain S1b	Isolation	***Kuo et al. 2015***
Labrenzia	*Chaetoceros* sp.	MBTDCMFRI S065	Isolation	***Sandhya et al. 2017***
Labrenzia	*Tetraselmis* sp.	MBTDCMFRI S082	Isolation	***Sandhya et al. 2017***
Labrenzia	*Nannochloropsis oceanica*	MBTDCMFRI S078	Isolation	***Sandhya et al. 2017***
Labrenzia	*Isochrysis galbana*	MBTDCMFRI S002	Isolation	***Sandhya et al. 2017***
Marinobacter	Hard Coral	Brazil	Amplicon	***Carlos et al. 2013***
Marinobacter	*Siderastrea siderea*	Horseshoe Reef, Bahamas	Isolation	***Gantar et al. 2011***
Marinobacter	*Porites lobata*	Persian/Arabian Gulf	Amplicon	***Hadaidi et al. 2017***
Marinobacter	*Porites lobata*	Red Sea	Amplicon	***Hadaidi et al. 2017***
Marinobacter	*Acropora hemprichii*	Al-Fahal Reef, Red Sea	Amplicon	***Jessen et al. 2013***
Marinobacter	*Pocillopora verrucosa*	Red Sea	Amplicon	***Lee et al. 2012***
Marinobacter	*Astreopora myriophthalma*	Red Sea	Amplicon	***Lee et al. 2012***
Marinobacter	*Stylophora pistillata*	Red Sea	Amplicon	***Lee et al. 2012***
Marinobacter	*Sarcophyton* sp.	Red Sea	Amplicon	***Lee et al. 2012***
Marinobacter	*Acropora valida*	Magnetic Island	Isolation	***Littman et al. 2009***
Marinobacter	*Acropora tenuis*	Magnetic Island	Isolation	***Littman et al. 2009***
Marinobacter	*Acropora millepora*	Magnetic Island	Isolation	***Littman et al. 2009***
Marinobacter	*Acropora millepora*	Great Barrier Reef	Isolation	***Morrow et al. 2017***
Marinobacter	*Orbicella faveolata*	Weimberg reef, Caribbean	Amplicon	***Roder et al. 2014***
Marinobacter	*Orbicella franksi*	Weimberg reef, Caribbean	Amplicon	***Roder et al. 2014***
Marinobacter	*Ctenactis echinata*	Al Quad Reef, Red Sea	Amplicon	***Roder et al. 2015***
Marinobacter	*Ctenactis echinata*	Abu Roma, Red Sea	Amplicon	***Roder et al. 2015***
Marinobacter	*Ctenactis echinata*	Shib Nazaar, Red Sea	Amplicon	***Roder et al. 2015***
Marinobacter	*Fungia granulosa*	Fsar Reef, Red Sea	Amplicon	***Rothig et al 2016***
Marinobacter	*Eguchipsammia*	Red Sea	Amplicon	***Rothig et al. 2017***

	fistula			
Marinobacter	*Rhizotrochus typus*	Red Sea	Amplicon	***Rothig et al. 2017***
Marinobacter	*Dendrophyllia* sp.	Red Sea	Amplicon	***Rothig et al. 2017***
Marinobacter	*Porites astreoides*	Florida Keys	Amplicon	***Sharp et al. 2012***
Marinobacter	*Porites astreoides*	Belize	Amplicon	***Sharp et al. 2012***
Marinobacter	*Pocillopora verrucosa*	Jeddah, Red Sea	Amplicon	***Ziegler et al. 2016***
Marinobacter	*Acropora hemprichii*	Jeddah, Red Sea	Amplicon	***Ziegler et al. 2016***
Marinobacter	*Acropora hyacinthus*	Ofu Island, American Samoa	Amplicon	***Ziegler et al. 2017***
Marinobacter	*Symbiodinium* A1	362 (NCMA 2458)	Isolation	***Frommlet et al. 2015***
Marinobacter	*Symbiodinium* A1	370 (NCMA 2467)	Isolation	***Frommlet et al. 2015***
Marinobacter	*Symbiodinium* A2	97	Isolation	***Frommlet et al. 2015***
Marinobacter	*Symbiodinium* A2	130	Isolation	***Frommlet et al. 2015***
Marinobacter	*Symbiodinium* B1	Pk702	Isolation	***Frommlet et al. 2015***
Marinobacter	*Symbiodinium* C2	203	Isolation	***Frommlet et al. 2015***
Marinobacter	*Nannochloropsis oceanic*	Ocean University of China	Isolation	***Han et al. 2016***
Marinobacter	*Chaetoceros gracili*	Ocean University of China	Isolation	***Han et al. 2016***
Marinobacter	*Navicula* sp.	Ocean University of China	Isolation	***Han et al. 2016***
Marinobacter	*Cylindrotheca fusiformis*	Ocean University of China	Isolation	***Han et al. 2016***
Marinobacter	*Pseudo-nitzschia* sp.	Ocean University of China	Isolation	***Han et al. 2016***
Marinobacter	*Pavlova* sp.	Ocean University of China	Isolation	***Han et al. 2016***
Marinobacter	*Amphidinium carterae*	Ocean University of China	Isolation	***Han et al. 2016***
Marinobacter	*Gymnodinium catenatum*	GCHU11	Isolation	***Amin et al. 2009***
Marinobacter	*Gymnodinium catenatum*	GCDE08	Isolation	***Amin et al. 2009***
Marinobacter	*Gymnodinium catenatum*	GCTRA14	Isolation	***Amin et al. 2009***
Marinobacter	*Gymnodinium catenatum*	CAWD101	Isolation	***Amin et al. 2009***
Marinobacter	*Gymnodinium catenatum*	YC499B15	Isolation	***Amin et al. 2009***
Marinobacter	*Gymnodinium catenatum*	GC21V	Isolation	***Amin et al. 2009***
Marinobacter	*Gymnodinium catenatum*	GCJP01	Isolation	***Amin et al. 2009***
Marinobacter	*Amphidinium carterae*	CCAP1102/7	Isolation	***Amin et al. 2009***
Marinobacter	*Amphidinium carterae*	CCAP1102/7	Isolation	***Amin et al. 2009***
Marinobacter	*Alexandrium tamarense*	CCAP 1119/4	Isolation	***Amin et al. 2009***
Marinobacter	*Scrippsiella trochoidea*	CCAP 1134/1	Isolation	***Amin et al. 2009***
Marinobacter	*Lingulodinium polyedrum*	CCAP 1121/2	Isolation	***Amin et al. 2009***
Marinobacter	*Gymnodinium microreticulatum*	I011	Isolation	***Amin et al. 2009***
Marinobacter	*Scrippsiella* sp.	CCMP 1073	Isolation	***Amin et al. 2009***
Marinobacter	*Alexandrium ostenfeldii*	CCMP 1773	Isolation	***Amin et al. 2009***
Marinobacter	*Prorocentrum minimum*	CCMP 1529	Isolation	***Amin et al. 2009***
Marinobacter	*Emiliania huxleyi*	AC475	Isolation	***Amin et al. 2009***
Marinobacter	*Emiliania huxleyi*	CCAP920/8	Isolation	***Amin et al. 2009***
Marinobacter	*Emiliania huxleyi*	CCAP 920/10	Isolation	***Amin et al. 2009***
Marinobacter	*Emiliania huxleyi*	AC472	Isolation	***Amin et al. 2009***
Marinobacter	*Emiliania huxleyi*	AC475	Isolation	***Amin et al. 2009***
Marinobacter	*Emiliania huxleyi*	CCMP 1516	Isolation	***Amin et al. 2009***
Marinobacter	*Coccolithus braarudi*	AC400	Isolation	***Amin et al. 2009***
Marinobacter	*Coccolithus braarudi*	AC392	Isolation	***Amin et al. 2009***

Marinobacter	*Pseudo-nitzschia* sp.	RUS	Isolation	***Amin et al. 2009***
Marinobacter	*Achnanthes* sp.	CCAP 1095/1	Isolation	***Amin et al. 2009***
Marinobacter	*Thalassosira pseudonana*	CCAP 1085/12	Isolation	***Amin et al. 2009***
Marinobacter	*Asterionellopsis glacialis*	CSIRO CS-372	Isolation	***Amin et al. 2009***
Marinobacter	*Fallacia carpentariae*	CSIRO CS-346	Isolation	***Amin et al. 2009***
Marinobacter	*Skeletonema costatum*	CCAP1077/7	Isolation	***Amin et al. 2009***
Marinobacter	*Gymnodinium catenatum*		Isolation	***Green et al. 2004***
Marinobacter	*Gymnodinium catenatum*	YC499B15	Isolation	***Green et al. 2006***
Marinobacter	*Gymnodinium catenatum*	GC21V	Isolation	***Green et al. 2006***
Marinobacter	*Alexandrium tamarense*	NEPCC 407	Isolation	***Green et al. 2006***
Marinobacter	*Aureococcus anophagefferens*	CCMP1708	Amplicon	***Baker et al. 2009***
Marinobacter	*Aureococcus anophagefferens*	CCMP1708	Amplicon	***Baker et al. 2009***
Marinobacter	*Aureococcus anophagefferens*	CCMP1784	Amplicon	***Baker et al. 2009***
Marinobacter	*Chaetoceros* sp.	MBTDCMFRI S065	Isolation	***Sandhya et al. 2017***
Marinobacter	*Nitzschia* sp.	MBTDCMFRI S092	Isolation	***Sandhya et al. 2017***
Marinobacter	*Navicula* sp.	MBTDCMFRI S043	Isolation	***Sandhya et al. 2017***
Marinobacter	*Dunaliella salina*	MBTDCMFRI S135	Isolation	***Sandhya et al. 2017***
Marinobacter	*Chlorella* sp.	MBTDCMFRI S072	Isolation	***Sandhya et al. 2017***
Marinobacter	*Nannochloropsis oceanica*	MBTDCMFRI S078	Isolation	***Sandhya et al. 2017***
Marinobacter	*Synechococcus* sp.	MBTDCMFRI S107	Isolation	***Sandhya et al. 2017***
Marinobacter	*Ostreococcus tauri*	RCC4221	Amplicon	***Lupette et al 2016***
Marinobacter	*Pseudopedinella elastica*	CCMP716	Amplicon	***Jasti et al 2005***
Marinobacter	*Alexandrium fundyense*	CB301	Amplicon	***Jasti et al 2005***
Marinobacter	*Pseudo-nitzschia pungens*	Santa Cruz Wharf, California	Isolation	***Sison-Mangus et al 2014***
Marinobacter	*Pseudo-nitzschia australis*	Santa Cruz Wharf, California	Isolation	***Sison-Mangus et al 2014***
Marinobacter	*Emiliania huxleyi*	CCAP 920/8	Isolation	***Green et al. 2015***
Marinobacter	*Emiliania huxleyi*	RCC 1214	Isolation	***Green et al. 2015***
Marinobacter	*Emiliania huxleyi*	RCC 1216	Isolation	***Green et al. 2015***
Marinobacter	*Coccolithus pelagicus* f. *braarudii*	RCC 1200	Isolation	***Green et al. 2015***
Marinobacter	*Coccolithus pelagicus* f. *braarudii*	RCC 1203	Isolation	***Green et al. 2015***
Chromatiaceae	*Porites lobata*	Persian/Arabian Gulf	Amplicon	***Hadaidi et al. 2017***
Chromatiaceae	*Porites lobata*	Red Sea	Amplicon	***Hadaidi et al. 2017***
Chromatiaceae	*Acropora hemprichii*	Al-Fahal Reef, Red Sea	Amplicon	***Jessen et al. 2013***
Chromatiaceae	*Porites cylindrica*	Milne Bay Island, Papua New Guinea	Amplicon	***Morrow et al. 2015***
Chromatiaceae	*Orbicella faveolata*	Weimberg reef, Caribbean	Amplicon	***Roder et al. 2014***
Chromatiaceae	*Orbicella franksi*	Weimberg reef, Caribbean	Amplicon	***Roder et al. 2014***
Chromatiaceae	*Eguchipsammia fistula*	Red Sea	Amplicon	***Rothig et al. 2017***
Chromatiaceae	*Rhizotrochus typus*	Red Sea	Amplicon	***Rothig et al. 2017***
Chromatiaceae	*Dendrophyllia* sp.	Red Sea	Amplicon	***Rothig et al. 2017***
Chromatiaceae	*Montastraea faveolata*	La Parguera, Caribbean	Amplicon	***Sunagawa et al. 2009***
Chromatiaceae	*Montastraea faveolata*	Caribbean	Amplicon	***Sunagawa et al. 2010***
Chromatiaceae	*Montastraea franksi*	Caribbean	Amplicon	***Sunagawa et al. 2010***
Chromatiaceae	*Diploria strigosa*	Caribbean	Amplicon	***Sunagawa et al. 2010***
Chromatiaceae	*Acropora palmata*	Caribbean	Amplicon	***Sunagawa et al. 2010***

Chromatiaceae	*Acropora cervicornis*	Caribbean	Amplicon	***Sunagawa et al. 2010***
Chromatiaceae	*Porites astreoides*	Caribbean	Amplicon	***Sunagawa et al. 2010***
Chromatiaceae	*Gorgonia ventalina*	Caribbean	Amplicon	***Sunagawa et al. 2010***
Chromatiaceae	*Acropora hemprichii*	Jeddah, Red Sea	Amplicon	***Ziegler et al. 2016***
Chromatiaceae	*Acropora hyacinthus*	Ofu Island, American Samoa	Amplicon	***Ziegler et al 2017***

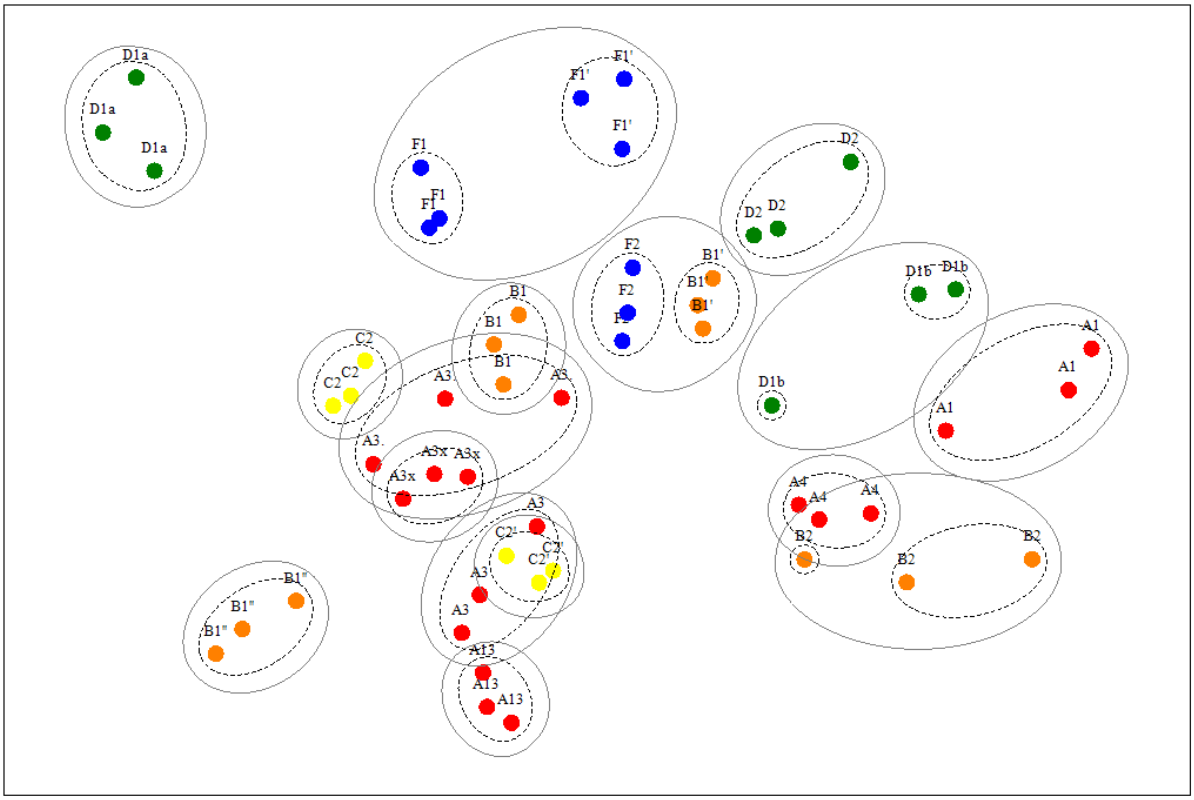

Figure S3.1 Two-dimensional non-metric MDS plot of bacterial communities associated with 18 Symbiodiniaceae types generated in PRIMER6. Bray Curtis similarity and a square root transformation were used. Clades are designated by colour, clade A: red, clade B: orange, clade C: yellow, clade D: green, clade F: blue. Clustered according to 60 (grey line) and 80% (dotted black line) similarity. 2D stress: 0.18.

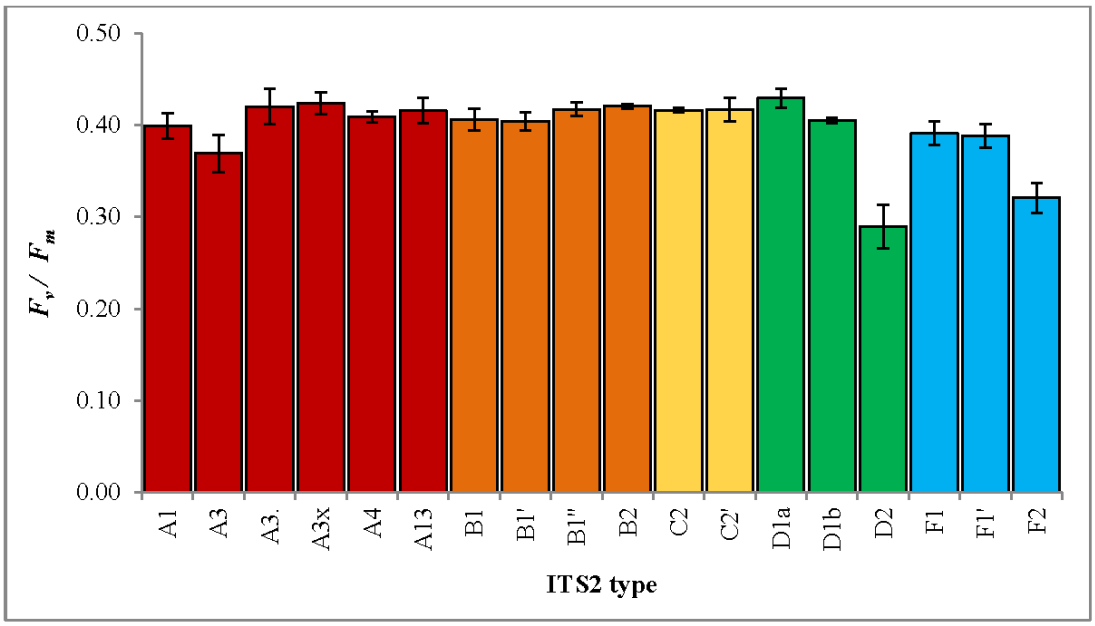

Figure S3.2 Mean F_v/F_m (± SE, n=3) of Symbiodiniaceae cultures maintained in exponential growth. Clades are designated by bar colour according to cladal identity (Red: A, Orange: B, Yellow: C, Green: D, Blue: F), ITS2 type is shown on the x-axis

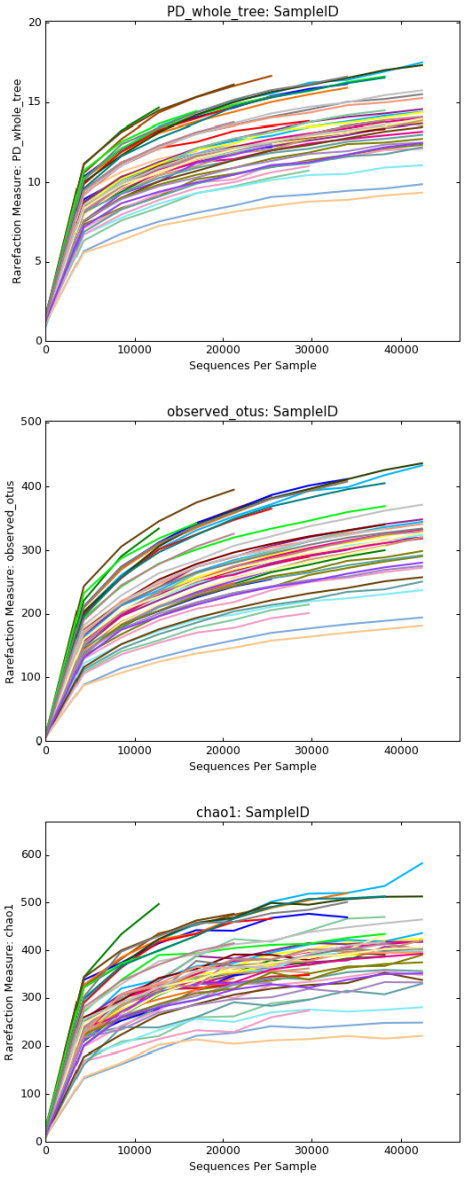

Figure S3.3 Rarefaction curves generated in QIIME using alpha_rarefaction.py. Data were then rarefied to 14000 sequences per sample.

Chapter 4

The volatilomes of Symbiodiniaceae-associated bacteria are influenced by chemicals derived from their algal partners

Author contributions: CA Lawson, JB Raina, JR Seymour and DJ Suggett conceived and designed the project, CA Lawson performed the experiments, analysed the data and produced figures. M Possell provided technical and data analysis assistance. CA Lawson wrote the paper. All authors edited the manuscript.

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Abstract

Biogenic volatile organic compounds (BVOCs) are a large group of molecules involved in trophic interactions, stress response and atmospheric chemistry. Although they have been extensively studied in terrestrial ecosystems, their identity and prevalence in the marine environment remains largely unexplored. Here I characterised the volatilome of two abundant marine bacteria that were previously identified as members of the core microbiome of Symbiodiniaceae (phylum: Dinoflagellata), the photosynthetic endosymbionts of reef building corals. To determine the influence of Symbiodiniaceae exudates on their associated bacteria, I incubated isolates of *Marinobacter adhaerens* and *Labrenzia* sp. in either Symbiodiniaceae culture filtrate or culture medium (control) and determined their volatilomes using GC-MS. The volatilome of *Labrenzia* sp. incubated in Symbiodiniaceae filtrate was significantly different and more diverse relative to the control. In contrast, the overall composition of the *M. adhaerens* volatilomes were consistent between treatment and control. Among the 35 compounds detected in both bacterial species, the dominant chemical functional groups were halogenated hydrocarbons, aromatic hydrocarbons and organosulfurs, some of which are known to play roles in inter-organism signalling, to act as antioxidants and as antimicrobials. This study provides new insights into the potential sources and diversity of marine BVOCs, uncovering a wide range of molecules that may play important physiological and ecological roles for these organisms, while also revealing the role of Symbiodiniaceae-associated bacteria in the emission of important climate-active gases.

Introduction

All organisms are capable of producing biogenic volatile organic compounds (BVOCs), a group of chemicals known for their ecological and physiological roles. BVOCs can function as infochemicals, allowing organisms to locate food sources (Runyon et al., 2006), warn conspecifics of danger (Frost et al., 2007) or provide defence from predators (De Moraes et al., 2001). These compounds can also act as antioxidants, preventing the

build-up of damaging reactive oxygen species (Loreto and Velikova, 2001; Sunda et al., 2002), and upon their release, they can influence atmospheric chemistry by forming secondary organic aerosols (Fu et al., 2010; Hallquist et al., 2009), reacting with greenhouse gases (Atkinson and Arey, 2003a) and thus impacting climate. Previous studies characterising BVOC production and emission have largely focussed on terrestrial organisms, and have established the physiological (e.g. stress response; Vickers et al., 2009), ecological (e.g. signalling; Runyon et al., 2006) and atmospheric (e.g. ozone depletion; Matsumoto, 2014) roles that BVOCs can play. However, the occurrence and potential roles of BVOCs in important marine ecosystems, such as coral reefs, has remained largely overlooked. Coral reefs are known to produce large concentrations of the sulfur BVOC, dimethyl sulfide (DMS), resulting in a large body of research on the potential antioxidant and atmospheric functions of this compound (Broadbent and Jones, 2004; Deschaseaux et al., 2014b; Hopkins et al., 2016). A recent study also identified the capacity of corals to produce other sulfur volatiles (methyl mercaptan, carbon disulfide, dimethyl disulfide, dimethyl trisulfide, thiirane, 2,4-dithiapentance, 1,2,4-trithiolane) as well as isoprene, a ubiquitous BVOC in terrestrial systems (Swan et al., 2016). However, the diversity of coral derived BVOCs remains to be fully explored and it is likely that the wide range of organisms living in symbioses with corals are partially responsible for the production of BVOCs across coral reef ecosystems.

Corals are now widely considered as holobionts - complex assemblages which include the cnidarian host, endosymbiotic algae (Symbiodiniaceae) and diverse microbial communities (Rohwer et al., 2002). Multiple mutualistic associations sustain the health of the coral holobiont (Muscatine and Porter, 1977; Rohwer et al., 2002; Little et al. 2004; Ziegler et al., 2019), with inter-organism chemical signalling likely to form an important foundation for these relationships (Garren et al., 2014; Koike et al., 2004; Raina et al., 2016; Tebben et al., 2011). Given their importance as signalling molecules in other environments (Runyon et al., 2006; Ryu et al., 2003; Shan et al., 2019; Sharifi and Ryu, 2018), BVOCs may play an important role in mediating interactions within the coral holobiont, by acting as infochemicals

and influencing stress responses. I recently performed a characterisation of the Symbiodiniaceae volatilome (total BVOCs), with a diverse suite of BVOCs identified, including compounds implicated in chemical signalling (e.g. benzaldehyde, methyl jasmonate) and stress tolerance (e.g. DMS) (**Chapter 2**). However, it is probable that these measurements have only scratched the surface of the full diversity of BVOCs produced by different members of the coral holobiont.

A central feature of the coral holobiont is the tripartite interaction between corals, their Symbiodiniaceae partners and associated bacterial assemblages. I recently identified putatively strong ecological links between Symbiodiniaceae and bacterial partners by characterising the core microbiome associated with 18 strains of Symbiodiniaceae (**Chapter 3**). I identified three bacterial operational taxonomic units (OTUs), belonging to the *Labrenzia* and *Marinobacter* genera and an unclassified Chromatiaceae, which occurred in all strains of Symbiodiniaceae (**Chapter 3**). Members of the *Marinobacter* and *Labrenzia* commonly develop associations with microalgae (Han et al., 2016; Sandhya et al., 2017), and *Marinobacter* species have previously been isolated from other Symbiodiniaceae cultures (Frommlet et al., 2015). Notably, *Labrenzia* species (which comprised up to 38.4% of the Symbiodiniaceae microbiome; **Chapter 3**) can synthesise the DMS precursor, dimethylsulfoniopropionate (DMSP) (Curson et al., 2017), a sulfur compound also abundantly produced by Symbiodiniaceae (Broadbent et al., 2002; Deschaseaux et al., 2014c).

Given the close associations between these bacteria and Symbiodiniaceae, I examined the impact of the microalgal exudate on the bacterial volatilomes. I specifically aimed to provide the first characterisation of the *Labrenzia* sp. 21p and *Marinobacter adhaerens* HP15 (hereafter referred to as *Labrenzia* sp. and *M. adhaerens*). volatilomes and to investigate the presence of putative inter-organism signalling BVOCs. I hypothesised that the presence of Symbiodiniaceae exudate, they will produce a greater diversity of BVOCs compared to controls incubated in Symbiodiniaceae growth medium

Methods

Bacterial isolation

A nonaxenic species of Symbiodiniaceae, *Breviolum minutum* (strain: UTSB), was grown in a sterile 1 L Schott bottle in IMK medium (in artificial seawater; Berges et al., 2001) (ARALAB incubator; 26°C ± 1.5°C; ca. 100 ± 10 µmol photons m^{-2} s^{-1} (LEDs) on a 12:12 hr light:dark cycle) (as per **Chapter 2 & 3**). Bacterial isolates from the *B. minutum* microbiome were subsequently obtained by plating 20 µL aliquots of *B. minutum* culture on Marine Agar (100%; Difco 2216). Plates were then incubated for 10 days at room temperature before visible individual colonies were picked, grown overnight in Marine Broth (Difco 2216) at 22°C under shaking (180 rpm; Ratek Orbital Mixer Incubator), and re-plated on Marine Agar until axenic. Subsequent individual colonies were picked and grown overnight in Marine Broth (22°C, 180 rpm). Following establishment of cultures, cells were preserved through the addition of glycerol (Sigma-Aldrich; 20% final concentration), snap frozen in liquid nitrogen and stored at -80°C. Small volumes of pure overnight cultures were centrifuged (1.5 mL at 7,000g for 10 minutes), before the supernatant was discarded and the pellets frozen (-20°C) for subsequent DNA extraction.

DNA extraction, 16S rRNA PCR and strain selection

To identify the bacterial isolates (25 in total; Table S1), their DNA was extracted and their 16S rRNA gene sequenced. Briefly, frozen bacterial pellets were resuspended in 200 µL of nuclease free water (Ambion, Austin, USA), heated at 95°C for 10 minutes to break cell walls, and centrifuged to pellet debris (10,000g for 5 minutes). The DNA in the resulting supernatant was amplified with PCR targeting the 16S rRNA gene using the primers: 27F-AGAGTTTGATCMTGGCTCAG and 907R-CCGTCAATTCMTTTRAGTTT. The PCR mixture comprised 1 µL DNA, 1 µL of each primer, 9.5 µL nuclease free water (Ambion, Austin, USA) and 12.5 µL of GoTaq colourless master mix (Promega, Madison, USA) for a total reaction volume of 25 µL. The PCR reaction conditions were as follows: 95°C for 3 minutes followed by 30 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 1.45 minutes,

and a final extension of 72°C for 7 minutes. The PCR products were visualised with electrophoresis on a 1% agarose gel strained with Gel Red (Biotium, Fremont, California) and analysed using Sanger sequencing at the Australian Genome Research Facility (AGRF). Sequences were identified using nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Table S1). Isolate 21p was identified as *Labrenzia* sp. (Genbank Accession number: MN305698), and displayed 97% sequence identity (BLASTN 2.9.0) to the *Labrenzia* sp. OTU that was previously identified as a core member of the Symbiodiniaceae microbiome (**Chapter 3**). None of the other bacterial isolates matched members of the Symbiodiniaceae core microbiome (**Chapter 3**). In lieu of other core microbiome isolates, I used *Marinobacter adhaerens* HP15, a model bacterium extensively studied in the context of its interactions with phytoplankton (Gärdes et al., 2010; Sonnenschein et al., 2012), and present in the Symbiodiniaceae core microbiome (**Chapter 3**). *M. adhaerens* HP15 displayed 97% sequence similarity to the *Marinobacter* sp. OTU previously identified as a core member of the Symbiodiniaceae microbiome (**Chapter 3**). To my knowledge, neither of these bacteria have previously had their volatilomes characterised and are currently not included in the microbial VOC database (mVOC; Lemfack et al., 2018).

Volatile sampling of Labrenzia sp. 21p and Marinobacter adhaerens

Glycerol stocks of *Labrenzia* sp. 21p and *M. adhaerens* HP15 were replated onto Marine Agar (100%; Difco 2216) and all bacterial biomass was resuspended in 300 mL of Marine Broth (Difco 2216). Bacteria cultures were grown overnight in a shaking incubator (KuhnerShaker X; room temperature; 90±10 µmol photons m^{-2} s^{-1}), before being centrifuged at 1,000g for 10 minutes. The supernatant was discarded, and the pellet washed by resuspension in IMK medium (Diago, Japan) (in artificial seawater; Berges et al., 2001). The process was repeated, before the cells were resuspended in either IMK or non-axenic Symbiodiniaceae culture filtrate (*B. minutum*; grown in IMK, n = 3, as above) and incubated for 4.5 hours prior to volatile sampling. This timing was selected based on a pilot study that identified additional compounds in bacterial cultures incubated for 4.5 hours in IMK medium

relative to axenic IMK medium. The IMK medium incubation was used as a reference to investigate the influence of Symbiodiniaceae culture filtrate on the bacterial volatilomes; hereafter the IMK incubation will be referred to as the "control". Symbiodiniaceae culture (*B. minutum*) was grown as described above, before triple filtration (5 µm, 2 µm, and 0.22 µm Isopore Membrane filters, Merck Millipore Ltd, Tullagreen, Ireland) using a peristaltic pump (Watson Marlow Sci-Q 323) in order to remove all algal cells and bacteria. This filtration was carried out, on average, 3.5 hours prior to use in the experiment.

Sampling of volatiles was staggered so that all samples received a 4.5 hour incubation period within either Symbiodiniaceae culture filtrate or the control. Following the incubation, technical duplicates were taken from each biological replicate for volatile sampling. Each technical duplicate (50 mL) was placed into a 100 mL gas tight vial and purged for 30 minutes (while under identical growth conditions) with instrument grade air (BOC Gases, Linde Group, Australia). Volatiles were captured on thermal desorption (TD) tubes (Markes Tenax TA) that were stored at 4°C until processing with GC-MS (< 2 weeks later). For processing, TD tubes were desorbed (ULTRA 2 & UNITY 2; Markes International Ltd, Llantrisant, UK) for 6 minutes at 300°C, then concentrated on a Tenax TA cold trap at -30°C. This concentrated sample was flash heated (300°C) and injected, at a flow rate of 2.3 mL min^{-1}, via a heated transfer line (150°C) onto a 7890A GC-MS (Agilent Technologies Pty Ltd, Melbourne) fitted with a BP1 capillary column (60 m × 0.32 mm, 1 µm film thickness; SGE Analytical Science Pty Ltd, Melbourne). Samples were run splitless to allow detection of trace compounds. The GC oven was heated at 35°C for 5 minutes, then ramped up 4°C min^{-1} to 160°C, and then 20°C min^{-1} to 300°C for 10 minutes. The GC-MS was coupled to a mass-selective detector (Model 5975C; Agilent) that was set to a scanning range of 35 – 300 amu.

Flow cytometry

Bacterial cell abundance was quantified in order to normalise BVOC concentrations. Aliquots (500 µL) of bacterial cultures were taken at the time of volatile sampling and fixed in

glutaraldehyde (Sigma-Aldrich; 2% final concentration). Bacterial abundance was quantified by staining the cells with SYBR Green (1:10,000 final dilution; Applied Biosystems, Foster City, California) and analysis on a CytoFLEX S (Beckman Coulter, Brea, California) flow cytometer with filtered MilliQ water as the sheath fluid. For each sample, forward scatter (FSC), side scatter (SSC), and green fluorescence (SYBR Green) were recorded. The samples were analysed at a flow rate of 25 µL min^{-1}, with bacterial cells discriminated according to SSC and SYBR Green fluorescence.

Data analysis

Chromatograms were stripped of common contaminating ions (73, 84, 147, 149, 207, 221, 262 and 281 m/z) using the Denoising function in OpenChrom (Wenig and Odermatt, 2010), before peak integration in ChemStation (Agilent Technologies Pty Ltd, Melbourne) with an initial threshold of 15 and an initial peak width of 0.068. The chromatograms were then processed in the MSeasyTkGUI package (Nicolè et al., 2012) in R version 3.5.3 (R Development Core Team, 2015) to cluster all putative compounds. Peaks were identified by manually comparing mass spectra against a commercial library (NIST08 library in NIST MS Search v.2.2f; NIST, Gaithersburg, MD). Blank media samples of both algal filtrate and IMK medium were run in conjunction with all analyses; the average values for compounds present in the blanks were subtracted from all samples. Trimethyl silanol was removed from the analysis as it was likely contamination resulting from the hydrolysis of dimethylpolysiloxane (GC column material) (Cella and Carpenter, 1994). Any compound that did not occur in at least 4 of the 6 samples (2 technical replicates per biological replicate) was removed. All data were normalised to the bacterial cell concentration (cells mL^{-1}).

PAST (PAleontological STatisitics; version 3.25) was used to generate Principal Component Analysis (PCA) and non-metric Multidimensional Scaling (nMDS) plots on fourth root transformed data (Hammer et al., 2001). Primer v6.1.14 was used to perform a PERMANOVA+ (v1.0.4) analysis (Anderson et al., 2008; Clarke and Gorley, 2006) with *p* values based on the Monte Carlo statistic (Hope, 1968). All statistical tests were run in IBM

SPSS Statistic (version 25). UC denotes an unclassified compound (the number following UC indicates the retention time of the compound if the chemical functional group could not be determined). All mass spectra files are available (Accession number MSV000084431, MassIVE, https://massive.ucsd.edu), to help future studies as databases continue to improve.

Results

Across the two bacterial strains (*Labrenzia* sp. and *M. adhaerens*) tested here, a total of 35 volatile compounds were detected. Half of these compounds were successfully identified, and a further 11% were assigned to a chemical functional group. Only six of the compounds detected from the two Symbiodiniaceae-associated bacteria examined here have previously been reported from other bacteria. These included acetone, DMS, dimethyl trisulfide (DTS), toluene, camphor and 2-ethylhexanal (mVOC2.0; Lemfack et al., 2018; as of 10/06/2019). The remaining 29 newly reported bacterial BVOCs included alcohols, aromatic hydrocarbons, esters, ethers, halogenated hydrocarbons, ketones and organosulfurs. The volatilomes of my two bacterial isolates were not only distinguished from each other, but each bacterium generated a different volatilome when incubated in Symbiodiniaceae filtrate relative to the control (nMDS, Stress = 0.1259; Figure 4.1A).

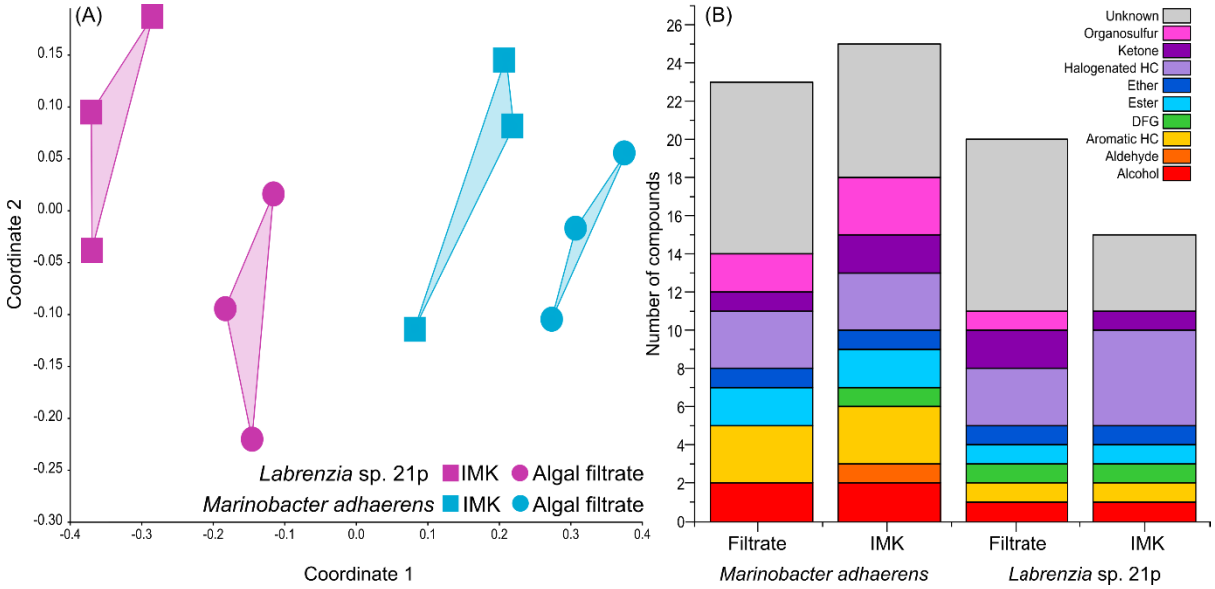

Figure 4.1 (A) Non-metric multidimensional scaling (nMDS; Bray-Curtis Similarity; Stress = 0.1055) analysis on the volatilomes of *Labrenzia* sp. 21p and *Marinobacter adhaerens* HP15 incubated either IMK medium or algal filtrate. (B) Chemical functional groups present in *Labrenzia* sp. 21p and *M. adhaerens* incubated in either IMK medium or algal filtrate (total number of volatile compounds present in at least two out of three replicates). HC: hydrocarbon; DFG: diverse functional groups.

The *Labrenzia* sp. volatilome was strongly influenced by the presence of Symbiodiniaceae filtrate, with significant differences in the overall structure of the volatilome (p = 0.014; PERMANOVA+; Table S4.2) and more BVOCs present in the filtrate compared to the control incubations (20 vs 15 compounds; Figure 4.1B). These differences were also confirmed using PCA, which identified the volatiles driving the strong separation between the two incubations. DMS was the leading cause of separation between the *Labrenzia* sp. control and filtrate volatilomes (Principal Component loading (PC)1 = 0.631), followed by acetone (PC1 = 0.405) and UC44.40 (PC2 = 0.409) (Figure 4.2A). Specifically, incubation of *Labrenzia* sp. in Symbiodiniaceae filtrate resulted in the production of one extra ketone and organosulfur, five additional unclassified compounds and two fewer halogenated hydrocarbons relative to the control (Figure 4.1B). Additionally, the relative quantities of nine compounds significantly differed among *Labrenzia* sp. cells incubated in the two media

types, including DMS, acetone and 1,2-15,16-diepoxyhexadecane (Figure 4.3). Only one of these compounds, 2-ethyl-3-hydroxyhexyl-2-methylpropanoate, was detected in higher levels in the control incubation, while the other eight compounds were present in higher quantities in the Symbiodiniaceae culture filtrate (Figure 4.3).

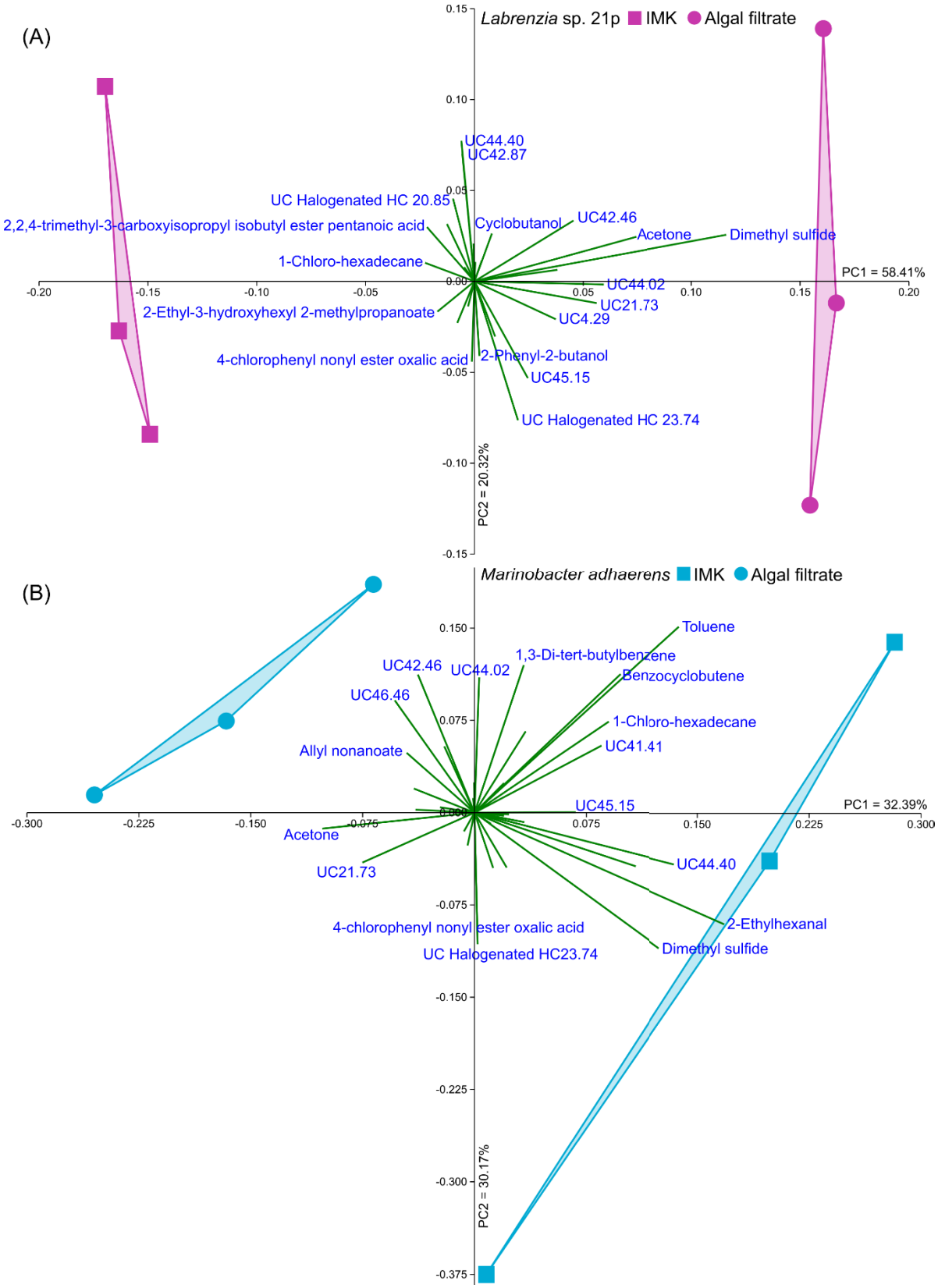

Figure 4.2 Principal component analyses (PCA; Bootstrap N = 1000) of (A) *Labrenzia* sp. 21p and (B) *Marinobacter adhaerens* HP15 incubated either IMK medium or Symbiodiniaceae filtrate.

Incubation of *M. adhaerens* in Symbiodiniaceae filtrate did not result in significant differences in the overall structure of the *M. adhaerens* (*p* = 0.163; PERMANOVA+; Table S4.2), nor a considerable change in the number of compounds detected relative to the control (23 BVOCs in filtrate, 25 in control) (Figure 4.1B). However, PCA did reveal some separation between the *M. adhaerens* volatilomes, with 2-ethylhexanal (PC1 = 0.445), UC44.40 (PC1 = 0.348), DMS (PC1 = 0.339, PC2 = -0.244) and toluene (PC1 = 0.307, PC2 = 0.421) driving this separation (Figure 4.2B). Moreover, there were specific differences in individual BVOCs, with the control incubation resulting in the production of the only aldehyde detected, an extra ketone and an extra organosulfur (Figure 4.1B). Additionally, the relative quantities of three BVOCs were significantly higher following incubation in the Symbiodiniaceae filtrate compared to the control, two of which were identified as acetone and allyl nonanoate (Figure 4.3). A further five compounds occurred in significantly lower quantities in the filtrate incubation, and included DMS, 2,2,4-trimethyl-3-carboxyisopropyl-isobutylester-pentanoic acid, 2-ethylhexanal, an UC organosulfur and UC44.40 (Figure 4.3). However, these differences in the quantities of individual BVOCs were not sufficient to significantly alter the overall structure of the *M. adhaerens* volatilome between culture filtrate and control.

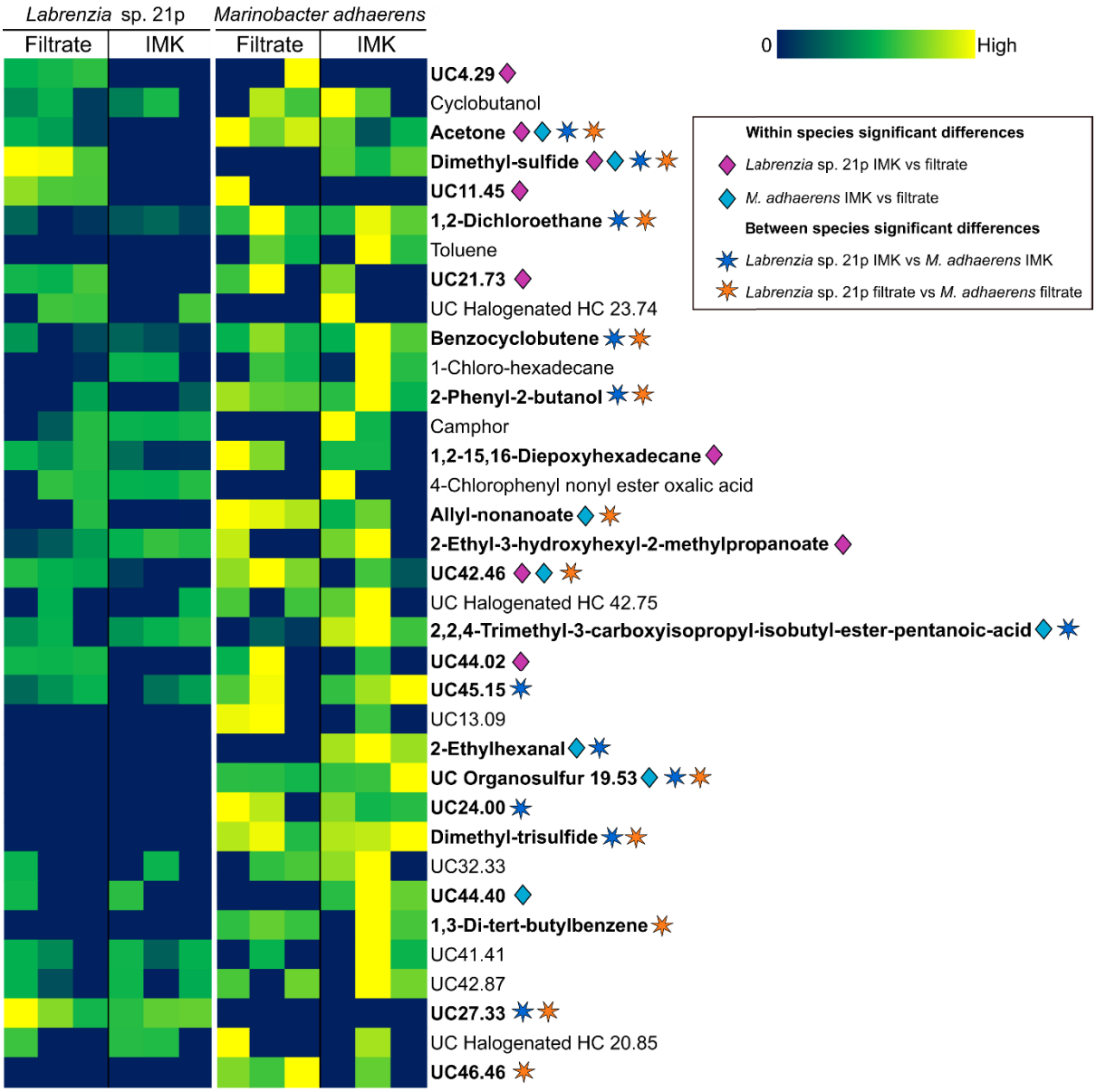

Figure 4.3. Heat map of all BVOCs present in at least two out of three replicates of *Labrenzia* sp. 21p and *Marinobacter adhaerens* HP15 incubated in either IMK medium or algal filtrate. Colour scale is specific to each compound. Significant differences (P<0.05; Kruskal Wallis; IBM SPSS version 25) are denoted with diamonds for within species differences (pink for *Labrenzia* sp. 21p IMK vs filtrate; blue for *M. adhaerens* IMK vs filtrate) and stars for between species differences (dark blue for *Labrenzia* sp. 21p IMK vs *M. adhaerens* IMK; orange for *Labrenzia* sp. 21p filtrate vs *M. adhaerens* filtrate). HC = Hydrocarbon, UC = Unclassified (the number following UC indicates the retention time of the compound).

The volatilomes of *Labrenzia* sp. and *M. adhaerens* cells also differed significantly to one another when incubated in Symbiodiniaceae culture filtrate (p = 0.007; PERMANOVA+; Table S4.2). DMS and UC27.33 were significantly over-represented in *Labrenzia* sp. relative to *M. adhaerens* (Figure 4.3). However, 10 compounds were detected in significantly higher levels in *M. adhaerens*, including acetone, 1,2-dichloroethane, benzocyclobutene, 2-phenyl-

2-butanol, allyl nonanoate, DTS, 1,3-di-tert-butylbenzene, UC organosulfur, UC42.46 and UC46.46 (Figure 4.3). Similarly, the control volatilomes of the *Labrenzia* sp. and *M. adhaerens* cells also differed significantly to one another (p = 0.014; PERMANOVA+; Table S4.2). This difference was driven by significantly higher amounts of UC27.33 in the *Labrenzia* sp. relative to the control and a further 11 BVOCs over-represented in *M. adhaerens*; these included acetone, DMS, 1,2-dichloroethane, benzocyclobutene, 2-phenyl-2-butanol, 2,2,4-trimethyl-3-carboxyisopropyl-isobutylester-pentanoic acid, 2-ethylhexanal, DTS, UC organosulfur 19.53, UC45.15 and UC24.00 (Figure 4.3). Neither species of bacteria showed any significant difference in cell abundance following incubation in either IMK medium or culture filtrate (Figure S4.1).

Discussion

Despite the important roles that BVOCs can play in enhancing the growth and survival of microorganisms (Ramirez et al., 2010; Ryu et al., 2003, 2004; Schmidt et al., 2015; Stotzky and Schenck, 1976), bacterial BVOC production and emission remains relatively unexplored. Numerous BVOCs have been detected from a range of bacteria and while we are currently unable to identify or determine the ecological relevance of them all, they have the potential to strongly influence the growth and success of neighbouring microorganisms. For example, the most abundant bacterium on Earth, *Pelagibacter* (SAR11), metabolises diatom derived BVOCs, which constitute a significant portion of the carbon released by phytoplankton cells (Moore et al., 2019). Furthermore, bacteria often live within or associated with a host and as such, bacterial emissions can interact and react with host emissions (Kai et al., 2018; Schmidt et al., 2015). Importantly, such associations can potentially lead to BVOCs being falsely attributed to the host and can also lead to the formation of new compounds resulting from the interaction of different BVOCs (Kai et al., 2018). The multitude of bacterial BVOCs that have been studied in terrestrial ecosystems have also highlighted the signalling nature of many of these BVOCs (Sharifi and Ryu, 2018). Following the characterisation of the Symbiodiniaceae volatilome and microbiome (**Chapter**

2 & 3), here I investigated the potential influence of the exudates from non-axenic Symbiodiniaceae cultures on the volatilomes of two members of the Symbiodiniaceae core microbiome.

Between *Labrenzia* sp. and *M. adhaerens*, a total of 35 BVOCs were detected. This BVOC richness is comparable to observations in other marine bacterial species. For example, 69 different BVOCs were detected in a liquid culture of the Actinobacteria *Streptomyces* sp. (Dickschat et al., 2005a), while 38 BVOCs were detected from two Proteobacteria grown on agar plates, *Loktanella* BIO-204 and *Dinoroseobacter shibae* DFL-27 (Dickschat et al., 2005b).

The range of BVOCs detected in this study varied significantly between species and the substrates that the bacteria were incubated in. The *Labrenzia* sp. volatilome differed significantly between cells incubated in Symbiodiniaceae culture filtrate and the control, with more BVOCs detected when the bacteria were inoculated in the Symbiodiniaceae filtrate. The quantities of all but one of the BVOCs that differed significantly between the *Labrenzia* sp. volatilomes were higher within the Symbiodiniaceae filtrate incubation. Among these were DMS, acetone, 1,2-15,16-diepoxyhexadecane and five unclassified BVOCs. The extract of the herbaceous plant *Artemisia annua* has been shown to contain 1,2-15,16-diepoxyhexadecane, but this compound has no known functions in microorganisms (Hameed et al., 2016). Acetone has previously been reported in 13 bacterial genera (mVOC2.0; Lemfack et al., 2018) and is thought to inhibit fungal growth (Amavizca et al., 2017; Stotzky and Schenck, 1976). Notably, acetone was also present in a mixture of bacterial volatiles that was shown to promote the growth of the microalga *Chlorella sorokiniana* (Amavizca et al., 2017). The number of halogenated hydrocarbons decreased in the *Labrenzia* sp. filtrate relative to the control, with some of these compounds thought to function as signalling molecules (Cabrita et al., 2010; Ohsawa et al., 2001; Paul and Pohnert, 2011) and quorum sensing inhibitors (Hentzer et al., 2002). The higher total numbers of BVOCs detected in the Symbiodiniaceae filtrate incubation relative to the control could suggest that *Labrenzia* sp. is capable of utilising Symbiodiniaceae-derived organic

molecules to produce a wider diversity of volatile compounds. Given the dominance of this bacterial genus in the Symbiodiniaceae core microbiome (Lawson et al., 2018) and its culturability, future experiments should identify the nature of the Symbiodiniaceae-*Labrenzia* relationship and the role(s) that this bacterium could fulfil.

The number of *M. adhaerens* derived BVOCs detected were equivalent when incubated in either Symbiodiniaceae culture filtrate or control (23 and 25, respectively), and the structure of the *M. adhaerens* volatilome did not differ significantly between conditions. As such, the presence of the chemicals produced by the Symbiodiniaceae host appear to have had little to no effect on the *M. adhaerens* volatilome. Despite no apparent influence from the Symbiodiniaceae culture filtrate, the *M. adhaerens* volatilome was still diverse, comprising a range of alcohols (2), aromatic hydrocarbons (3), esters (2), ethers (1), halogenated hydrocarbons (3), ketones (1) and organosulfurs (2). The aromatic hydrocarbon toluene was detected in both *M. adhaerens* incubations. This compound has previously been detected in the volatilomes of 20 different bacterial genera (mVOC 2.0; Lemfack et al., 2018) and is also produced by both higher plants and microalgae (Misztal et al., 2015). The enzyme responsible for toluene biosynthesis (PhdB) was recently identified in bacteria (Beller et al., 2018), and while the biological function of this molecule remains uncertain, it has been proposed to serve as an infochemical and/or to play a role in stress response (Misztal et al., 2015). Dimethyl trisulfide (DTS) was also detected in all *M. adhaerens* samples under both incubation conditions. This compound has previously been detected in 34 bacterial genera (mVOC 2.0; Lemfack et al., 2018) and has been shown to have a strong antifungal activity (Fernando et al., 2005), while also playing a role in inter-organism signalling (Thibout et al., 1995). *Marinobacter* has previously been reported to consume volatile hydrocarbons (Ali et al., 2015), yet to the best of my knowledge no studies have reported BVOC production by this genus. Given the close association of this bacterial genus with phytoplankton, its production of the diverse range of BVOCs reported here may contribute to the complex inter-organism signalling underpinning these relationships.

DMS is an important sulfur molecule known to act as an antioxidant (Sunda et al., 2002) and as a signalling molecule (Seymour et al., 2010). DMS was a strong contributor to the differentiation between the volatilomes of *Labrenzia* sp. 21p cells, as it was not detected in the control incubation. This is particularly interesting as *Labrenzia aggregata* is capable of synthesising the DMS precursor, DMSP (Curson et al., 2017). Detection of DMS solely in the filtrate incubation may indicate that *Labrenzia* sp. 21p was preferentially using Symbiodiniaceae-derived DMSP to form DMS. Surprisingly, DMS was detected in the *M. adhaerens* control volatilome. Given that IMK medium only contains inorganic sulfur and no DMSP, the presence of DMS in this sample provides the first evidence that *M. adhaerens* may have the capacity to biosynthesize DMSP. Furthermore, the absence of the DMSP-producing gene *dsyB* in the *M. adhaerens* HP15 genome (Table S4.3) potentially points to the existence of alternative production pathways and a more widely distributed capacity to produce DMSP among marine bacteria.

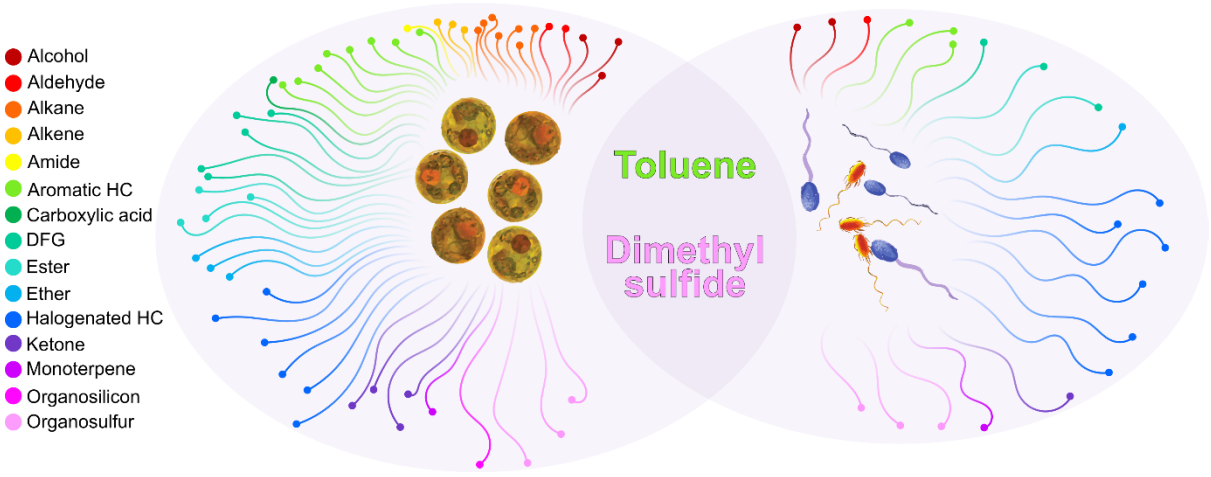

Figure 4.4. Functional chemical groups produced by Symbiodiniaceae (left oval) and *Marinobacter adhaerens* and *Labrenzia* sp. 21p (right oval) and the compounds that were present in both (overlap). Only compounds detected in Symbiodiniaceae during ambient conditions (26°C) were included. Compounds had to be present in at least four (out of six) technical replicates in at least one species. Unclassified BVOCs were excluded from this figure, see Table S4.4 for a full list of BVOCs present in Symbiodiniaceae and bacteria.

Further comparing the BVOC emissions of *Labrenzia* sp. 21p and *M. adhaerens* here with those previous identified from Symbiodiniaceae cultures (**Chapter 2**) demonstrated that only two compounds, DMS and toluene, were common to both bacteria and Symbiodiniaceae volatilomes (Figure 4.4). The ubiquitous nature of these BVOCs in the *Labrenzia* sp. and *M. adhaerens* volatilomes, and their similar putative functions, suggests that they might be critical for the physiology of both Symbiodiniaceae and their core bacteria. Excluding DMS and toluene, a further 33 BVOCs were unique to the bacterial volatilomes, highlighting the capacity of these bacteria to produce a wide variety of BVOCs.

In summary, I have demonstrated that two members of the Symbiodiniaceae core microbiome, *M. adhaerens* and *Labrenzia* sp., are capable of producing a diverse range of BVOCs, with the structure of the volatilome varying with the availability of Symbiodiniaceae-derived nutrients. My experiments demonstrate the importance of marine bacteria as a source of BVOCs and provide evidence that BVOC production may play a role in the complex chemical interplay between bacteria and their hosts – and thus between microbes that are critical to sustaining the health and productivity of coral reef ecosystems. Identification of the production of several key compounds such as DTS, toluene and a range of halogenated BVOCs, provides direction for future work specifically targeting and quantifying the occurrence and potential functions of these chemicals in marine ecosystems.

Acknowledgments

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Supplementary Material

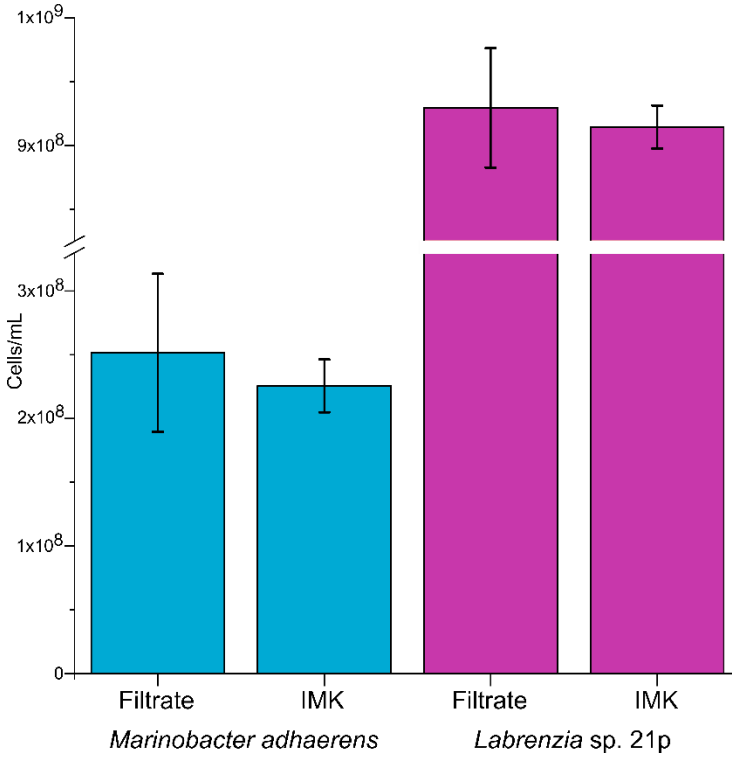

Figure S4.1. Cell density (mean cells mL^{-1} ± standard error) of *Labrenzia* sp. 21p and *Marinobacter adhaerens* incubated in either IMK medium or algal filtrate.

Table S4.1. Taxonomic identity of bacterial isolates from Symbiodiniaceae cultures – based on nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Isolate 21p (bold) was selected for further studies.

Isolate	Closest taxonomic relative	Class	Base pair alignment	Sequence identity	Host culture
1C	Roseovarius sp. strain THAF9 16S ribosomal RNA gene, partial sequence, GenBank: MG996617.1	Alphaproteobacteria	802/805	99%	*Cladocopium* sp. (rt203)
3P	Labrenzia sp. strain CAU 1498 16S ribosomal RNA gene, partial sequence, GenBank: MK053918.1	Alphaproteobacteria	794/794	100%	*Breviolum minutum*
4C	Roseovarius sp. strain THAF9 16S ribosomal RNA gene, partial sequence, GenBank: MG996617.1	Alphaproteobacteria	800/804	99%	*Cladocopium* sp. (rt203)
5P	Roseovarius sp. strain THAF9 16S ribosomal RNA gene, partial sequence, GenBank: MG996617.1	Alphaproteobacteria	799/799	100%	*Cladocopium* sp. (rt203)
6P	Labrenzia sp. strain CAU 1498 16S ribosomal RNA gene, partial sequence, GenBank: MK053918.1	Alphaproteobacteria	793/793	100%	*Breviolum minutum*
7P	Labrenzia alexandrii strain SCSIO_43768 16S ribosomal RNA gene, partial sequence, GenBank: MH283852.1	Alphaproteobacteria	794/794	100%	*Breviolum minutum*
8P	Labrenzia sp. strain CAU 1498 16S ribosomal RNA gene, partial sequence, GenBank: MK053918.1	Alphaproteobacteria	799/799	100%	*Breviolum minutum*
9P	Labrenzia alexandrii strain SCSIO_43768 16S ribosomal RNA gene, partial sequence, GenBank: MH283852.1	Alphaproteobacteria	798/798	100%	*Breviolum minutum*
10P	Rhodobacterales bacterium JB-21 16S ribosomal RNA gene, partial sequence, GenBank: KP265961.1	Alphaproteobacteria	793/793	99%	*Cladocopium* sp. (rt203)
11P	Rhodobacterales bacterium JB-21 16S ribosomal RNA gene, partial sequence, GenBank: KP265961.1	Alphaproteobacteria	795/795	100%	*Cladocopium* sp. (rt203)
12P	Labrenzia alexandrii strain SCSIO_43768 16S ribosomal RNA gene, partial sequence, GenBank: MH283852.1	Alphaproteobacteria	795/795	100%	*Breviolum minutum*
13P	Labrenzia alexandrii strain SCSIO_43768 16S ribosomal RNA gene, partial sequence, GenBank: MH283852.1	Alphaproteobacteria	795/795	100%	*Breviolum minutum*
15P	Mameliella sp. LZ-28 16S ribosomal RNA gene, partial sequence, GenBank: MK099814.1	Alphaproteobacteria	783/784	99%	*Durusdinium trenchii*
16(100)	Rhodobacterales bacterium JB-21 16S ribosomal RNA gene, partial sequence, GenBank: KP265961.1	Alphaproteobacteria	792/792	100%	*Cladocopium* sp. (rt203)

17(100)	Rhodobacterales bacterium JB-21 16S ribosomal RNA gene, partial sequence, GenBank: KP265961.1	Alphaproteobacteria	791/791	100%	*Cladocopium* sp. (rt203)
18P	Labrenzia alexandrii strain SCSIO_43768 16S ribosomal RNA gene, partial sequence, GenBank: MH283852.1	Alphaproteobacteria	794/794	100%	*Breviolum minutum*
19P	Labrenzia alexandrii strain SCSIO_43768 16S ribosomal RNA gene, partial sequence, GenBank: MH283852.1	Alphaproteobacteria	794/794	100%	*Breviolum minutum*
20P	Labrenzia sp. strain CAU 1498 16S ribosomal RNA gene, partial sequence, GenBank: MK053918.1	Alphaproteobacteria	800/803	99%	*Breviolum minutum*
21P	**Labrenzia alexandrii strain SCSIO_43768 16S ribosomal RNA gene, partial sequence, GenBank: MH283852.1**	**Alphaproteobacteria**	**796/796**	**100%**	***Breviolum minutum***
22P	Maribacter flavus strain KCTC 42508 16S ribosomal RNA, partial sequence, NCBI Reference Sequence: NR_144593.1	Flavobacteriia	836/836	100%	*Breviolum minutum*
3k10	Maribacter flavus strain KCTC 42508 16S ribosomal RNA, partial sequence, NCBI Reference Sequence: NR_144593.1	Flavobacteriia	846/846	100%	*Cladocopium* sp. (rt203)
4k10	Maribacter flavus strain KCTC 42508 16S ribosomal RNA, partial sequence, NCBI Reference Sequence: NR_144593.1	Flavobacteriia	842/842	100%	*Cladocopium* sp. (rt203)
5k10	Uncultured bacterium clone HXF_1_41 16S ribosomal RNA gene, partial sequence, GenBank: KJ814148.1	NA	839/839	100%	*Cladocopium* sp. (rt203)
1k100	Rhodobacterales bacterium JB-21 16S ribosomal RNA gene, partial sequence, GenBank: KP265961.1	Alphaproteobacteria	784/784	100%	*Cladocopium* sp. (rt203)
2k100	Rhodobacterales bacterium JB-21 16S ribosomal RNA gene, partial sequence, GenBank: KP265961.1	Alphaproteobacteria	789/790	99%	*Cladocopium* sp. (rt203)

Table S4.2. Pair-wise PERMANOVA tests between *Labrenzia* sp. 21p and *Marinobacter adhaerens* incubated in either Symbiodiniaceae filtrate or IMK medium. Analysis performed in PRIMER v6.1.14 (and PERMANOVA+ v1.0.4) (Anderson et al., 2008; Clarke and Gorley, 2006), data were fourth root transformed and the resemblance plot used Bray-Curtis similarity. Bold lettering indicates significant difference ($P < 0.05$). Monte Carlo P values were used given the low level of replication ($n = 3$) (Hope, 1968).

Groups	t	P value (Monte Carlo)
Labrenzia filtrate vs. *Labrenzia* IMK	2.3981	**0.013**
Marinobacter filtrate vs. *Marinobacter* IMK	1.5599	0.114
Labrenzia filtrate vs. *Marinobacter* filtrate	2.9954	**0.008**
Labrenzia IMK vs. *Marinobacter* IMK	2.8019	**0.016**

Table S4.3. BLAST result of querying *dsyB* (GenBank: KT989543.1; Length = 1023; 31) against the *Marinobacter adhaerens* HP15 genome (KEGG entry: T01922; Length = 4421911; 35).

Query	**Genome**	**Base pair alignment**	**Percentage of alignment**	**Identities**
dsyB	*M. adhaerens*	39/1023	3.81%	32/39

Table S4.4. All compounds and their functional chemical groups produced by *Marinobacter adhaerens* and *Labrenzia* sp. 21p and Symbiodiniaceae (**Chapter 2**). Only compounds detected in Symbiodiniaceae during ambient conditions (26°C) were included. Compounds had to be present in at least four (out of six) technical replicates in at least one species (for each Symbiodiniaceae and bacteria species three biological replicates were used and two technical replicates take from each biological replicate). UC = unclassified, HC = hydrocarbon.

Labrenzia* sp. 21p & *Marinobacter adhaerens		**Symbiodiniaceae**	
Compound ID	Functional group	Compound ID	Functional group
2-Phenyl-2-butanol	Alcohol	1-Hexadecanol, 2-methyl-	Alcohol
Cyclobutanol	Alcohol	Cyclooctyl alcohol	Alcohol
		UC alcohol	Alcohol
2-Ethylhexanal	Aldehyde	Nonanal	Aldehyde
		UC Aldehyde	Aldehyde
		Heptadecane, 9-octyl-	Alkane
		Hexadecane	Alkane
		Pentane	Alkane
		UC Alkane	Alkane
		UC Alkane	Alkane
		Hexane, 2,3-dimethyl-	Alkane
		Octadecane, 6-methyl-	Alkane
		1-Pentene, 2,4,4-trimethyl-	Alkene
		2,4-Dimethyl-1-heptene	Alkene
		Squalene	Alkene
		l-Alanine ethylamide, (S)-	Amide
1,3-Di-tert-butylbenzene	Aromatic HC	[2.2]Paracyclophane	Aromatic HC
Toluene	Aromatic HC	Benzene, 1-ethyl-3-methyl-	Aromatic HC
Benzocyclobutene	Aromatic HC	Ethylbenzene	Aromatic HC
		Naphthalene, 2-methyl-	Aromatic HC
		Phenol, 2,4-bis(1,1-dimethylethyl)-	Aromatic HC
		Toluene	Aromatic HC
		UC Aromatic hydrocarbon	Aromatic HC
		Styrene	Aromatic HC
		Nonanoic acid	Carboxylic acid
2-Ethyl-3-hydroxyhexyl 2-methylpropanoate	DFG	2-Propanol, 1,3-dimethoxy-	DFG
		4-Fluoro-3-trifluoromethylbenzoic acid, eicosyl ester	DFG
		Benzaldehyde	DFG
		Methyl jasmonate	DFG
		Oxime-, methoxy-phenyl-	DFG
Allyl nonanoate	Ester	1-Propanol, 2,2-dimethyl-, benzoate	Ester
2,2,4-trimethyl-3-carboxyisopropyl isobutyl ester pentanoic acid	Ester	UC Ester	Ester
		UC Ester	Ester
		UC Ester	Ester
1,2-15,16-Diepoxyhexadecane	Ether	1-Methoxy-3,5-dimethyl-cyclohexene	Ether
		Benzene, 1-[1,1-dimethylethyl]-4-[2-propenyloxy]-	Ether
		UC Ether	Ether
UC Halogenated HC 42.75	Halogenated HC	3-Trifluoroacetoxypentadecane	Halogenated HC
4-chlorophenyl nonyl ester oxalic acid	Halogenated HC	Halogenated organosilicon	Halogenated HC

UC Halogenated HC 20.85	Halogenated HC	Propane, 1,2-dichloro-	Halogenated HC
UC Halogenated HC 23.74	Halogenated HC	UC Halogenated hydrocarbon	Halogenated HC
1,2-Dichloroethane	Halogenated HC	UC Halogenated hydrocarbon	Halogenated HC
1-Chloro-hexadecane	Halogenated HC	UC Halogenated hydrocarbon	Halogenated HC
Acetone	Ketone	2-Butanone, 3,3-dimethyl-	Ketone
		UC benzoquinone	Ketone
		UC Ketone	Ketone
		UC Ketone	Ketone
Camphor	Monoterpene	Azulene	Monoterpene
		Silanediol, dimethyl-	Organosilicon
Dimethyl trisulfide	Organosulfur	Disulfide, dimethyl	Organosulfur
UC Organosulfur 19.53	Organosulfur	UC Organosulfur	Organosulfur
Dimethyl sulfide	Organosulfur	Dimethyl sulfide	Organosulfur
UC4.29	Unclassified	UC_13.09	Unclassified
UC11.45	Unclassified	UC_17.25	Unclassified
UC13.09	Unclassified	UC_18.34	Unclassified
UC21.73	Unclassified	UC_24.96	Unclassified
UC24.00	Unclassified	UC_26.31	Unclassified
UC27.33	Unclassified	UC_26.79	Unclassified
UC32.33	Unclassified	UC_26.79	Unclassified
UC41.41	Unclassified	UC_27.81	Unclassified
UC42.46	Unclassified	UC_28.26	Unclassified
UC42.87	Unclassified	UC_30.06	Unclassified
UC44.02	Unclassified	UC_31.50	Unclassified
UC44.40	Unclassified	UC_33.16	Unclassified
UC45.15	Unclassified	UC_34.06	Unclassified
UC46.46	Unclassified	UC_36.69	Unclassified
		UC_38.08	Unclassified
		UC_38.58	Unclassified
		UC_38.65	Unclassified
		UC_39.00	Unclassified
		UC_39.36	Unclassified
		UC_39.59	Unclassified
		UC_40.45	Unclassified
		UC_40.78	Unclassified
		UC_41.89	Unclassified
		UC_42.02	Unclassified
		UC_42.37	Unclassified
		UC_42.46	Unclassified
		UC_45.18	Unclassified
		UC_45.20	Unclassified
		UC_40.63	Unclassified
		UC_46.85	Unclassified

Chapter 5

Volatile trade-offs amongst reef building corals: heat stress decreases coral volatilome diversity

Author contributions: CA Lawson, JB Raina, JR Seymour and DJ Suggett conceived and designed the project, CA Lawson performed the experiments, analysed the data and produced figures. M Possell provided technical assistance. E Deschaseaux and V Hreiben performed the isoprene analysis. CA Lawson wrote the paper. All authors edited the manuscript.

In preparation for *Global Change Biology*:

*****Lawson, C.A., Seymour, J.R., Deschaseaux, E., Hreiben, V., Possell, M., Raina, J.B. and Suggett, D.J., Final Draft. Volatile trade-offs amongst reef-building corals: thermal stress negatively affects the diversity of the coral volatilome. Intended Journal, Global Change Biology.–*Author list to be finalised.

Abstract

Productive tropical ecosystems emit large quantities of biogenic volatile organic compounds (BVOCs), these compounds are involved in stress response, pathogen and grazing defences, inter-species communications and climate regulation. Coral reefs produce some of the highest concentrations of the BVOC, dimethyl sulfide (DMS). However, recent work has demonstrated that both Symbiodiniaceae and their associated bacteria produce diverse volatilomes, suggesting that reef-building corals likely produce significant quantities of other, previously overlooked BVOCs that may play critical roles in coral ecology and their stress tolerance. Here I used volatilomics to examine the BVOC production capacity of two common, heat sensitive, reef-building corals, *Acropora intermedia* and *Pocillopora damicornis*, during an experimental heat stress event. A total of 88 BVOCs were detected, but the chemical richness of both volatilomes decreased with thermal stress, with 41 and 62% reductions in BVOC richness observed in *A. intermedia* and *P. damicornis* respectively. Additionally, none of the detected BVOCs significantly increased in abundance during thermal stress in *P. damicornis*, revealing that thermal stress decreases both the diversity and quantities of BVOCs within coral volatilomes. Two brominated BVOCs: bromoform and chlorodibromomethane, were ubiquitous across species and temperature, indicating not only that they may be physiologically important for corals, but also that reefs have previously been an unrecognised source of these ozone-degrading compounds. Isoprene, a BVOC linked to stress protection in higher plants, did not vary significantly during stress for either coral. However, significantly more of the isoprene oxidation product, methyl vinyl ketone, was produced in *P. damicornis* while no DMS was detected. These results support the previously hypothesised trade-off between the abundance of the antioxidant functioning BVOCs, isoprene and DMS, in marine organisms. We reveal that coral reefs are a novel source of volatile metabolites that could prove central to the healthy functioning of reef ecosystems. Furthermore, we show for the first time how thermal stress reduces the

chemical diversity of the coral volatilome and reveals the prevalence of climatically active BVOCs, regardless of temperature.

Introduction

Coral reefs are exceptionally diverse ecosystems sustained by symbiotic interactions occurring within the coral holobiont – the diverse consortium of organisms living in association with a coral host (including the endosymbiotic algae from the family Symbiodiniaceae and complex communities of prokaryotes) (Robbins et al., 2019; Rohwer et al., 2002; Thompson et al., 2015). Metabolic resources are tightly exchanged amongst the various coral holobiont constituents, thereby enabling corals to thrive in otherwise nutrient poor waters (Ceh et al., 2013; Falkowski et al., 1984; Muscatine and Hand, 1958; Rädecker et al., 2015; Robbins et al., 2019; Stanley, 2006; Wegley et al., 2007). However, environmental perturbations destabilise the effectiveness of resource exchange and compromise the stability of these symbiosis (Pogoreutz et al., 2017; Rädecker et al., 2015). Coral bleaching is a general response to stress whereby corals expel their endosymbiotic algae (Hughes et al., 2017; Oakley and Davy, 2018; Suggett and Smith, 2011; Weis, 2008) and recent mass bleaching events have resulted in the loss of more than 30% of the corals on the Great Barrier Reef (GBR) (Hughes et al., 2017, 2018). While changes in coral reef health are commonly measured through multiple metrics, including coral cover, habitat structure, species diversity, fish abundance and functional redundancies (Hughes et al., 2018, 2019; Ostrander et al., 2000; Pratchett et al., 2008), identifying shifts in reef biochemistry, indicative of metabolic dysfunction, may provide early signatures of ecosystem perturbations.

Within terrestrial environments, ecosystem-scale disruptions are accompanied by changes in the emissions of volatile metabolites (biogenic volatile organic compounds – BVOCs), which represent the end products of many biological processes (Loreto and Schnitzler, 2010; Peñuelas, 2008; Peñuelas and Llusià, 2003), and hence can be directly linked to the functional status of an ecosystem. Stress events can lead to the synthesis of

BVOCs not normally produced to support stress tolerance and can also result in up or downregulation of BVOC production (Loreto et al., 2006; Loreto and Schnitzler, 2010; Possell and Loreto, 2013). For example, isoprene, the BVOC most commonly emitted by higher plants, can scavenge harmful reactive oxygen species produced during stressful conditions (Sharkey and Singsaas, 1995; Velikova et al., 2006; Vickers et al., 2009b) and enhanced isoprene emission is frequently correlated with increases in temperature in many plant species (Guidolotti et al., 2019). In addition, various other BVOCs can signify stress responses (Loreto and Schnitzler, 2010; Sunda et al., 2002), and are implicated in a wide range of ecophysiological roles that govern organism fitness, such as inter-organism signalling (Loivamäki et al., 2008; Runyon et al., 2006) and defence against pathogens (Kasal-Slavik et al., 2017).

The broad consortium of BVOCs that is ultimately produced by any organism has been termed its 'volatilome' (D'Alessandro, 2006; Steinke et al., 2018). Examining and monitoring an organism by assessing its volatilome is gaining increased attention as samples can be taken non-destructively yet provide key insight into the diversity of functional responses at play. Plant volatilomes are proving to be highly diverse (comprised of hundreds of BVOCs; Jud et al., 2018), but whether such similarly large diversity extends to marine organisms is currently unexplored.

The Great Barrier Reef (GBR) has long been known as a source of aerosols that can be derived from BVOCs (Bigg and Turvey, 1978). Some of these BVOCs can also play a role in climate regulation through the formation of cloud condensation nuclei, which can decrease temperature locally by increasing albedo (Laothawornkitkul et al., 2009; Sporre et al., 2019), while others can increase the residence time of greenhouse gases (Carpenter et al., 2012; Curci et al., 2009; Kulmala et al., 2004; Peñuelas and Staudt, 2010). High irradiance levels have been linked to the accumulation of significant levels of BVOCs above the GBR (Cropp et al., 2018; Jackson et al., 2018). The most widely studied of these BVOCs is dimethyl sulfide (DMS), which is present in high concentrations in corals (Broadbent and

Jones, 2004; Hopkins et al., 2016; Swan et al., 2016), has a role in climate dynamics (Ayers and Gras, 1991) and impacts organism tolerance to stress (Deschaseaux et al., 2014a; Hopkins et al., 2016; Sunda et al., 2002). However, DMS is likely just one of many compounds emitted by reef-building corals. Recent work has highlighted that corals can also produce isoprene as well as several volatile sulfur compounds (Swan et al., 2016), yet the full volatilome of reef-building corals remains uncharacterised. Identifying the full extent of the volatile molecules produced by corals is crucial to fully understand their physiological response to stress, and to provide accurate estimates of BVOC emissions for climate modelling.

After exploring the volatilome of coral algal endosymbionts (Symbiodiniaceae; **Chapter 2**) and associated bacteria (*Labrenzia* and *Marinobacter*; **Chapter 4**), in this chapter I describe, for the first time, the volatilome of two common GBR reef-building corals, *Acropora intermedia* and *Pocillopora damicornis* during a simulated heat stress event. I hypothesised that (a) the two species of corals have distinct volatilomes while sharing a few 'core' compounds, and (b) that these volatilomes shift in response to thermal stress. By investigating the whole coral volatilome, addressing these hypotheses demonstrates how production by individual corals potentially scales to previous reef-based observations, and identifies the potential importance of certain BVOCs during stress.

Methods

Sample site and coral collection

The experimental design used here mimicked previous coral thermal stress experiments performed on corals at Heron Island, which is situated on the southern Great Barrier Reef (Gardner et al., 2017; Leggat et al., 2019; Nitschke et al., 2018; Petrou et al., 2018). Briefly, corals were collected from the reef flat (1-2m depth) on the southern side of Heron Island (23.44°S, 151.91°E; Figure 5.1). Five healthy colonies of *A. intermedia* and *P. damicornis* were sampled and 10 fragments were collected from each colony. Fragments were transferred to Heron Island Research Station (The University of Queensland) into 350

L holding tanks with continuous reef water flow-through. Fragments were split into nubbins (see Figure S5.1 for detailed information on coral collection) that were fixed onto glass microscope slides with Selleys Epoxy (Selleys Pty Ltd., Australia) and left to acclimate in holding tanks overnight. Nubbins from each fragment were then split into two aquaria (~70 L each), representing one control and one treatment (yielding a total of five aquaria for each control and treatment). All aquaria were maintained with continuous flow of ambient lagoon seawater to allow nubbins to acclimate for a further three days. All holding tanks and experimental aquaria were kept under neutral density shade cloth to minimise extreme daily light conditions (see Figure 5.2a for light conditions).

Figure 5.1. Map of eastern Australia (A) indicating the location of Heron Reef and Island (B). Corals were collected on the reef flat within the red section.

Heat stress experiment

The thermal stress experiment ran for 12 days. Following acclimation, treatment aquaria were gradually ramped-up in temperature by ~1°C per day, resulting in a temperature increase from ~27.5°C to 32°C over a period of five days (Figure 5.2a). Treatment aquaria were then maintained at 31.63 ± 0.08°C for six days (mean ± standard error; Figure 5.2a). On the sixth day, BVOCs were sampled from all treatment and control nubbins. In order to artificially increase the temperature, the reef water flow through was diverted to a sump (200 L) that was heated with two custom built, thermostat controlled

heaters (± 0.1°C; 300W) before flowing into all treatment tanks at a flow rate matching control aquaria. Control aquaria were maintained with continuous reef water flow-through with no manual temperature alterations for the duration of the experiment (27.65 ± 0.69°C; mean ± standard deviation; Figure 5.2a).

Coral physiological status was monitored daily (dawn) using Pulse Amplitude Modulated (PAM) fluorometry (Diving PAM, Walz GmbH, Effeltrich, Germany; settings: MI: 8, Gain: 4, SI: 8, SW: 0.8, D: 3) (as per Suggett et al., 2012). Water temperature and light intensity (LUX) in all aquaria were measured every 5 minutes for the duration of the thermal stress experiment (HOBO pendant loggers, Onset Computer Corp., Bourne, MA, USA). The experiment was terminated after the F_v/F_m of heat-treated corals started to significantly decrease, indicating the onset of sub-lethal stress (Figure 5.2b & c).

Volatilome sampling

Live coral nubbins fixed on microscope slides were placed in 500mL gas tight chambers filled with 300 mL aquarium water (to ensure the entire nubbin was immersed). Chambers were placed in a water bath, with temperature and light matching their experimental conditions (HYDRA; Aquaillumination, Iowa, USA). Samples for characterisation of the volatilome were then collected as per **Chapters 2 & 4**, whereby chambers were purged for 30 minutes with instrument grade air (BOC Gases, Linde Group, Australia) while the outlet was collected on thermal desorption (TD) tubes (Tenax TA; Markes International Ltd, Llantrisant, UK; see Figure S5.2), that were capped immediately post-purge and stored at 4°C. Blanks containing seawater from the setup (from control and heat treated conditions) and hardened Selleys epoxy were run in conjunction with all analysis. TD tubes were desorbed within two weeks using an automated thermal desorption unit (ULTRA 2 & UNITY 2; Markes International Ltd, Llantrisant, UK) for 6 minutes at 300°C and concentrated on a Tenax TA cold trap held at -30°C before flash heating (300°C) and injection (via a heated transfer line; 150°C) onto a 7890A GC-MS (Agilent Technologies Pty Ltd, Melbourne). The GC-MS was fitted with a BP1 capillary column (60 m x 0.32 mm, 1 µm

film thickness; SGE Analytical Science Pty Ltd, Melbourne) at a flow rate of 2.3 mL min^{-1} and run splitless. To allow for complete desorption, the GC oven was heated at 35°C for 5 minutes then 4°C min^{-1} to 160°C then 20°C min^{-1} to 300°C for 10 minutes. The GC was coupled to a mass-selective detector (Model 5975C; Agilent Technologies Pty Ltd, Melbourne) that was set to a scanning range of 35 – 250 amu.

Isoprene sampling

Isoprene had not previously been detected with our GC-MS set up (e.g. **Chapter 2 & 4**), potentially due to its especially high reactivity and because of its very low concentration. Given the ubiquitous nature of isoprene in terrestrial systems and its potential roles in stress tolerance, additional samples were taken for targeted isoprene analysis. Coral nubbins for isoprene analysis were preserved prior to the temperature ramping (day 2), as well as upon reaching 32°C (day 7) and after being held at 32°C for five days (day 12) (Figure 5.2a). Nubbins were removed from their epoxy holds and placed in 100 mL amber crimp cap vials (Chromalytic Technology, Boronia, Victoria) with 70 mL of water from the aquaria and sealed with PTFE/White Silicone septa caps (Agilent Technologies Pty Ltd, Melbourne) before adding 300 µL of saturated mercuric chloride solution (AJAX Chemicals, Cheltenham, Victoria). Isoprene concentrations were determined using a gas chromatograph (GC) (Agilent Technologies 6890N) with a mass selective detector (MSD) (Agilent Technologies 5973N) fitted with a Gerstel multipurpose sampler (MPS 2XL). From each vial, 1 mL of headspace was cumulatively extracted and injected onto a Tenax liner, kept at 20°C, three times. After the third injection, the GC inlet was held at 20°C on solvent vent mode for 30 seconds, before the temperature was ramped to 280°C at 720°C min^{-1} to release the isoprene onto the capillary column (ZB-5, 60 m x 0.25 mm x 0.25 µm), at which point the inlet was switched to splitless mode. The GC oven was held at 50°C for 7 minutes and the MDS was held at 280°C in single ion monitoring mode with selected ion mass of 53, 67, 68. Solvent delay was set for 4 minutes; total run time including sample injection was 13 minutes. A 6 point calibration was performed, ranging from 29-1428 pM of isoprene, the

limits of detection and quantification in this set up were 15 pM and 285 pM, respectively. Blanks of aquarium water were run in conjunction with all analyses.

Coral Symbiodiniaceae cell counts and surface area calculation

Following all volatilome sampling, coral nubbins were air-picked to remove all tissue and Symbiodiniaceae cells. Cell counts were performed using standard methods as previously described (**Chapter 2**; Gardner et al., 2019). Following isoprene sampling, nubbins were cleaned. All coral skeletons were dried overnight (65°C; BINDER oven; model FD260; Tuttlingen, Germany) and surface area was calculated using a single wax dipping method (Veal et al., 2010).

Data analysis

Common contaminating ions (73, 84, 147, 149, 207 and 221 m/z) were removed using the Denoising function in OpenChrom (Wenig and Odermatt, 2010). Using the PARAFAC2 based deconvolution and identification system (PARADISe; Johnsen et al., 2017) peaks were resolved and assessed. PARADISe uses an integrated NIST database (NIST14 library in NIST MS Search v.2.2f; NIST, Gaithersburg, MD) to assign compounds to peaks, compound identity was further checked by manually comparing mass spectra against NIST14. Samples of aquaria water were run as blanks in conjunction with all analyses; the average values for compounds present in the blanks were subtracted from all samples. Any compound that did not occur in at least two samples was considered contamination and removed. UC denotes an unclassified compound (the number following UC indicates the retention time of the compound if the functional group could not be determined). Univariate General Linear Models were used to check for significant differences between treatments and species, if the data did not meet the test's assumptions, the Kruskal-Wallis test was used instead (IBM SPSS Statistics, version 25). Bubble plots were created with ggplot2 (Wickham, 2016) in RStudio. Principal Component Analysis (PCA) was carried out with PAST (PAleontological STatisitics; version 3.25; Hammer, Harper, and Ryan 2001) on glog-transformed data (Durbin et al., 2002). Due to equipment malfunctions, one biological

replicate was lost from each *A. intermedia* control and stress treatment and the *P. damicornis* control (n = 4), and two from the *P. damicornis* stress treatment (n = 3). Trimethyl silanol and dimethylsilanediol were removed from the analysis as they are suspected contamination from dimethylpolysiloxane (GC column material) hydrolysis (Cella and Carpenter, 1994).

Results

Both *A. intermedia* and *P. damicornis* experienced significant declines in photochemical efficiency (F_v/F_m; dimensionless) following exposure to increased temperatures (Figure 5.2b & c). Values of F_v/F_m for both species were reduced under heat stress compared to the control by 10.86% (*A. intermedia*) and 9.22% (*P. damicornis*) by day 12 of the experiment (following 5 days maintained at 31.63 ± 0.08°C) (Figure 5.2b & c). Symbiodiniaceae cell density did not differ significantly between the control and heat stress treatment throughout the experiment for *A. intermedia* (Figure 5.2c). In contrast, Symbiodiniaceae density within *P. damicornis* was significantly reduced in the heat stress treatment compared to the control, by 33% on day 7 and 71% on day 12 (Figure 5.2b).

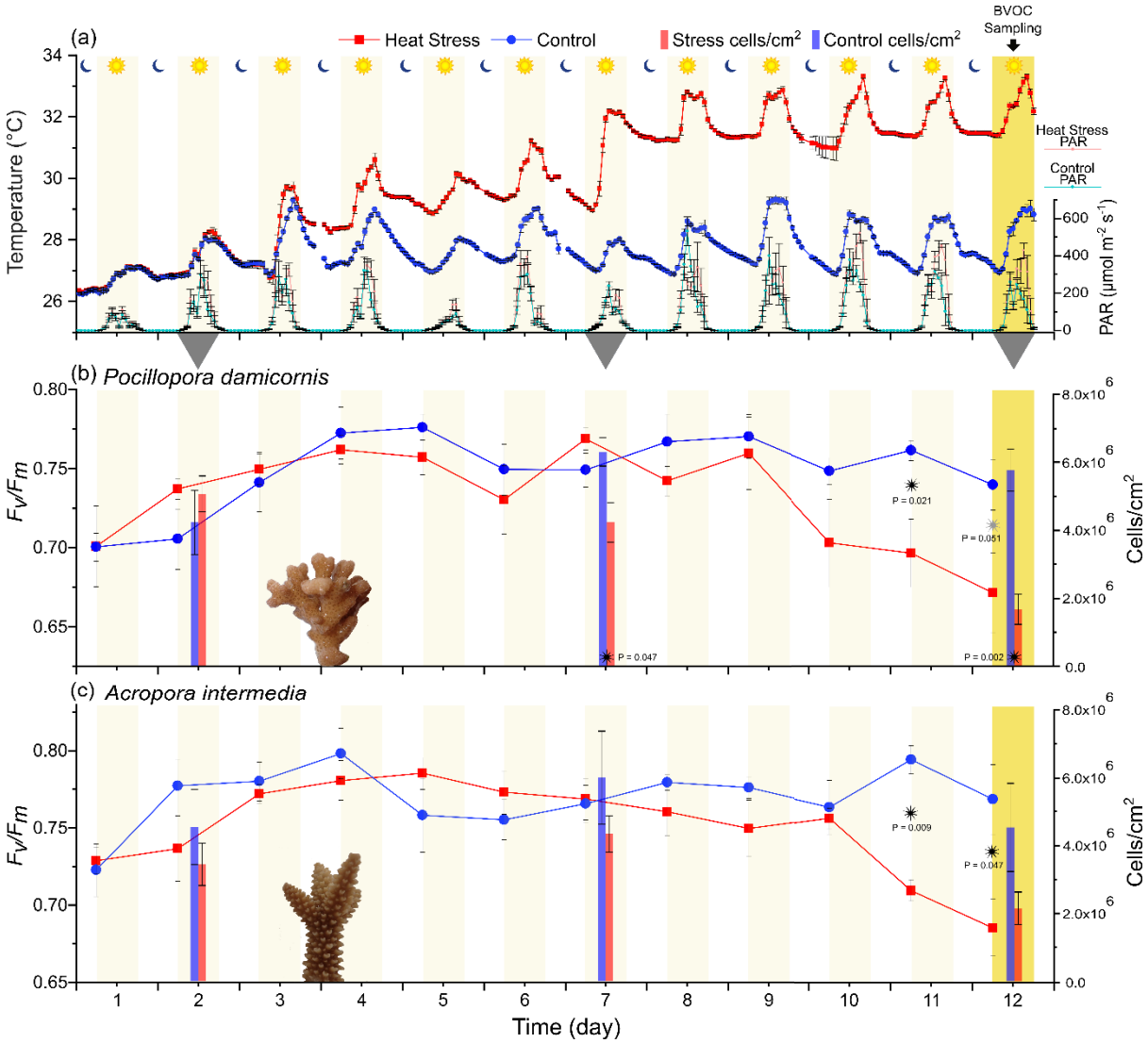

Figure 5.2. (a) Mean temperature (hourly mean ± standard error) and light (hourly mean ± standard error) profile in control and treatment aquaria, grey triangles indicate isoprene sampling. Photochemical efficiency (F_v/F_m; mean ± standard error; dimensionless; n = 5; PAM fluorometry) and cell density (cells cm^{-2}; mean ± standard error) for (b) *Pocillopora damicornis* and (c) *Acropora intermedia* throughout the heat stress experiment. Significant differences are indicated with stars and probability values.

Isoprene concentrations did not differ significantly between treatment or time for either coral species (Repeated Measures ANOVA; Figure 5.3). In addition to the targeted isoprene analysis, 87 BVOCs were detected across *A. intermedia* and *P. damicornis* heat stress and control conditions. The volatilome composition under control conditions did not differ significantly between the two corals (PERMANOVA; p = 0.113; Figure S5.3). Within this untargeted analysis, 19 BVOCs were common to control conditions of both coral species (present in 100% of samples) and included aldehydes, alkanes, an aromatic hydrocarbon,

carboxylic acid, esters and halogenated hydrocarbons (Figure 5.4a; Table S5.1). However, the quantities of seven BVOCs differed significantly between *A. intermedia* and *P. damicornis* in the controls, these included DMS (p = 0.01; Kruskal Wallis), methyl vinyl ketone (MVK; p = 0.05; univariate GLM), geranyl acetone (p = 0.01; univariate GLM) and tridecane (p = 0.04; univariate GLM) (Figure 5.5). Quantities of geranyl acetone, DMS and tridecane were all significantly higher in *A. intermedia* (p < 0.05), while only MVK was detected in significantly higher quantities in *P. damicornis* (Figure 5.5). Only three compounds, bromoform, chlorodibromomethane and an unclassified halogenated hydrocarbon were detected in 100% of samples across both species and treatments, and as such were defined as core members of the coral volatilome (Figure 5.4a; Table S5.1).

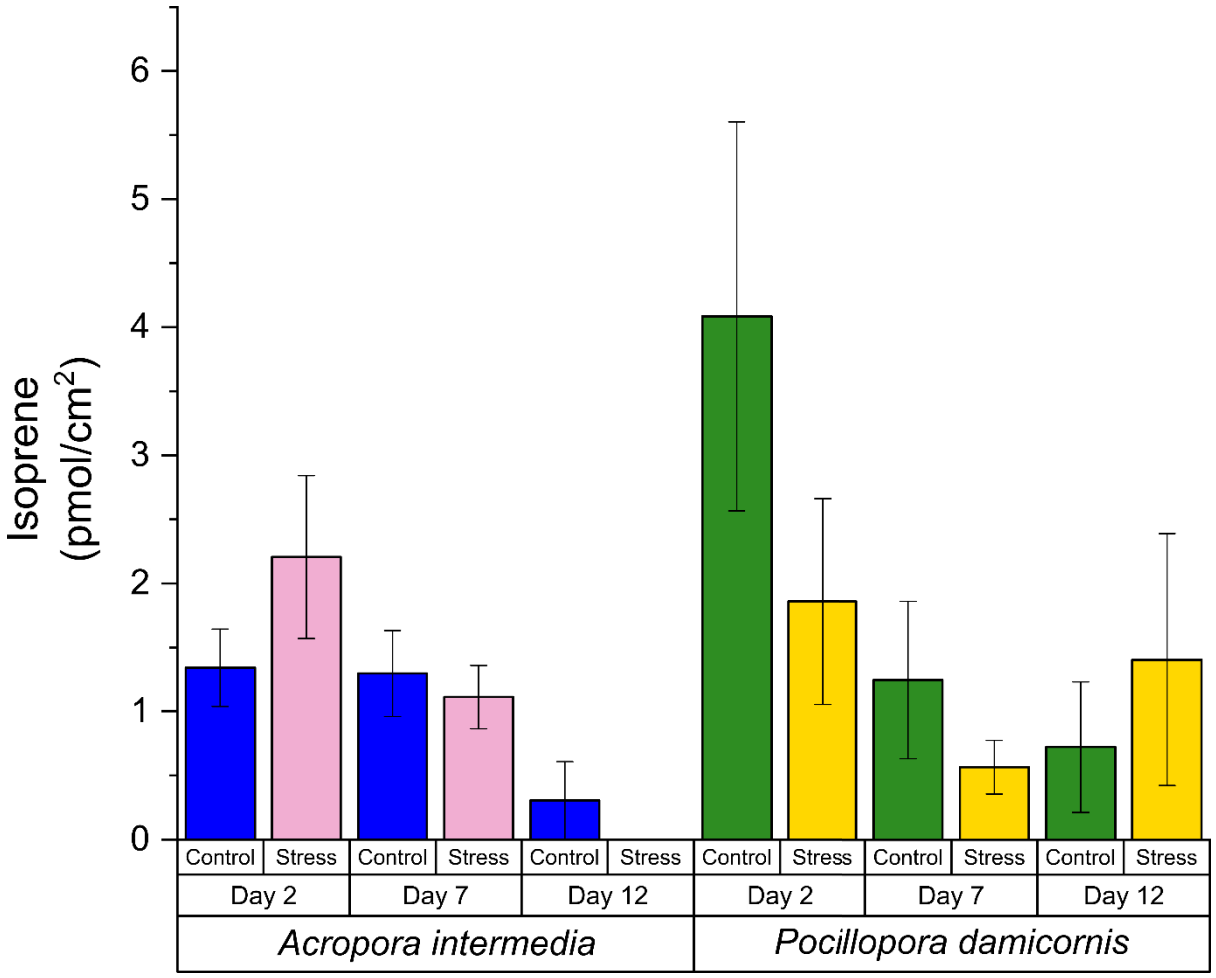

Figure 5.3. Isoprene (pmols cm^{-2}, mean ± standard error) concentrations in *Acropora intermedia* and *Pocillopora damicornis* before any thermal ramping (day 2), after reaching 32°C (day 7) and after being held at 32°C for five days (day 12). Controls were maintained in ambient seawater for the duration of the experiment. No significant differences were detected between treatment or time for either species (Repeated Measures ANOVA). N = 3 for *A. intermedia* and N = 4 for *P. damicornis*.

Similarly to under control conditions, the volatilome composition of the heat stressed *P. damicornis* did not significantly differ from the heat stressed *A. intermedia* (PERMANOVA; $p = 0.674$; Figure S5.3). However, thermal stress significantly altered the volatilome of each species (PERMANOVA; $p < 0.05$; Figure S5.3), reducing the diversity of BVOCs emitted by corals. Furthermore, there was a sharp decrease in the number of BVOCs shared by both species, this number dropped from 19 to 8 BVOCs, which included halogenated hydrocarbons, unclassified compounds and a monoterpene (limonene) (Figure 5.4a; Table S5.1). Following thermal stress, both species exhibited significant decreases in the quantities of multiple BVOCs, seven of which were shared between *P. damicornis* and *A. intermedia.* These included acetic acid, 2(E)-decanal, heptanal, 5-methylhexan-5-olide, nonane, UC28.66 and 4-ethylbenzoic-acid-2,2-dimethylpropyl ester.

A total of 79 BVOCs were detected in control *A. intermedia* samples, which were dominated by aromatic hydrocarbons and ketones (Figure 5.4a). During stressed conditions, *A. intermedia* produced fewer BVOCs (46 BVOCs) and no alkanes, carboxylic acids, ethers or halogenated organosulfurs were detected, while aromatic hydrocarbons and ketones remained the dominant chemical functional groups (Figure 5.4a). Principal component analysis (PCA) showed clear clustering of replicates and significant separation (PERMANOVA; $p = 0.022$; Figure S5.3) between heat stressed and control samples of *A. intermedia* (Figure 5.4c). This differentiation between control and stressed *A. intermedia* samples was largely driven by nonanal (Principal Component (PC1) loading = 0.22), benzaldehyde (PC1 loading = -0.14), MVK (PC2 loading = 0.21) and DMS (PC2 = -0.24). The quantities of 22 BVOCs differed significantly between the *A. intermedia* heat stress and control samples (Figure 5.5). The majority of these were detected in significantly lower amounts during stress conditions while only three BVOCs (benzaldehyde, P = 0.03; univariate GLM; 2-ethyltoluene, P = 0.04; Kruskal Wallis & UC25.19, P = 0.01; univariate GLM) were significantly higher under heat stress (Figure 5.5).

Under control conditions, 76 BVOCs were detected in *P. damicornis*. Aromatic hydrocarbons and ketones were the dominant chemical functional groups and this pattern did not change during stressed conditions, but the total number of detected BVOCs decreased by 62% (to 29 BVOCs). Furthermore, no alkanes, alkenes, carboxylic acids, esters or halogenated organosulfurs were detected in the heat stressed *P. damicornis*. Control and heat stress *P. damicornis* volatilomes showed significantly different compositions (PERMANOVA; $p = 0.025$; Figure S5.3) with a PCA (Figure 5.4b) revealing the major drivers of this separation to be methyl isobutyl ketone (PC1 loading = 0.22), acetic acid (PC1 loading = 0.21), tetradecane (PC2 loading = 0.24) and m-ethyltoluene (PC2 loading = 0.22). Additionally, no BVOCs showed significantly higher concentrations in *P. damicornis* during thermal stress however, 11 BVOCs were detected in significantly lower concentrations (Figure 5.5).

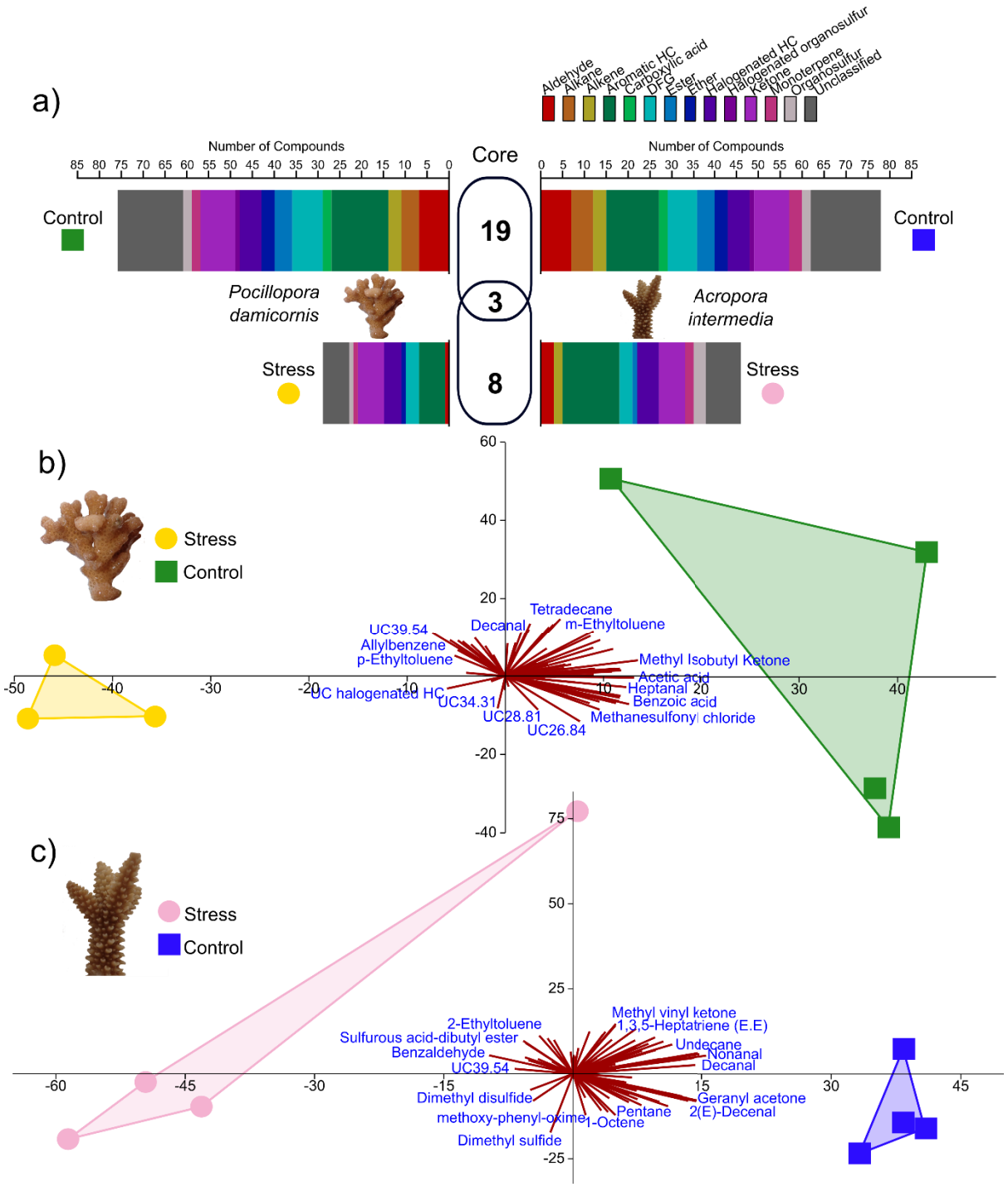

Figure 5.4. a) Chemical functional groups (total number of volatile compounds present - in at least two biological replicates) present in *Acropora intermedia* and *Pocillopora damicornis* during control (27.65 ± 0.69°C) and stressed (31.63 ± 0.08°C) conditions and the core volatiles that are shared between them (present in 100% of samples, see Table S5.1 for full list of core BVOCs). Principal component analysis (PCA; Bootstrap N = 1000) of (b) *Pocillopora damicornis* (PC1 = 38.38%, PC2 = 21.96%) and (c) *Acropora intermedia* (PC1 = 39.93%, PC2 = 22.37%) under control (27.65 ± 0.69°C) and stressed (31.63 ± 0.08°C) conditions.

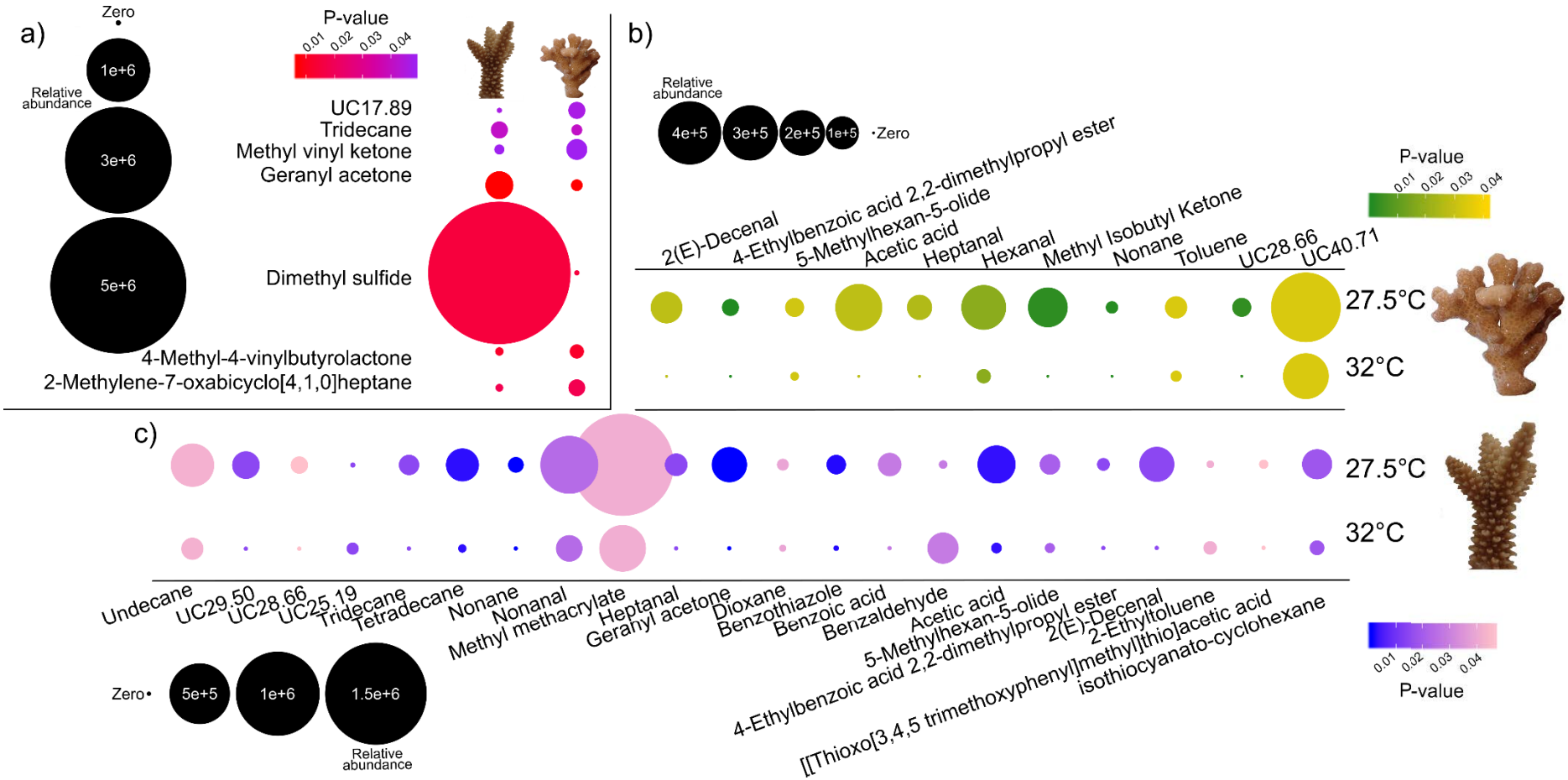

Figure 5.5. (a) Significant differences between *Acropora intermedia* and *Pocillopora damicornis* under ambient temperature (27.65 ± 0.69°C). Significant differences between control (27.65 ± 0.69°C) and stressed (31.63 ± 0.08°C) conditions for *P. damicornis* (b) and *A. intermedia* (c). Significance was tested with a univariate general linear model or a Kruskal Wallis test (if assumptions were not met).

Discussion

Anomalous thermal stress events are increasing in frequency and severity, which is driving dramatic declines in coral health and abundance (Donner et al., 2017; Heron et al., 2016; Hughes et al., 2017). Heat stress experiments targeting reef-building corals have been performed for over 20 years to identify key processes potentially enhancing their tolerance to thermal anomalies. These experiments have demonstrated complex and specific responses by the cnidarian host, endosymbiotic algae and associated bacteria (Abrego et al., 2008; Desalvo et al., 2010; Dove et al., 2006; Downs et al., 2000; Kenkel et al., 2013; Littman et al., 2010; Petrou et al., 2018). Recently, metabolomics has demonstrated that the coral host and endosymbiotic algae experience significant shifts in the production of stable metabolites in response to thermal stress (Hillyer et al., 2016, 2017, 2018; Petrou et al., 2018; Sogin et al., 2016). Here, we expand on these recent metabolomic approaches by exploring volatile metabolomes (volatilome) and take an important step forward to understanding the capacity of corals to produce a diverse range of BVOCs and how this changes under conditions of thermal stress. We examined two common reef-building corals that have been significantly impacted by thermal stress on the GBR, *Acropora intermedia* and *Pocillopora damicornis* (Hughes et al., 2017), and exposed them to an acute thermal stress event. Between these two species, we detected isoprene with a targeted analysis and a further 87 BVOCs with untargeted analysis. Our results provide the first insight on the production of BVOCs by reef building corals and how these compounds vary during heat stress. They reveal that many identified compounds may play potentially important – but previously unrecognised – roles in coral physiology. In addition, our results reveal that thermal stress dramatically decreases the chemical diversity, quantity and functional potential of these important compounds.

The “core volatilome” of the two coral species, regardless of temperature, consisted of two brominated compounds, bromoform, and chlorodibromomethane, and an unclassified halogenated hydrocarbon. The apparent ubiquitous nature of halogenated hydrocarbons in these corals is particularly interesting since they were also detected in Symbiodiniaceae

(**Chapter 2**) and Symbiodiniaceae associated bacteria (**Chapter 4**). However, two members of this core volatilome, bromoform and chlorodibromomethane (and in fact any brominated compounds) were not previously been detected in Symbiodiniaceae (**Chapter 2**) or their associated bacteria (**Chapter 4**). Bromoform is one of the most abundant forms of brominated hydrocarbons and has strong potential to react and deplete atmospheric ozone, which can impact the atmospheric radiation budget (Hossaini et al., 2015; Saiz-Lopez et al., 2012). Marine micro and macroalgae are known sources of bromoform (Bondu et al., 2008; Lim et al., 2017; Manley et al., 1992). The function of these halogenated compounds remains uncertain, yet they are thought to play a role in mitigating oxidative stress (Abrahamsson et al., 2003; Goodwin et al., 1997). This is particularly relevant as various reactive oxygen species are known to be a key driver of coral bleaching (Smith et al., 2005; Weis, 2008). However, it is notable that we found no significant change in halogenated hydrocarbons between control and heat stress conditions. The ubiquitous presence of these compounds regardless of environmental state and physiological condition might suggest an alternate functional role. For example, brominated compounds can function in the chemical defence of the red alga, *Corallina pilulifera,* from parasitic microbes (Ohsawa et al., 2001). It is therefore possible that coral holobionts produce these brominated BVOCs to aid in protection against harmful microbes. It is imperative we resolve the defence mechanisms that corals use against pathogens and predators, and how effective these systems are following damaging stress events. The finding of core volatiles that are conserved under the onset of heat stress provides bearing for future work to elucidate the functional roles for these specific BVOCs.

Nineteen BVOCs were shared between *A. intermedia* and *P. damicornis* under control conditions; these included aldehydes, alkanes and esters. However, under heat stress conditions, 16 of these BVOCs were not detected. It is possible that these BVOCs are no longer detected during stress because they potentially react with ROS, or their synthesis is down-regulated under stress conditions. Amongst the 16 BVOCs that were absent during

thermal stress, were two nitrogen-containing BVOCs, methoxyphenyl-oxime (MPO) and benzothiazole. MPO is classed as an oxime (R_1R_2C=NOH), a chemical group recently recognised as highly bioactive, likely playing roles as infochemicals, regulating growth and scavenging reactive oxygen species (Sørensen et al., 2018). Benzothiazole (C_5H_7NS) contains both nitrogen and sulfur and is produced by higher plants (Le Bozec and Moody, 2009) and bacteria, where it can function as a strong fungicide (Fernando et al., 2005). The absence of these nitrogen-containing molecules during thermal stress is particularly notable, given the hypothesis that excess nitrogen and the concurrent shift to a phosphate-limited state, may be a cause for coral holobiont breakdown and subsequent bleaching (Pogoreutz et al., 2017; Rädecker et al., 2015).

Only eight compounds were ubiquitously present among thermally stressed corals, including halogenated hydrocarbons and a single monoterpene (limonene). Limonene was detected in all stressed coral samples and while it was also detected in control corals, it was not present in all biological replicates. This compound is frequently detected in terrestrial ecosystems (global emission of limonene is ~11.4 Tg $year^{-1}$; Guenther et al., 2012) and its concentration significantly increases in higher plants following thermal stress (Copolovici et al., 2012). Limonene has also been quantified in five species of macroalgae (Tokarek et al., 2019) and its production was light dependent in a range of microalgae (diatoms, prymnesiophytes, cryptophytes & dinoflagellates; Meskhidze et al., 2015). Emissions of limonene are particularly notable, as in the presence of ozone, this compound is a significant source of atmospheric aerosols (Draper et al., 2015). Furthermore, limonene is a monoterpene, a class of BVOCs that are extremely common higher plant volatiles, frequently influenced by physiological stress and often involved in chemical signalling (Loreto et al., 1998; Riedlmeier et al., 2017). Given its important functions in higher plants, the universal presence of limonene in stressed corals highlights the need to fully characterise its role in corals.

Species of *Acropora* are particularly sensitive to thermal stress (Hoogenboom et al., 2017; Hughes et al., 2017; Marshall and Baird, 2000) and produce copious amounts of DMS (Broadbent et al., 2002; Broadbent and Jones, 2004; Hopkins et al., 2016; Swan et al., 2016). Here we demonstrate that DMS is not the sole BVOC produced by *A. intermedia*; this species can produce a diverse range of volatiles, 22 of which changed significantly during heat stress. The vast majority of these (19 out of 22) were detected in significantly lower amounts during stressed conditions, with only three compounds – benzaldehyde, 2-ethyltoluene and UC25.19 – that were significantly higher during stress. Benzaldehyde was detected in Symbiodiniaceae (**Chapter 2**), can function as an infochemical (Yang et al., 2018) and can increase in response to thermal stress (Misztal et al., 2015). Ethyltoluene has previously been detected from a marine invertebrate (*Platynereis dumerilii*) and is thought to function as a pheromone (Zeeck et al., 1991). The occurrence of significantly higher concentrations of two different putative infochemicals in stressed *A. intermedia* is particularly notable as previous work demonstrated that specific infochemicals can be used by pathogenic bacteria to target stressed corals (Garren et al., 2014).

Overall, the number of BVOCs detected in *A. intermedia* during stress decreased relative to control conditions. This change was consistent with the patterns observed in *P. damicornis*, but there was a more dramatic reduction during stress in *P. damicornis.* Fewer BVOCs changed significantly in *P. damicornis* between control and heat stress (11) and all of these were significantly lower in stressed *P. damicornis*. Toluene was among the compounds that significantly decreased during stress and recent work has shown toluene emissions are tightly coupled to photosynthesis, increasing with temperature before reaching a threshold and subsequently declining (Misztal et al., 2015). Toluene has been reported in macroalgae (Tokarek et al., 2019), furthermore its emission was seen to correlate with the abundance of *Emiliania huxleyi* and it has been suggested that it may play a role in chemical communication (Misztal et al., 2015). Notably, we did not detect any DMS in *P. damicornis*,

which is consistent with previous work (Exton et al., 2015) and indicates the variable production of DMS in coral reef ecosystems.

Isoprene accounts for approximately half of global terrestrial BVOC emissions (~ 500Tg $year^{-1}$; Guenther et al., 2012) and its emission is tightly coupled to the thermal stress response of higher plants. For example, isoprene is used to protect the photosynthetic apparatus during the onset of thermal stress and the associated build-up of reactive oxygen species, likely via interacting with and strengthening thylakoid membranes (Behnke et al., 2007; Sharkey and Yeh, 2001; Singsaas et al., 1997; Siwko et al., 2007; Vickers et al., 2009a, 2009b) or by allowing excess energy to dissipate (Sanadze, 2017; Sanadze et al., 2016). Isoprene has previously been detected in samples of *Acropora aspera* under ambient conditions (Swan et al., 2016), and in Symbiodiniaceae species under both ambient conditions and heat stress (Exton et al., 2013, 2015). Yet no significant variation in isoprene concentration was detected between our two coral species relative to thermal stress. However, we did detect two of the most common oxidation products of isoprene, methyl vinyl acrylate (MVK) and methacrolein (Cappellin et al., 2019). MVK was detected in significantly higher amounts in control *P. damicornis* than *A. intermedia* though there were no other significant patterns between treatments or species for these compounds. Nevertheless, their presence suggests that more real-time analysis of isoprene (e.g. Proton Transfer Reaction-Mass Spectrometry; Cappellin et al., 2019) may aid efforts to determine if more subtle patterns of isoprene emission occur in corals during thermal stress. A trade-off has recently been hypothesized between DMS and isoprene synthesis as both compounds have antioxidant functions (Dani and Loreto, 2017; Exton et al., 2015). Here, we only detected DMS in *A. intermedia*, but significantly more of the isoprene break-down product, MVK, was present in *P. damicornis*. While we did not detect different DMS concentrations between control and heat stress in *A. intermedia*, DMS was highly variable in the heat stress biological replicates – ranging from undetectable to 85% higher than the control mean. These complex patterns highlight the variable nature of these BVOCs, even within a single

species, highlighting the need for more replication and time-resolved sampling in future studies.

In contrasting the volatilomes of *P. damicornis* and *A. intermedia* under ambient conditions with six species of Symbiodiniaceae (**Chapter 2**) and two species of Symbiodiniaceae-associated bacteria (**Chapter 4**), we uncover two BVOCs that are ubiquitous – toluene and DMS (Figure 5.6, Table S5.2). While the coral volatilomes do include contributions from endosymbiotic Symbiodiniaceae and bacteria, we know that each component is individually capable of producing the precursor of DMS (Alcolombri et al., 2015; Curson et al., 2017; Raina et al., 2013). The Symbiodiniaceae volatilome shared a further six BVOCs with the coral volatilome, which comprised nonanal, benzaldehyde, pentane, methoxyphenyl-oxime, azulene and dimethyl disulfide (Figure 5.6, Table S5.2). While it is critical to understand the individual components of the coral holobiont, the lack of overlap between the volatilomes shown here demonstrates the necessity of examining the holobiont as a whole. This becomes especially relevant as novel compounds can be formed from the emitted BVOCs from each component (Kai et al., 2018), thus directly impacting the net emission from the holobiont – which has implications for trophic interactions that utilise these chemical signatures and their overall fluxes to the atmosphere.

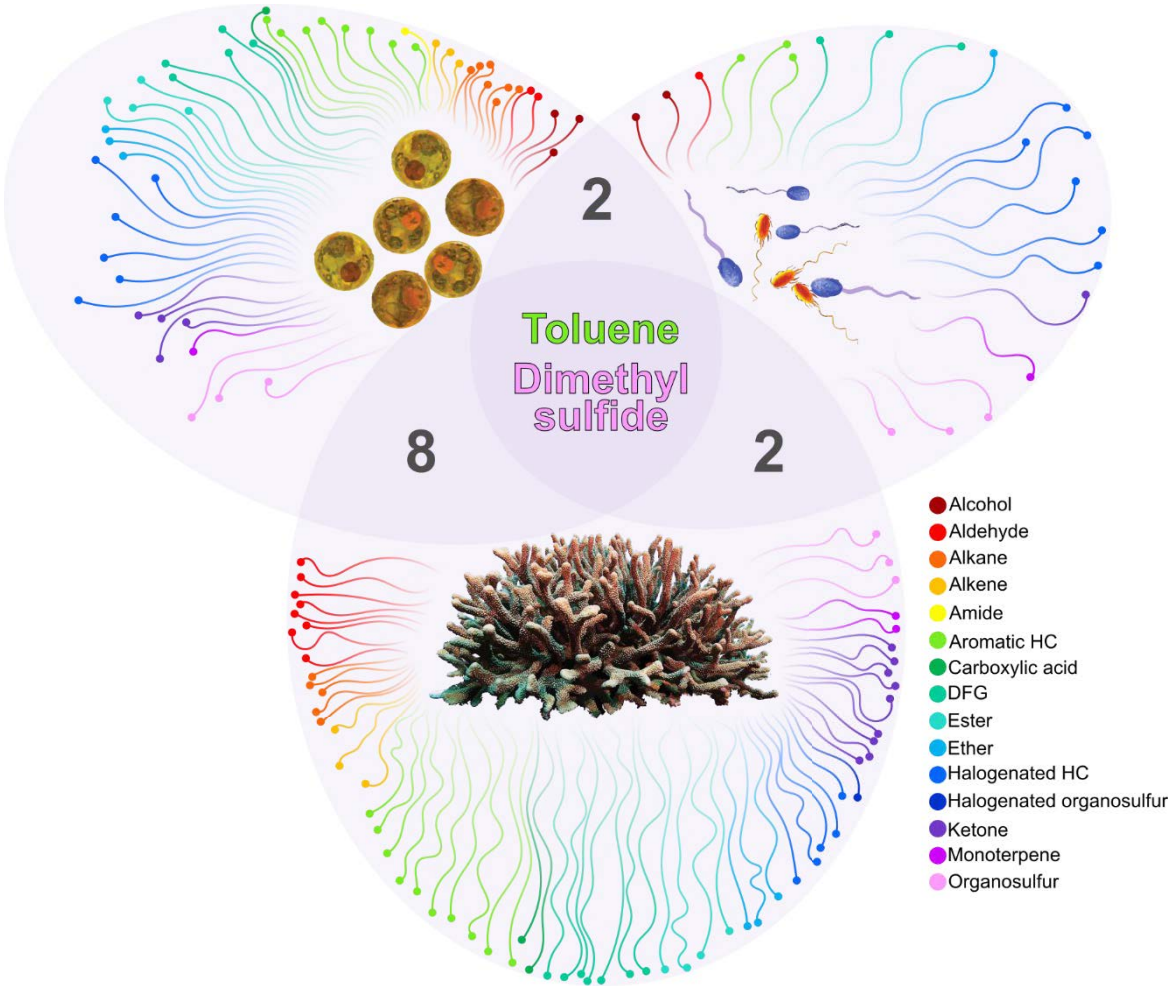

Figure 5.6. Functional chemical groups produced by Symbiodiniaceae (upper left oval), Symbiodiniaceae-associated bacteria (upper right oval) and coral (bottom oval) and the shared BVOCs (overlap). Only compounds detected in Symbiodiniaceae and corals during ambient conditions (26°C) were included. BVOCs had to be present in at least three replicate coral samples and in at least one species. Unclassified BVOCs were excluded from this figure; see Table S5.2 for a full list of BVOCs present in corals, Symbiodiniaceae and bacteria.

Tropical regions are especially important in regards to their BVOC emissions as stronger convection forces result in fast transport of BVOCs to the upper atmosphere (Randel and Jensen, 2013) and these biomes are recognised as key areas of focus for BVOC production and emission research. This is enforced by the potential roles BVOCs can play in thermal tolerance (Loreto and Schnitzler, 2010; Peñuelas and Llusià, 2003; Peñuelas and Staudt, 2010). Our work provides the first information on the coral volatilome and indicates that corals could be a substantial source of brominated compounds. We also demonstrate the complex and diverse nature of the coral volatilome, which is complemented

by our previous work on Symbiodiniaceae and their associated bacteria (**Chapter 2 & 4**). Coral reefs face ecological tipping points worldwide and we need to consider their volatile emissions in light of potential methods proposed to mitigate bleaching events (e.g. cloud seeding, misting; Latham et al., 2013). Coral volatilomics represents a key frontier, especially given the potential these BVOCs have to influence stress response and survival.

Acknowledgments

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Supplementary material

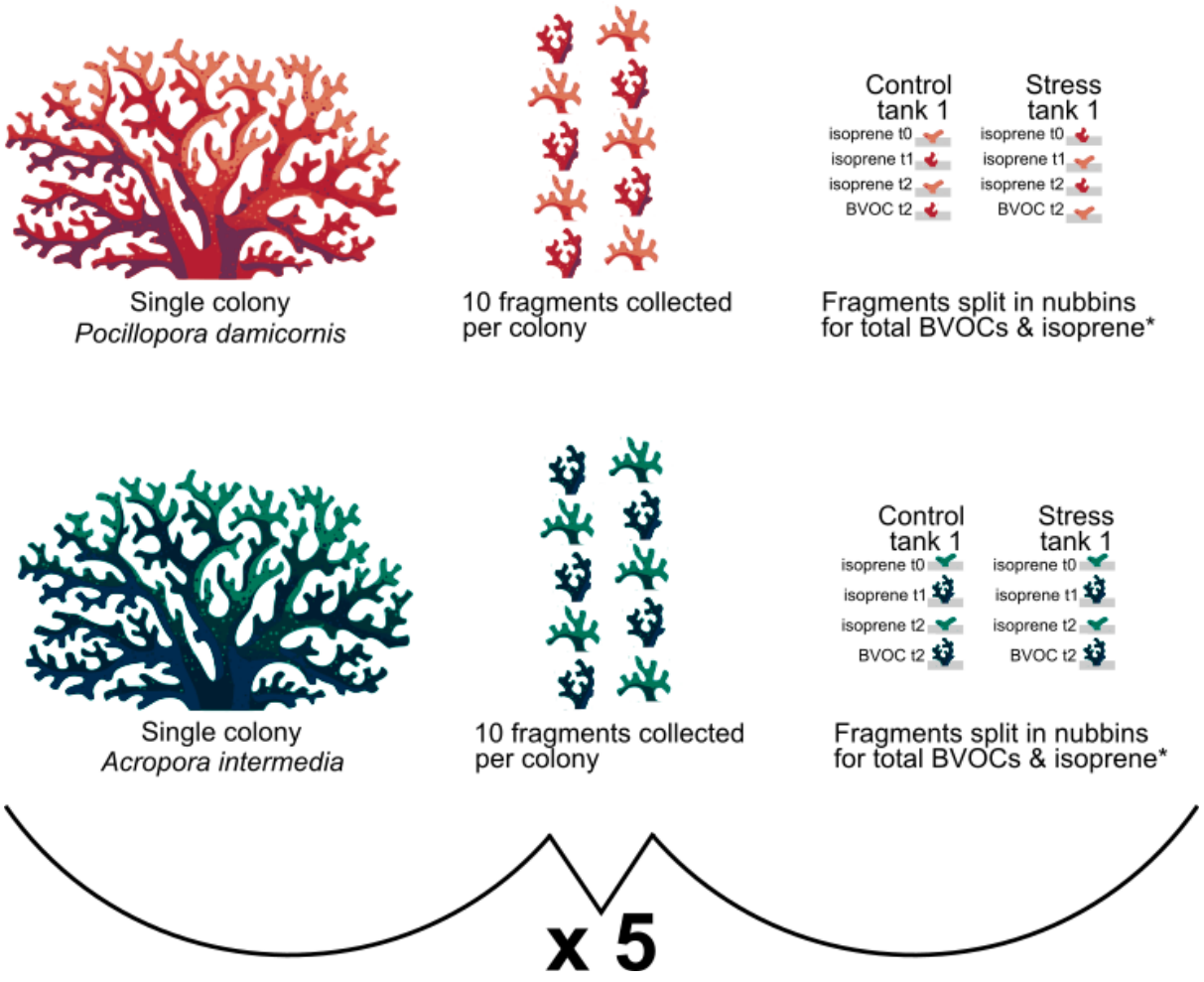


Figure S5.1. Coral collection on the Heron Island reef flat. Five *Acropora intermedia* and *Pocillopora damicornis* colonies were sampled. Ten fragments were collected from each colony, which were split into nubbins that were used for total BVOCs and isoprene samples. These nubbins were split into control and stress tanks, resulting in five control and five stress tanks. BVOC = biogenic volatile organic compounds. *Additional nubbins were taken from the ten fragments for data not presented in this work. All fragments < 10cm.

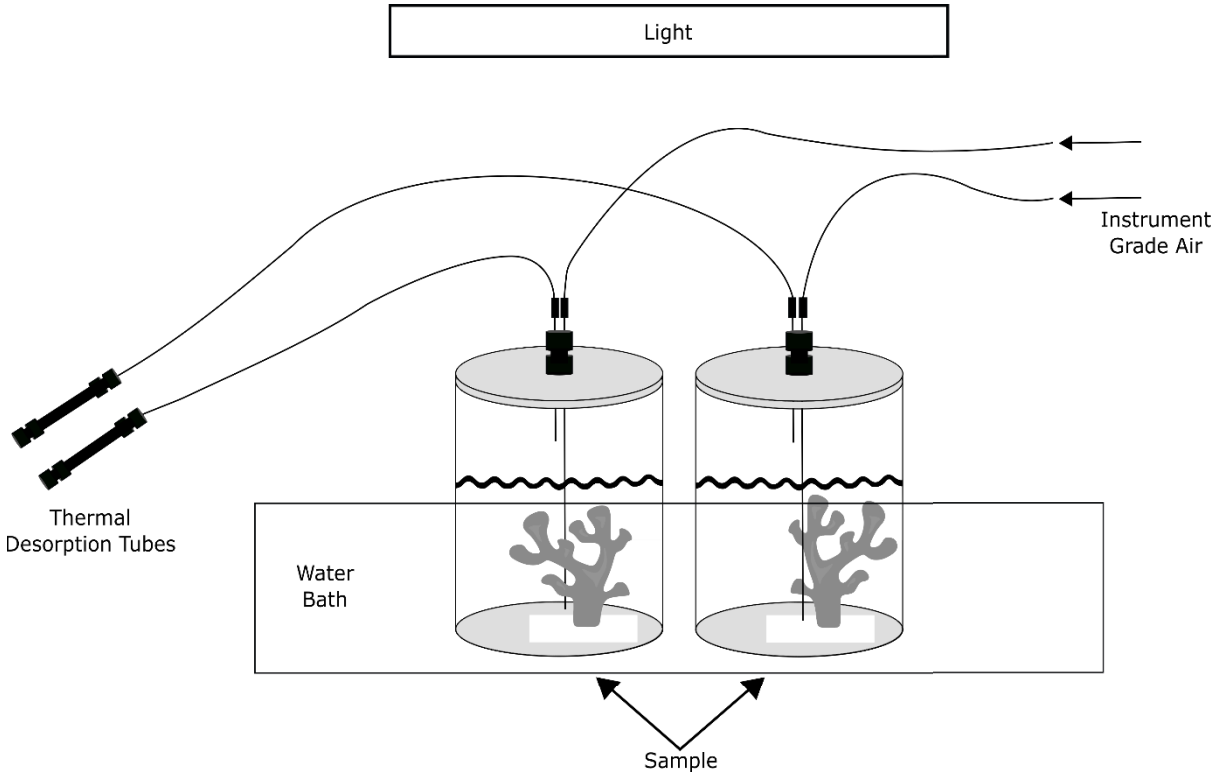
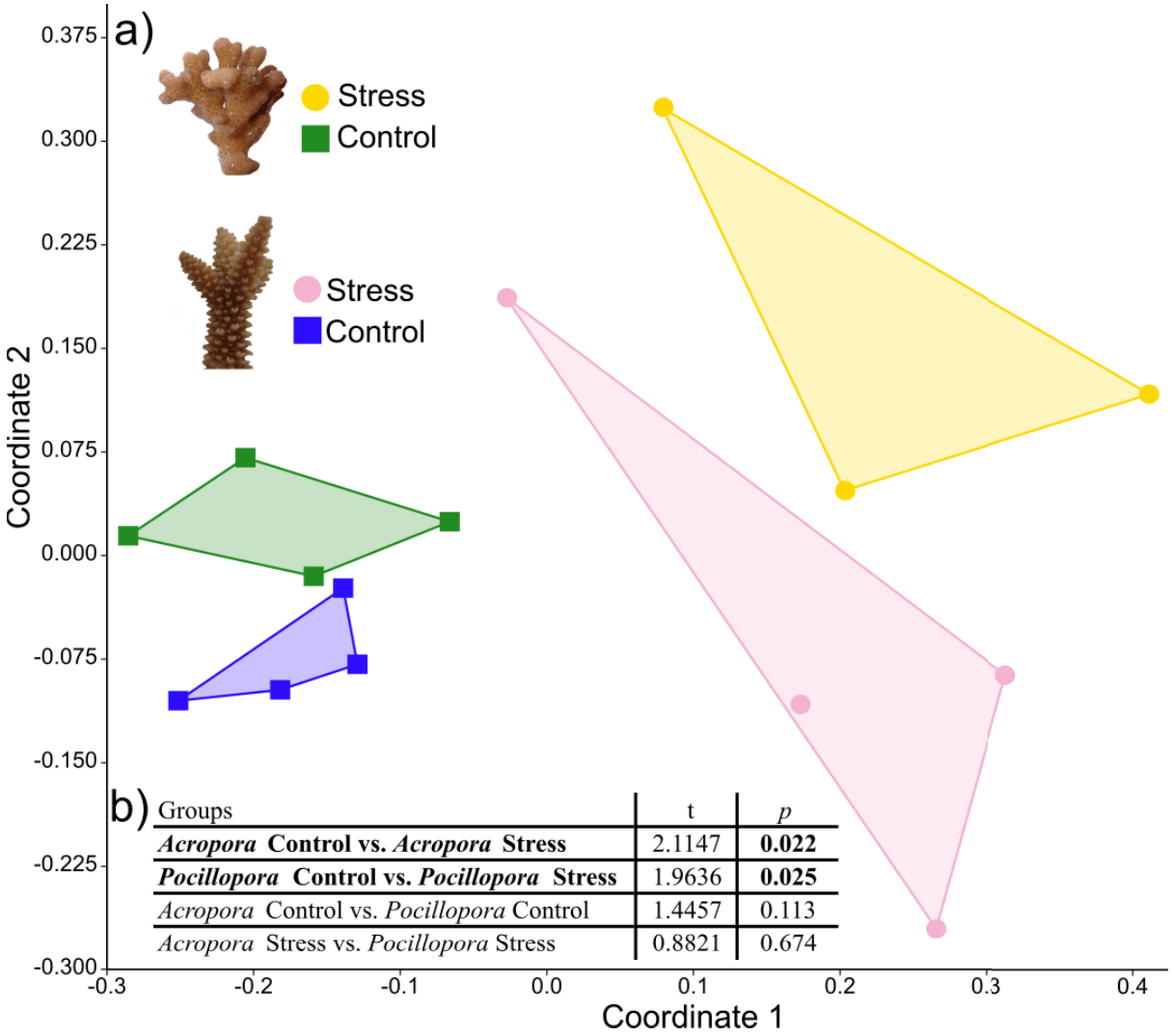


Figure S5.2. Schematic of volatile sampling set up. Coral nubbins were placed in gas tight chambers and maintained under growth conditions while undergoing a 30 minute purge of instrument grade air (BOC Gases, Linde Group, Australia). The outlet of this purge was passed over Markes thermal desorption tubes (Tenax TA).



b)

Groups	t	*p*
***Acropora* Control vs. *Acropora* Stress**	2.1147	**0.022**
***Pocillopora* Control vs. *Pocillopora* Stress**	1.9636	**0.025**
Acropora Control vs. *Pocillopora* Control	1.4457	0.113
Acropora Stress vs. *Pocillopora* Stress	0.8821	0.674

Figure S5.3. a) Non-metric multidimensional scaling (nMDS; Bray-Curtis Similarity) analysis and b) PERMANOVA analysis on the volatilomes of *Acropora intermedia* and *Pocillopora damicornis* under control and stress conditions.

Table S5.1. List of core BVOCs that are present in 100% of all samples, 100% of control samples and 100% of stressed samples. HC = hydrocarbon, DFG = diverse functional groups.

All samples	
ID	**Functional group**
Bromoform	Halogenated HC
Chlorodibromomethane	Halogenated HC
UC halogenated HC	Halogenated HC
Control samples	
2(E)-Decenal	Aldehyde
Heptanal	Aldehyde
Hexanal	Aldehyde
Methacrolein	Aldehyde
Nonane	Alkane
(Z)-3-Octene	Alkene
Toluene	Aromatic HC
Acetic acid	Carboxylic acid
Benzothiazole	DFG
Methoxyphenyl-oxime	DFG
4-Ethylbenzoic acid 2.2-dimethylpropyl ester	Ester
4-Methyl-4-vinylbutyrolactone	Ester
5-Methylhexan-5-olide	Ester
Methyl methacrylate	Ester
Bromoform	Halogenated HC
Chlorodibromomethane	Halogenated HC
UC halogenated HC	Halogenated HC
UC10.77	Unclassified
UC40.71	Unclassified
Stressed samples	
Bromoform	Halogenated HC
Chlorodibromomethane	Halogenated HC
UC halogenated HC	Halogenated HC
UC halogenated HC	Halogenated HC
Limonene	Monoterpene
UC25.19	Unclassified
UC36.75	Unclassified
UC39.54	Unclassified

Table S5.2. All compounds and their functional chemical groups produced by Symbiodiniaceae (Chapter 2), *Marinobacter adhaerens* and *Labrenzia* sp. 21p (Chapter 4) and *Pocillopora damicornis* and *Acropora intermedia*. Only compounds detected in corals and Symbiodiniaceae during ambient conditions were included. Compounds had to be present in at least three biological replicates for corals in at least one species. Compounds had to be present in at least four (out of six) technical replicates in at least one species (for each Symbiodiniaceae and bacteria species three biological replicates and two technical replicates take from each biological replicate). UC = unclassified, HC = hydrocarbon. *denotes a BVOC present in both coral and Symbiodiniaceae. § denotes a BVOC present in bacteria, Symbiodiniaceae and corals.

Labrenzia* sp. 21p & *Marinobacter adhaerens		**Symbiodiniaceae**		***Pocillopora damicornis* & *Acropora intermedia***		
Compound ID	**Chemical functional groups**	**Compound ID**	**Chemical functional groups**	**Compound ID**	**Chemical functional groups**	
2-Phenyl-2-butanol	Alcohol	1-Hexadecanol, 2-methyl-	Alcohol			
Cyclobutanol	Alcohol	Cyclooctyl alcohol	Alcohol			
		UC alcohol	Alcohol			
2-Ethylhexanal	Aldehyde	Nonanal	Aldehyde	Nonanal	Aldehyde	*
		Benzaldehyde	Aldehyde	Benzaldehyde	Aldehyde	*
		UC Aldehyde	Aldehyde	Undecanal	Aldehyde	
				2(E)-Decenal	Aldehyde	
				Decanal	Aldehyde	
				Heptanal	Aldehyde	
				Hexanal	Aldehyde	
				Methacrolein	Aldehyde	
		Pentane	Alkane	Pentane	Alkane	*
		Heptadecane, 9-octyl-	Alkane	Tetradecane	Alkane	
		Hexadecane	Alkane	Tridecane	Alkane	
		UC Alkane	Alkane	Nonane	Alkane	
		UC Alkane	Alkane	Undecane	Alkane	
		Hexane, 2,3-dimethyl-	Alkane			
		Octadecane, 6-methyl-	Alkane			
		1-Pentene, 2,4,4-trimethyl-	Alkene	1.3.5-Heptatriene (E.E)	Alkene	
		2,4-Dimethyl-1-heptene	Alkene	(Z)-3-Octene	Alkene	
		Squalene	Alkene	1-Octene	Alkene	
		l-Alanine ethylamide, (S)-	Amide			
Toluene	Aromatic HC	Toluene	Aromatic HC	Toluene	Aromatic HC	§
Benzocyclobutene	Aromatic HC	[2.2]Paracyclophane	Aromatic HC	2-Ethyl-p-xylene	Aromatic HC	
1,3-Di-tert-butylbenzene	Aromatic HC	Benzene, 1-ethyl-3-methyl-	Aromatic HC	3-ethyl-o-xylene	Aromatic HC	
		Ethylbenzene	Aromatic HC	Allylbenzene	Aromatic HC	
		Naphthalene, 2-methyl-	Aromatic HC	Hemimellitene	Aromatic HC	
		Phenol, 2,4-bis(1,1-dimethylethyl)-	Aromatic HC	m-Ethyltoluene	Aromatic HC	
		UC Aromatic hydrocarbon	Aromatic HC	1-Methylcyclohexa-2.4-diene	Aromatic HC	
		Styrene	Aromatic HC	1-methyl-Naphthalene	Aromatic HC	
				Benzene	Aromatic HC	

				2-ethyl-m-xylene	Aromatic HC
		Nonanoic acid	Carboxylic acid	Acetic acid	Carboxylic acid
				Benzoic acid	Carboxylic acid
2-Ethyl-3-hydroxyhexyl 2-methylpropanoate	DFG	methoxy-phenyl-oxime	DFG	methoxy-phenyl-oxime	DFG
		2-Propanol, 1,3-dimethoxy-	DFG	2-Acetyl-5-methylfuran	DFG
		4-Fluoro-3-trifluoromethylbenzoic acid, eicosyl ester	DFG	isothiocyanato-cyclohexane	DFG
		Methyl jasmonate	DFG	[[Thioxo[3.4.5 trimethoxyphenyl]methyl]thio]acetic acid	DFG
				1-methoxy-2-propanol	DFG
				Benzothiazole	DFG
				Isovanillin - TBDMS derivative	DFG
Allyl nonanoate	Ester	1-Propanol, 2,2-dimethyl-, benzoate	Ester	4-Ethylbenzoic acid 2.2-dimethylpropyl ester	Ester
2,2,4-trimethyl-3-carboxyisopropyl isobutyl ester pentanoic acid	Ester	UC ester	Ester	4-Methyl-4-vinylbutyrolactone	Ester
		UC Ester	Ester	5-Methylhexan-5-olide	Ester
		UC ester	Ester	Methyl methacrylate	Ester
1,2-15,16-Diepoxyhexadecane	Ether	1-Methoxy-3,5-dimethyl-cyclohexene	Ether	2-ethyl-5-methyl-Furan	Ether
		Benzene, 1-[1,1-dimethylethyl]-4-[2-propenyloxy]-	Ether	2-methylene-7-oxabicyclo[4.1.0]heptane	Ether
		UC Ether	Ether	Dioxane	Ether
UC Halogenated HC 42.75	Halogenated HC	3-Trifluoroacetoxypentadecane	Halogenated HC	Bromodichloromethane	Halogenated HC
4-chlorophenyl nonyl ester oxalic acid	Halogenated HC	Halogenated organosilicon	Halogenated HC	Bromoform	Halogenated HC
UC Halogenated HC 20.85	Halogenated HC	Propane, 1,2-dichloro-	Halogenated HC	Chlorodibromomethane	Halogenated HC
UC Halogenated HC 23.74	Halogenated HC	UC Halogenated hydrocarbon	Halogenated HC	UC halogenated HC	Halogenated HC
1,2-Dichloroethane	Halogenated HC	UC Halogenated hydrocarbon	Halogenated HC	UC halogenated HC	Halogenated HC
1-Chloro-hexadecane	Halogenated HC	UC Halogenated hydrocarbon	Halogenated HC		
				Methanesulfonyl chloride	Halogenated organosulfur
Acetone	Ketone	2-Butanone, 3,3-dimethyl-	Ketone	2.2.4-Trimethyl-3-pentanone	Ketone
		UC benzoquinone	Ketone	Geranyl acetone	Ketone
		UC Ketone	Ketone	Methyl Isobutyl Ketone	Ketone
		UC Ketone	Ketone	Methyl vinyl ketone	Ketone
				1-Acetylcyclohexene	Ketone
				2-Butanone	Ketone
				2-Cyclopenten-1-one	Ketone
				Methylacetylacetone	Ketone

				UC ketone	Ketone	
Camphor	Monoterpene	Azulene	Monoterpene	Azulene	Monoterpene	*
				Limonene	Monoterpene	
Dimethyl sulfide	Organosulfur	Dimethyl sulfide	Organosulfur	Dimethyl sulfide	organosulfur	§
Dimethyl trisulfide	Organosulfur	Disulfide, dimethyl	Organosulfur	dimethyl disulfide	organosulfur	*
UC Organosulfur 19.53	Organosulfur	UC Organosulfur	Organosulfur	Sulfurous acid-dibutyl ester	organosulfur	
UC4.29	Unclassified	UC_13.09	Unclassified	UC10.51	Unclassified	
UC11.45	Unclassified	UC_17.25	Unclassified	UC10.77	Unclassified	
UC13.09	Unclassified	UC_18.34	Unclassified	UC17.89	Unclassified	
UC21.73	Unclassified	UC_24.96	Unclassified	UC25.19	Unclassified	
UC24.00	Unclassified	UC_26.31	Unclassified	UC28.66	Unclassified	
UC27.33	Unclassified	UC_26.79	Unclassified	UC28.81	Unclassified	
UC32.33	Unclassified	UC_26.79	Unclassified	UC29.50	Unclassified	
UC41.41	Unclassified	UC_27.81	Unclassified	UC32.93	Unclassified	
UC42.46	Unclassified	UC_28.26	Unclassified	UC34.31	Unclassified	
UC42.87	Unclassified	UC_30.06	Unclassified	UC36.37	Unclassified	
UC44.02	Unclassified	UC_31.50	Unclassified	UC36.75	Unclassified	
UC44.40	Unclassified	UC_33.16	Unclassified	UC40.71	Unclassified	
UC45.15	Unclassified	UC_34.06	Unclassified			
UC46.46	Unclassified	UC_36.69	Unclassified			
		UC_38.08	Unclassified			
		UC_38.58	Unclassified			
		UC_38.65	Unclassified			
		UC_39.00	Unclassified			
		UC_39.36	Unclassified			
		UC_39.59	Unclassified			
		UC_40.45	Unclassified			
		UC_40.78	Unclassified			
		UC_41.89	Unclassified			
		UC_42.02	Unclassified			
		UC_42.37	Unclassified			
		UC_42.46	Unclassified			
		UC_45.18	Unclassified			
		UC_45.20	Unclassified			
		UC_40.63	Unclassified			
		UC_46.85	Unclassified			

Chapter 6

General Discussion: synthesis of results, future directions and conclusions

Summary

The specific work presented here has yielded new understanding of tropical coral BVOC production capacity, conclusively establishing corals, their endosymbiotic algae and associated bacteria as sources of a multitude of ecologically and atmospherically important BVOCs. Within this final chapter, I synthesise the results presented throughout this thesis to further discuss the importance of these findings towards understanding trophic interactions, coral survival and the biogeochemical implications of tropical coral reefs undergoing climate change.

What BVOC capacity does the coral holobiont possess?

The coral holobiont is a biologically diverse meta-organism that includes microalgae, bacteria, archaea, fungi and viruses, all engaged in resource exchange. Within this diversity lies a previously unknown capacity to produce a wide range of ecologically, physiologically and climatically relevant BVOCs. Throughout this thesis, I have demonstrated the capacity of the i) coral endosymbionts, Symbiodiniaceae (**Chapter 2**), ii) their associated bacteria (**Chapter 4**) and iii) the coral holobiont (**Chapter 5**) to produce and emit a wide diversity of BVOCs.

I detected 32 BVOCs by screening five species of Symbiodiniaceae (*Symbiodinium linucheae, Breviolum psygmophilum, Durusdinium trenchii, Effrenium voratum, Fugacium kawagutii*) covering diverse life histories from endosymbiont to obligate free living. By further increasing the mass-spectrometer scanning range for a subsequent thermal stress experiment on *Cladocopium goreaui* and *D. trenchii*, I was able to detect a total of 72 BVOCs. Across all of these Symbiodiniaceae species, I detected eight chemical functional groups: alcohols, aldehydes, alkanes, alkenes, aromatic hydrocarbons, esters, halogenated hydrocarbons and organosulfurs (**Chapter 2**).

After identifying the Symbiodiniaceae microbiome (**Chapter 3**), I isolated some of its core members to assess their production of volatile compounds. I detected 35 BVOCs across the volatilomes of *Marinobacter adhaerens* and *Labrenzia* sp., two members of the

Symbiodiniaceae core microbiome (**Chapter 4**). I consistently detected five chemical functional groups between these two bacteria: alcohols, aromatic hydrocarbons, esters, ethers and halogenated hydrocarbons.

The coral holobiont yielded the largest number of BVOCs, with 88 compounds detected from *Acropora intermedia* and *Pocillopora damicornis*. These compounds belonged to twelve chemical functional groups: aldehydes, alkanes, alkenes, aromatic hydrocarbons, carboxylic acids, esters, ethers, halogenated hydrocarbons, ketones, monoterpenes and organosulfurs. This systematic evaluation, from microbe to holobiont, revealed increasingly complex metabolic networks as biological complexity increases. Given that previous targeted studies have only reported a handful of coral derived BVOCs (Deschaseaux et al., 2014c; Exton et al., 2015; Swan et al., 2016), the untargeted approach used in this thesis reveals the impressive diversity of coral BVOC production.

Thermal stress drives species-specific responses in Symbiodiniaceae and the coral holobiont

Thermal stress events are increasing in frequency and severity in tropical reef ecosystems, driving mass coral bleaching events on a global scale (Heron et al., 2016; Hughes et al., 2017) that are dramatically shifting ecosystem community structures. Many studies have attempted to understand the thermal stress response of coral (Gardner et al., 2017; Hillyer et al., 2016, 2017, 2018; Nitschke et al., 2018; Petrou et al., 2018; Sogin et al., 2016) and Symbiodiniaceae (Deschaseaux et al., 2014a; McGinley et al., 2012; Robison and Warner, 2006; Suggett et al., 2008), yet the effects of thermal stress and declining health on the volatilome of corals and their symbionts has been entirely overlooked.

I examined the volatilomic response of thermally tolerant (*D. trenchii*) and sensitive (*C. goreaui*) Symbiodiniaceae (**Chapter 2**) and identified their contrasting responses: the tolerant species exhibited an increase in its volatilome richness during thermal stress, while the sensitive species exhibited a decrease (Figure 6.1a). Such an outcome likely reflects different metabolic pathways operating in response to sub-optimal temperature exposure

(e.g. Gierz et al., 2017; Levin et al., 2016). Furthermore, 43 individual BVOCs experienced significant changes in abundance across both Symbiodiniaceae during heat stress. Out of these 43 compounds that differed significantly, the vast majority increased in abundance (96% significantly increased in *D. trenchii* and 94% in *C. goreaui*; Figure 6.1b). Only DMS significantly decreased in *D. trenchii* and UC42.37 in *C. goreaui* (**Chapter 2**). Conversely, the opposite occurred when coral holobionts were subjected to thermal stress. Out of the 33 volatiles that responded significantly to thermal stress, 100% in *P. damicornis* and 86% in *A. intermedia* significantly decreased (the only exceptions were benzaldehyde, 2-ethyltoluene and UC25.19, which significantly increased in *A. intermedia* during thermal stress) (**Chapter 5**; Figure 6.1b). In addition, both coral species displayed a greater decrease in volatilome richness following heat stress than the Symbiodiniaceae in isolation (76 to 29 in *P. damicornis*; 78 to 46 in *A. intermedia*; Figure 6.1a).

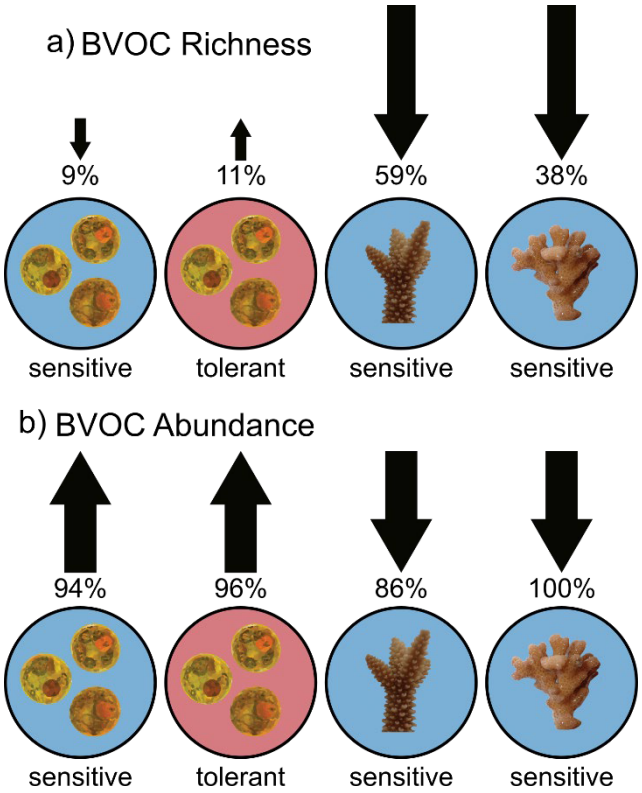


Figure 6.1 Changes (%) in BVOC a) richness and b) abundance following thermal stress in Symbiodiniaceae (Chapter 2; *Cladocopium goreaui* – sensitive; *Durusdinium trenchii* – tolerant) and coral (Chapter 5; *Acropora intermedia* and *Pocillopora damicornis* – both sensitive). Percentage changes in BVOC abundance are calculated as the proportional change out of the total number of significant differences per species.

While the consequences of heat stress on coral BVOC emission contrast with those for Symbiodiniaceae cells, it is plausible that corals decrease BVOC quantities since the complex and tightly coupled nature of the coral holobiont is more capable of utilising BVOCs produced by its symbionts. For example, some *inorganic* compounds (e.g. H_2O_2) are considered to be important signalling molecules that move between symbiont and coral tissues (e.g. Hawkins and Davy, 2012; Smith et al., 2005; Tchernov et al., 2011), and it is possible that BVOCs also act as important infochemicals that are produced and degraded amongst the holobiont components. In order to further understand the impact of Symbiodiniaceae on the BVOC emissions of the holobiont, future studies should characterise the volatilome of symbiotic and aposymbiotic cnidarians (e.g. *Aiptasia* sp.).

Infochemicals, stress response and climate regulation: putative functions of the coral volatilome

Out of the 155 BVOCs detected throughout this thesis, 99 were identified and many of these compounds are known to have relevant biological and climatic functions. Across the identified compounds, 41 are implicated in a range of atmospheric processes and can influence climate, 39 are involved in response to stress, 28 are putative signalling molecules and 44 have antimicrobial properties (Figure 6.2). This demonstrates that coral holobionts and their constituents are capable of producing a functionally diverse array of BVOCs, which together would appear to represent the net outcome of holobiont metabolic processes.

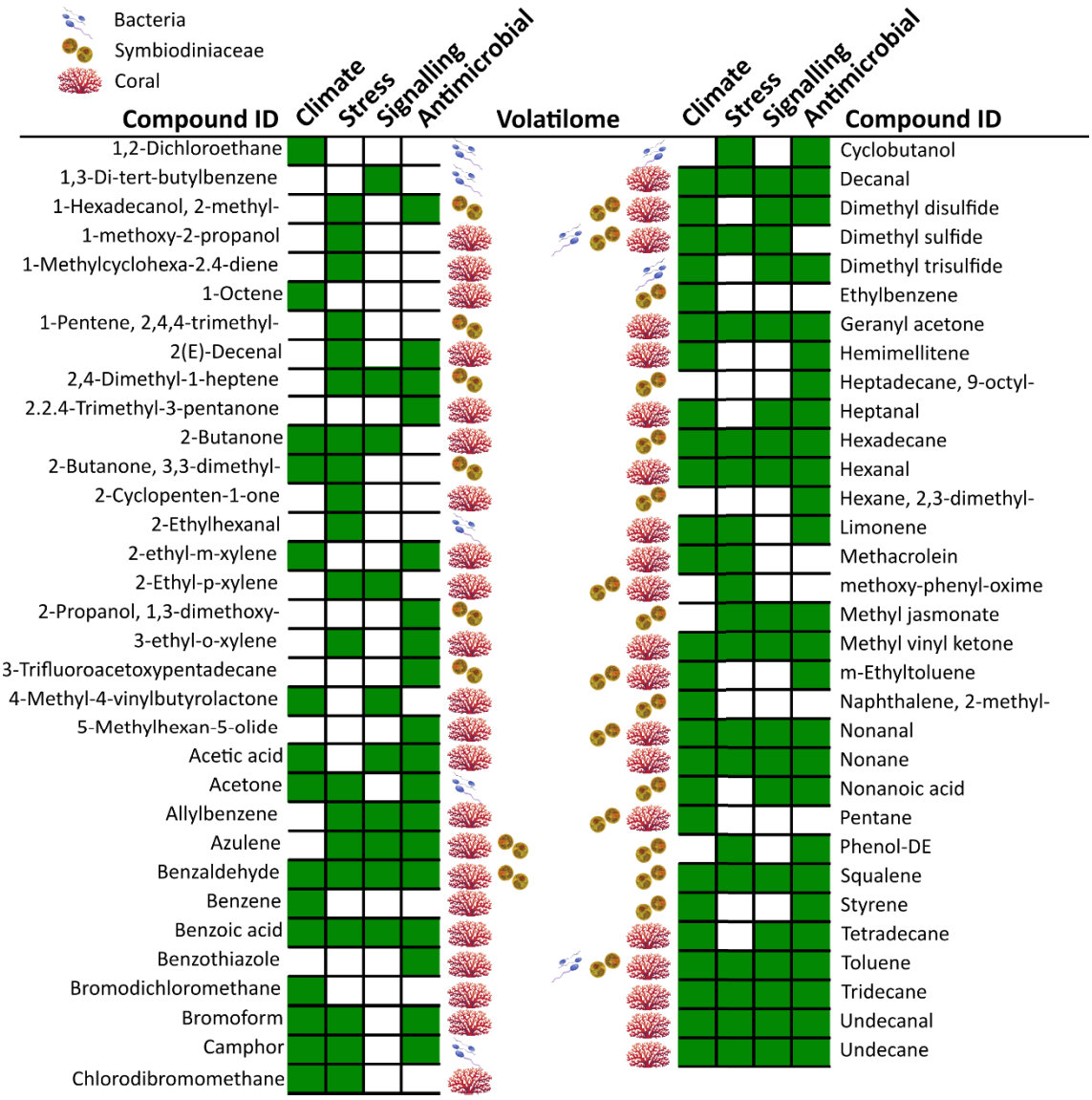


Figure 6.2 All compounds (presence indicated by green shading) detected in the volatilomes of Symbiodiniaceae (Chapter 2), bacteria (Chapter 4) coral (Chapter 5), their overlap and their putative functions in climate, stress response, chemical signalling and as antimicrobials. This figure excludes unidentified BVOCs and BVOCs that could not be assigned a putative function. See Table S6.1 for referencing and notes.

The bacterial volatilome contained fewer compounds than the ones derived from the Symbiodiniaceae species and corals, an outcome that is perhaps unsurprising given that the bacteria were pure isolates whereas both Symbiodiniaceae and corals contained complex microbial communities. Out of the volatile compounds detected from bacteria, 22.9% are putatively involved in (i) signalling, (ii) stress response, (iii) antimicrobial defence or (iv) climate regulation (Figure 6.2; Figure 6.3). In comparison, 31.5% of the Symbiodiniaceae volatilome and 56.8% of the coral volatilome are possibly involved in these functions (Figure

6.3). In addition, 2.9% of the bacterial volatilome, 6.3% of the Symbiodiniaceae volatilome and 16.2% of the coral volatilome was comprised of molecules that can carry out multiple functions and that are implicated in all four functional categories (Figure 6.3). As such the coral holobiont maintains the greatest pool of volatiles that are putatively functionally versatile within physiological, ecological and climatic contexts.

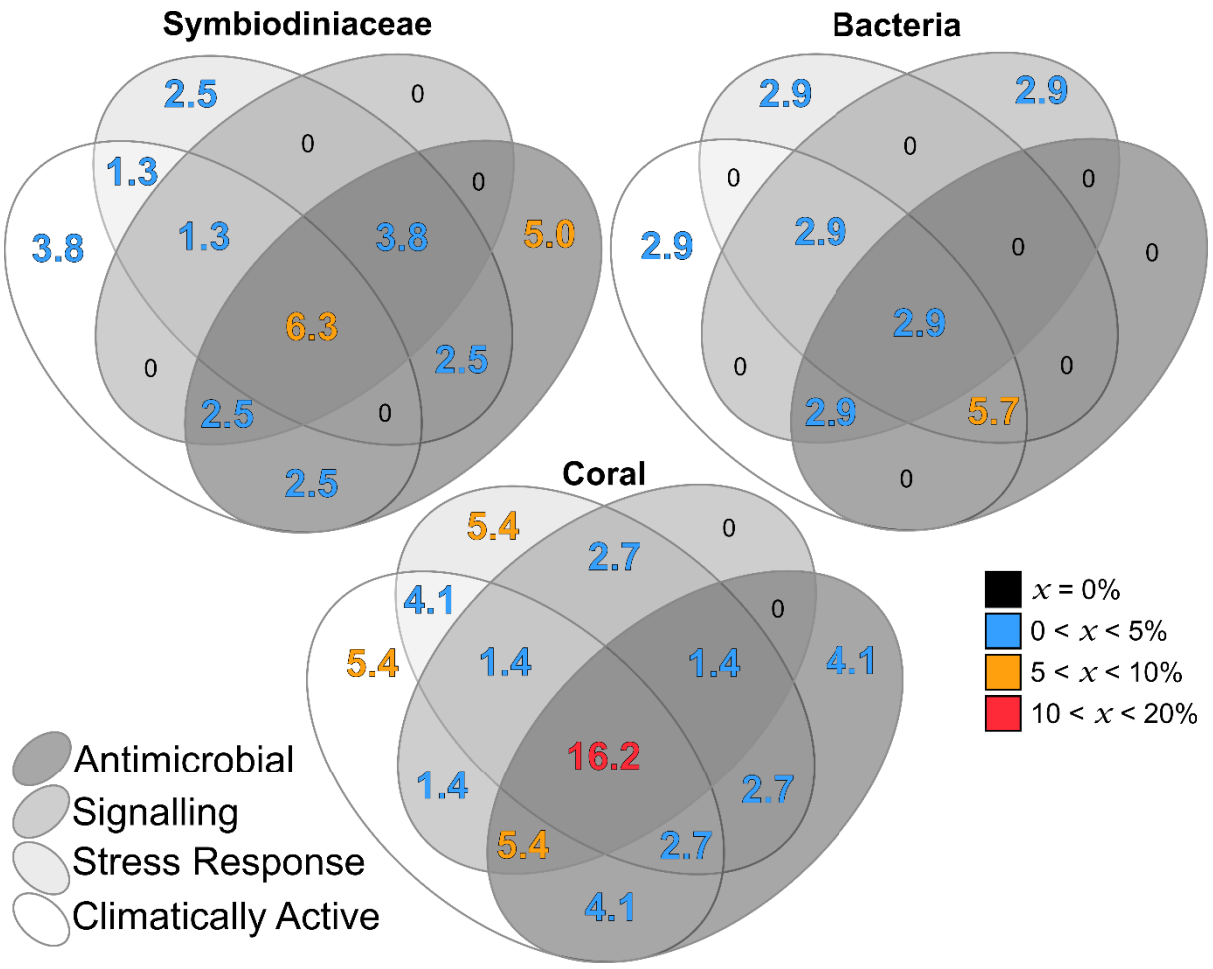


Figure 6.3 Percentage of BVOCs identified from Symbiodiniaceae, bacteria and coral with putative functions in climate, stress response, chemical signalling and as antimicrobials. Functions were assigned based on the literature, see Table S6.1 for details.

Examining the potential roles of these various BVOCs, and how they differ across taxa raises a key issue: how do BVOCs influence coral fitness and how will this change as future environmental and climatic forces impact coral communities? I have shown that the abundance of many BVOCs significantly vary with thermal stress (**Chapter 2**; **Chapter 5**), but it is unlikely that all of these compounds will be involved in alleviating stress. Some may

result from cellular breakdown (e.g. C6 compounds, Loreto et al., 2006) and as such, might not directly help in the stress response, yet they may then indirectly influence coral survival by serving alternate roles. As shown in terrestrial ecosystems, they could be functioning as signalling molecules – potentially detrimental to the coral by serving as chemoattractants to pathogenic microbes. Increased temperatures have been shown to increase the chemotactic response of pathogenic bacteria towards coral mucus (Garren et al., 2016), which is known to release BVOCs (Broadbent and Jones, 2004). During thermal stress, corals may attempt to counteract this by producing more antimicrobials in an effort to stem any possible infections, yet it is likely that all these responses are delicately balanced and continued disruption by stress may ultimately lead to the disruption of this symbiotic system. Moreover, the production and emission of these molecules can further influence reef ecosystems by forming atmospheric aerosols that can scatter sunlight and cool local temperatures (Albrecht, 1989; Hallquist et al., 2009). Alternatively, some BVOCs (e.g. benzene; see Figure 6.2 and Table S6.1) can react with OH or NO_x radicals and influence the abundance and residence time of greenhouse gases, thereby warming the local climate (Atkinson and Arey, 2003a). Given that I have provided the first step in identifying potential – and previously overlooked – BVOCs of interest from corals, (and hence likely involved in the overall BVOC flux from coral reefs), the potential implications of coral BVOC emissions are vast and clearly warrant more attention.

Future directions

Throughout this thesis, I consistently detected BVOCs that were not successfully identified (36% of all volatiles detected). Similar issues have been reported in metabolomic analyses (Matthews et al., 2018) and further characterisation and quantification of currently unclassified marine BVOCs is a key frontier in marine volatilomics. Across the data chapters presented here, numerous unclassified BVOCs were key drivers of biological patterns, clearly suggesting that these molecules may have important functional roles. The creation of a marine specific BVOC database should be a priority, and will allow *a priori* research to

select target BVOCs of interest in order to quantify their abundance in corals and response to changing temperature.

This first view of coral volatilomics highlights some key volatiles that should receive immediate attention. For example, the prevalence of brominated compounds detected from the coral holobiont, regardless of temperature, should be examined – quantitative studies could examine the overall flux of these ozone-degrading BVOCs from coral reefs. Additionally, the aromatic hydrocarbon, toluene, was consistently detected in bacteria, Symbiodiniaceae and corals (**Chapter 2, 4, 5**). The bacterial enzyme responsible for toluene biosynthesis has recently been identified (Beller et al., 2018) and given the bacterial associations with Symbiodiniaceae and corals, it is possible that the ubiquity of toluene is derived from bacterial production. Bacteria can be chemo-attracted to toluene (Vardar et al., 2005) and natural ecosystems are a newly recognised source of this BVOC (Misztal et al., 2015). These findings highlight the potential importance of this compound and the need for future work to examine its biological sources and functions. Moreover, toluene, brominated BVOCs and many other compounds detected throughout this thesis, have suspected functions as signalling molecules (Figure 6.2; Table S6.1), facilitating complex trophic interactions. Further work should be dedicated to uncovering the specific functional roles of the most abundant BVOCs and which organisms they affect in the holobiont. Moreover, it is important to determine if specific combinations of BVOCs are required to elicit a response or if only a handful of key dominant BVOCs suffice; and how pervasive are these signals between locations and coral species? What proportion of BVOCs are involved in signalling within the holobiont and how many are released, acting as cues for other organisms? These are important questions to answer if we are to fully understand the functioning of reef ecosystems and their inter-connectivity.

Production and emission of the dominant terrestrial BVOC, isoprene, is starting to receive attention in coral reef ecosystems (Swan et al., 2016). Despite the putative role of this BVOC in thermal tolerance (Sharkey et al., 2008; Velikova et al., 2011; Vickers et al., 2009a), I detected no significant change in isoprene concentrations in response to thermal

stress in *A. intermedia* or *P. damicornis* (**Chapter 5**). However, given the high reactivity of isoprene and its documented roles in the thermal tolerance of higher plants, further targeted analysis should explore the production of isoprene by corals using on-line and real-time detection processes capable of capturing highly volatile and dynamic trace gases (e.g. Proton Transfer Reaction-Mass Spectrometry). Moreover, by coupling such targeted analysis with DMS measurements, we could start to determine if there is a potential trade-off between these two antioxidants, as was hypothesised for marine phytoplankton (Dani and Loreto, 2017). While DMS has received vast attention in marine systems, some uncertainty remains regarding the extent of its production and prevalence on coral reefs. This is evidenced by the complete lack of detection of this BVOC in the *P. damicornis* volatilome (**Chapter 5**) – highlighting that its abundance can be highly variable between coral species.

Concluding remarks

Although marine tropical regions have previously been identified as 'hotspots' of BVOC emissions, the work presented in this thesis represents the first thorough exploration of coral reef emissions. The use of volatilomics has revealed that coral holobionts and their constituents are capable of producing a diverse range of BVOCs. Within this diversity lies a significant novel source of compounds that have the capacity to impact atmospheric chemistry, trophic interactions and stress tolerance of tropical ecosystems. As coral reefs continue to face numerous threats, understanding the metabolic mechanisms enabling coral reef functioning and resilience is critical. Only then can we hope to successfully manage and protect these fragile and precious ecosystems.

Supplementary Material

Table S6.1 All compounds detected in the volatilomes of Symbiodiniaceae (Chapter 2), bacteria (Chapter 4) coral (Chapter 5), their overlap and their putative functions in climate, stress response, chemical signalling and as antimicrobials. This table excludes unidentified BVOCs.

Origin	Compound ID	Climatically active	Notes	Stress Response	Notes	Signalling	Notes	Anti - microbial	Notes
Sym & Bacteria & Coral	Dimethyl sulfide	Ayers and Gras 1991	Forms CCN	Sunda et al 2002; Deschaseaux et al 2014	Antioxidant	Seymour et al 2010	Chemoattractant to bacteria	-	-
Coral	isothiocyanato-cyclohexane	-	-	-	-	-	-	-	-
Coral	(Z)-3-Octene	-	-	-	-	-	-	-	-
Coral	[[Thioxo[3.4.5 trimethoxyphenyl]methyl]thio]acetic acid	-	-	-	-	-	-	-	-
Sym	[2.2]Paracyclophane	-	-	-	-	-	-	-	-
Bacteria	1,2-15,16-Diepoxyhexadecane	-	-	-	-	-	-	-	-
Bacteria	1,2-Dichloroethane	Wallington et al 1996	Reacts with OH	-	-	-	-	-	-
Bacteria	1,3-Di-tert-butylbenzene	-	-	-	-	Cotes et al 2015	Insect parasites avoid fungi patches producing this and other infochemicals	-	-
Coral	1.3.5-Heptatriene (E.E)	-	-	-	-	-	-	-	-
Coral	1-Acetylcyclohexene	-	-	-	-	-	-	-	-
Bacteria	1-Chloro-hexadecane	-	-	-	-	-	-	-	-
Sym	1-Hexadecanol, 2-methyl-	-	-	Panrong, Chunyan and Kebin 2007	Associated with drought stress in tea leaves	-	-	Ugur et al. 2009	Antimicrobial as part of an extract
Coral	1-methoxy-2-propanol	-	-	Scutareanu et al 2003	Associated with herbivore grazing of trees	-	-	-	-
Sym	1-Methoxy-3,5-dimethyl-cyclohexene	-	-	-	-	-	-	-	-
Coral	1-Methylcyclohexa-2.4-diene	-	-	Joó et al. 2011	Increased following heat stress/grazing pressure	-	-	-	-
Coral	1-methyl-naphthalene	-	-	-	-	-	-	-	-
Coral	1-Octene	atkinson and arey 2003 chemical reviews	Reacts with O3	-	-	-	-	-	-

Sym	1-Pentene, 2,4,4-trimethyl-	-	-	Colville et al. 2012	Ageing/desiccation of seeds	-	-	-	-
Sym	1-Propanol, 2,2-dimethyl-, benzoate	-	-	-	-	-	-	-	-
Coral	2(E)-Decenal	-	-	Mohekar et al. 2017; Biswas et al 2011	Stress response of stink bugs; decreased with salt stress	-	-	Quintana-Rodriguez et al 2018	Antifungal
Bacteria	2,2,4-trimethyl-3-carboxyisopropyl isobutyl ester pentanoic acid	-	-	-	-	-	-	-	-
Sym	2,4-Dimethyl-1-heptene	-	-	Weldegergis et al 2014	Increased in drought and grazing stressed plants	Coppola et al. 2017	Indirect defence through inter-specific communication between plants following aphid infestation	Selvin et al. 2009	Demonstrated no antimicrobial activity
Coral	2.2.4-Trimethyl-3-pentanone	-	-	-	-	-	-	Yabesh et al. 2018	Antibacterial properties from plant extract
Coral	2-Acetyl-5-methylfuran	-	-	-	-	-	-	-	-
Coral	2-Butanone	atkinson and arey 2003 chemical reviews	reacts with criegee intermediates	Blande, Holopainen and Li 2010	Herbivore induced stress response in plants	-	-	-	
Sym	2-Butanone, 3,3-dimethyl-	atkinson and arey 2003 chemical reviews	Reacts with OH	Henry et al. 2007	Increase in drought stressed plants	-	-	-	-
Coral	2-Cyclopenten-1-one	-	-	Stintzi et al. 2001	Decline following leaf injury, may be involved in defence gene regulation	-	-	-	-
Bacteria	2-Ethyl-3-hydroxyhexyl 2-methylpropanoate	-	-	-	-	-	-	-	-
Coral	2-ethyl-5-methyl-Furan	-	-	-	-		-	-	-
Bacteria	2-Ethylhexanal	-	-	Liu et al. 2017	Elevated in grazer stressed plants	-	-	-	-
Coral	2-ethyl-m-xylene	Kallio et al. 2006	Identified in atmospheric aerosols above coniferous forest	-	-	-	-	Morah and Ashipu 2017	Component of an essential oil that inhibits mircobial growth
Coral	2-Ethyl-p-xylene	-	-	Wang et al. 2006	Concentration declines in wheat roots with aluminium stress	Latreche, Rahmania 2010	Appears in leaves with britte leaf disease	-	-
Coral	2-methylene-7-oxabicyclo[4.1.0]heptane	-	-	-	-	-	-	-	-
Bacteria	2-Phenyl-2-butanol	-	-	-	-	-	-	-	-
Sym	2-Propanol, 1,3-dimethoxy-	-	-	-	-	-	-	Alrumman 2017	Major component dry leaf gel that inhibits some gram-positive bacteria

Coral	3-ethyl-o-xylene	-	-	Rinnan et al. 2004	Increased emissions following ozone damage	-	-	Yildirim 2017	Component of an essential oil that inhibits mircobial growth
Sym	3-Trifluoroacetoxypentadecane	-	-	-	-	-	-	Hussein 2016	Component of leaf extract that inhibits microbial growth
Bacteria	4-chlorophenyl nonyl ester oxalic acid	-	-	-	-	-	-	-	-
Coral	4-Ethylbenzoic acid 2.2-dimethylpropyl ester	-	-	-	-	-	-	-	-
Sym	4-Fluoro-3-trifluoromethylbenzoic acid, eicosyl ester	-	-	-	-	-	-	-	-
Coral	4-Methyl-4-vinylbutyrolactone	Nørgaard et al. 2014	Reaction product/ozone precursor	-	-	Kotze 2012	Component of VOC emissions that attracts gall midges	-	-
Coral	5-Methylhexan-5-olide	-	-	-	-	-	-	Lee et al. 2009	Component of oil with antimicrobial properties against marine pathogens
Coral	Acetic acid	atkinson and arey 2003 chemical reviews	reacts with criegee intermediates	-	-	Schulz and Dickschat, 2007	Attractive to mexican fruit flies	Ryssel et al 2009; Giorgio et al 2015	Antibacterial; Antifungal
Bacteria	Acetone	Singh et al. 1994	Photolyses, producing free radicals	Cordis et al. 1994	Associated with oxidative stress	-	-	Stotzky and Schenk, 1976	Inhibits fungal growth, no effect on bacteria
Bacteria	Allyl nonanoate	-	-	-	-	-	-	-	-
Coral	Allylbenzene	-	-	Khakdan et al 2017	Fluctuates with drought stress	Qualley and Dudareva 2008	Phenylpropenes are a major constituent of VOCs involved in plant defence and reproduction	-	-
Sym & Coral	Azulene	-	-	Figueiredo et al. 2008	Herbicides reduce emitted azulene in a plant	Roshchi- and Yashin 2014	Likely invoilved in mediating plant alllelopathy	Li et al. 2010	Anti-inflammatory, antifungal effects
Sym & Coral	Benzaldehyde	brun et al 2016	SOA	Ullah et al. 2014	Antioxidant	Klaschka 2009	pheromone	Ullah et al 2014	Antimicrobial, insecticidal
Coral	Benzene	brun et al 2016; atkinson and Arey 2003 chem reviews	SOA; reacts with OH, O3, NO3	-	-	-	-	-	-
Sym	Benzene, 1-[1,1-dimethylethyl]-4-[2-propenyloxy]-	-	-	-	-	-	-	-	-
Bacteria	Benzocyclobutene	-	-	-	-	-	-	-	-
Coral	Benzoic acid	Zhang et al 2004	forms SOA	Elad 1992	antioxidant	Klaschka 2009	phytoalexin - inhibits parasites	Elad 1992	antifungal
Coral	Benzothiazole	-	-	-	-	-	-	Fernando et al 2005	antifungal

Coral	Bromodichloromethane	Yokochi et al 2005	reacts with OH and impacts ozone	-	-	-	-	-	-
Coral	Bromoform	Yokochi et al 2005; Barrie et al 1988; saiz-lopex et al 2012; hossaini et al 2015	reacts with OH and degrades ozone; undergoes photolysis	Mtolera et al 2010	increased with high light	-	-	Ohsawa et al 2001	antimicrobial - gets rid of parasitic microbes (diatoms)
Bacteria	Camphor	Atkinson and Arey 2003 AE	reacts with OH, O3, NO3	Juteau et al 2002	antioxidant as a part of an essential oil mixture	-	-	Juteau et al 2002	antimicrobial as part of essential oil extracts
Coral	Chlorodibromomethane	Yokochi et al 2005	reacts with OH and impacts ozone	Mtolera et al 2010	increased with high light	-	-	-	-
Bacteria	Cyclobutanol	-	-	Koksal et al 2015	absent during salt stress	-	-	Ara et al 2013	antimicrobial as part of extract
Sym	Cyclooctyl alcohol	-	-	-	-	-	-	-	-
Coral	Decanal	Atkinson and Arey 2003 chem reviews	reacts with NO3	Liu et al 2012	antioxidant	Joythi et al 2002	attracts wasps	Liu et al 2012	antimicrobial
Sym & Coral	dimethyl disulfide	thesis Horne 2018	reacts with OH and NO3 radicals	-	-	Podskalska et al 2009; Meldau et al 2013	attracts beetles ; promotes plant growth	Ossowicki et al 2017	antifungal
Bacteria	Dimethyl trisulfide	thesis Horne 2018	reacts with OH and NO3 radicals	-	-	Podskalska et al 2009	attracts beetles	Fernando et al 2005; Ossowicki et al 2017	antifungal
Coral	Dioxane	-	-	-	-	-	-	-	-
Sym	Ethylbenzene	Atkinson and Arey 2003 chem reviews	reacts with OH	-	-	-	-	-	-
Coral	Geranyl acetone	Fruekilde et al 1998	reacts with ozone	Kask et al 2016	respond to heat stress	Pinto-Zevallos et al 2018	herbivore induced - attracts predators	Akin et al 2010	antibacterial as part of essential oil mixture
Coral	Hemimellitene	Atkinson and Arey 2003 chem reviews	reacts with NO3 radicals and OH radicals	-	-	-	-	Kivcak et al 2007; Wang et al 2013	antibacterial as part of essential oil mixture; antifungal as part of a mixture
Sym	Heptadecane, 9-octyl-	-	-	-	-	-	-	Musa et al 2015	antibacterial as part of essential oil mixture
Coral	Heptanal	atkinson and arey 2003; paulson et al 2006	reacts with NO3 radicals; undergoes photolysis	-	-	Joythi et al 2002	attracts wasps	Yayli et al 2005	antibacterial as part of essential oil mixture
Sym	Hexadecane	atkinson and arey 2003 chemical reviews; atkinson 2003 ACP	reacts with OH	Aisha Hussein Saleh Abou Zeid 2002 Food Chemistry	decreased with (chemical) stress in Corchorus olitorius (shrub	Jiang et al 2015	mediates ovipositioning	Karabay-Yavasoglu et al 2007	antimicrobial as part of essential oil extracts
Coral	Hexanal	Atkinson and Arey 2003 chem reviews	reacts with OH	Goulitquer et al 2009	abundance responds to stress in kelp	Joythi et al 2002	attracts wasps	Setzer et al 2004	antibacterial

Sym	Hexane, 2,3-dimethyl-	-	-	-	-	-	-	Hasannezhad et al 2016	antibacterial as part of essential oil mixture
Coral	Isovanillin - TBDMS derivative	-	-	-	-	-	-	-	-
Sym	l-Alanine ethylamide, (S)-	-	-	-	-	-	-	-	-
Coral	Limonene	Draper et al 2015	SOA	Copolovici et al., 2012	increases with thermal stress	-	-	Giorgio et al 2015	antimicrobial
Coral	Methacrolein	Gierczak et al 1997	reacts with OH; precursor to photochemical pollutants	Jardine et al 2013; kessler et al 2006	coupled to biotic and abiotc stress; herbivore induced	-	-	-	-
Coral	Methanesulfonyl chloride	-	-	-	-	-	-	-	-
Sym & Coral	methoxy-phenyl-oxime	-	-	Wang et al 2014; tang et al 2015	herbivore induced; increases in response to methyl salicylate (triggers plant defence response)	-	-	-	-
Coral	Methyl Isobutyl Ketone	-	-	-	-	-	-	-	-
Sym	Methyl jasmonate	-	-	Yoon et al 2009	mitigates salt stress	Farmer and Ryan 1990. Xu et al 1994	induces plant defence response	Ayala-Zavala et al 2008	Antimicrobial
Coral	Methyl methacrylate	-	-	-	-	-	-	-	-
Coral	Methyl vinyl ketone	Gierczak et al 1997	reacts with OH; precursor to photochemical pollutants	Jardine et al 2013	coupled to biotic and abiotc stress	Almeras et al 2003	activate defensive genes	Herrington et al 1985	antifungal
Coral	Methylacetylacetone	-	-	-	-	-	-	-	-
Sym & Coral	m-Ethyltoluene (or benzene, 1-ethyl-3-methyl)	Andino et al 1996	reacts with OH	-	-	-	-	Jerkovic et al 2012	part of essential oil mixture
Sym	naphthalene, 2-methyl-	wu et al 2015	reacts with OH	-	-	-	-	-	-
Sym & Coral	Nonanal	atkinson and arey 2003 chemical reviews; bowman et al 2003	reacts with NO3 radicals and OH radicals	Goulitquer et al 2009; framptom et al 1999	abundance responds to stress in kelp; increased in human lungs following ozone exposure	Syed and Leal 2009; joythi et al 2002	mosquito & wasp attractant	fernando et al 2005; bisignano et al 2001	antifungal and antibacterial
Coral	Nonane	atkinson and arey 2003 chemical reviews; atkinson 2003 ACP	reacts with OH	Aisha Hussein Saleh Abou Zeid 2002 Food Chemistry ; Zuo et al 2012	decreased with (chemical) stress in Corchorus olitorius (shrub); decreased with salt stress in chlamydomonas	Zuo et al 2012 - mayne	part of a mixture from stressed chlamy cells that triggered a response in healthy chlamy cells	Saroglou et al 2007; fernando et al 2005	antifungal, antibacterial - part of mixture
Sym	Nonanoic acid	Rossignol et al 2016	photolysis and reacts with OH	-	-	Ponnusamy et al 2008; schultz et al 1983	mediates ovipositioning; mosquito repellant	Aneja et al 2005; vahedi et al 2011	Antifungal; part of antibacterial essential oil

Sym	Octadecane, 6-methyl-	-	-	-	-	-	-	-	-
Sym & Coral	Pentane	atkinson 2003 ACP	reacts with OH	-	-	-	-	-	-
Sym	Phenol, 2,4-bis(1,1-dimethylethyl)-	-	-	varsha et al 2015; yoon et al 2006	antioxidant	-	-	Varsha et al 2015; Gong et al 2015	antifungal
Sym	Propane, 1,2-dichloro-	-	-	-	-	-	-	-	-
Sym	Squalene	Athanasiadis et al 2016	SOA, reactive with OH	Biswas and Chakraborty 2013	antioxidant	Dutton et al 2002	herbivore induced	Biswas and Chakraborty 2013	antimicrobial
Sym	Styrene	brun et al 2016	SOA	-	-	-	-	Wu et al 2015	antifungal
Coral	Sulfurous acid-dibutyl ester	-	-	-	-	-	-	-	-
Coral	Tetradecane	atkinson 2003 ACP	reacts with OH	-	-	eisner et al 1977	pheromone in insects	ozdemir et al 2004; wu et al 2015	antibacterial, antifungal
Sym & Bacteria & Coral	Toluene	brun et al 2016; atkinson and arey 2003 CR	SOA and reacts with OH	misztal et al 2015	coupled to photosynthesis	misztal et al 2015	putative signalling	Wu et al 2015	antifungal
Coral	Tridecane	atkinson 2003 ACP	reacts with OH	pellegrini et al 2012	decrease after exposure to ozone	krall et al 1999; fraga et al 2017	pheromone in insects	ajilogba and babalola 2019	antibacterial
Coral	Undecanal	eliason et al 2004	forms CCN	pellegrini et al 2012	decrease after exposure to ozone	vanickova et al 2012; dweck et al 2010	pheromone in insects	kubo et al 2004	antibacterial
Coral	Undecane	atkinson and arey 2003 chemical reviews; atkinson 2003 ACP	reacts with OH	Aisha Hussein Saleh Abou Zeid 2002 Food Chemistry ; Zuo et al 2012	decreased with (chemical) stress in Corchorus olitorius (shrub); decreased with salt stress in chlamydomonas	Cardenas et al 2012	pheromone in insects	Saroglou et al 2007	antibacterial

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