Lipid based adaptive traits of a subtropical and temperate coral promoting survival in high-latitude reefs

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Certificate of Original Authorship

I, Laura Marie La Motta, declare that this thesis is submitted in fulfilment of the requirements for the award of Master of Science (Research), in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Thesis Abstract

Sydney Harbour is situated in a major biogeographic transition zone on Australia's east coast. This area is characterised by an overlap of temperate and subtropical species, and has been proposed as a haven for subtropical species at their poleward range limits, such as coral (e.g., *Pocillopora aliciae*) against the warming oceans occurring due to climate change. However, Sydney Harbour is not immune to ocean warming, and subtropical residents must tolerate dynamic conditions including periods of low temperature, changes in nutrient concentrations and high seasonal variation associated with temperate environments. Consequently, how subtropical species will populate and persist in biogeographic zones remains unresolved but is an area of increasing focus, as the influx of new species can compromise resident temperate species as they compete for space and resources. Furthermore, as oceans warm, temperate species are projected to be pushed closer to their upper thermal limits in this region. As such, determining the thermotolerance of sympatric subtropical and temperate coral species in biogeographic transition zones can help understand the factors that influence coral range dynamics and facilitate more accurate predictions of coral species composition in high-latitude reefs under a changing climate. The aim of my thesis was to determine lipid based adaptive traits that govern survivability of two coral species with different distributions living at the extent of their thermal optima in Sydney.

Pocillopora aliciae is a small-range subtropical coral, endemic to the subtropical east coast of Australia, with the core of its distribution in Northern New South Wales (NSW) and its poleward range limit in Sydney (34ºS). Despite this, range edge populations of *P. aliciae* in temperate Sydney waters are not only surviving, but thriving, whereas more northerly coral populations in Solitary Islands Marine Park near Coffs Harbour (31ºS) have been found to be impacted by heat stress. *Plesiastrea versipora* is a widely distributed temperate coral, with populations ranging from Western Australia, down to Tasmania (40ºS), and along the temperate and subtropical coast of NSW in eastern Australia, including Sydney. However, *P. versipora* corals have been impacted by heat stress, with mass bleaching (colour loss as a result of symbiont expulsion) of Sydney *P. versipora* during the 2016 global heatwave event, indicating this species is abiding at its upper thermal limits and may be further affected as warming intensifies. The differences in thermotolerance of these coral species may be driven by species composition of associated

Symbiodiniaceae, endosymbiotic algae living within coral tissue, that have been found to help govern the thermotolerance and resilience of coral to environmental stressors. The thermotolerance of Symbiodiniaceae has been proposed to be a genotypic response, with trends across species and genera, but it is becoming more evident that genetic variation does not explain all phenotypic diversity in Symbiodiniaceae. Hence, recent studies have attempted to determine other cellular mechanisms driving thermotolerance and adaptation to environmental change in Symbiodiniaceae, including lipid remodelling.

Previous studies have observed that microalgae can adapt by remodelling their lipid composition and abundances, especially within membranes, as a response to altered environmental conditions, allowing longer-term survival under stress, via a process called homeoviscous adaptation. Within the biofuel industry for instance, changes to the external culturing environment of algae have been shown to induce lipid remodelling, such as increased abundances of target lipids under nutrient deprivations and increased temperatures. In Symbiodiniaceae, homeoviscous adaptation has previously been observed, including alterations to specific lipids, but a wholistic understanding of lipid remodelling across a wide temperature regime, and under altered nutrient concentrations had not yet been established. In Chapter 2, to determine whether Symbiodiniaceae were able to remodel their lipid profile under sublethal environmental stress, and to identify significant lipids and trends within lipid remodelling, I analysed the lipid profile of three genera of Symbiodiniaceae (*Durusdinium*, *Cladocopium* and *Breviolum*), representing Symbiodiniaceae found in corals differing in geographical distribution, when cultured across a wide temperature regime (16 ºC – 31 ºC) and under altered nutrient concentrations (Nitrates (N) and Phosphate (P)). As anticipated, all cultures grown under nutrient (N, P) limited conditions exhibited a higher abundance of storage lipids, especially triacylglycerols. Notably, we also found evidence that Symbiodiniaceae are capable of diverting lipid synthesis pathways when key nutrients are depleted. Under phosphorus limitation, where membrane phospholipids were unable to be synthesised (PC), there was as an increase in vitamin-A fatty ester (VAE) abundance across all Symbiodiniaceae species. This suggests a diversion in lipid synthesis from phosphate based to isoprenoid based, a process described in fungi, but until now, never described within algae. Upon testing both cold and heat stress on Symbiodiniaceae, we also found sublethal heat stress (31 ºC) has a more profound effect on lipid remodelling than decreased temperatures. Across all

Symbiodiniaceae, we found increases in oxidised lipids, particularly oxidised phosphatidylinositol (OxPI), within cultures grown at 31 ºC. This indicates an increase in reactive oxygen species (ROS) abundance and oxidative stress to the cell, further supported by increased cell size within *Cladocopium* and *Durusdinium* species. Overall findings indicate Symbiodiniaceae can remodel their lipid profile under varied environmental conditions, and, if similar mechanisms occur when *in hospite*, may be a strategy allowing corals occupying high latitude reefs to adapt to high temperature fluctuation and decreased temperatures in these cooler range-edge environments.

The sympatric corals, *P. aliciae* and *P. versipora*, represent coral species with differing geographical distributions inhabiting the same geographical niche in Sydney, at the lower and upper limits of their thermal capacity, respectively. As lipid remodelling in Symbiodiniaceae under temperature increases and decreases has been observed, this mechanism may be similarly driving adaptation to thermal stress within these coral species. To determine whether corals abiding at their thermal limits are able to remodel their lipid profile as an adaptive mechanism contributing to survivability, in my second investigation (Chapter 3), I utilised the coral bleaching automated stress system (CBASS) in a novel experimental design incorporating both temperature increases and decreases (11 ºC – 32 ºC) to assess photophysiology (F_v/F_m) changes and test for lipidome changes after the short-term stress assay was applied. Although findings indicated lipid remodelling in host and Symbiodiniaceae did not occur under the acute thermal stress, likely reflecting their evolved capacity to survive high daily variation in temperatures, we did observe lipid remodelling within both coral species, and their associated Symbiodiniaceae, across a seasonal scale. In control samples, membrane lipids (PC, ST, VAE), were found in higher proportional abundances in winter, which could potentially indicate a mechanism allowing for membrane stability during cooler seasonal temperatures. We also observed increased proportional abundances of glycerolipids (DGDG, MGDG) within *P. aliciae* Symbiodiniaceae compared to *P. versipora*. This may suggest a species-specific adaptation of *P. aliciae* in occupying an area where light levels may be reduced compared to core populations in Northern NSW. Nevertheless, my results show that acute short-term temperature stress methods may not be suitable to assess lipid-based mechanisms of thermotolerance in high-latitude corals,

which future studies should consider in further research on lipid remodelling within corals in marginal reef environments. In Chapter 4, I consider my data chapter findings collectively, and discuss the new knowledge my thesis has provided, whilst also discussing challenges and future directions of research.

Collectively, my findings illustrate some potential traits of *P. aliciae* and *P. versipora* that allow sympatric coral species from differing geographical distributions to thrive at their thermal range limits in Sydney. In doing so, this thesis delivered new insights on lipid remodelling within coral and associated Symbiodiniaceae as an adaptive trait that can influence survivability in high-latitude reefs and under a changing climate. I highlight the importance of developing an enhanced understanding of homeoviscous adaptation within both coral and Symbiodiniaceae in helping predict future reef trajectories as well as contribute to conservation efforts under a changing climate and demonstrate the potential capacity of corals living at their thermal extent to adapt to environmental change via lipid remodelling.

Thesis Structure

This thesis is submitted as a Thesis by Compilation, and is comprised by a combination of chapters, and published/publishable work. This thesis consists of a general introduction (**Chapter 1**), two data chapters (**Chapters 2 and 3**) in the form of a journal manuscript for peer review, and a synthesis chapter (**Chapter 4**). At the time of thesis submission, one data chapter (**Chapter 2**) was published as a manuscript within Frontiers in Protistology and one data chapter (**Chapter 3**) in preparation for submission.

Chapter 1: General introduction and background literature

Chapter 2: This chapter has been published as a manuscript.
La Motta, LM, Padula, MP, Sommer, B, Camp, EF, and Matthews, JL (2024) Diversity of lipid profiles of Symbiodiniaceae under temperature and nutrient stress. *Front. Protistol*, 2:1320353 *doi: 10.3389/frpro.2024.1320353* *

Chapter 3: This chapter is presented as a full article prepared for journal submission.
La Motta, LM, Olander, A, Padula, MP, Sommer, B, Camp, EF, and Matthews, JL. Comparative lipidomics analysis of a temperate and a subtropical coral under acute temperature stress. *

Chapter 4: General discussion, synthesis of results from both data chapters and recommendations for future research/ pathways of study.

*Please see title page Chapter 2 and 3 for signed authorship declarations.

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Chapter 1: General introduction and thesis outline

1.1 The Poleward Shift of Corals

Coral reef ecosystems are one of the most ecologically diverse environments in the world, despite occupying only a small proportion of the ocean floor (Reaka-Kudla, 1997). These ecosystems hold immense ecological importance, functioning as breeding grounds, habitat, and nurseries for a wide array of aquatic species, as well as protecting coastal communities against shoreline erosion (Moberg & Folke, 1999). They also hold high socio-economic value, generating a revenue of over US$30 billion per year, globally, and over US$2 billion per year in Australia, from tourism alone (Pendleton et al., 2019; Spalding et al., 2017; Spurgeon, 1992). However, climate change and other anthropogenic pressures have been detrimental to these areas, with a plethora of studies detailing significant rates of decline in coral coverage over recent years, and significant further loss projected for the future (Eddy et al., 2021; Hughes, Kerry, et al., 2017; Pandolfi et al., 2011). Globally, many species, including corals and associated organisms, are responding to the effects of climate change by migrating poleward, where temperatures are cooler (Pecl et al., 2017; Yamano et al., 2011). Corals are naturally occurring along tropical to temperate transition zones, in marginal conditions at their poleward range limits (Veron, 1993), but in some high-latitude regions where temperatures are cooler and nutrient conditions (Nitrogen and Phosphorus) altered (Lønborg et al., 2021; Messer et al., 2021; Takahashi et al., 1993) compared to the tropics, the abundance of some corals increased in recent years (Booth and Sear 2018) and some have migrated (Baird et al., 2012; Yamano et al., 2011). These areas, known as biogeographic transition zones, are characterised by an overlap of species with different thermal affinities and biogeographic distributions (i.e., tropical, subtropical, temperate) occupying the same geographical niche, and are sometimes hypothesised to be areas of refuge allowing for the poleward range expansion of certain species as conditions in their existing ranges become less favourable, such as due to increasing temperatures (Beger et al., 2014; Malcolm et al., 2010).Yet, in occupying new environments, these corals now reside at the lower limits of their thermal optima, and potentially in areas differing in light availability, nutrient availability and increased seasonal and short-term variation (Sommer et al., 2018). Additionally, corals occurring in high-latitude regions tend to have lower species richness and be

genetically isolated from origin populations, potentially increasing susceptibility to changes in environmental condition, and risking population mortality (Abrego et al., 2021; Evans et al., 2021; McIlroy et al., 2019)

1.2 Sydney; a Biogeographic Transition Zone

Sydney, on the South-East Coast of New South Wales (NSW, 34ºS), is situated along the subtropical-to-temperate transition zone of Eastern Australia. As the East Australian Current (EAC) intensifies, ocean temperatures along the East Coast of Australia have been exposed to more rapid warming compared to other regions (Hobday et al., 2006; Hobday & Pecl, 2014; Scanes et al., 2020). This in turn has resulted in some tropical and subtropical organisms extending their distribution poleward (e.g., tropical herbivorous fishes) and occurring within this biogeographic transition zone (Vergés et al., 2014). Although some expansions of corals have been documented along the east coast of Australia (Baird et al., 2012; Booth & Sear, 2018), northern NSW coral assemblages have not tropicalised since the 1990s (Mizerek et al., 2021), contrasting with range shifts and tropicalisation occurring in Japan, where in some regions, corals and tropical fish assemblages now occupy areas previously inhabited by temperate seaweed (Fujita, 2010; Kumagai et al., 2018; Vergés et al., 2014).

Pocillopora aliciae (Figure 1.1A), a scleractinian coral endemic to subtropical NSW (Schmidt-Roach et al 2013), has been observed, with increasing frequency, dominating the rocky substrate near Sydney's North Head at a depth of 8-12 meters (Booth & Sear, 2018), where it occurs at its poleward range limit (Wells, 1955). As a branching coral, *P. aliciae*, has brought a new structural complexity to the coral fauna of Sydney, which is dominated by soft corals, such as *Dendronepthya australis* and *Carijoa* sp., as well as the plating coral *Coscinaraea mcneilli*, and the encrusting coral, *Plesiastrea versipora* (Steinberg et al., 2024; Wells, 1962). Increased structural complexity within reefs has been found to enhance coral recovery and support a higher diversity of marine organisms (Graham & Nash, 2013; Mills et al., 2023). This can be seen with *P. aliciae*, as an assemblage of tropical reef fish not historically recorded in the area, including *Plectroglyphidodon dickii*, *Dascyllus reticulatus*, *Chaetodon auriga* and *Thalassoma lunare* have been observed on the coral, yet not within areas lacking *P. aliciae* corals (Booth & Sear, 2018; O'Connell et al., 2023). Furthermore, as a brooding coral, meaning that developed larvae are

released and settle within a close vicinity of parent colonies, populations of *P. aliciae* that reside in NSW could be genetically isolated from upstream populations, ultimately limiting genetic diversity and increasing vulnerability to variation in environmental conditions (Lachs et al., 2021; Schmidt-Roach et al., 2013).

Despite this, temperate Sydney currently seems to offer a refuge for *P. aliciae* against climate change (*sensu* Keppel et al., 2012). Endemic Northern NSW populations experienced severe bleaching (the breakdown of the coral-Symbiodiniaceae symbiosis) during the 2016 heat stress event (Kim et al., 2019), with marked declines in abundance (Lachs et al 2021) and limited recovery three years after bleaching (Sommer et al., 2024). Nevertheless, there is no documented evidence of populations bleaching in Sydney. As such, further coral expansion and population growth around Sydney is anticipated, especially if strengthening of the East Australian Current (EAC) and warming events continue as predicted (Davis et al., 2023; González-Pech et al., 2022). However, making definitive predictions on further expansion of *P. aliciae* is difficult, as traits determining capacity to withstand colder temperatures and higher seasonal variation, as well as altered nutrient availability, topology, and seawater biochemistry, remains unknown. Factors contributing to the ability of corals to withstand environmental change, such as genetic variation, physiological plasticity, Symbiodiniaceae assemblage and metabolic function are known to vary between species and geographical distribution (Camp et al., 2018; Rogers, 2013; Rossi et al., 2018), and as such, comparing traits between *P. aliciae* at its lower thermal limit and a temperate coral species near its upper thermal limit, such as *Plesiastrea versipora*, may highlight specific mechanisms allowing for survival of species with different thermal affinities and geographic distributions in temperate Sydney.

P. versipora (Fig 1.1B) is a plating scleractinian coral with subtropical and temperate distribution in Australia (Juszkiewicz et al., 2022), also found on rocky platforms around 5-12 meters depth in Sydney Harbour and surrounds. Molecular analyses have determined Sydney populations to be genetically isolated, yet reproductively active, facilitating reproduction and growth without external propagule input (Madsen et al., 2014; Rodriguez-Lanetty & Hoegh-Guldberg, 2002). Previously identified *P. versipora* populations in tropical regions of Australia have been reclassified instead as *Plesiastrea peroni* (Juszkiewicz et al., 2022). As a temperate

species, *P. versipora* is resilient to colder temperatures found in high-latitude reefs (Tuckett & Wernberg, 2018), but not so much under higher temperatures, as Sydney populations bleached during the 2016 marine heat wave (Goyen et al., 2019). Yet, this study on *P. versipora* also found fast and widespread recovery, with over half of affected populations returning to colour within a 3-month period. Whilst this indicates strong recovery of *P. versipora* post-stress, evidence suggests that even once corals return to colour, their reproductive performance and immune response may remain impaired (Briggs et al., 2024; Muller et al., 2018). Nevertheless, with heatwaves expected to increase in frequency and intensity (Trancoso et al., 2020), repetitive bleaching may occur, which could lead to mortality and affect their recovery potential in the future (Brown et al., 2002; Brown & Barott, 2022). Overall, high-latitude biogeographic transition zones may only be a temporary haven for subtropical species against climate change, but native populations could soon be overwhelmed (Dixon et al., 2022), with predictions that future environmental conditions will exceed historical conditions across the entire distributional ranges of many reef corals including high-latitude specialists by 2050 (Kim et al., 2023). Previous studies suggest that variation in coral assemblages along the tropical-to-temperate transition in eastern Australia may be regulated by species-specific response to stress and trait-mediated environmental filtering (Sommer et al., 2014, 2018). To gain valuable knowledge on further colonisation potential of cryptic corals, and what the future reefs of NSW may look like, it is essential to develop a better understanding of the physiological factors which influence plasticity and fitness of corals at their poleward range limits. As the mutualistic association with symbiotic algae (Symbiodiniaceae) is an important relationship that can underpin coral survivability (Baker, 2003) , developing an understanding of the traits increasing plasticity and fitness within Symbiodiniaceae associated with these corals is essential.

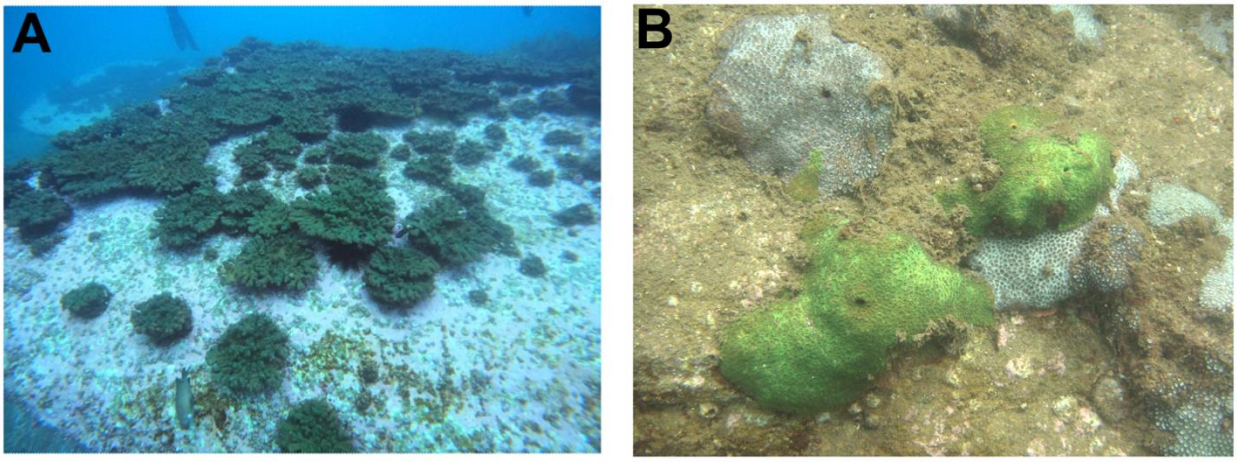

Figure 1.1: *Pocillopora aliciae* colonies (-33.799, 151.300) (A) and *Plesiastrea versipora* colonies (-33.826, 151.264) (B)

1.3 Symbiodiniaceae

Mutualistic associations between cnidarian hosts and their endosymbiotic dinoflagellate algae (Symbiodiniaceae) underpin the formation and expansion of coral reefs and provide a mechanism for resilience and adaptation to environmental change (Davy et al., 2012; Lee et al., 2022). However, nutrient limitation, light availability and temperature extremes are known to disrupt the stability of the coral-Symbiodiniaceae symbiosis, ultimately increasing susceptibility to coral bleaching and mortality (Blanckaert et al., 2023; Ezzat et al., 2016; Marangoni et al., 2021; Morris et al., 2019). There are 11 named genera of Symbiodiniaceae, however *Symbiodinium* (Clade A), *Breviolum* (Clade B), *Cladocopium* (Clade C), *Durusdinium* (Clade D), *Foraminifera* (Clade G), and *Halluxium* (Clade H) are most frequently associated with corals (Pratomo et al., 2022). The species composition and abundance of Symbiodiniaceae communities differs among corals, especially between species and across localities, with both genus level and fine scale variability in Symbiodiniaceae composition influencing coral plasticity, adaptive capability to stressors and ultimately, long term survival (de Souza et al., 2022; Sampayo et al., 2008). Furthermore, studies demonstrate Symbiodiniaceae play an important role in coral acclimation and long term survival under changes in environmental condition (Marhoefer et al., 2021; Wang et al., 2023) and across environmental extremes (Camp et al., 2019;Camp et al., 2020) and considering the role of Symbiodiniaceae is thus important in developing an understanding on how corals, especially those residing at the limits of their environmental optima, might be impacted by altered environmental conditions.

Thermotolerance in corals is known to be determined, in part, by the thermotolerance of their Symbiodiniaceae (Baker et al., 2004), and a large proportion of studies on coral adaptive traits in corals have focused on the resident Symbiodiniaceae thermotolerance (Suggett et al., 2015, 2017). Species within the genus *Durusdinium* are well evidenced to be thermally tolerant (Silverstein et al., 2017), with species such as *D. trenchii* and *D. glynni* linked to resilience of corals under heat stress (Palacio-Castro et al., 2023; Russnak et al., 2021). *Cladocopium* is the most abundant and diverse genus as well as a known generalist Symbiodiniaceae (Cunning & Baker, 2013; Thornhill et al., 2014), meaning this genus occupies a range of diverse coral species across a wide geographical distribution, and has the potential to adapt to a wider range of environmental conditions as predicted by climate change (Butler et al., 2023). This is supported by findings of *Cladocopium thermophilum*, a clade C3 species of Symbiodiniaceae, to be the prevalent symbiont in the Persian/Arabian Gulf, also known as the world's hottest sea, rather than a traditionally tolerant *Durusdinium* spp. (Hume et al., 2015). The dominant Symbiodiniaceae within the subtropical endemic *P. aliciae* was found to be *Cladocopium* sp. (C75h) (González-Pech et al., 2022), whereas the temperate Sydney coral, *P. versipora*, has been found to be dominated by Symbiodiniaceae *Breviolum* sp. (B18). *Breviolum* is a less explored clade of Symbiodiniaceae, often found in corals from temperate environments, and as such has been found to be susceptible to heat stress (Johnston et al., 2023; Russnak et al., 2021). This could in part explain why under heat stress, *P. aliciae* endemic to subtropical NSW, may outperform *P. versipora*, whose Symbiodiniaceae community is dominated by *Breviolum* sp. (González-Pech et al., 2022). Additionally, plasticity within the coral-algal symbiosis also seems to be a strategy for increased resilience to stress, with the switching of dominant Symbiodiniaceae (*Cladocopium* to *Durusdinium*) observed between optimal and marginal reef environments (Camp et al., 2018; Hennige et al., 2010; Ros et al., 2021). Symbiont shuffling is not without its detriments, with research finding trade-offs in growth for resilience to environmental stress (Wang et al., 2023), however, this is not always the case (Matthews et al., 2020). Besides associations with unique symbionts, survival in marginal systems is mediated by plasticity in energy acquisition, primarily via altered trophic strategies (e.g. increased heterotrophy due to decrease autotrophy) (Camp et al., 2018). Whether acquisition mode (trophic strategy) and storage of resources is altered to support survival remains untested but may be an

adaptive mechanism allowing for increased coral survivability when nutrient availability and requirements change.

1.4 The Lipidome

Coral survivability has been found to be linked to an exchange of nutrients, including lipids, with Symbiodiniaceae and other members of the holobiont (Matthews, Raina, et al., 2020). Lipids play an important role within the cell, specifically within energy storage, cellular signalling and membrane permeability (Chen et al., 2017; Díaz-Almeyda et al., 2011). The accumulation and composition of lipids within coral and Symbiodiniaceae membranes may be altered under changes to environmental condition, a term coined homeoviscous adaptation (Cirino et al., 2021; Ernst et al., 2016). Lipid remodelling has been utilised extensively within the biofuel industry, with manipulations to environmental condition, specifically nitrogen limitation and increased temperatures, influencing the abundance and composition of lipids within microalgae (Chokshi et al., 2017; Hu et al., 2008). For corals abiding in marginal reef environments, reliance on lipids as a main energy source within Symbiodiniaceae may facilitate survival under extreme environmental conditions (Alessi et al., 2024; Grottoli et al., 2017), suggesting that higher abundances of storage lipids may allow for longer term survivability and increased recovery potential of corals within marginal environments (Rodrigues & Grottoli, 2007).

As climate change intensifies, oceans are expected to become further nutrient (N,P) limited (Marinov et al., 2010; Zhang et al., 2022). Historically, there has been contention as to whether nitrogen or phosphorus limit production within oceans (Fennel & Laurent, 2018; Smith, 1984), yet, regardless of the main limiting nutrient, reduced concentrations of both nitrogen or phosphorus can impact Symbiodiniaceae cell proliferation and potentially disrupt the coral-algae symbiosis (Cui et al., 2022; Morris et al., 2019). Glycerolipids, such as triacylglycerols (TG), are known to be important storage lipids (Hu et al., 2008), and have been found to increase in microalgae under nitrogen limitation (Fakhry & El Maghraby, 2015). Similar trends have been observed within Symbiodiniaceae, with increased storage lipid accumulation in response to nitrogen limitation (Jiang et al., 2014), and increased temperatures (Rosset et al., 2019). In corals, phosphate limitation can promote bleaching (Rosset et al., 2017), enforced by findings that heterotrophic uptake rates of phosphorus increased under thermal stress to maintain health

(Ezzat et al., 2016). Within algae, P-limitation has resulted in increased glycerolipids in a freshwater species, *Monodus subterraneus* (Khozin-Goldberg & Cohen, 2006), and substitutions from phospholipids within membranes as well as acidification of membrane vesicles in *Emiliania huxleyi* (Shemi et al., 2016). As such, the limitation of phosphates may be detrimental to coral reefs under climate change, yet the cellular processes affected by P-limitation remains unknown. Lin (2023) details the biogeochemical and physiological processes that must be investigated in order to develop an understanding of how predicted P-limitation could affect phytoplankton populations. One such process mentioned is "omics", inclusive of metabolomics, proteomics and lipidomics. Applying these to Symbiodiniaceae and coral research will aid in generating further knowledge on how altered environmental conditions can shape the lipid profile and contribute to resilience and survivability of corals.

The abundance of storage lipids within corals has also been found to be impacted by thermal bleaching events, with reduced storage lipid accumulation in heat-stressed samples observed (Grottoli et al., 2004). This suggests reliance of corals on the usage of energy stores and increased heterotrophy (Kochman et al., 2021) as mechanisms of persistence under stress, and enforces the notion that higher storage lipid abundance facilitates longer term survival (Liu et al., 2022). Similarly, corals under heat stress have been shown to down-regulate genes related to the metabolism and transport of lipids, which can be linked to the destabilisation of nutrient cycling between host and symbiont (Rädecker et al., 2021). This suggests the potential of lipids as biomarkers of stress, with changes in abundance and composition of certain lipids potentially indicative the breakdown of coral symbiosis. This is supported by findings implying the potential role of oxidised lipids as biomarkers indicating thermal stress (Botana et al., 2022). Indeed, oxidative stress and the presence of reactive oxygen species (ROS) within Symbiodiniaceae have been linked to increased temperatures, such as those predicted under climate change, and can cause oxidative damage to the cell (Amario et al., 2023). Research in eukaryotes suggests an increase of ROS can be sourced back to increases in the oxidation of Fatty Acids (OxFA) (Rosca et al., 2012), yet despite the implications this could have within the coral-algae symbiosis, oxidised lipids remain under-researched within the coral holobiont. Additionally, medical research has determined the capacity of ether-linked lipids as a powerful antioxidant, capable of reducing ROS and oxidative stress (Dean & Lodhi, 2018). Despite this, there have been no

studies in Symbiodiniaceae or coral targeting presence, or abundance of ether-lipids under environmental stress, which may be important lipid biomarkers in resilience under climate change. Recent evidence also suggests the importance of inositol as a biomarker within the cellular signalling pathway between coral host and Symbiodiniaceae (Matthews et al., 2018), with breakdowns in the phosphatidylinositol (PI) lipid pathways potentially impacting the coral-algal symbiosis and precursing hyperthermal bleaching events (Rosic et al., 2015).

Cold water bleaching can occur when temperatures drop significantly (Hoegh-Guldberg et al., 2005; Rich et al., 2022), and corals undergoing cold stress appear to have similar physiological responses to those affected by heat stress (Saxby et al., 2003). Corals residing at their poleward range limits in subtropical and temperate regions face temperatures below their environmental optima and close to their lower-bleaching thresholds (Bellworthy & Fine, 2021). Despite limited research on the impacts of cold stress on corals, the current evidence suggests differences lie in acclimation potential. For example, Roth et al (2012) found that initial cold stress had a profound impact on growth and photophysiology of a branching coral, *Acropora yongei*, but acclimation occurred over a few weeks with improvements to physiology observed. Inversely, under increased temperatures, the corals exhibited higher resilience under short-term stress, but prolonged exposure resulted in bleaching. The cellular mechanisms that drove these responses in *A. yongei* were not investigated, but cellular metabolism and metabolic composition of the coral holobiont could have played a role. For example, cellular membranes are comprised mostly of phospholipids, such as phosphatidylcholine (PC), and Fatty Acids (FA's) (Hąc-Wydro & Wydro, 2007), and temperature affects membrane permeability by increasing or decreasing lipid packing. The lipid composition of cell membranes could thus influence resilience to temperature changes (Menegol et al., 2017; Valledor et al., 2013). There has been a link between decreases in temperature and lipid unsaturation (Holm et al., 2022), with increased double bonds in fatty acids allowing for maintenance of permeability within membranes under temperature drops (Los & Murata, 2004). However, under heat stress, results appear to be determined by symbiont genus, with *Durusdinium* exhibiting similar unsaturation of membrane lipids seen under cold stress, whereas *Cladocopium* symbionts exhibited an increased saturation and higher membrane rigidity under rising temperatures (Oakley et al., 2022).

Overall, lipid composition and abundance are important mechanisms driving coral and associated symbiont function under stress events predicted by climate change and tropicalisation of high-latitude reefs (Sikorskaya et al., 2022). Yet, further research is needed to identify specific lipids and classes that may aid in long-term survivability and resilience under stress, as well as lipids that may be biomarkers indicative of stress prior to physiological changes such as disruptions to the coral-algae symbiosis and eventual coral bleaching and mortality.

1.5 Thesis Roadmap, Aims and Objectives:

As with every organism, corals require sufficient nutrition for optimal health and persistence, especially under environmental changes. However, there is currently a gap in the understanding of what constitutes the optional nutrition (i.e., the balance of nutrients to meet the nutritional needs) that allow corals to survive. Lipids are critical nutrients for coral, supporting their growth, reproduction, and immune function (Farre et al., 2010). Previous research has demonstrated the importance of lipids in minimising cellular stress when environmental conditions change in both coral and Symbiodiniaceae, which may enable corals to persist when environmental conditions change (Botana et al., 2022; Solomon et al., 2020). However, how specific lipids determine coral health and resilience remains unknown. This is a fundamental gap in our ability to understand coral resilience and ultimately understand how corals ecosystems will respond to climate change. Thus, the overall aim of this thesis is to identify the lipid-based adaptive traits that govern survivability of two coral species with different distributions living at the extent of their thermal optima in Sydney. In doing so, I hope to contribute to an ever-growing knowledge bank that can be used for coral protection, restoration, management, and decision making.

To address this overarching aim, two data chapters address the following research questions:

Aim 1, Chapter 2: To determine whether Symbiodiniaceae representing a range of different genera with described differences in physiological optima can adapt to sublethal environmental shifts via lipid remodelling.

In corals, lipid synthesis is primarily performed by the resident Symbiodiniaceae (Trench, 1979), therefore, symbiont lipid composition and adaptability may play a key role in coral energetics and resilience to environmental change, but how symbiont lipids determine coral health and

resilience remains largely unknown. Lipid composition differs between Symbiodiniaceae species in culture (Rosset et al., 2017), and evidence suggests Symbiodiniaceae are able to modulate the lipid composition of cells and membranes to maintain cell stability and adapt to environmental alterations (Botana et al., 2022; Tchernov et al., 2004). Variation in the nutrient composition has been shown to increase photosynthetic efficiency and lipid accumulation in algal biofuel production (Chokshi et al., 2017). Research shows that microalgae are able to change their lipid composition (lipid energy stores and/or important structural lipids) to survive changes in nutrient composition (Wang et al., 2019). Whether Symbiodiniaceae are able to do the same is unknown, but might provide them with the capacity to better withstand shifting nutrient baselines as projected by climate change. In Chapter 2, we exposed cultured Symbiodiniaceae (genera *Cladocopium, Breviolum and Durusdinium)* that originated from corals found in both temperate and subtropical regions to a range of temperatures (16 ºC to 31 ºC) and nutrient concentrations (N and P limitation) to determine whether Symbiodiniaceae representing a range of different genera with described differences in physiological optima can adapt to sublethal environmental shifts via lipid remodelling. Symbiodiniaceae growth and photophysiology were measured tri-weekly over a two-week period, before screening via high-throughput lipidomics to examine if and how culture conditions alter symbiont lipid composition.

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This study examined lipid remodelling as an adaptive strategy of Symbiodiniaceae to heat and nutrient stress, but whether these strategies occur *in hospite*, and whether the lipidome is similarly affected by environmental shifts in coral tissue remains unknown.

Aim 2, Chapter 3: To reveal the lipid characteristics of corals contributing to survival at their environmental limits.

As lipid composition directly affects membrane motion and fluidity, which are determined by the size and saturation levels of Fatty Acid (FA) chains of polar lipids (polyunsaturated FAs tend to

increase membrane fluidity, whereas saturated FAs have the opposite effect (Hąc-Wydro & Wydro, 2007), modifications to cellular lipid composition is a potential means for corals and/or symbionts to rapidly acclimate to environmental change, but this has never before been investigated. Closing this knowledge gap can be achieved by comparing the lipid profiles of corals at the extent of their poleward range limits (*P. aliciae)* and of temperate corals close to their warm-range boundary (*P. versipora*) under short-term intense temperature fluctuations. Chapter 3 builds upon the knowledge from cultured Symbiodiniaceae lipidomics (Chapter 2) by comparing photophysiological and metabolomic responses to extreme high and low temperatures (11 ºC-32 ºC) using an acute temperature stress experimental design (CBASS) in a widely distributed subtropical-temperate coral (*P. versipora*) and the small-range subtropical endemic coral (*P. aliciae*) at its poleward range limit in temperate Sydney. The lipid profiles of both the coral host tissues and their associated Symbiodiniaceae communities were examined in isolation to determine how cellular lipid composition can support corals living at the extent of their thermal optima. This revealed lipid profile characteristic of corals with the greatest capacity to persist under future climate conditions, and determine lipid profile dynamics of Symbiodiniaceae *in hospite* (Davies et al., 2023).

This chapter is presented as a fully drafted article prepared for submission to a peer-reviewed journal: La Motta, LM, Olander, A, Padula, MP, Sommer, B, Camp, EF, and Matthews, JL. Comparative lipidomics analysis of a temperate and a subtropical endemic coral under short term temperature stress.

Finally, the information addressed in the above aims are considered within Chapter 4, where I aggregate and review findings within both data Chapters, address the potential implications of lipid remodelling in coral persistence, specifically *P. aliciae* and *P. versipora*, representing sympatric coral species with differing distributions, under environmental stress, and identify future directions of research. . In doing so, I address potential lipid-based adaptive traits of *P. aliciae* and associated Symbiodiniaceae that may allow this subtropical coral to persist in temperate Sydney, and *P. versipora* and associated Symbiodiniaceae at the upper thermal limits of distribution.

Chapter 2: Diversity of lipid profiles of Symbiodiniaceae under temperature and nutrient stress

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2.1 Abstract

Lipid-based survival mechanisms allow microalgae to occupy wide geographical ranges and survive abiotic stress. The protist Symbiodiniaceae are globally distributed from temperate to tropical environments, and establish mutualisms with numerous hosts, including cnidarians. The ability for these dinoflagellates to maintain cellular function under wide ranging environmental conditions will influence the survival and geographic distribution of their hosts. One mechanism that microalgae utilise to adapt to environmental changes is lipid remodeling, such as increased saturation of membranes to maintain the structural integrity under temperature changes, and lipid accumulation when nutrient availability decreases. Whether Symbiodiniaceae utilise lipid remodeling to adapt to sublethal environmental change is yet to be resolved. This study examines the effects of temperature (16 °C to 31 °C), and nitrogen (N) and phosphorus (P) availability, on the lipid composition and physiology of cultured Symbiodiniaceae (from genera *Breviolum, Cladocopium* and *Durusdinium*) isolated from temperate or tropical environments. Glycerolipids, particularly triacylglycerols, increased while cell size decreased under N- and NP-nutrient limited cultures, across all Symbiodiniaceae species. P-limitation caused a decrease in phosphatidylcholine, an important membrane lipid, and saw an increase in isoprenol lipids. This suggests a diversion of phosphorus from phospholipid membranes to the biosynthesis of membrane-stabilizing isoprenes. Reduced photophysiology under P-limitation in all Symbiodiniaceae further supports evidence that P-limitation induced stress in these Symbiodiniaceae cells. As expected, growth rate was reduced in all Symbiodiniaceae at temperature extremes (31 °C). Significant increases in oxidised lipids, particularly oxidised phosphatidylinositol, and a reduction in ether-linked phospholipids in cultures grown at 31 °C, suggests increased reactive oxygen species (ROS) abundance in these cells. In addition, at 31

°C, *D. trenchii* and both *C. goreaui* spp. cell size increased, a common sign of ROS accumulation, cell cycle arrest and necrosis. The observed increases in lipid energy storage (triacylglycerols and isoprenoids) under nutrient stress, as well as ROS-mitigation via lipid remodeling leading to increases in saturated fatty acids and oxidised lipids under temperatures stress, suggest Symbiodiniaceae can remodel their lipids to adapt to environmental shifts. If similar mechanisms occur *in hospite*, this could be an adaptive strategy for coral holobionts under a changing climate.

2.2 Introduction

Marine microalgae possess remarkable plasticity and adaptability to abiotic stressors and have evolved a variety of physiological strategies allowing them to survive in a wide range of habitats, including regulation of lipid biosynthesis (Fakhry & El Maghraby, 2015; Holm et al., 2022). Lipids are found within all cells, encompassing a range of functions including energy storage, cell signalling and structure (Santos & Preta, 2018; van Meer et al., 2008). The accumulation of lipids involved in energy storage, such as triacylglycerols, has been observed in many microalgae species under varied environmental conditions, such as nitrogen and phosphate starvation (Jiang et al., 2014; Yang et al., 2018), light wavelength and intensity, temperature (Dickinson et al., 2017), carbon dioxide levels (Ortiz Montoya et al., 2014), and increased salinity (Zhu et al., 2016). Some microalga taxa can cope with decreased available phosphate by remodelling polar-lipid membranes from phospholipids to phosphate-free lipids, such as betaine lipids (Cañavate et al., 2017). Temperature stress induced changes within the saturation of membrane glycolipids and fatty acids in order to maintain cell and thylakoid membrane stability (Tian et al., 2022).

Symbiodiniaceae, a family of dinoflagellate microalgae, associate with numerous hosts across large geographic ranges, including other protists and cnidarians such as reef building corals (LaJeunesse et al., 2018). Symbiodiniaceae are genetically and functionally diverse, with different functional types based on thermal tolerance, and photo physiological performance most frequently studied (Sampayo et al., 2008). Within studies to resolve functional differences between species of Symbiodiniaceae across environmental gradients, it has been found Symbiodiniaceae lipid composition can differ between species (Sikorskaya et al., 2021), and

strong evidence suggests that Symbiodiniaceae might modulate the lipid composition in order to maintain cell stability and adapt to environmental alterations (Bachok et al., 2006; Tchernov et al., 2004). Indeed, studies have observed the effects of heat shock (34 °C) on the lipid profile of cultured Symbiodiniaceae from the genera *Durusdinium, Breviolum* and *Cladocopium*, which presented increased saturation of fatty acids and higher abundances of energy-rich triacylglycerols, supporting lipid-remodeling survival strategies under stress conditions (Botana et al., 2022). It is clear Symbiodiniaceae can change their lipid profiles, but how this occurs across broader environmental conditions and stress remains unresolved. Membranes rich in unsaturated fatty acids (UFAs) are more susceptible to oxidation by reactive oxygen species (ROS) (Su et al., 2019). Under abiotic stress induced by temperature or light, accumulation of ROS causes oxidative stress and damage to Symbiodiniaceae cells, evidenced by disruptions to the saturated:unsaturated ratio of membranes, increased oxidation of lipids (Koch et al., 2017; Rezayian et al., 2019) and reduced concentrations of unsaturated fatty acids (UFAs) (Ayala et al., 2014). Increase in the biosynthesis of saturated fatty acids (SFAs) and lipogenesis intermediates was observed in endosymbiotic *Breviolum minutum* after 6 days of heat stress (Hillyer et al., 2017), which may reflect alterations to structural lipids in order to maintain chloroplast structure and function. Symbiodiniaceae species-specific lipid compositions and alterations to the lipid profile under stress have been recorded, with total recomposition of lipids observed in more thermosensitive species (*Cladocopium*) and higher digalactosyldiacylglycerol:monogalactosyldiacylglycerol (DGDG:MGDG) ratios, indicating high photophysiological functioning in thermally tolerant *Durusdinium* under heat stress (Rosset et al., 2019; Sikorskaya et al., 2021). Studies to date have investigated lipid shifts beyond inhabitable ranges, but their ability to adapt to environmental shifts via lipid remodelling is still unknown. It is therefore important to understand how lipid remodeling strategies differ between Symbiodiniaceae representing different genera, stress tolerances, and geographic distributions (e.g., isolated from temperate versus tropical regions). Nevertheless, Symbiodiniaceae lipid remodeling under wide ranging temperatures and nutrient concentrations remains under-researched despite being an important metric in other algae.

Mutual exchange of nutrients of functional significance between Symbiodiniaceae and corals plays a vital role in governing overall coral performance and survival, including fatty acids and

lipids (Matthews et al., 2017, 2018). Lipids are critical for both coral and Symbiodiniaceae energy stores, growth and reproduction, the ability to minimise cellular stress, as well as acting as essential interpartner signalling molecules in this symbiosis (Chen et al., 2017). Corals are able to synthesise lipids, however, benefit from lipids provided by the resident Symbiodiniaceae, particularly those they cannot synthesise themselves (such as some polyunsaturated fatty acids) (Treignier et al., 2008). Therefore, species specific symbiont lipid composition and adaptability may also play a key role in coral energetics and resilience to environmental stressors (Boulotte et al., 2016; Sampayo et al., 2008). For example, nutrient limitation, alterations to the N:P ratio, and temperature extremes are known to disrupt the stability of some coral-Symbiodiniaceae symbioses, ultimately increasing susceptibility to coral bleaching and mortality (Blanckaert et al., 2023; Ezzat et al., 2016; Rosset et al., 2017). As nutrient concentrations and temperature can affect microalgae lipid composition (Gao et al., 2023; Holm et al., 2022), the plasticity of Symbiodiniaceae lipid composition, and thus what is translocated to the coral hosts, could determine the susceptibility of coral-Symbiodiniaceae associations to climate change and potential for the poleward range shifts of corals (Nielsen & Petrou, 2023).

Studying Symbiodiniaceae *in vitro* is commonly used to assess differences in performance under both thermal and nutrient stress (Dilernia et al., 2023; Wong et al., 2021). In this study we tested cultured Symbiodiniaceae of the genera *Cladocopium*, *Breviolum* and *Durusdinium* that originated from corals found in both tropical and subtropical regions in eastern Australia. We exposed these Symbiodiniaceae to a variety of temperatures (16°C to 31°C) and nutrient regimes (N and P limitation) (Khan et al., 2018) in order to determine whether Symbiodiniaceae are able to adapt to sublethal environmental shifts via lipid remodeling. This could provide an adaptive strategy for corals as climate pressures continue to change environmental conditions.

2.3.0 Methods and Materials

2.3.1 Symbiodiniaceae Cultures

Four Symbiodiniaceae cultures originally isolated from cnidarians ranging from tropical to temperate distributions were selected from existing stocks at the University of Technology Sydney. These were *Durusdinium trenchii* (ITS2 D1a, culture ID: SCF082, isolated from *Acropora muricata* and previously recorded to be thermally tolerant (LaJeunesse et al., 2018), two *Cladocopium goreaui* isolates, Hetero -W (ITS2 C1, culture ID: AIMS-aten-C1-

WSY, also isolated from *Acropora tenuis*, and previously recorded to be more thermally tolerant), and Hetero-M (ITS2 C1, culture ID: SCF055, isolated from *Acropora tenuis*, and previously recorded to be more thermally sensitive (Levin et al., 2016; Ros et al., 2020), and *Breviolum psygmophilum* (ITS2: B2, culture ID: PVB18B, isolated from the temperate coral *Plesiastrea versipora*) (Camp et al., 2022). Each Symbiodiniaceae species was sub-cultured ($N = 2$ per Symbiodiniaceae species, one allocated to the temperature experiment and one to the nutrient limitation experiment) by adding 5 mL of original cultures in 45 mL of autoclaved and filter sterilised (0.22 µm) artificial seawater (ASW) and F/2 media. Cultures were grown for one month (to achieve a minimum cell density of at least 106 cells/mL) at 26 ºC, with an irradiance of 85 ± 15 µmol photons m-2 s-1 (Philips TLD 18W/54 fluorescent tubes, 10 000 K) on a 12h:12h light:dark cycle.

2.3.2 Symbiodiniaceae cell density and size

An aliquot of 184 µL was collected from each culture, photophysiological performance analysed (see Symbiodiniaceae Photophysiology below) and fixed with 16 µL of 25% glutaraldehyde for direct use in flow cytometry analysis (CytoFLEX S, Beckman Coulter, CA, United States). Symbiodiniaceae cell concentration was assessed by relative cell chlorophyll fluorescence (650 nm) using the flow cytometer gating strategy shown in Supplementary Figure S2.1. The gating strategy was formed based on the control of each experiment, accounting for differences in gates for each experiment. Several blanks were run prior to sampling to blank correct. Relative cell size was determined from the same data using a calibration curve generated using Forward Scatter Width (FSC-HW) of unstained polystyrene microspheres of known sizes (1-15 µm diameter) (Molecular Probes Calibration Kit F-13838), run parallel to the experimental samples.

2.3.3 Nutrient Experiment

Each Symbiodiniaceae species (N = 4 replicates per species per nutrient treatment, total N = 64) was exposed to four nutrient treatments; the control (standard ASW F/2) and three nutrient-limited treatments. Nutrient limitation was achieved by reducing the amount of nitrates and phosphates added to 25% of the original concentration as per CSIRO F/2 (Guillard, 1975)(N-limited (ASW F/2 media, N at 25%), P-Limited (ASW F/2 media, P at 25%) and both N:P Limited (ASW F/2 media, N and P at 25%)). ASW + F/2 media, in which the stock cultures were

reared, is nutrient (N&P) enriched well above what is found in seawater (Berges et al., 2001). For the purposes of this study, nutrient limitation refers to a rapid reduction in N and P relative to the stock ASW + F/2 media concentrations. Media nutrient (N and P) concentrations were confirmed prior to culture mixing via a Gallery Discrete Analyzer (ThermoFisher, Supplementary Table 2.1).

From each subculture allocated to the nutrient treatment (generated as above), 2×10^6 cells were collected per replicate (N = 4), centrifuged at 2,000 × g for 5 min at 26 °C and rinsed twice with ASW to remove residual media solution. Cells were resuspended in 100 mL ASW + F/2 media in sterile culture flasks and placed in Eppendorf Innova Stackable Shaking Incubators with an irradiance of 85 ± 15 µmol photons m-2 s-1 (photosynthetic LED lightbank on a 12h:12h light:dark cycle) and manually shaken once per day to prevent sticking. Temperature was maintained at 26 °C (+/- 0.9 °C) for the 14-day period, flasks were randomly distributed within the incubators and rearranged twice per week to account for any slight differences in temperature or light. Flow-cytometric cell abundance was conducted for each Symbiodiniaceae culture every 3-4 days over the 14-day period.

2.3.4 Temperature Experiment

From each subculture allocated to the temperature experiment, 10^6 cells were collected based on flow cytometry cell density results, centrifuged at 2,000 × g for 5 minutes, and then resuspended in 100 mL ASW + F/2 media in sterile culture flasks. Replicates of each Symbiodiniaceae species (N = 4 per species per temperature treatment, total N = 64) were placed directly into four Eppendorf Innova Stackable Shaking Incubators representing each temperature treatment (16 °C, 21 °C, 26 °C and 31 °C) and maintained (+/- 0.9 °C) for the entire experimental duration. Temperatures were chosen to ensure Symbiodiniaceae remained viable after the 14-day period yet pushed to the extents of their thermal optima based on results from a pilot experiment (12-33 °C). Each incubator had an irradiance of 85 ± 15 µmol photons m-2 s-1 (photosynthetic LED lightbank on a 12h:12h light:dark cycle) and cultures were manually shaken once per day to prevent sticking. Replicates were randomly distributed within the incubators and rearranged twice per week. Flow-cytometric cell abundance was conducted for each Symbiodiniaceae culture every 3-4 days over the 14-day period.

2.3.5 Symbiodiniaceae growth

Specific growth rates (μ) were calculated from the linear regression of the natural log of the flow cytometry cell densities versus time during the exponential growth phase of cultures. Standard error of μ was calculated from μ values from biological replicates (N = 4 per treatment per Symbiodiniaceae species) over the exponential growth period determined to be between 7-11 days in nutrient-treated cultures, and between 4-11 days in temperature treatments. Percentage change in growth was calculated as the difference between μtreatment and μcontrol divided by μcontrol. PERMANOVA (Permutational Multivariate Analysis of Variance, 999 permutations) tests were used to assess variation in exponential growth rates between treatments across each Symbiodiniaceae species in both temperature and nutrient experimentation. All data was transformed using Log(X+1). Due to the small sample size, Montecarlo p-values were used. Pair-wise tests were then undertaken to determine where significance occurred.

2.3.6 Symbiodiniaceae photophysiology

Photophysiological performance of Symbiodiniaceae was assessed using a Closed FluorCam FC 800-C. Photophysiological analyses occurred every 3-4 days. 184 μL of each sample was pipetted into a 96 U-bottom well plate and low light (ca. 5-10 μmol photons m-2 s-1) acclimated for at least 30 min prior to measurements. The saturating pulse was delivered using a blue LED excitation source (450 nm) with an intensity of 2930 μE for a duration of 800 ms. All fluorescence yields were adjusted for baseline fluorescence using ASW + F/2. Light response protocols were used to derive the maximum dark-adapted yield of PSII photochemical efficiency (F_v/F_m, dimensionless), which served as a proxy for tracking cellular health. F_v/F_m was compared across treatments over time, and data analysed using Repeated Measures ANOVA (RMANOVA). Significance was determined using pairwise tests undergoing Bonferroni corrections to account for multiple tests on the same dataset. A one-way ANOVA was utilised to determine whether cell size differed significantly by the final timepoint of this study, with Tukeys post hoc test employed to test for differences between treatments. Where data did not meet assumptions of normality, Kruskal-Wallis One-Way ANOVA was utilised to test for variation in size, followed by a Wilcoxon rank-sum test with p-value adjustment to determine

where significant variance occurred. Graphical plots of physiological and photophysiological data were generated using RStudio (2023.06.0 + 421).

2.3.7 Sampling for lipidomics analysis

At 14 days, after cell density, size counts and photophysiology measurements were taken. A total density of 10^6 (temperature experiment) or 10^7 (nutrient experiment) Symbiodiniaceae cells were then concentrated by centrifugation at 2,000 × g for 5 min at 26 °C, the media discarded, and pellets resuspended in 500 µL of ASW before snap freezing in liquid nitrogen. Samples were stored at -80 °C prior to lipid extraction. Cell densities differed due to insufficient growth of the higher temperature cultures to reach 10^7 cells per culture.

2.3.8 Lipid extraction and analysis via UHPLC-MS/MS

All steps were performed at 4 °C and using LC-MS grade glassware. Lipid extraction was based on the methanol/methyl-tert-butyl ether (MTBE) extraction and phase separation protocol for high-throughput lipidomics (Matyash et al., 2008). Symbiodiniaceae samples were thawed on ice, centrifuged at 3000 × g for 5 min at 4 °C, and the ASW supernatant discarded. To each pellet, 300 µL of 100% methanol (at -20 °C) and 5 µL of EquiSPLASH LIPIDOMIX mass spec standard (Avanti Polar Lipid 330707), and ~25 µg acid-washed glass beads were added. Cells were lysed using a bead mill for 3 min at 30 Hz. Samples were incubated on ice for 10-minutes, vortexed twice during this time. 1,000 µL of MTBE was added, samples were vortexed for 30 s and shaken for 1-hour at 1000 x rmp at 4 °C in a rotary shaker. To prepare for phase separation, 250 µL of ultra-pure water was added, samples incubated on ice for 10-minutes, and centrifuged at 1,000 × g for 10 min at 4 °C. 900 µL of the organic layer was collected, dried under a nitrogen stream, and stored at -80 °C until analysis. Sample blanks (N = 8) were undertaken alongside each run.

Prior to analysis, samples were resuspended in 100 µL 2:1 isopropanol methanol (IPA : MeOH) and transferred to autosampler glass vials with a 125 µL glass insert. A pooled sample was created to assist with compound analysis, by combining 5 µL of each sample into a single tube and 100 µL of this pooled sample transferred to an autosampler vial with a 125 µL glass insert. 5 µL injections of each sample was processed via Liquid Chromatography Mas-Spectrometry

(Themo Orbitrap LC-MS) in positive and negative ion mode. Each sample was run as per methodology developed by Violi (2022), in positive mode under acidic chromatographic conditions, and in negative mode with neutral chromatographic conditions using an Agilent 1290 UPLC system and Waters ACQUITY UPLC CSH C18 Column (130Å, 1.7 µm, 2.1 mm X 150 mm). The column oven was set to 65°C for both methods. The positive mode method had a flow rate of 0.4 mL/min with mobile phase A consisting of 60:40 CH3CN : Water + 10 mM Ammonium formate + 0.1% Formic acid and B consisting of 90:10 IPA : CH3CN + 10mM Ammonium formate + 0.1% formic acid. For 2 electrospray ionization (H-ESI) and data acquisition parameters see Supplementary Tables 2.2, 2.3. The negative mode method had a flow rate of 0.4 mL/min with mobile phase A consisting of 60:40 CH3CN: Water + 10 mM Ammonium acetate and B consisting of 90:10 IPA : CH3CN + 10mM Ammonium acetate. Both methods used the same gradient of the following solvent B: 0.00 min 30%, 2.00 min 30%, 2.50 min 50%, 13.00 min 85%, 13.50 min 99%, 15.00 min 99%, 15.10 min 30%, 18.00 30%. Separated lipids were then ionised into the source of a Thermo Q-Exactive Plus mass spectrometer. Data was acquired via DDA topN and scan range was set to *m/z* 200 – 1200, with the only difference between the methods being the polarity (positive or negative mode). Every 9 samples a blank and a QC were injected. When analysing negative mode temperature data within the LC-MS, a system error occurred, compromising several of the samples. These replicates were removed from both negative and positive datasheets, creating an N of 3 for some treatments.

Raw lipid spectral data was exported to .raw files and processed in MS-Dial (version 5.2) (Tsugawa et al., 2015), for peak alignment, blank correction and lipid identification against the LipidMaps LipidFinder (V2) and Structure Database (LMSD) with an accurate mass tolerance (MS) of 0.05 Da for MS1 and 0.075 for MS2. Any unmatched lipids and lipids with an identification score of <80% were removed. Peak areas for each lipid were exported and normalised to the relative peak area of the internal standards. Data was arranged into all lipids and lipid sub-class (using LipidMaps ontology and summed relative abundance) data for further analysis. Samples within temperature treatments underwent further normalization to cell density, as some cultures within 31 °C and 16 °C treatments did not reach the cell volume required. All lipid data underwent cube root transformation and scaling through mean centring in MetaboAnalyst (v5.0). Variability in lipid abundance and composition within Symbiodiniaceae

across all treatments were analysed using Principal Component Analysis (PCA) to determine relationships and clustering between the treatments. PERMANOVA permutational MANOVA (999-permuations) was employed using Montecarlo p-value (P_{mc} <0.05) and pairwise testing to highlight variance between treatments in the PRIMER software (V7.0). Variability between individual lipids was tested using an ANOVA with Fischer's post hoc tests, using a false discovery rate (FDR) of < 0.05. A heatmap was created to visualise all significant lipids and sub-classes, created in MetaboAnalyst using Euclidean distance and ward clustering.

2.4.0 Results

2.4.1 Symbiodiniaceae Physiology

Nutrient experiment:

The exponential growth rates of *D. trenchii* and *C. goreaui (HW)* did not vary significantly between treatments (Figures 2.1A, B). However, the exponential growth rates of N and NP-Limited treatments of *B. psygmophilum* were approximately 2% lower than the control group (Supplementary Table 2.4, (P_{mc}=0.037, (P_{mc}=0.001), but P-limited cultures did not differ significantly. Within *C. goreaui (HM)*, both P- and NP-Limited culture growth rates decreased by 2% on average compared to controls (Figure 2.1; Supplementary Table S2.4, (P_{mc}=0.033, (P_{mc}=0.048).

Nutrient concentration affected cell size across the 14-day period, with nutrient treated cultures exhibiting, at the final sampling point, changes in sizes compared to controls across most Symbiodiniaceae species (Figure 2.1E). NP-Limited cultures of *D. trenchii* were close to 4% smaller than control cultures (Figure 2.1E; Supplementary Table S2.5, ANOVA p=0.028). A similar trend occurred in *C. goreaui (HM)* cultures grown in N-Limited media, with cell size approximately 3% smaller compared to controls (Figure 2.1E; Supplementary Table S2.5, ANOVA p=0.001), and within *B. psygmophilum* which exhibited approximately 4% lower average cell size in both N and N-P limited treatments compared against the control (Figure 2.1E; Supplementary Table S2.5, ANOVA p=0.001). Meanwhile, *B. psygmophilum* P-limited cells increased in size relative to controls (Figure 2.1E; Supplementary Table S2.5, ANOVA p=0.003). In *C. goreaui (HW*), none of the treatments changed significantly relative to the control (Figure 2.1E; Supplementary Table S2.5).

Across all species, P-Limited cultures had the lowest effective quantum yield of PSII (F_v/F_m) by the end of the 14-day period, dropping below 0.55 across all Symbiodiniaceae species (Figure 2.2; Supplementary Table S2.6). The photophysiology of *D. trenchii*, *B. psygmophilum*, and *C. goreaui (HM)* N and NP-Limited cultures remained stable relative to the control, while P-Limited cultures declined significantly by 23%, 20% and 12%, respectively (Figures 2.2A, C, D; Supplementary Table S2.6, ANOVA $p<0.001$). The photosynthetic performance of N-Limited *C. goreaui (HW)* cultures resembled the control, remaining stable throughout the study period, while P-Limited and NP- Limited *C. goreaui (HW)* cultures dropped on average by 24% and 7% by the 14th day compared to control cultures (Figure 2.2B; Supplementary Table S2.6, ANOVA $p<0.01$).

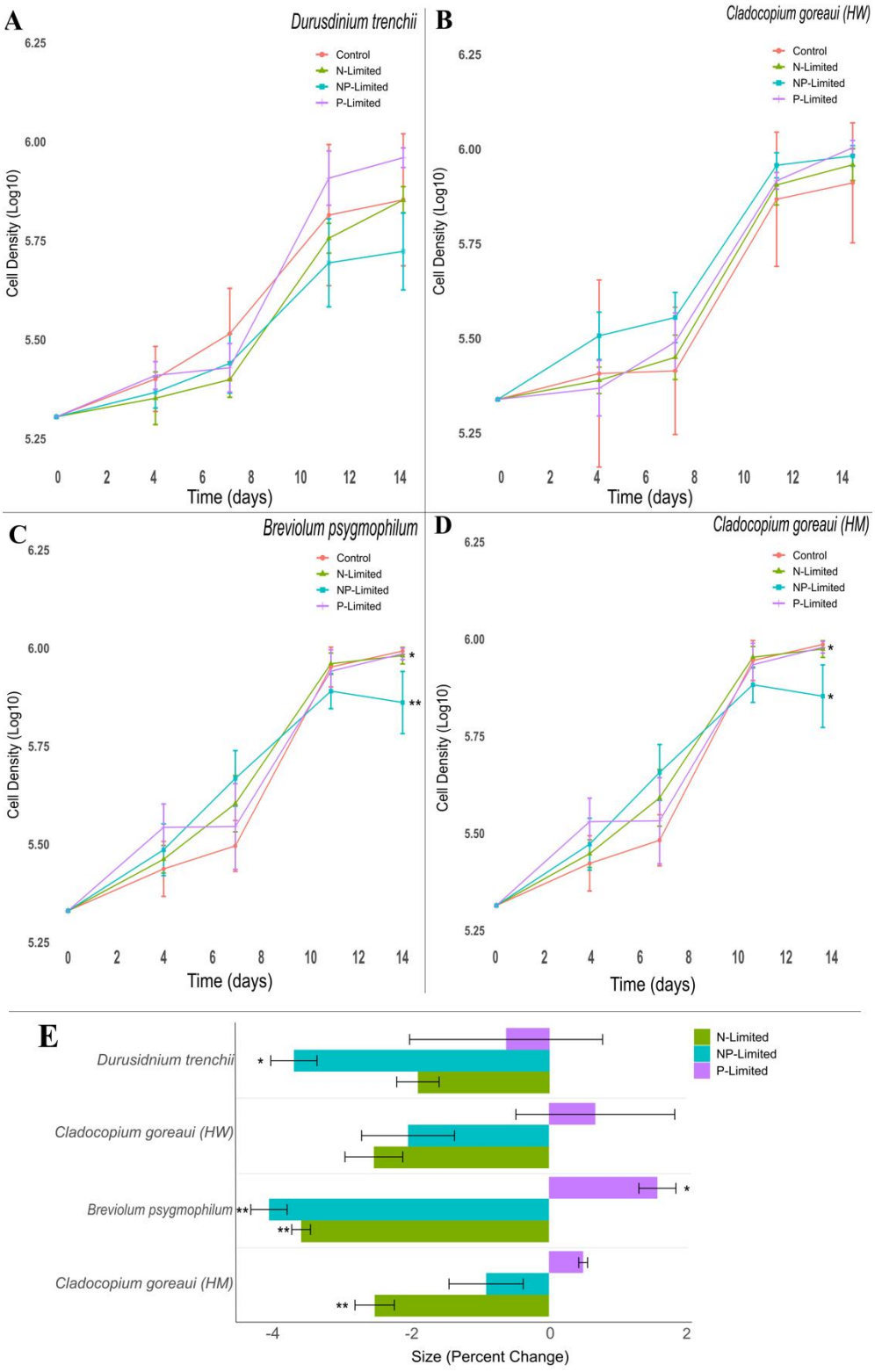

Figure 2.1 Symbiodiniaceae cell density and size under different nitrogen and phosphate concentrations. Symbiodiniaceae cell densities (ln) for *Durusdinium trenchii* **(A)**, *Cladocopium goreaui HW* **(B)**, *Breviolum psygmophilum* **(C)**, and *Cladocopium goreaui HM* **(D)** across the sample period for each nutrient treatment (control

nutrient concentrations, and in N- (green), P- (purple), and NP-limited (blue) media). PERMANOVA permutational MANOVA (PERMANOVA) (P_{mc}) significance shown using *(P_{mc} =<0.05) and **(P_{mc}=<0.001). **(E)**: Variation in cell size for each Symbiodiniaceae across nutrient treatments. One-way ANOVA and Tukeys post hoc significance shown using *(P=<0.05) and **(P=<0.001). Values are means and error bars are SEM. N=4 per treatment, per species.

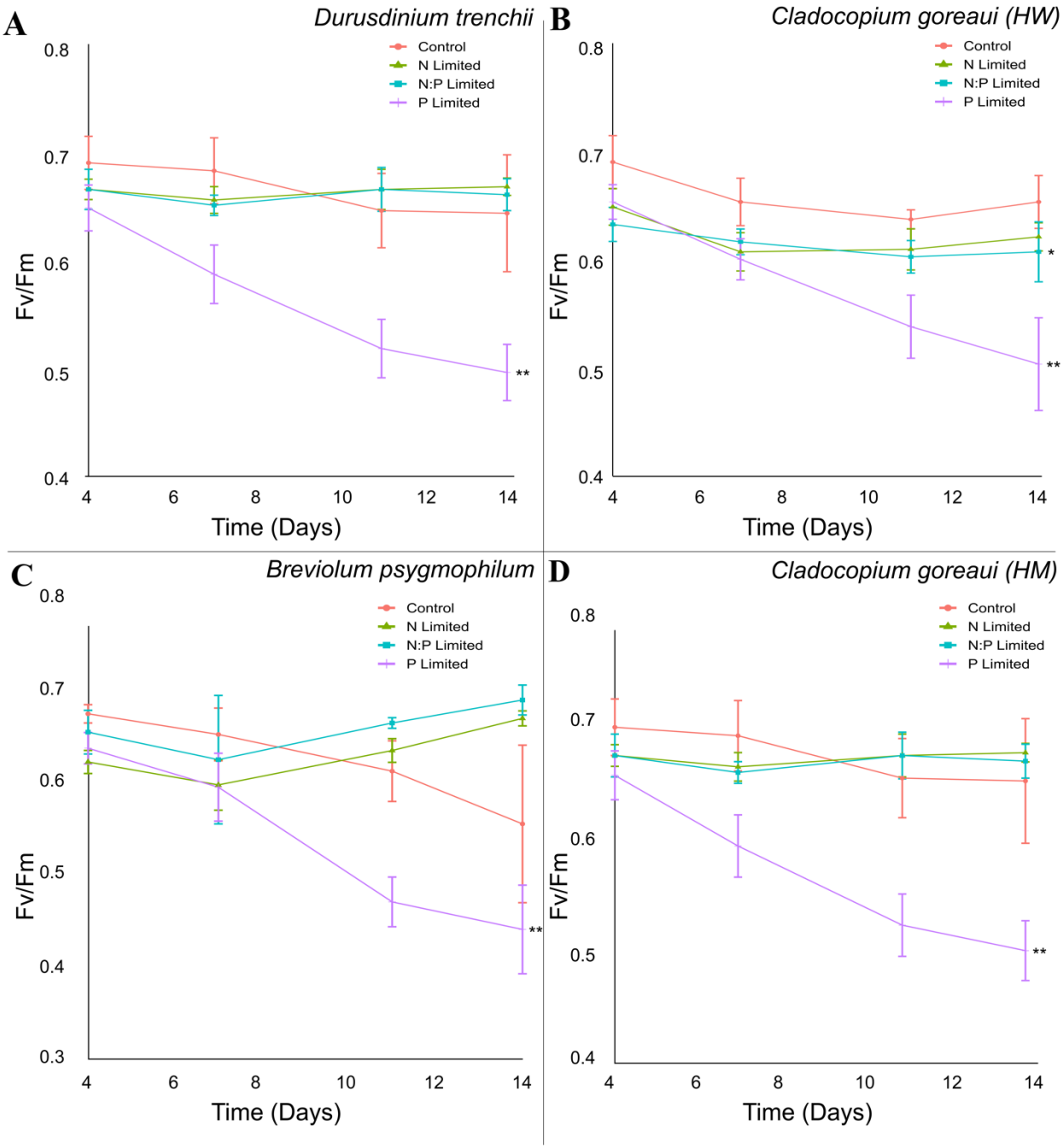

Figure 2.2 Symbiodiniaceae effective quantum yield of photosystem II under different nitrogen and phosphate concentrations. F_v/F_m (dimensionless) measurements of *Durusdinium trenchii* **(A)**, *Cladocopium goreaui*

HW **(B)**, *Breviolum psygmophilum* **(C)**, and *Cladocopium goreaui HM* **(D)** cultures grown in control nutrient concentrations, and in N- (green), P- (purple), and NP-limited (blue) media, taken every 3-4 days. Values are means and error bars are SEM, N=4 per treatment, per species. Repeated Measures ANOVA (RMANOVA) significance shown using *(P =<0.05) and **(P =<0.001). N=4 per treatment, per species.

Temperature Experiment:

All Symbiodiniaceae species exhibited similar trends in growth, with cultures grown at 16 °C and 31 °C displaying a reduced growth rate compared against cultures grown at 21 °C and 26 °C (Figure 2.3). At the lowest (16 °C) and highest (31 °C) temperatures, the exponential growth rate of *D. trenchii* cultures were on average 10% and 16% lower, respectively, compared to controls (Figure 2.3A; Supplementary Table S2.7, (P_{mc}= 0.001). This trend was also observed at 16°C and 31°C in *B. psygmophilum* with exponential growth rates 10% and 17% lower than control cultures (Figure 2.3C; Supplementary Table S2.7, (P_{mc}=0.002, (P_{mc}=0.001), and *C. goreaui (HW*), with exponential growth rates 6% and 10% lower than control cultures (Figure 2.3B; Supplementary Table S2.7, (P_{mc}=0.03, (P_{mc}=0.012). The growth rate of *C. goreaui (HM)* cultures grown at 31°C was 11% lower than that of control samples (Figure 3D; Supplementary Table S2.7, (P_{mc}=0.043), but those grown at 16˚C were not significantly different to controls. Meanwhile, *C. goreaui (HW)* grown at 21 °C had, on average, an exponential growth rate 8% higher than the control (Figure 2.3B; Supplementary Table S2.4, (P_{mc}=0.007).

Symbiodiniaceae cell size was also affected by high temperature (Figure 2.3E), with relative cell size in cultures grown at 31 °C approximately 13%, 25% and 26% larger, respectively, than control samples at the final timepoint in *D. trenchii*, *C. goreaui (HW)* and *C. goreaui (HM)* (Figure 2.3E; Supplementary Table S2.8, p-Tukey<0.001). *C. goreaui (HM)* at 26 °C also increased in size, although by only 0.45% (Figure 2.3E; Supplementary Table S2.8). The cell size of *B. psygmophilum* did not vary significantly across treatments, and there was no other significant difference in size across other treatments (Figure 2.3E; Supplementary Table S2.8). Effective quantum yield of PSII (F_v/F_m) appeared to be unaffected by high temperature after 14 days in all species except *C. goreaui (HM)*, which had significantly elevated F_v/F_m relative to the control for the 16 °C, 21 °C and 31 °C treatments at the final timepoint (Supplementary Figure S2.2D; ANOVA p=0.02, p<0.001, p=0.002). Although RMANOVA revealed large variances in F_v/F_m across the sample period for each culture, all species maintained effective quantum yield

values within what is considered a healthy range of Symbiodiniaceae (Supplementary Figure S2.2; Supplementary Table S2.9 (Carballo-Bolaños et al., 2019; McRae et al., 2023).

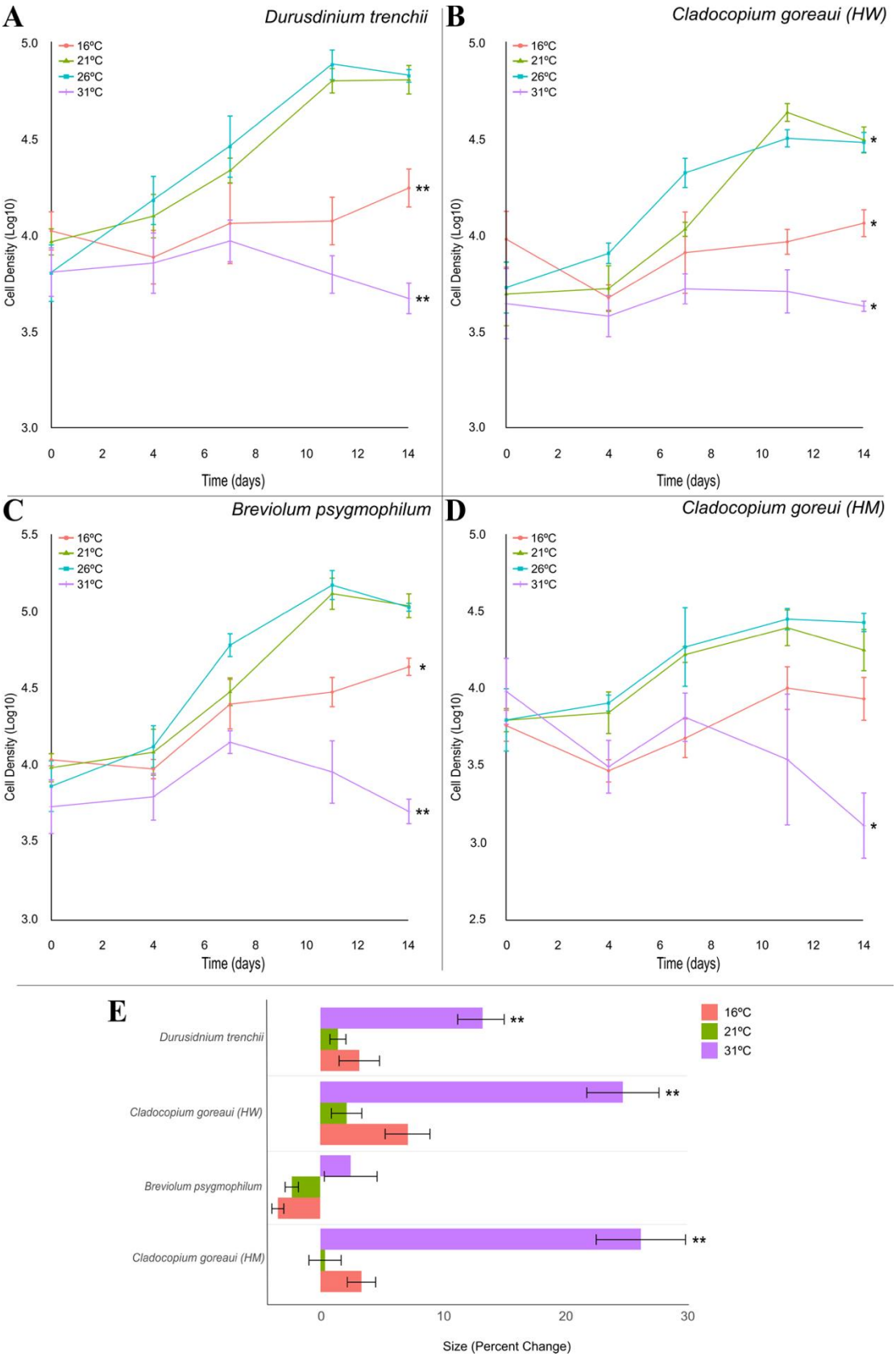
A
Durusdinium trenchii
B
Cladocopium goreaui (HW)
C
Breviolum psygmophilum
D
Cladocopium goreui (HM)
16ºC
21ºC
26ºC
31ºC
Cell Density (Log10)
Time (days)
E
Durusidnium trenchii
Cladocopium goreaui (HW)
Breviolum psygmophilum
Cladocopium goreaui (HM)
Size (Percent Change)

Figure 2.3 Symbiodiniaceae cell density and size under different temperatures. Symbiodiniaceae cell densities (ln) for *Durusdinium trenchii* (A), *Cladocopium goreaui HW* (B), *Breviolum psygmophilum* (C), and *Cladocopium goreaui HM* (D) across the sample period for each temperature treatment (16 °C (red), 21 °C (green), Controls; 26 °C (purple), 31 °C (blue). PERMANOVA permutational MANOVA (PERMANOVA) (P_{mc}=) significance shown using *(P_{mc}= =<0.05) and **(P_{mc}= =<0.001). (E) Variation in cell size for each Symbiodiniaceae across temperature treatments. One-way ANOVA and Tukeys post hoc significance shown using *(P=<0.05) and **(P=<0.001). Values are means and error bars are SEM. N=4 per treatment, per species.

2.4.2 Lipid diversity and abundance

Nutrient experiment:

Principal component analysis (PCA) revealed patterns in lipid abundance when Symbiodiniaceae were exposed to changes in nutrient concentration (Figure 2.4). For *D. trenchii*, the majority of the separation occurred across PC1, explaining 55.6% of the variance, and the greatest separation can be seen between P-Limited and N-Limited treatments, mainly driven by fatty acids (FA). N and NP-Limited samples clearly overlapped across both PC1 and PC2 (13.8%), with separation driven mainly by triacylglycerols (TG) (Figure 2.4A). Within *C. goreaui (HW)*, there was distinct separation on PC1 (35.4%), primarily occurring between control and NP-Limited treatments, whereas P and N-Limited cultures overlapped (Figure 4B). P- and N-Limited samples were also separated from C and NP-Limited cultures along PC2 (19.2%), with main drivers seemingly FA and hexosylceramides (HexCer) (Figure 2.4B). *B. psygmophilum* also followed a similar pattern to *C. goreaui (HW)* along PC1 (31.1%), with complete separation between control and NP-Limited cultures. P and N-Limited samples were also completely separated across PC2 (22%) (Figure 2.4C). Most of the separation in *C. goreaui (HM)* occurred between control and all nutrient treated samples across PC1 (39.2%), mainly driven by TG (Figure 2.4D). There was some overlap between N and NP-Limited samples, yet both showed distinct separation from P-Limited samples across PC1 (39.2%). Along PC2 (23.2%), there was separation between N and NP-Limited *C. goreaui (HM)* cultures (Figure 2.4D). In both *B. psygmophilum* and *C. goreaui (HM)* NP-Limited cultures, cirsiliol (4',5-dihydroxy-3',6,7-trimethoxy-flavone) was also a driver of separation from controls (Figures 2.4C, D).

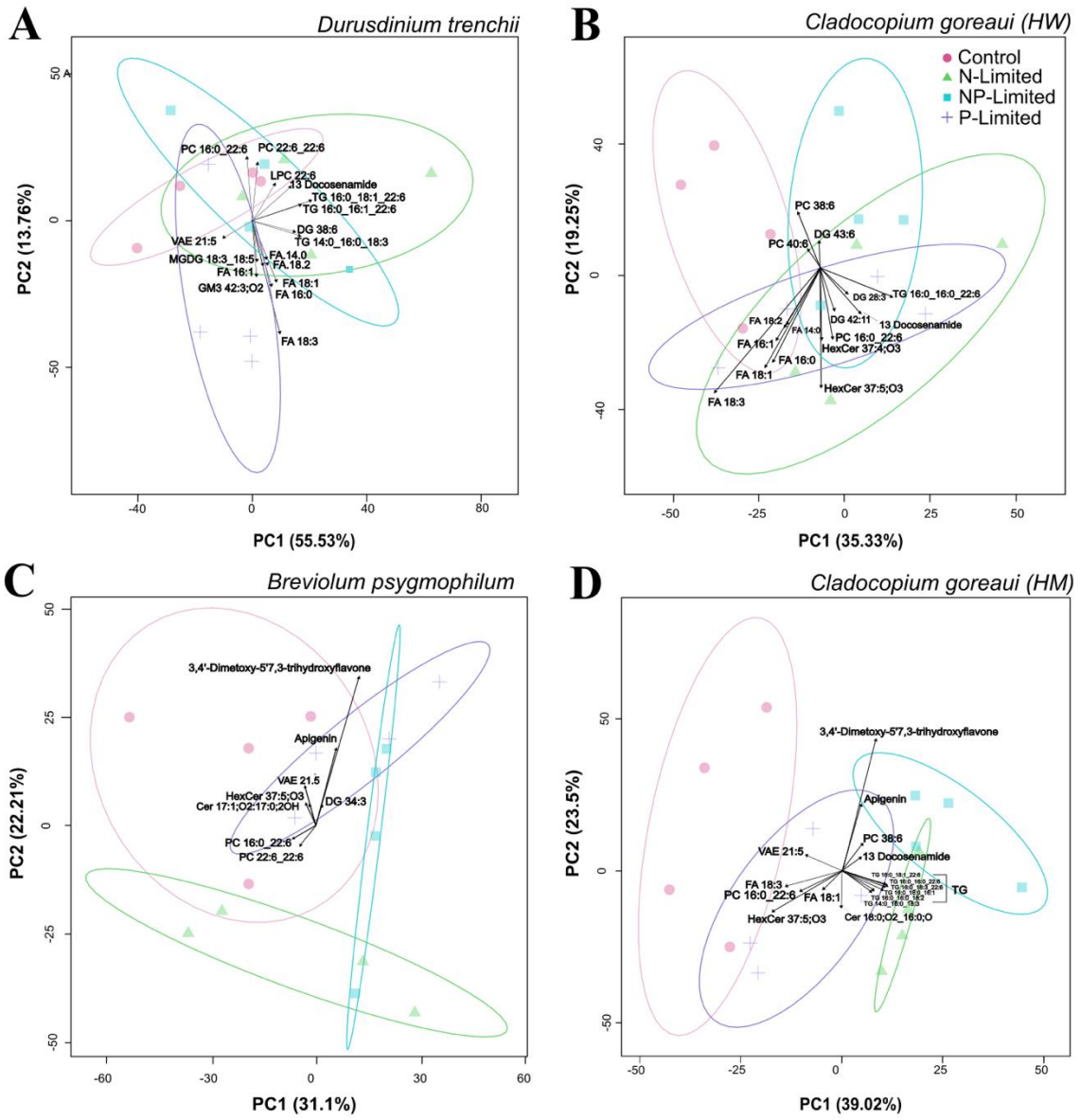

Figure 2.4 Principal Component Analysis with lipid sub-class biplot of lipid abundance under different nitrogen and phosphate concentrations. Separation of treatments based on lipid composition in *Durusdinium trenchii* **(A)**, *Cladocopium goreaui* HW **(B)**, *Breviolum psygmophilum* **(C)**, and *Cladocopium goreaui* HM **(D)** across the sample period for each nutrient treatment (control nutrient concentrations, and in N- (green), P- (purple), and NP-limited (blue) media). *N*=4 per treatment per species.

For *B. psygmophilum*, 4 lipid subclasses were significantly changed under N and NP-Limited conditions (vitamin-a fatty esters (VAE), digalactosyldiacylglycerol (DGDG), Ceramide and Hexosylceramide hydroxy fatty acid-sphingosine (Cer_HS, HerCer_HS)), which decreased in relative abundance from control conditions (Figure 2.5A; Supplementary Table S2.10, ANOVA

FDR<0.01). Under P and NP-Limited conditions PC decreased in relative abundance (Supplementary Table S2.10, ANOVA FDR<0.001) and n-acyl ornithine (NAOrn) changed significantly under nitrogen limited conditions (Figure 2.5A; Supplementary Table S2.10, ANOVA FDR=0.018). Within *C. goreaui (HM)*, 2 lipid subclasses changed significantly under N and NP-Limited treatments (DGDG, VAE) (Figure 2.5B; Supplementary Table S2.10, ANOVA FDR=0.01). Under NP and P-Limited cultures, phosphatidylcholine (PC) relative abundance decreased significantly (Supplementary Table S2.10, ANOVA FDR=0.009), and under all nutrient treated cultures, triacylglycerols (TG) relative abundance increased significantly against controls, but especially under N and NP-Limitation (Figure 2.25B; Supplementary Table S2.10, ANOVA FDR=0.01). There was no overall change in lipid subclasses between nutrient treatments in either *D. trenchii* or *C. goreaui (HW)*.

Individual lipid profiles revealed 11 specific lipids significantly changed in *D. trenchii* across nutrient treatments relative to controls, belonging to five sub-classes in total (Figure .6A). Across nutrient treatments, *C. goreaui (HW)* had 57 significant lipids from 10 different sub-classes (Figure 2.6B) and *B. psygmophilum* had 75 significant lipids from 14 differing sub-classes. *C. goreaui (HM)* had the highest variation in lipid composition when grown under changes in nutrient composition with a total of 142 significant lipids from 21 different sub-classes (Figures 2.6C, D; Supplementary Table S2.6). Of these, 39% [22/57; *C. goreaui (HW)*], 42% [60/142; *C. goreaui (HM)*] and 28% (21/75; *B. psygmophilum*) belong to the TG subclass of lipids, all of which were higher in relative abundance under N and NP-limited treatments. Meanwhile, lipids belonging to the diacylglycerol (DG) subclass showed the opposite, with a decrease in relative abundance across all N- and NP- limited Symbiodiniaceae cultures relative to controls and represented 18% [10/57; *C. goreaui (HW)*], 17% [24/142; *C. goreaui (HM)*] and 15% (11/75; *B. psygmophilum*) of significantly different lipids. No TG or DG lipids were significantly different in the nutrient limited *D. trenchii* cultures. VAE lipids appeared in all four Symbiodiniaceae species and were commonly higher in relative abundance in P-limited cultures, relative to controls. There was no clear trend in the saturated to unsaturated fatty acid ratio (SFA/UFA) within *D. trenchii* and *B. psygmophilum*, but all nutrient treatments within both *C. goreaui* spp. exhibited slightly higher SFA/UFA, indicating a higher proportion of SFA (Supplementary Table S2.11). In addition, SFA relative abundance was increased in NP-Limited treatments of *C.*

goreaui (HM), and P-Limited *B. psygmophilum* treatments (Supplementary Table S2.11). Of all significant lipids, VAEs accounted for 36% (4/11; *D. trenchii*), 21% [12/57; *C. goreaui (HW)*], 11% [16/142; *C. goreaui (HM)*] and 21% (16/75; *B. psygmophilum*) of significantly different lipids. A total of 9 lipids (DGDG39:10, DGDG19:5_20:5, DGDG 40:9|DGDG18:3_22:6, DGDG42:11|DGDG20:5_22:6, PC36:6, PC44:12|PC22:6_22:6, VAE18:5, VAE20:4, VAE22:4 and VAE24:6) from 3 sub-classes (DGDG, PC and VAE) were found to be significantly different between treatments across all four Symbiodiniaceae species (Figure 2.5; FDR<0.05). Total lipid profiles were found to have undergone significant alterations from controls in *C. goreaui (HM), C. goreaui (HW)* and *B. psygmophilum* (P_{mc}=0.001, P_{mc}=0.011, P_{mc}=0.006), whereas nutrient stress was not shown to similarly influence the total lipid profile in *D. trenchii* (P_{mc}=0.083).

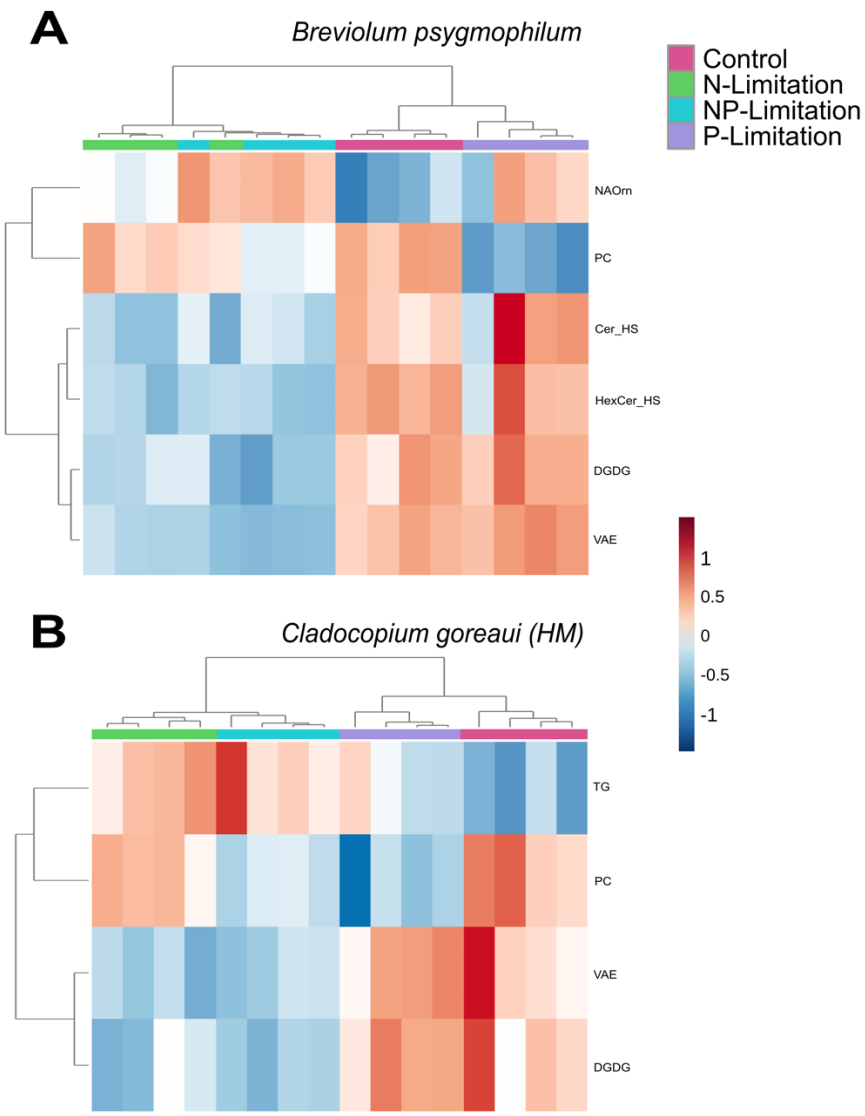

Figure 2.5 Symbiodiniaceae lipid sub-class relative abundance heatmap under different nutrient concentrations as determined by an ANOVA FDR with Fishers pos hoc in *Breviolum psygmophilum* **(A)** and *Cladocopium goreaui HM* **(B)** across the sample period for each nutrient treatment (control nutrient concentrations, and in N- (green), P- (purple), and NP-limited (blue) media). *N*=4 per treatment per species. Significant lipid sub-classes inclusive of ceramide hydroxy fatty acid-sphingosine, (Cer), hexosylceramide hydroxyfatty acid-sphingosine (HexCer), vitamin A fatty acid ester (VAE), digalactosyldiacylglycerol (DGDG), phosphatidylcholine (PC), n-acyl ornithine (NAOrn) and triacylglycerol (TG).

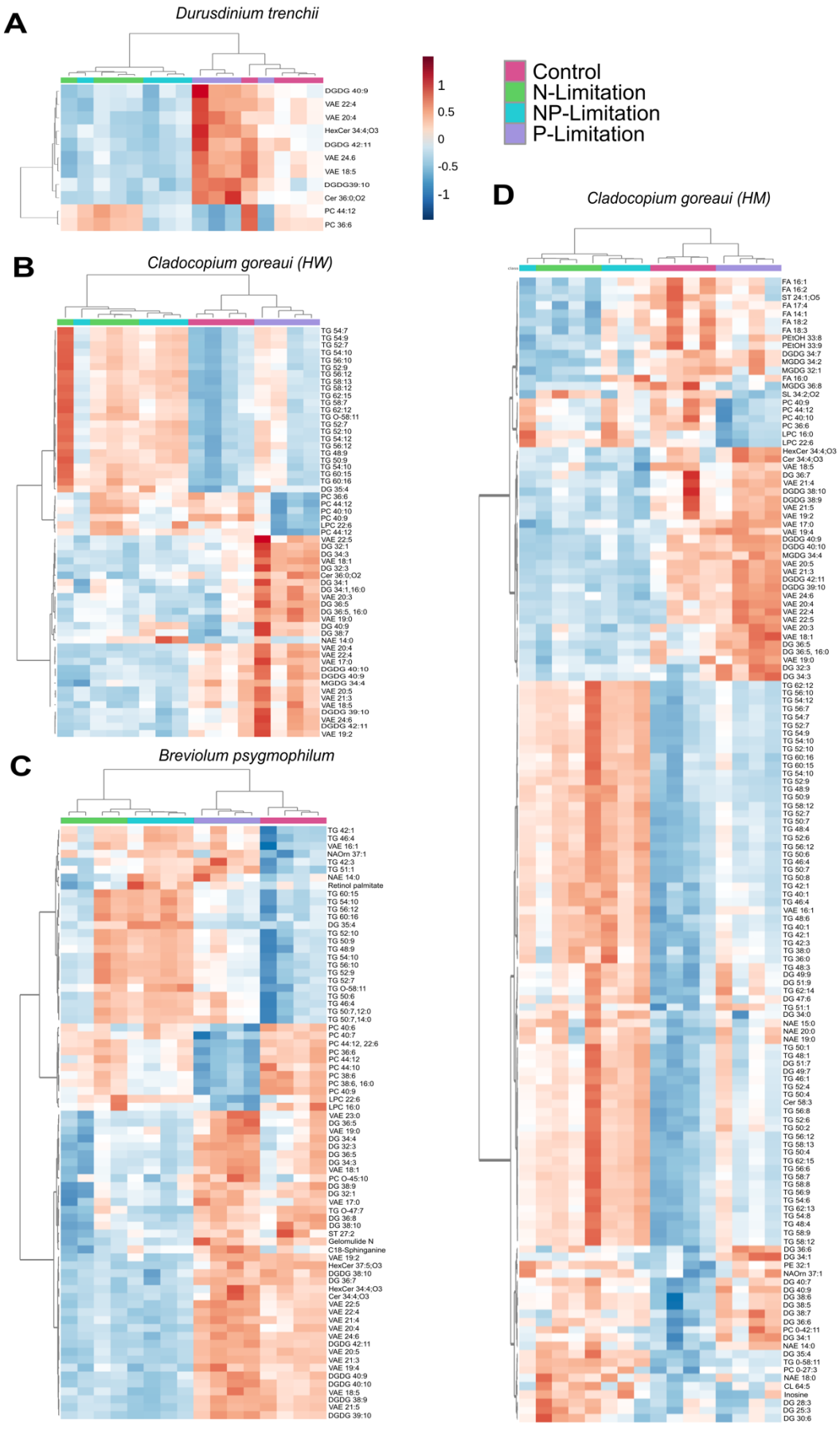
A
Durusdinium trenchii
DGDG 40:9
VAE 22:4
VAE 20:4
HexCer 34:4;O3
DGDG 42:11
VAE 24:6
VAE 18:5
DGDG39:10
Cer 36:0;O2
PC 44:12
PC 36:6
1
0.5
0
-0.5
-1
Control
N-Limitation
NP-Limitation
P-Limitation
B
Cladocopium goreaui (HW)
TG 54:7
TG 54:9
TG 52:7
TG 54:10
TG 56:10
TG 52:9
TG 56:12
TG 58:13
TG 58:12
TG 62:15
TG 58:7
TG 62:12
TG O-58:11
TG 52:7
TG 52:10
TG 54:12
TG 56:12
TG 48:9
TG 50:9
TG 54:10
TG 60:15
TG 60:16
DG 35:4
PC 36:6
PC 44:12
PC 40:10
PC 40:9
LPC 22:6
PC 44:12
VAE 22:5
DG 32:1
DG 34:3
VAE 18:1
DG 32:3
Cer 36:0;O2
DG 34:1
DG 34:1,16:0
VAE 20:3
DG 36:5
DG 36:5, 16:0
VAE 19:0
DG 40:9
DG 38:7
NAE 14:0
VAE 20:4
VAE 22:4
VAE 17:0
DGDG 40:10
DGDG 40:9
MGDG 34:4
VAE 20:5
VAE 21:3
VAE 18:5
DGDG 39:10
VAE 24:6
DGDG 42:11
VAE 19:2
C
Breviolum psygmophilum
TG 42:1
TG 46:4
VAE 16:1
NAOm 37:1
TG 42:3
TG 51:1
NAE 14:0
Retinol palmitate
TG 60:15
TG 54:10
TG 56:12
TG 60:16
DG 35:4
TG 52:10
TG 50:9
TG 48:9
TG 54:10
TG 56:10
TG 52:9
TG 52:7
TG O-58:11
TG 50:6
TG 46:4
TG 50:7,12:0
TG 50:7,14:0
PC 40:6
PC 40:7
PC 44:12, 22:6
PC 36:6
PC 44:12
PC 44:10
PC 38:6
PC 38:6, 16:0
PC 40:9
LPC 22:6
LPC 16:0
VAE 23:0
DG 36:5
VAE 19:0
DG 34:4
DG 32:3
DG 36:5
DG 34:3
VAE 18:1
PC O-45:10
DG 38:9
DG 32:1
VAE 17:0
TG O-47:7
DG 36:8
DG 38:10
ST 27:2
Gelomulide N
C18-Sphinganine
VAE 19:2
HexCer 37:5;O3
DGDG 38:10
DG 36:7
HexCer 34:4;O3
Cer 34:4;O3
VAE 22:5
VAE 22:4
VAE 21:4
VAE 20:4
VAE 24:6
DGDG 42:11
VAE 20:5
VAE 21:3
VAE 19:4
DGDG 40:9
DGDG 40:10
VAE 18:5
DGDG 38:9
VAE 21:5
DGDG 39:10
D
Cladocopium goreaui (HM)
FA 16:1
FA 16:2
ST 24:1;O5
FA 17:4
FA 14:1
FA 18:2
FA 18:3
PEtOH 33:8
PEtOH 33:9
DGDG 34:7
MGDG 34:2
MGDG 32:1
FA 16:0
MGDG 36:8
SL 34:2;O2
PC 40:9
PC 44:12
PC 40:10
PC 36:6
LPC 16:0
LPC 22:6
HexCer 34:4;O3
Cer 34:4;O3
VAE 18:5
DG 36:7
VAE 21:4
DGDG 38:10
DGDG 38:9
VAE 21:5
VAE 19:2
VAE 17:0
VAE 19:4
DGDG 40:9
DGDG 40:10
MGDG 34:4
VAE 20:5
VAE 21:3
DGDG 42:11
DGDG 39:10
VAE 24:6
VAE 20:4
VAE 22:4
VAE 22:5
VAE 20:3
VAE 18:1
DG 36:5
DG 36:5, 16:0
VAE 19:0
DG 32:3
DG 34:3
TG 62:12
TG 56:10
TG 54:12
TG 56:7
TG 54:7
TG 52:7
TG 54:9
TG 54:10
TG 52:10
TG 60:16
TG 60:15
TG 54:10
TG 52:9
TG 48:9
TG 50:9
TG 58:12
TG 52:7
TG 50:7
TG 48:4
TG 52:6
TG 56:12
TG 50:6
TG 46:4
TG 50:7
TG 50:8
TG 42:1
TG 40:1
TG 46:4
VAE 16:1
TG 48:6
TG 40:1
TG 42:1
TG 42:3
TG 38:0
TG 36:0
TG 48:3
DG 49:9
DG 51:9
TG 62:14
DG 47:6
TG 51:1
DG 34:0
NAE 15:0
NAE 20:0
NAE 19:0
TG 50:1
TG 48:1
DG 51:7
DG 49:7
TG 46:1
TG 52:4
TG 50:4
Cer 58:3
TG 56:8
TG 52:6
TG 50:2
TG 56:12
TG 58:13
TG 50:4
TG 62:15
TG 56:6
TG 58:7
TG 58:8
TG 56:9
TG 54:6
TG 62:13
TG 54:8
TG 48:4
TG 58:9
TG 58:12
DG 36:6
DG 34:1
PE 32:1
NAOm 37:1
DG 40:7
DG 40:9
DG 38:6
DG 38:5
DG 38:7
DG 36:6
PC O-42:11
DG 34:1
NAE 14:0
DG 35:4
TG O-58:11
PC O-27:3
NAE 18:0
CL 64:5
Inosine
DG 28:3
DG 25:3
DG 30:6

Figure 2.6 Symbiodiniaceae lipid accumulation and relative abundance heatmap under different nutrient concentrations as determined by an ANOVA FDR with Fishers *post hoc* in *Durusdinium trenchii* **(A)**, *Cladocopium goreaui* HW **(B)**, *Breviolum psygmophilum* **(C)**, and *Cladocopium goreaui* HM **(D)** across the sample period for each nutrient treatment (control nutrient concentrations, and in N- (green), P- (purple), and NP-limited (blue) media). $N = 4$ per treatment per species.

Temperature experiment:

For *D. trenchii*, the majority of individual lipid separation occurred on PC1, with 71.8% between the control (26 °C), and both temperature extremes (31 °C and 16 °C). Separation in 31°C samples were mainly driven by sphingomyelin (SM), cholesterol (ST) and n-arachidonoyl taurine (NATau), whereas the main driver in separation for 16 °C samples was ceramide hydroxy fatty acid-sphingosine (Cer-HS). There was strong overlap between samples grown under control temperatures and 21 °C (Figure 2.7A). For *C. goreaui (HW)*, separation was more obvious along PC2 (24.7%), where samples grown at 31 °C were distinctly separated from all other cultures, seen to be similarly driven by ST, and NATau, but also phosphatidylcholine (PC) and the fatty amide; docosenamide. There was also slight separation between the 26 °C and 21 °C samples, and the 16°C samples across PC1 (60.5%) (Figure 2.7B). There was clear separation (PC1; 74.1%), between *B. psygmophilum* cultures grown at 31 °C versus 21 °C and 26 °C, again mainly driven by SM, docosenamide and NATau, and cultures grown at 16°C overlapping all treatments. There was also distinct separation between 21 °C and 26 °C (PC2; 16.7%) for *B. psygmophilum*, which was not observed in the other Symbiodiniaceae species (Figure 2.7C). *C. goreaui (HM)* revealed separation between 31°C and all other cultures along PC1 (75.8%), with SM, ST, docosenamide and NATau again driving this change, while 16 °C was also separated from 26 °C and 21 °C along PC2 (14%) (Figure 2.7D). Separation of 16 °C treatments across all species seemed to be driven by the lack of any specific lipid abundance, grouping closely with control and 21 °C treatments.

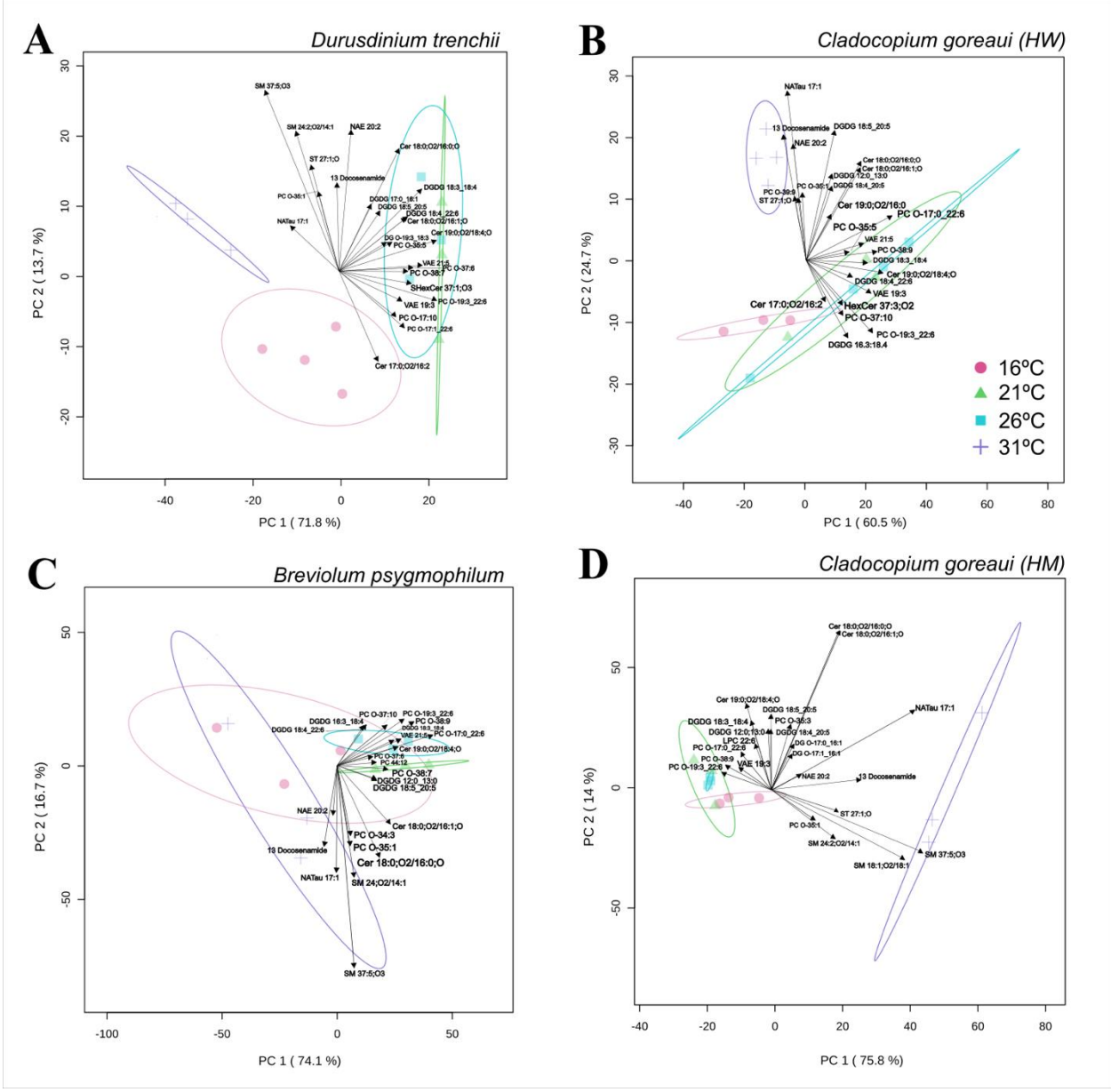

Figure 2.7 Principal Component Analysis with lipid sub-class biplot of lipid abundance under different temperatures. Separation of treatments based on lipid composition in *Durusdinium trenchii* **(A)**, *Cladocopium goreaui* HW **(B)**, *Breviolum psygmophilum* **(C)**, and *Cladocopium goreaui* HM **(D)** across the sample period for each temperature treatment [control temperature 26 °C; (purple), 16 °C (red), 21 °C (green), and 31 °C (blue)]. *N* varied between 3-4 per treatment per species.

Within *D. trenchii*, 56 individual lipids were significantly differently from control temperatures from 23 different subclasses (Figure 2.8A). *C. goreaui (HW)* had 63 significant lipids from 21 different subclasses, *B. psygmophilum* had 36 significant lipids from 17 subclasses, and *C.*

goreaui (HM) had a total of 55 lipids differing significantly from control samples from a total of 21 sub-classes (Figure 2.8). Across all four Symbiodiniaceae, there were 6 lipids across 5 subclasses that significantly differed between treatments (CL 74:6|CL15:0:24:0_16:1_19:5, LPE O-20:5, NAGly 21:0;O, PC O-37:6, PC. O-41:9|PC O19:3:22:6, VAE22:4). Cardiolipins (CL) increased in relative abundance in all 31 °C treatments, as did lysophosphatidylethanolamine (LPE), and n-acyl glycine (NAGly) (Supplementary Table S2.7, ANOVA FDR=<0.025). Ether-linked phosphatidylcholine (Ether-PC) and VAE decreased in relative abundance in both 16 °C and 31°C treatments. There was also an increased presence of oxidised lipids, such as oxidised phosphatidylinositol (OxPI) and oxidised fatty acids (OxFA) across all 31°C treatments, and, within both *C. goreaui* species, an increased relative abundance of ether-linked phospholipids (Ether-PC). The SFA/UFA ratio increased from controls within all Symbiodiniaceae species grown under 31 °C as well as 16° C, whereas controls and 21 °C were mostly similar (Supplementary Table S2.12). Additionally, there was a 61%, 69%, 74%, and 23% decrease in ether-linked lipids within *D. trenchii*, *C. goreaui (HW)*, *B. psygmophilum*, and *C. goreaui (HM)* treatments 16 °C, respectively, and a 77%, 55%, 83% and 18% decrease within the same Symbiodiniaceae under 31 °C treatments. Both *C. goreaui* spp. saw a slight increase in ether-linked lipids at 21 °C, whereas *D. trenchii* and *B. psygmophilum* exhibited the inverse.

When grouped into sub-classes, *D. trenchii* showed significant differences in 21 lipid subclasses, *C. goreaui (HW)* and (HM) showed 10, and *B. psygmophilum* 12 (Figure 2.9; Supplementary Table S2.13). Most lipid subclasses followed similar patterns across all Symbiodiniaceae, including NAGly and PG which were relatively more abundant in 31°C treatments (Figure 2.9; Supplementary Table S2.13). In all species except *B. psygmophilum*, DG relative abundance was also significantly increased in 31 °C treatments, whereas uniquely, *B. psygmophilum*, exhibited an increase in ceramide beta-hydroxy fatty acid-sphingosine (Cer-BS) as temperatures increased, with a low concentration in 16 °C and 21 °C samples, and increased in controlled (26 °C) and 31 °C treatments (Figure 2.9; Supplementary Table S2.13, FDR< 0.01). *C. goreaui (HM)* also exhibited a unique pattern, with highest relative abundances of PC at 16 °C, which showed an inverse relationship with temperature (Figure 2.9; Supplementary Table S2.13, FDR< 0.01). Total lipid profiles were found to have undergone significant alterations from controls in *C. goreaui (HM), C. goreaui (HW)* and *D. trenchii* (P_{mc}=0.001, P_{mc}=0.009, P_{mc}=0.001), whereas

nutrient stress was not shown to similarly influence the total lipid profile in *B. psygmophilum* (P_{mc}=0.093).

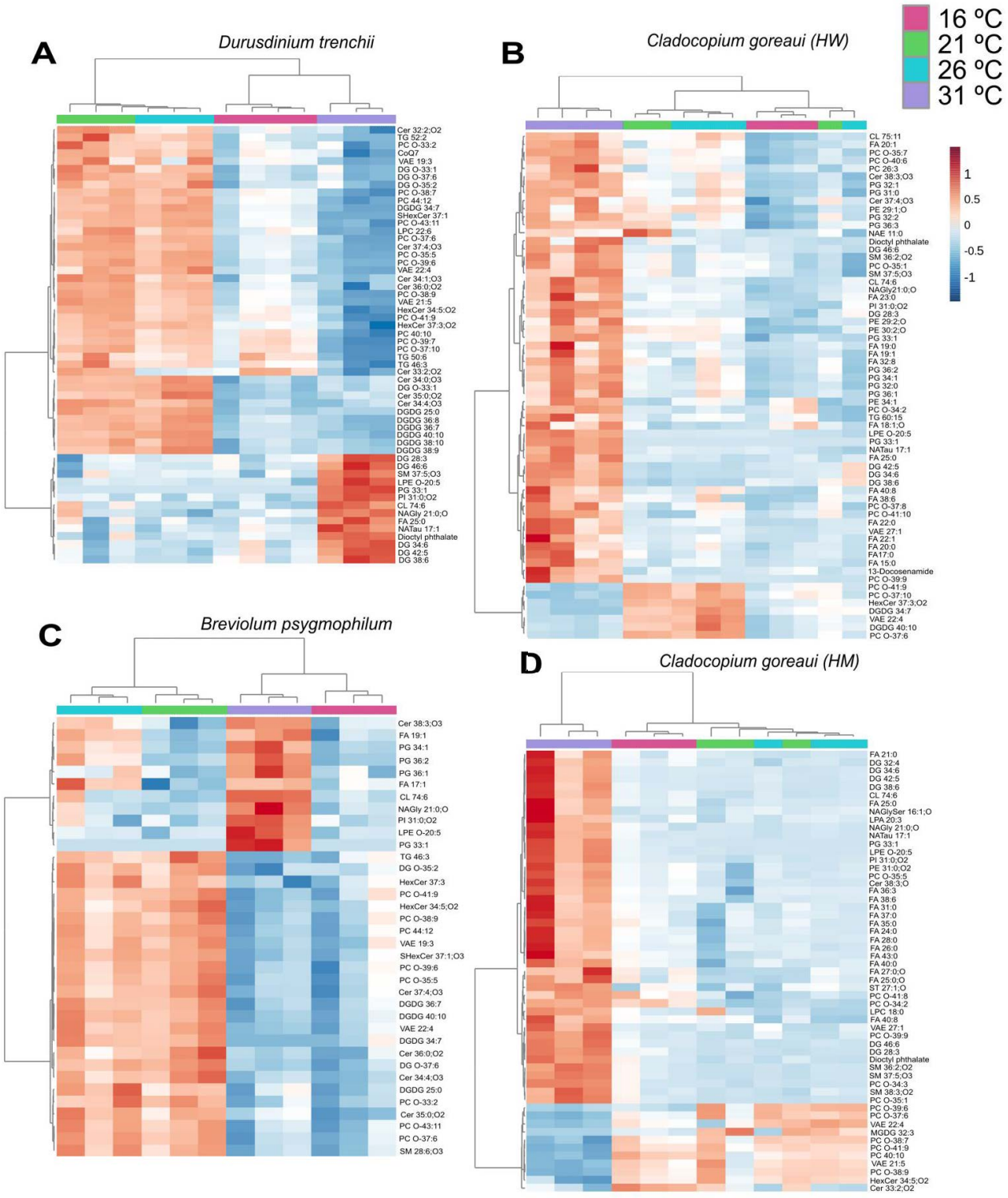

Figure 2.8 Symbiodiniaceae lipid accumulation and relative abundance heatmap under different temperatures as determined by an ANOVA FDR with Fishers *post hoc* in *Durusdinium trenchii* **(A)**, *Cladocopium*

goreaui HW **(B)**, *Breviolum psygmophilum* **(C)**, and *Cladocopium goreaui* HM **(D)** across the sample period for each temperature treatment [control temperature (26 °C; purple), 16 °C (red), 21 °C (green), and 31 °C (blue)]. *N* varied between 3-4 per treatment per species.

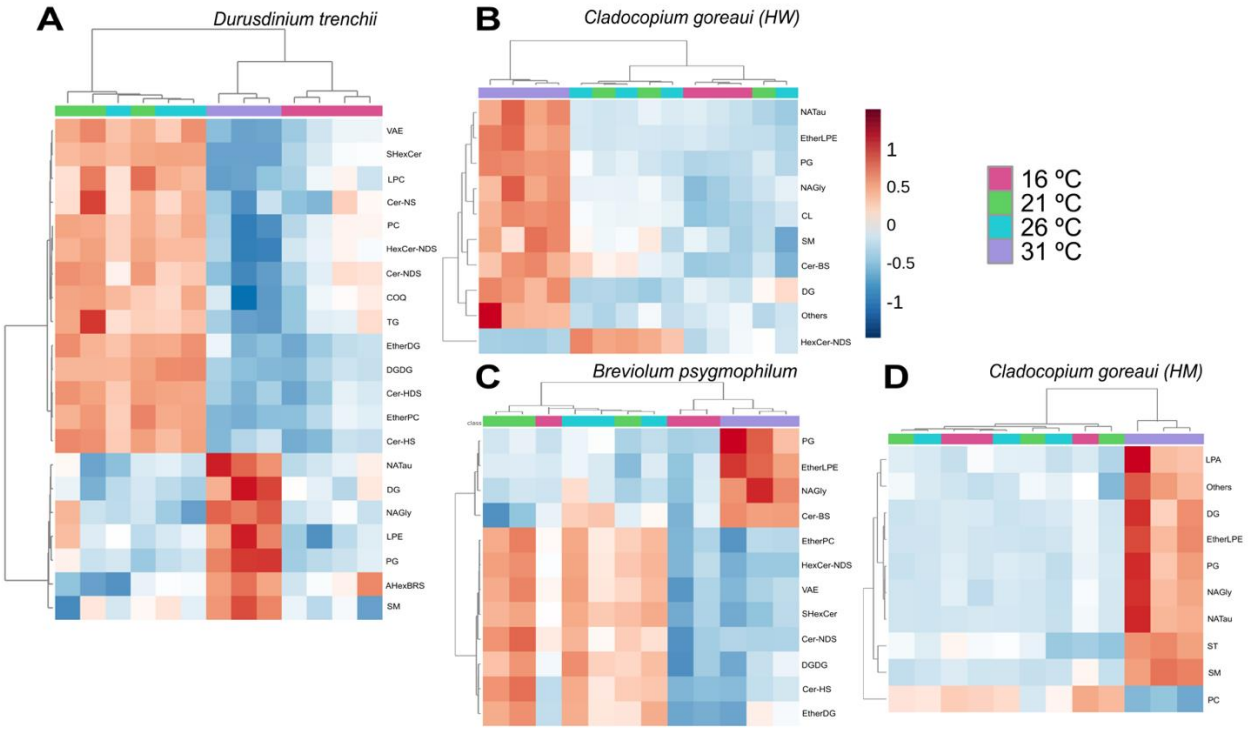

Figure 2.9 Symbiodiniaceae lipid sub-class abundance heatmap under different temperatures as determined by an ANOVA FDR with Fishers *post hoc* in *Durusdinium trenchii* **(A)**, *Cladocopium goreaui* HW **(B)**, *Breviolum psygmophilum* **(C)**, and *Cladocopium goreaui* HM **(D)** across the sample period for each temperature treatment [control temperature (26 °C; purple), 16 °C (red), 21 °C (green), and 31 °C (blue)]. *N* varied between 3-4 per treatment per species.

2.5 Discussion

Microalgae can remodel their lipid composition in response to changes in temperature and nutrient concentration (Fakhry & El Maghraby, 2015; Liu et al., 2022). The results here suggest Symbiodiniaceae are similarly able to remodel their lipids, including shifting from the biosynthesis of phospholipids to cell membrane-stabilizing isoprenoids in phosphorus limited conditions, increased storage of energy-rich triacylglycerols under nitrogen limitation, and an increase in saturated fatty acid abundance and phosphatidylinositol oxidation under high

temperatures. This raises an intriguing possibility that Symbiodiniaceae lipid plasticity, and in turn what is being translocated to the coral host when *in hospite*, could determine the resilience of coral-Symbiodiniaceae associations.

Phosphorus limitation induced a significant reduction in the relative abundance of phosphatidylcholine (PC) lipids exhibited by all Symbiodiniaceae cultures grown in P-limited and NP-limited media (Figure 2.6). PC are the most abundant lipid class of cell membranes, and as such, a reduction in relative abundance can lead to a change in the composition and structure of cellular membranes (Kanno et al., 2007). It has been well established that phosphorus is a limiting nutrient in marine environments (Correll, 1999). For instance, in other microalgae, when phosphorus becomes less available for uptake, membranes biosynthesis shifts from phospholipid headgroups to non-phosphorus headgroups, such as diacylglycerolcarboxyhydroxymethylcholine, (DGCC); and sulfoquinovosyldiacylglycerol (SQDG) (Lowenstein et al., 2021) and betaine lipids (Martin et al., 2011; Van Mooy et al., 2009). In Symbiodiniaceae *in hospite*, imbalances in the N:P ratio resulted in shifts in the phosphatidylglycerol:sulphoquinovosyldiacylglycerol (PG:SQDG) ratio (Wiedenmann et al., 2013). By reducing the biosynthesis of phosphorus-containing lipids in membranes, remaining nutrients are able to be conserved for essential cellular functions (Dörmann & Benning, 2002). Phosphorus is a major component of DNA, and thus essential for reproduction, the transmission of chemical energy (ATP) within the cell, and photosynthesis (Brembu et al., 2017; Li et al., 2016). Phosphorus limitation can affect photosynthetic capacity by suppressing photosynthetic efficiency in PSII, as reported for diatoms [e.g., *Thalassirosira weissflogii* (Liu et al., 2011)], and green algae [e.g., *Dunaliella tertiolecta* (Geider et al., 1998)]. While cell density remained unchanged across all Symbiodiniaceae cultures in this study, photosynthetic capacity declined, suggesting a possible diversion of phosphorus from photosynthesis to central metabolism and cell replication, as evidenced by the stable growth rates.

Coinciding with the decrease in PC concentrations in P-Limited cultures, vitamin-a fatty esters (VAE), a type of isoprenol lipid, increased under phosphorus limitation in all Symbiodiniaceae (Figure 2.6), and as a class significantly increased in *B. psygmophilum* and *C. goreaui (HM)* cultures (Figure 2.6; Supplementary Table S2.10). Isoprenoids have been shown to be important

components of membrane biosynthesis within algae and cyanobacteria (Lohr et al., 2012; Pattanaik & Lindberg, 2015) and are involved in human cell membrane structure and function (Dingle & Lucy, 1965). Isoprenes are derived from isoprenoids and are thought to possess antioxidant capabilities (Loreto et al., 2001) and stabilise plant cell membranes under stress (Fares et al., 2008). Isoprenoid biosynthesis requires high amounts of phosphorylated intermediates, but decreasing extracellular phosphate has been linked to the diversion of phosphate to isoprenoid synthesis to stabilise membranes (Jordan et al., 2019; Wadhwa et al., 2018). The increase of VAE coinciding with the decrease in PC may therefore indicate the diversion of phosphorus from phospholipid to isoprenoid synthesis to support cell viability under phosphorus deprivation in Symbiodiniaceae. However, whether such potential redirection in pathway activity is involved in either maintaining cell homeostasis, or is a physiological consequence to phosphorous stress, is unclear. Additionally, within *B. psygmophilum* and *C. goreaui (HM)* cultures, the polyphenol, cirsiliol, was shown to be a driver of separation in NP-Limited cultures (Figures 2.4C, D). Cirsiliol has been found to have several biological functions, notably including antioxidative capabilities within plants (Carlini et al., 2022). However, due to high sample variability within treatments, cirsiliol relative abundance was not detected as significant.

This is therefore a potential lipid remodeling strategy to maintain Symbiodiniaceae populations despite low phosphate levels. Furthermore, phosphorus reduction has been shown to inhibit cell division, but not cell growth for the phytoplankton, *Emiliania huxleyi* (Romano et al., 2015) and dinoflagellate *Amphidinium carterae* (Li et al., 2016). This is further reflected in the increase in cell size and stability in growth rate observed for P-limited Symbiodiniaceae cultures tested here. As a subclass, isoprenoids (VAE) significantly increased for NP- and P-limited *B. psygmophilum* and *C. goreaui (HM)* cultures (Figure 2.6; Supplementary Table S2.10), but not for *D. trenchii* or *C. goreaui (HW)* cultures. Although individual VAEs were significantly increased in these cultures, encompassing 43% of significant P-limited lipids in *D. trenchii* (Figure 2.6A), 20% in *C. goreaui (HW)* (Figure 6B), 21% of significant P-limited lipids in *B. psygmophilum* (Figure 2.6C), and 11% significant P-limited lipids in *C. goreaui (HM)* (Figure 5). To date, comparative analysis of different Symbiodiniaceae species under phosphorus stress is not well understood, but as *Breviolum* sp. and *C. goreaui (HM)* are known to be more susceptible to thermal stress

than *D. trenchii* or *C. goreaui (HW)* (Dang et al., 2019; Russnak et al., 2021), temperature stress tolerance mechanisms may also extend to nutrient stress. It is possible that the increase in the isoprenoid class of lipids in stress sensitive species is a mechanism to stabilise cell membranes under the increased physiological stress in these cultures - a cellular response not experienced by *D. trenchii* or *C. goreaui (HW)*. Indeed, cultures of the relatively 'stress tolerant' species *D. trenchii* had the highest growth rate under phosphorus limitation and cell sizes similar to the control cultures, suggesting no morphological stress response, however, lipid remodeling was observed indicating a response to phosphorous stress (Figure 2.1A; Supplementary Table S2.4, Supplementary Table S2.5).

Glycerolipids were found to be increasingly abundant in all nutrient limited cultures. Glycerolipids encompass a range of lipids, including monoacylglycerol (MG), DG, and TG, and serve essential roles in cellular signalling and as precursors to other biological active molecules (mainly MG and DG) and energy storage (mainly TG). Except for. *D. trenchii*, TGs encompassed 28-42% of significant lipids within each Symbiodiniaceae species (Figure 2.6), in which they were consistently more abundant in N-limited cultures. An increase in TG response under N limitation has been well documented in microalgae (Fakhry & El Maghraby, 2015), and is hypothesised to provide increased energy stores to support cells experiencing nutrient stress (Hu et al., 2008). Indeed, Symbiodiniaceae are thought to be well adapted to N starvation and can divert resources from growth to maintaining functional photophysiology (Rosset et al., 2017). Research within other organisms have also suggested that accumulation of TG may serve an important function in the stress response, aiding in the prevention of lipotoxicity from increased abundances of specific lipids, such as DG, observed within plants (Lu et al., 2020) and yeast (Kurat et al., 2006). These results suggest a similar function may occur within Symbiodiniaceae. Furthermore, increases in TG abundance has been linked to the capacity to maintain photosynthetic activity under nitrogen limitation (Fattore et al., 2021). Indeed, photophysiology in N-Limited cultures remained stable throughout the 14-day period across all Symbiodiniaceae species whereas P-Limited cultures exhibited greatly reduced F_v/F_m. Conversely, P-limitation increased the abundance of DGs relative to the controls (Figure 2.6). Under phosphorus deprivation, increases in DG accumulation within plant cells have been shown to coincide with a decrease in PC, hypothesised to be due to hydrolysis of PC within the

phosphatidylcholine-specific phospholipase C (PC-PLC) pathway under nutrient stress (Cocco et al., 2015; Jouhet et al., 2003). The same could be occurring within Symbiodiniaceae. Glycerolipids in the thylakoid membranes are characterised by the presence of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG), and the absence of PC (Reue & Brindley, 2008; Tchernov et al., 2004). Nitrogen limitation resulted in a decrease in the relative abundance of specific DGDGs in all Symbiodiniaceae cultures, total DGDGs in *C. goreaui (HM)* and *B. psygmophilum*, and specific MGDGs in *C. goreaui (HW)* and (HM) (Figures 2.5, 2.6, Supplementary Table S2.10). Glyceroglycolipids are also important in cell membranes (Kalisch et al., 2016), and may therefore reflect the reduced cell size observed across N and NP-limited samples across *D. trenchii*, *B. psygmophilum* and *C. goreaui (HM)*(Figure 2.1; Supplementary Table S2.5, $p<0.028$). Similar decreases in cell size were observed within the diatom *Stephanodiscus minutulus* (Lynn et al., 2000) and other microalgal species (Jia et al., 2015; Latsos et al., 2020). Meanwhile, the relative abundance of DGDG and MGDG lipids increased under P limitation (Figure 2.6). High concentrations of DGDG and MGDG can support photosynthetic growth under phosphorus limitation (Awai et al., 2007), which corroborates our results as growth rates of P-limited cultures were not reduced in all cultures bar *C. goreaui (HM)* (Figure 2.1, Supplementary Table S2.4).

Alterations to temperature was also shown to induce lipid remodeling within Symbiodiniaceae. Such remodeling may have assisted culture survival over the 14 days, despite an increase in the abundance of oxidised lipids, and significantly reduced growth rate across all Symbiodiniaceae cultures. Across all Symbiodiniaceae, fatty acids (FA) increased in cultures grown at 31°C, of which a higher proportion of SFA:UFA compared to controls were found in all Symbiodiniaceae species (Figure 2.8; Supplementary Table S2.12), supporting previous findings for *D. trenchii* and *Cladocopium* sp. (Rosset et al., 2019). Temperature increase leads to elevated abundance of reactive oxygen species (ROS) in microalgae, which, if it exceeds the antioxidant capacity, can induce oxidative stress, lipotoxicity and cell mortality (Verma et al., 2021). Unsaturated fatty acids are more susceptible to ROS attack than saturated fatty acids (Bacellar & Baptista, 2019), therefore, increased saturation of membrane-bound fatty acids can serve to maintain microalgal cell stability during heat stress (Los & Murata, 2004). ROS accumulation has been shown with

increased temperature in *Cladocopium* sp. (McGinty et al., 2012) and *D. trenchii* (Scharfenstein et al., 2023), and higher abundance of ROS has also been linked to increases in cell size (Amario et al., 2023; Lima et al., 2022). Indeed, *D. trenchii* and *C. goreaui (HM & HW)* cell sizes increased when grown at 31 °C (Figure 3) in this study, indicating potential cellular swelling prior to cell cycle arrest and necrosis as a result of heat stress (Dunn et al., 2004). N-acyl amino acids (NAAA), are long chained fatty acids linked to an amino acid through an amide bond, and are important within signalling pathways (Battista et al., 2019). NAAA, inclusive of NAE, NAGly, *N*-acyl glycine serine (NAGlyser) and N-arachidonoyl taurine (NATau), increased in all cultures grown at 31 °C (Figure 2.9). There have been no studies on NAAA within microalgae, however these molecules have been suggested to function within the immune response, and potentially induce apoptosis within marine macrophage cells (Takenouchi et al., 2012). The increased relative abundance of NAAA exhibited by cultures grown at 31 °C, in addition to increase in cell size (Figure 2.3; Supplementary Table S2.8) suggests these lipids may serve a similar role within Symbiodiniaceae, yet further studies are essential to confirm. Despite the cell size increases, cell growth and photophysiology remained stable, suggesting the increased SFA : UFA ratio served to support cell stability under the higher temperature ranges.

Unsaturation of fatty acids within microalgae actively occurs under decreasing temperatures, and is known to increase membrane permeability, and is considered important in maintaining photosynthetic capacity in thylakoid membranes under cold temperatures (Ferrer-Ledo et al., 2023; Holm et al., 2022). An increased abundance of unsaturated fatty acids under cold stress has been previously recorded in *Cladocopium* sp. exposed to 16 ºC for 4 days (Oakley et al., 2022). When temperatures were decreased to 21 ºC, we observed an increase in the abundance of unsaturated fatty acids (and a decrease in the SFA:UFA ratio) across all species, supporting previous physiological responses in microalgae and Symbiodiniaceae (Oakley et al., 2022). However, at the coldest temperature (16 ºC), the SFA:UFA ratio increased compared to control cultures across all species (Supplementary Table S2.12). Longer term exposure (i.e., entire growth cycle) to cold temperatures could increase the level of ROS due to the increased solubility of O2 (Ermilova, 2020), which may have resulted in the relative increase in saturated fatty acids in order to maintain cell stability (Ayala et al., 2014; Su et al., 2019). Betaine lipids are a family of glycerolipids linked to DGs and are considered crucial to low temperature adaptation in other microalgae (Murakami et al., 2018). While the analysis of betaine lipids

requires an alternative extraction process to that used here due to their chemical composition, DGs are components, and their abundance could be linked to betaine lipid abundance. DGs were more abundant at 16 °C versus the control in all Symbiodiniaceae, suggesting betaine lipids may also provide a lipid remodelling mechanism of protection against cold temperatures in Symbiodiniaceae. Symbiodiniaceae specific production of betaine lipids has been previously recorded (Sikorskaya et al., 2023), and thus may explain why DGs were significantly higher in relatively stress tolerant strains *D. trenchii* and *C. goreaui (HW)* and the temperate Symbiodiniaceae *B. psygmophilum*, but not the tropical, stress sensitive *C. goreaui (HW)*. Previous studies on *D. trenchii* and *B. psygmophilum* noted a distinct lack of change in F_v/F_m when grown under conditions colder than optimum (Dilernia et al., 2023), which may imply that the slightly slowed growth rate exhibited by these cultures in comparison to controls may not indicate stress, but slowed metabolic processes as seen in bacteria (Zhang & Gross, 2021). In order to resolve this current knowledge gap, future studies should be conducted on Symbiodiniaceae physiology and photophysiological response as temperatures are reduced below 16 °C, as well as specifically targeting betaine lipid composition.

Over-abundance of ROS under temperature stress can result in photosystem damage and alterations to thylakoid membranes, lipid peroxidation, and an increase in the abundance of oxidised lipids generated by free (Amario et al., 2023; Botana et al., 2022; Xing et al., 2022). Here, there was an increased relative abundance of oxidised phosphatidylinositol (OxPI) across all Symbiodiniaceae species grown at 31 °C (Figure 2.8). Phosphatidylinositol (PI) is an important secondary messenger in eukaryotic cells, inducing cellular changes (Berridge & Irvine, 1989), and PI signalling pathways (Capelluto, 2013), as found in the slime mold *Dictystelium* (Stephens & Irvine, 1990). Thermal stress has caused large increases in the relative abundance of inositols in cnidarians (González-Pech et al., 2022; Hillyer et al., 2017), and cultured Symbiodiniaceae (Klueter et al., 2015). The PI pathway, activated through the phosphorylation of inositol, has been observed in Symbiodiniaceae from genera *Symbiodinium*, *Breviolum*, *Cladocopium* and *Durusdinium* (Rosic et al., 2015). This resembles pathways found in the parasitic taxon Apicomplexa (Ashley et al., 2023), which use PI to impair host cell signalling, preventing host immune responses and allowing for infection (Lüder et al., 2009). This has led to inositol having a predicted function as an important signalling molecule in the cnidarian-

Symbiodiniaceae symbiosis (González-Pech et al., 2022; Matthews et al., 2017, 2018). Oxidised derivatives of phosphatidylinositol, OxPI, are less well understood, with studies identifying the presence of OxPI in plants (Nilsson et al., 2014) but not yet able to determine specific function. The presence of oxidised phospholipids has been linked to oxidative damage and increases in ROS (Fellows et al., 2023), potentially impacting membrane structure and permeability, and leading to eventual cell apoptosis (Gaschler & Stockwell, 2017). OxPI and other oxidised phospholipids have not been well explored in algae but could indicate increases in ROS and oxidative stress due to temperature increase. ROS have been shown to leak from Symbiodiniaceae cells, implying that when *in hospite* would transfer to the coral host (Nielsen & Petrou, 2023), and corals are known to bleach under increased oxidative stress (Lesser, 1997). Therefore, it is possible that increases in relative abundances of OxPI as a result of temperature stress could potentially disrupt signalling between Symbiodiniaceae and coral host when *in hospite* and contribute to the breakdown of the symbiosis.

This study demonstrates the homeoviscous adaptation of Symbiodiniaceae species under variation to temperature and nutrient concentration. Reef building corals acquire some (mainly PUFAs and fatty acid precursors) of their lipids from endosymbiotic Symbiodiniaceae (A. Imbs et al., 2010; Matthews et al., 2018), which serve as vital energy sources and key inter-partner signalling molecules (Matthews et al., 2017). Therefore, Symbiodiniaceae lipid remodelling can have significant influence on their physiology that in turn cascades to their host. This is especially important as warming temperatures are causing the increased occurrence of coral bleaching (Hughes et al., 2017) and poleward shift of coral species (Yamano et al., 2011). As bleached corals have been found to consume greater amounts of lipid stores for longer term survival under stress (Liu et al., 2022), it is important to develop upon this knowledge. Future studies may also want to consider further studying homeoviscous adaptation of Symbiodiniaceae under cold stress and how this might mediate poleward range shifts to cooler regions.

2.6 Supporting Information

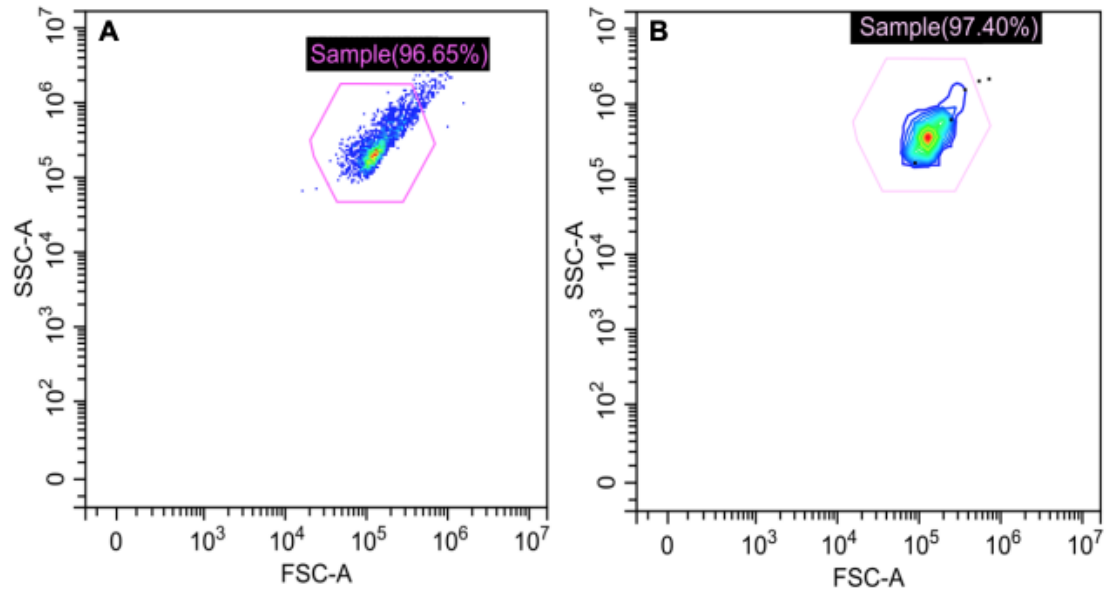

Figure S2.1 Flow cytometry gating strategy for Symbiodiniaceae cell physiology in (A) nutrient experimentation and (B) temperature experimentation. Symbiodiniaceae sp. population characterised according to front scatter (FSC) and side scatter (SSC)

Table S2.1 Concentration of P (mg P/L) and N (mg N/L) in ASW media between the control and nutrient treatments

Treatment	Concentration P (mg P/L)	Concentration N (mg N/L)
Control (ASW F/2)	1.13	12.72
N-Limited (ASW N 25%)	1.12	3.81
NP-Limited (ASW N 25% P 25%)	0.29	3.54
P-Limited (ASW P 25%)	0.36	13.32

Table S2.2 H-ESI conditions for all Lipid methods

Source Condition	Lipid (+)	Lipid (-)
Sheath condition	30	30
Aux Gas	10	10
Sweep	1	1
Source Temp	400	400
Heat Gas	300	0
Voltage	4	4
RF	50	50

Table S2.3 General data acquisition parameters for all methods used.

Scan Parameters	
Default charge	1
Full MS	
Resolution	70,000
AGC target	3.00E+06
Maximum IT	100 ms
dd-MS	
Resolution	17,500
AGC target	1.00E+05
Maximum IT	60 ms
Loop Count/ Top N	10
Isolation Window	1 *m/z*
dd settings	
Minimum AGC target	8.00E+03
Exclude isotope	On
Dynamic Exclusion	2.0 s

Table S2.4 PERMANOVA permutational MANOVA (P_{mc}) in mean exponential growth within nutrient treatments compared against the control for each Symbiodiniaceae species. Pairwise testing post hoc detailing any significance between Symbiodiniaceae growth rates

	Symbiodiniaceae sp.	Treatment	Mean exponential u	SE	% change vs control	Sig. diff vs Control	Post Hoc
A	*D. trenchii*	Control	0.211	0.004	-	-	
	D. trenchii	N-Limited	0.213	0.001	1.099	-	B, D
	D. trenchii	NP-Limited	0.209	0.001	-1.847	-	B, D
	D. trenchii	P-Limited	0.218	0.001	4	-	B, D
B	*C. goreaui HW*	Control	0.135	0.002	-	-	
	C. goreaui HW	N-Limited	0.135	0.001	-0.095	-	A, C
	C. goreaui HW	NP-Limited	0.133	0.002	-1.368	-	A, C
	C. goreaui HW	P-Limited	0.137	0	2.948	-	A, C
C	*B. psygmophilum*	Control	0.217	0.001	-	-	
	B. psygmophilum	N-Limited	0.213	0.001	-1.839	0.037	B, D
	B. psygmophilum	NP-Limited	0.208	0.002	-2.369	0.001	B, D
	B. psygmophilum	P-Limited	0.215	0.002	3.215	-	B, D
D	*C. goreaui HM*	Control	0.137	0.001	-	-	
	C. goreaui HM	N-Limited	0.137	0.001	-0.327	-	A, C
	C. goreaui HM	NP-Limited	0.135	0.001	-1.656	0.033	A, C
	C. goreaui HM	P-Limited	0.134	0.001	-0.188	0.048	A, C

Table S2.5 One-Way ANOVA and Tukeys post hoc showing variance in cell size at the final timepoint within nutrient treatments compared against the control for each Symbiodiniaceae species.

Symbiodiniaceae Sp.	Treatment	Mean size	SE	% change from control	Post hoc
D. trenchii	Control	5.63357698	0.03335255	-	-
D. trenchii	N-Limited	5.52698054	0.01714915	-1.892162631	-
D. trenchii	NP-Limited	5.42665448	0.01872042	-3.673021577	0.028
D. trenchii	P-Limited	5.59837328	0.07812347	-0.624890684	-
C. goreaui (HW)	Control	5.6342936	0.01794841	-	-
C. goreaui (HW)	N-Limited	5.49258304	0.02336216	-2.515143325	-
C. goreaui (HW)	NP-Limited	5.52026228	0.03762768	-2.023879553	-
C. goreaui (HW)	P-Limited	5.67182629	0.06430477	0.666147316	-
B. psygmophilum	Control	5.56639435	0.01522095	-	-
B. psygmophilum	N-Limited	5.36825039	0.00750933	-3.55964661	<0.001
B. psygmophilum	NP-Limited	5.34254183	0.01453465	-4.021499493	<0.001
B. psygmophilum	P-Limited	5.65310473	0.0147809	1.557747703	0.003
C. goreaui (HM)	Control	5.3604572	0.01749174	-	-
C. goreaui (HM)	N-Limited	5.2262711	0.01518021	-2.503258581	0.001
C. goreaui (HM)	NP-Limited	5.31208571	0.02854453	-0.902376257	-
C. goreaui (HM)	P-Limited	5.38679279	0.00338185	0.49129374	-

Table S2.6 Effective quantum yield of PSII (F_v/F_m) under nutrient treatments across Symbiodiniaceae species. Mean F_v/F_m values, standard error and percentage difference from controls were taken from the final sampling point. RMANOVA and pairwise t-test with Bonferroni correction P-value for post hoc.

Symbiodiniaceae Sp.	**Treatment**	**Mean Final F_v/F_m**	**SE**	**% Diff. from control**	**post hoc**
D. trenchii	Control	0.645	0.02753785	-	-
D. trenchii	N-Limited	0.67	0.00408248	3.875968992	-
D. trenchii	NP-Limited	0.6625	0.0075	2.713178295	-
D. trenchii	P-Limited	0.495	0.01322876	-23.25581395	<0.001
C. goreaui (HW)	Control	0.675	0.01322876	-	-
C. goreaui (HW)	N-Limited	0.64	0.00707107	-5.185185185	-
C. goreaui (HW)	NP-Limited	0.625	0.015	-7.407407407	0.012
C. goreaui (HW)	P-Limited	0.5125	0.02322893	-24.07407407	<0.001
B. psygmophilum	Control	0.555	0.04291464	-	-
B. psygmophilum	N-Limited	0.67	0.00408248	20.72072072	-
B. psygmophilum	NP-Limited	0.69	0.00816497	24.32432432	-
B. psygmophilum	P-Limited	0.44	0.0241523	-20.72072072	<0.001
C. goreaui (HM)	Control	0.625	0.01554563	-	-
C. goreaui (HM)	N-Limited	0.6275	0.0075	0.4	-
C. goreaui (HM)	NP-Limited	0.675	0.00957427	8	-
C. goreaui (HM)	P-Limited	0.5475	0.01436141	-12.4	<0.001

Table S2.7 PERMANOVA permutational MANOVA (P_{mc}) in mean exponential growth within nutrient treatments compared against the control for each Symbiodiniaceae species. Pairwise testing post hoc detailing any significance between Symbiodiniaceae growth rates

	Symbiodiniaceae sp.	Treatment	Mean u expo	SE	%change vs control	Post Hoc	Symbiodiniaceae sp.
A	*D. trenchii*	16 ºC	0.131	0.001	-10.29	0.001	C
	D. trenchii	21 ºC	0.147	0.003	0.31		B, C
	D. trenchii	26 ºC	0.146	0.001	-		C
	D. trenchii	31 ºC	0.123	0.002	-15.68	0.001	-
B	*C. goreaui HW*	16 ºC	0.135	0.001	-6.429	0.003	D
	C. goreaui HW	21 ºC	0.156	0.002	8.088	0.007	A, D
	C. goreaui HW	26 ºC	0.144	0.002	-		C
	C. goreaui HW	31 ºC	0.13	0.003	-10.083	0.012	-
C	*B. psygmophilum*	16 ºC	0.141	0.002	-10.289	0.002	A
	B. psygmophilum	21 ºC	0.157	0.002	-0.214		A, D
	B. psygmophilum	26 ºC	0.157	0.002	-		A, B, D
	B. psygmophilum	31 ºC	0.13	0.003	-16.887	0.001	-
D	*C. goreaui HM*	16 ºC	0.144	0.002	1.357		A, B
	C. goreaui HM	21 ºC	0.143	0.003	0.438		B, C
	C. goreaui HM	26 ºC	0.142	0.001	-		C
	C. goreaui HM	31 ºC	0.127	0.006	-11.138	0.043	-

Table S2.8 One-Way ANOVA and Tukeys post hoc showing variance in cell size at the final timepoint within temperature treatments compared against the control for each Symbiodiniaceae species.

Symbiodiniaceae sp.	Treatment	Mean u expo	SE	%change vs control	Post Hoc
D. trenchii	16 ºC	6.209914365	0.09983028	3.188306566	-
D. trenchii	21 ºC	6.104392848	0.03915273	1.434886802	-
D. trenchii	26 ºC	6.018040775	0.02036759	-	-
D. trenchii	31 ºC	6.818678562	0.11497744	13.30396082	<0.001
C. goreaui (HW)	16 ºC	6.406625103	0.10803203	7.195743405	-
C. goreaui (HW)	21 ºC	6.106273962	0.07437223	2.170263789	-
C. goreaui (HW)	26 ºC	5.976566699	0.04264171	-	-
C. goreaui (HW)	31 ºC	7.459869576	0.17683865	24.81864508	<0.001
B. psygmophilum	16 ºC	5.786932531	0.02957696	-3.468113083	-
B. psygmophilum	21 ºC	5.855548389	0.03254303	-2.32353117	-
B. psygmophilum	26 ºC	5.994840374	0.03162134	-	-
B. psygmophilum	31 ºC	6.145060733	0.12979544	2.505827506	-
C. goreaui (HM)	16 ºC	6.047332402	0.06801504	3.368549992	-
C. goreaui (HM)	21 ºC	5.872657566	0.07720567	0.382789772	-
C. goreaui (HM)	26 ºC	5.850263356	0.04262226	-	-
C. goreaui (HM)	31 ºC	7.38749149	0.2140732	26.2762211	<0.001

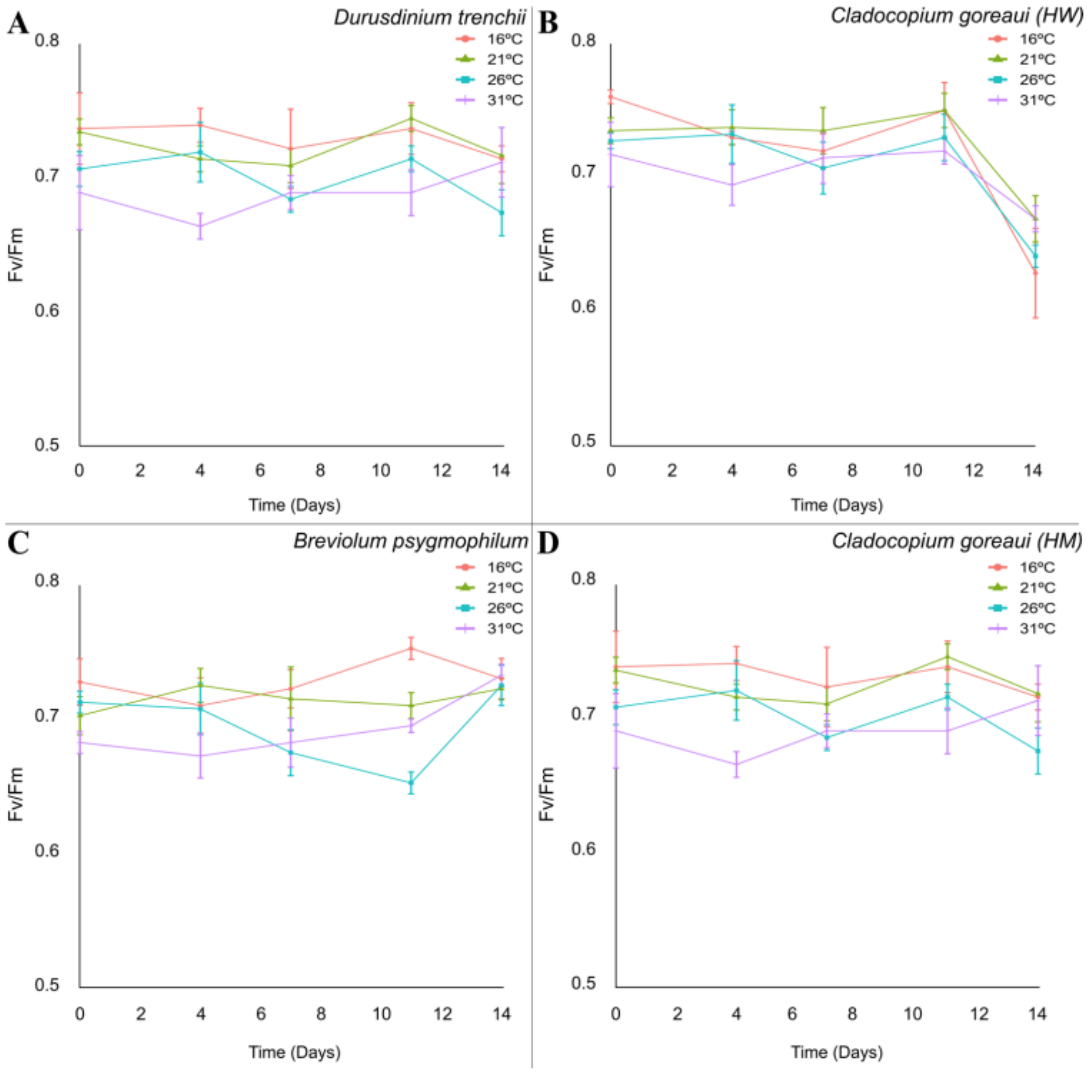

Figure S2.2 Symbiodiniaceae effective quantum yield of photosystem II under different temperatures. F_v/F_m (dimensionless) measurements of *D. trenchii* (A), *C. goreaui-HW* (B), *B. psygmophilum* (C) and *C. goreaui-HM* (D) cultures grown in temperature treatment (16 ºC (red), 21 ºC (green), Controls (26 ºC, purple), 31 ºC (blue)). N varied between 3-4 per treatment per species. Values are means and error bars are SEM, N=4 per treatment, per species

Table S2.9 Effective quantum yield of PSII (F_v/F_m) under temperature treatments across Symbiodiniaceae species. Mean F_v/F_m values, standard error and percentage difference from controls were taken from the final sampling point. RMANOVA and pairwise t-test with Bonferroni correction P-value for post hoc over time and ANOVA with Tukeys Post Hoc for final sampling point.

Symbiodiniaceae Sp.	Treatment	Mean Final F_v/F_m	SE	Post hoc (RMANOVA)	Post hoc (ANOVA)
D. trenchii	16 ºC	0.7125	2.182784	<0.001	-
D. trenchii	21 ºC	0.715	1.957673	<0.001	-
D. trenchii	26 ºC	0.6725	0.89856	-	-
D. trenchii	31 ºC	0.71	2.238967	<0.001	-
C. goreaui (HW)	16 ºC	0.6175	2.234258	0.003	-
C. goreaui (HW)	21 ºC	0.6575	1.701519	<0.001	-
C. goreaui (HW)	26 ºC	0.63	0.669379	-	-
C. goreaui (HW)	31 ºC	0.6575	1.277736	-	-
B. psygmophilum	16 ºC	0.7275	1.048934	<0.001	-
B. psygmophilum	21 ºC	0.72	1.33695	<0.001	-
B. psygmophilum	26 ºC	0.7225	1.559972	-	-
B. psygmophilum	31 ºC	0.73	0.618813	-	-
C. goreaui (HM)	16 ºC	0.67	0.956777	<0.001	0.02
C. goreaui (HM)	21 ºC	0.6975	2.643285	<0.001	<0.001
C. goreaui (HM)	26 ºC	0.615	2.169837	-	
C. goreaui (HM)	31 ºC	0.68	1.168346	0.01	0.002

Table S2.10 Lipids Subclasses differing significantly from controls across nutrient treatments in Symbiodiniaceae species (ANOVA False Discovery Rate (FDR, <0.05) and Fishers LSD post hoc)

Symbiodiniaceae	SubClass	Class	FDR	Fisher's LSD
B. psygmophilum	**VAE**	Isoprenoids	8.84E-07	C - N; C - NP; P - N; P - NP
B. psygmophilum	**PC**	Glycerophosphocholines	8.78E-06	C - NP; C - P; N - NP; N - P; NP - P
B. psygmophilum	**DGDG**	Digalactosyldiacylglycerol	0.000807	C - N; C - NP; P - N; P - NP
B. psygmophilum	**HexCer_HS**	Neutral glycosphingolipids	0.00165	C - N; C - NP; P - N; P - NP
B. psygmophilum	**Cer_HS**	Ceramides	0.01789	C - N; C - NP; P - N; P - NP
B. psygmophilum	**NAOrn**	Fatty amides	0.01789	N - C; NP - C; P - C
C. goreaui (HM)	**PC**	Glycerophosphocholines	0.00868	C - NP; C - P; N - NP; N - P
C. goreaui (HM)	**DGDG**	Digalactosyldiacylglycerol	0.0102	C - N; C - NP; P - N; P - NP
C. goreaui (HM)	**VAE**	Isoprenoids	0.0102	C - N; C - NP; P - N; P - NP
C. goreaui (HM	**TG**	Triradylglycerols	0.0102	N - C; NP - C; P - C; N - P; NP - P

Table S2.11 SFA/UFA ratio for each nutrient treatment

Symbiodiniaceae	Treatment	Mean SFA	Mean UFA	SFA/UFA
D. trenchii	Control	68.5432473	50.7409469	1.35084683
D. trenchii	N-Limited	77.0238714	58.814689	1.30960263
D. trenchii	NP-Limited	77.9426968	61.9513972	1.25812654
D. trenchii	P-Limited	93.1711982	70.1379403	1.32839941
C. goreaui (HW)	Control	69.8565106	57.358784	1.21788688
C. goreaui (HW)	N-Limited	75.1371012	58.5688418	1.28288522
C. goreaui (HW)	NP-Limited	63.374022	30.9148013	2.04995728
C. goreaui (HW)	P-Limited	72.9448825	58.1753374	1.25387983
B. psygmophilum	Control	61.288926	35.4497036	1.72889812
B. psygmophilum	N-Limited	74.3423145	45.7115698	1.62633475
B. psygmophilum	NP-Limited	59.2357417	36.5729593	1.61965952
B. psygmophilum	P-Limited	61.8305074	32.2482962	1.91732633
C. goreaui (HM)	Control	80.9930033	67.9531405	1.19189493
C. goreaui (HM)	N-Limited	90.0394026	50.8115749	1.77202542
C. goreaui (HM)	NP-Limited	49.2239128	29.9065731	1.64592288
C. goreaui (HM)	P-Limited	73.4139092	51.7365968	1.41899378

Table S2.12 SFA/UFA ratio for each temperature treatment

Symbiodiniaceae	Treatment	Mean SFA	Mean UFA	SFA/UFA
D. trenchii	16 ºC	0.1254574	0.0240669	5.21286011
D. trenchii	21 ºC	0.32095779	0.10224958	3.13896447
D. trenchii	26 ºC	0.18245411	0.03762134	4.84975044
D. trenchii	31 ºC	0.37950737	0.05571404	6.8117006
C. goreaui (HW)	16 ºC	0.15530379	0.0282608	5.49537897
C. goreaui (HW)	21 ºC	0.15586052	0.04779474	3.26103893
C. goreaui (HW)	26 ºC	0.18753481	0.05855773	3.20256294
C. goreaui (HW)	31 ºC	0.43126133	0.08241255	5.23295693
B. psygmophilum	16 ºC	0.18178127	0.02575985	7.05676688
B. psygmophilum	21 ºC	0.12669415	0.02887184	4.38815672
B. psygmophilum	26 ºC	0.16576172	0.06218601	2.66557909
B. psygmophilum	31 ºC	0.22140451	0.06627444	3.34072248
C. goreaui (HM)	16 ºC	0.25611142	0.05525195	4.63533699
C. goreaui (HM)	21 ºC	0.29051182	0.08875723	3.27310596
C. goreaui (HM)	26 ºC	0.20304746	0.04689313	4.33000453
C. goreaui (HM)	31 ºC	2.40147866	0.47405972	5.06577245

Table S2.13 Lipids Subclasses differing significantly from controls across temperature treatments in Symbiodiniaceae species (ANOVA False Discovery Rate (FDR, <0.05) and Fishers LSD post hoc)

Symbiodiniaceae	Subclass	FDR	Fisher's LSD
D. trenchii	**SHexCer**	2.5E-05	21 - 16; 26 - 16; 16 - 31; 21 - 31; 26 - 31
D. trenchii	**DGDG**	5.9E-05	21 - 16; 26 - 16; 21 - 31; 26 - 31
D. trenchii	**EtherPC**	1.1E-04	21 - 16; 26 - 16; 16 - 31; 21 - 31; 26 - 31
D. trenchii	**VAE**	1.1E-04	21 - 16; 26 - 16; 16 - 31; 21 - 31; 26 - 31
D. trenchii	**Cer-HS**	2.0E-04	21 - 16; 26 - 16; 21 - 31; 26 - 31
D. trenchii	**HexCer-NDS**	2.7E-04	21 - 16; 26 - 16; 16 - 31; 21 - 31; 26 - 31
D. trenchii	**PG**	2.7E-04	31 - 16; 31 - 21; 31 - 26
D. trenchii	**Cer-HDS**	2.9E-04	21 - 16; 26 - 16; 21 - 31; 26 - 31
D. trenchii	**PC**	4.0E-04	21 - 16; 26 - 16; 16 - 31; 21 - 31; 26 - 31
D. trenchii	**Cer-NDS**	4.5E-04	21 - 16; 16 - 31; 21 - 26; 21 - 31; 26 - 31
D. trenchii	**EtherDG**	4.5E-04	21 - 16; 26 - 16; 21 - 31; 26 - 31
D. trenchii	**LPC**	1.1E-03	21 - 16; 26 - 16; 16 - 31; 21 - 31; 26 - 31
D. trenchii	**NAGly**	1.3E-03	31 - 16; 21 - 26; 31 - 21; 31 - 26
D. trenchii	**DG**	1.9E-03	31 - 16; 31 - 21; 31 - 26
D. trenchii	**TG**	3.6E-03	21 - 16; 16 - 31; 21 - 31; 26 - 31
D. trenchii	**NATau**	3.7E-03	31 - 16; 31 - 21; 31 - 26
D. trenchii	**LPE**	6.8E-03	31 - 16; 31 - 21; 31 - 26
D. trenchii	**COQ**	7.0E-03	21 - 16; 26 - 16; 16 - 31; 21 - 31; 26 - 31
D. trenchii	**SM**	4.3E-02	31 - 16; 31 - 21; 31 - 26
D. trenchii	**Cer-NS**	4.3E-02	21 - 16; 21 - 31; 26 - 31
D. trenchii	**AHexBRS**	4.5E-02	31 - 21; 31 - 26
C. goreaui (HW)	**PG**	4.3E-06	26 - 16; 31 - 16; 31 - 21; 31 - 26
C. goreaui (HW)	**EtherLPE**	4.3E-06	31 - 16; 31 - 21; 31 - 26
C. goreaui (HW)	**CL**	4.4E-06	21 - 16; 26 - 16; 31 - 16; 31 - 21; 31 - 26
C. goreaui (HW)	**NAGly**	4.8E-05	21 - 16; 26 - 16; 31 - 16; 31 - 21; 31 - 26
C. goreaui (HW)	**NATau**	5.4E-05	31 - 16; 31 - 21; 31 - 26
C. goreaui (HW)	**Others**	2.8E-03	31 - 16; 31 - 21; 31 - 26
C. goreaui (HW)	**DG**	4.7E-03	31 - 16; 31 - 21; 31 - 26
C. goreaui (HW)	**Cer-BS**	1.3E-02	31 - 16; 31 - 21; 31 - 26
C. goreaui (HW)	**HexCer-NDS**	2.9E-02	21 - 16; 26 - 16; 21 - 31; 26 - 31
C. goreaui (HW)	**SM**	2.9E-02	31 - 16; 31 - 21; 31 - 26
B. psygmophilum	**EtherLPE**	5.0E-03	31 - 16; 31 - 21; 31 - 26
B. psygmophilum	**Cer-HS**	5.0E-03	21 - 16; 26 - 16; 21 - 31; 26 - 31
B. psygmophilum	**NAGly**	5.0E-03	31 - 16; 31 - 21; 31 - 26
B. psygmophilum	**HexCer-NDS**	5.0E-03	21 - 16; 26 - 16; 21 - 31; 26 - 31
B. psygmophilum	**PG**	5.0E-03	31 - 16; 31 - 21; 31 - 26
B. psygmophilum	**SHexCer**	5.0E-03	21 - 16; 26 - 16; 21 - 31; 26 - 31

B. psygmophilum	**Cer-BS**	1.7E-02	26 - 16; 31 - 16; 26 - 21; 31 - 21
B. psygmophilum	**EtherPC**	2.3E-02	21 - 16; 26 - 16; 21 - 31; 26 - 31
B. psygmophilum	**VAE**	3.2E-02	21 - 16; 26 - 16; 21 - 31; 26 - 31
B. psygmophilum	**DGDG**	3.6E-02	21 - 16; 26 - 16; 21 - 31; 26 - 31
B. psygmophilum	**EtherDG**	3.7E-02	21 - 16; 26 - 16; 21 - 31; 26 - 31
B. psygmophilum	**Cer-NDS**	3.7E-02	21 - 16; 26 - 16; 21 - 31; 26 - 31
C. goreaui (HM)	**SM**	1.9E-04	31 - 16; 31 - 21; 31 - 26
C. goreaui (HM)	**EtherLPE**	1.6E-03	31 - 16; 31 - 21; 31 - 26
C. goreaui (HM)	**NATau**	6.5E-03	31 - 16; 31 - 21; 31 - 26
C. goreaui (HM)	**NAGly**	6.5E-03	31 - 16; 31 - 21; 31 - 26
C. goreaui (HM)	**PG**	6.5E-03	31 - 16; 31 - 21; 31 - 26
C. goreaui (HM)	**PC**	6.5E-03	16 - 31; 21 - 31; 26 - 31
C. goreaui (HM)	**DG**	6.6E-03	31 - 16; 31 - 21; 31 - 26
C. goreaui (HM)	**Others**	1.6E-02	31 - 16; 31 - 21; 31 - 26
C. goreaui (HM)	**LPA**	2.2E-02	31 - 16; 31 - 21; 31 - 26
C. goreaui (HM)	**ST**	2.2E-02	31 - 16; 31 - 21; 31 - 26

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2.9 Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Chapter 3: Comparative lipidomics analysis of a temperate and a subtropical coral under acute temperature stress.

Prepared as a fully drafted manuscript for submission:

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List of Abbreviations

Abbreviation	Name	Class
AHexCer	Acylhexosylceramide	Glycosphingolipids
ASM	Acylsphingomyelin	Phosphosphingolipids
Cer-BS	Ceramide beta-hydroxy fatty acid-sphingosine	Ceramides
Cer-HDS	Ceramide hydroxy fatty acid-dihydrosphingosine	Ceramides
Cer-HS	Ceramide hydroxy fatty acid-sphingosine	Ceramides
Cer-NDS	Ceramide non-hydroxyfatty acid-dihydrosphingosine	Ceramides
Cer-NS	Ceramide non-hydroxyfatty acid-sphingosine	Ceramides
CL	Cardiolipin	Glycerophosphoglycerophosphoglycerols
DG	Diacylglycerol	Diradylglycerols
DGDG	Digalactosyldiacylglycerol	Glycosyldiradylglycerols
FA	Free fatty acid	Fatty acids
FAHFA	Fatty acid ester of hydroxyl fatty acid	Fatty esters
HexCer_HS	Hexosylceramide hydroxyfatty acid-sphingosine	Glycosphingolipids
HexCer-NDS	Hexosylceramide non-hydroxyfatty acid-dihydrosphingosine	Glycosphingolipids
LPA	Lysophosphatidic acid	Glycerophosphates
LPC	Lysophophatidylcholine	Glycerophosphocholines
LPE	Lysophosphatidylethanolamine	Glycerophosphoethanolamines
MG	Monoacylglycerol	Monoradylglycerols
MGDG	Monogalactosyldiacylglycerol	Glycosyldiradylglycerols
NAE	N-acyl ethanolamines	Fatty amides
NAOrn	N-acyl ornithine	Fatty amides
NATau	N-acyl taurine	Fatty amides
NAGly	N-acyl glycine	Fatty amides
NAGlyser	N-acyl glycyl serine	Fatty amides
PC	Phosphatidylcholine	Glycerophosphocholines
PE	Phosphatidylethanolamine	Glycerophosphoethanolamines
PG	Phosphatidylglycerol	Glycerophosphoglycerols

PI	Phosphatidylinositol	Glycerophosphoinositols
PS	Phosphatidylserine	Glycerophosphoserines
SHexCer	Sulfatide	Glycosphingolipids
SM	Sphingomyelin	Phosphosphingolipids
ST	Sterol	Sterols
TG	Triacylglycerol	Triradylglycerols
TG-EST	Triacylglycerol estolides	Triradylglycerols
VAE	Vitamin-A fatty ester	Isoprenoids

3.1 Abstract

Climate change has triggered shifts in coral distributions, leading to increased abundance of corals at the poleward boundaries of their optimal habitats. While high latitude temperate environments may offer refuge for subtropical corals escaping heat stress in their core habitats, they are faced with new challenges such as cooler winter temperatures, greater seasonal variation, and changes in nutrient and light levels. Lipids play a crucial role in facilitating organismal adaptation to shifting environmental conditions by maintaining membrane stability, supporting cellular functions, and providing energy. Yet, the specific mechanisms of lipid remodelling in corals at their current environmental limits remain poorly understood. In this study, we used the Coral Bleaching Automated Stress System (CBASS) to investigate the effects of acute temperature stress (ranging from 11 ºC to 32 ºC) on the photophysiology (F_v/F_m) and lipid composition of both temperate coral (*Plesiastrea versipora*) near its northern distributional limit and a subtropical endemic coral at its poleward range limit (*Pocillopora aliciae*). Our research represents the first known application of CBASS on temperate corals. Contrary to expectations, we observed minimal impacts on photophysiology and limited lipid remodelling across temperatures. These findings suggest that corals in high latitude regions may exhibit minimal stress response to acute short-term low or high temperature stress due to their natural exposure to high short-term and seasonal temperature fluctuations. Although overall lipid profiles remained stable under acute temperature stress, shifts in lipid proportions between winter (17 ºC) and summer (24 ºC) indicate potential lipid remodelling mechanisms that facilitate survival amidst high seasonal temperature variations. Notably, both coral species and their associated Symbiodiniaceae showed increased abundances of membrane lipids (such as Phosphatidylcholine (PC), Sterols and Prenols) during winter, potentially enhancing cellular structure and permeability in colder conditions. Furthermore, the higher proportions of glycerolipids (digalactosyldiacylglycerol) observed in *P. aliciae* Symbiodiniaceae during winter may contribute to the support of thylakoid membranes, thereby bolstering photophysiology under colder temperatures and reduced light levels. These findings underscore potential mechanisms enabling corals to thrive at the lower limits of their environmental ranges, offering insights that could inform future conservation efforts for subtropical corals amidst ongoing climate change.

3.2 Introduction

Climate change and local environmental pressures are driving marine species to extend their ranges poleward to stay within the bounds of their environmental optima (Hastings et al., 2020). These areas, known as biogeographic transition zones, are characterised by an overlap of tropical, subtropical and temperate organisms, as well as a high proportion of unique biodiversity with cryptic and endemic species cohabitating the same environmental niche (Beger et al., 2014). Australia's coastal New South Wales is considered an important biogeographic transition zone where tropical, subtropical and temperate species overlap at the limits of distributions and environmental tolerances, including corals at their poleward range limits (Booth & Sear, 2018; Sommer et al., 2014; Veron, 1993). For corals and other warm-affinity taxa to migrate poleward, they must have the capacity to tolerate altered environmental conditions, including nutrient concentration, pH, dissolved oxygen, temperature and light levels (Lønborg et al., 2021; Nati et al., 2021). While subtropical-to-temperate transition zones are sometimes heralded as refugia (*sensu* Keppel et al., 2012) for warm-affinity species from climate change, temperate corals near their upper thermal limits in Sydney have been found to succumb to bleaching under heat stress (e.g., *Plesiastrea versipora*, Goyen et al., 2019). Thus, as sea surface temperatures are continuing to rise, identifying the adaptive capacities of coral species with different thermal affinities and distributions will help inform strategies to protect ecosystems and functions (Dixon et al., 2022). *Pocillopora aliciae* is a scleractinian branching coral endemic to subtropical NSW (Schmidt-Roach et al 2013), that has become more prevalent on the rocky substrate of temperate Sydney (34 ºS) in recent years, where it occurs at its poleward range limit (Booth and Sear, 2018). Yet, the extent to which *P. aliciae* can expand in Sydney and further poleward is unknown. Whilst *P. aliciae* might be finding refuge from ocean warming on temperate rocky reefs, understanding the mechanisms that underly its physiological performance at its poleward range limit, and of *P. versipora*, will support adaptive management and help inform the future of high-latitude reef environments.

Achieving this will require the following:

1. evaluating the thermotolerance for *P. aliciae* and *P. versipora* when exposed to acute short-term extreme temperature increases and decreases, and
2. determining the adaptive mechanisms that mediate the persistence of corals with different distributions in high-latitude reefs

Recent evidence indicates that reef building corals, such as pocilloporids, have the capacity to acclimate quickly to slight environmental change (Marhoefer et al., 2021), driven by the capacity for local adaptation, or phenotypic plasticity of individuals (Torda et al., 2017). Additionally, due to high seasonal and daily temperature fluctuations in temperate reefs (Clarke & Gaston, 2006), corals that inhabit these areas tend to favour short-term resilience over long-term performance (Cant et al., 2023), and harbour a higher resilience to bleaching than corals in areas where temperatures are more stable (Safaie et al., 2018). However, these areas are becoming increasingly threatened by climate change (Soler et al., 2022), which may result in the loss of temperate species, such as temperate seaweeds, and facilitate abundance increases of subtropical and temperate corals in high-latitude regions (Tuckett et al., 2017). Ex situ experimentation has shown that the subtropical-temperate coral *Plesiastrea versipora,* under temperatures reflecting in situ conditions, will outcompete *P. aliciae* when in close proximity, however under prolonged increased temperatures (26 ºC, 6 days), the opposite occurs (González-Pech et al., 2022). Furthermore, *P. versipora* have been documented to bleach extensively during heat stress events in Sydney, while there is no documented evidence of *P. aliciae* bleaching at its poleward range limit in Sydney (Goyen et al., 2019). Nevertheless, *P. aliciae* has experienced severe bleaching and mortality at the core of its distributional range in northern NSW (Kim et al 2019, Lachs et al 2021). Cold water bleaching is less explored than bleaching from heat stress (Hoegh-Guldberg et al., 2005; Saxby et al., 2003), however with corals migrating poleward, there has been an emergence of corals residing at the lower limit of their thermal optima and consequently more at risk to cold water bleaching (Bellworthy & Fine, 2021). Additionally, thermotolerance of corals has been associated with the species composition of Symbiodiniaceae, symbiotic algae that form partnerships with coral, providing oxygen, amino acids and lipids in return for protection and nutrients (Nitrogen, Phosphorus, CO_2)(Goreau, 1959; Martinez et al., 2022; Muscatine & Porter, 1977). The dominant Symbiodiniaceae species in *P. aliciae* in Sydney is *Cladocopium* sp. (Type C75h), whereas in *P. versipora*, *Breviolum* sp. (Type B18) is dominant (González-Pech et al., 2022). As such, observing coral physiological and associated Symbiodiniaceae photophysiological responses to both heat and cold temperature stress could provide valuable knowledge on the thermal tolerance of corals living at the extent of their environmental optima.

One physiological mechanism that organisms can employ to adapt to environmental shifts is alterations to their metabolism, such as the remodelling of lipid and fatty acid composition within cellular membranes to increase cell stability under changing temperatures (Sinensky, 1974). This process is known as homeoviscous adaptation, where changes to accumulation and composition of lipids can be a sign of resilience to stressors (Rodrigues & Grottoli, 2007). For corals living at the limits of their optima, modifications to lipid composition may be an important means that allows acclimation to changed conditions, but this remains unresolved. Symbiodiniaceae tolerance to stress governs coral response (Cunning & Baker, 2013), and Symbiodiniaceae are thus often used in studies that examine coral response to environmental change. For example, lipid remodelling in response to elevated temperature stress has been studied in Symbiodiniaceae, revealing increases in storage lipids, such as triacylglycerols and other glycerolipids, as well as lipid membrane remodelling, as potential mechanisms of resilience (Botana et al., 2022; Tchernov et al., 2004). Although underexplored, cold stress similarly influences the lipid profile in Symbiodiniaceae, with findings demonstrating lipid unsaturation at low temperatures as a mechanism of survival (Holm et al., 2022; Oakley et al., 2022). Lipid profiles differ between Symbiodiniaceae species, and evidence suggests that corals acquire some of their lipids from Symbiodiniaceae (Rosset et al., 2021). This suggests that Symbiodiniaceae with lipid profiles that could contribute to thermotolerance may in turn allow for more resilient corals (Rosset et al., 2021; Sikorskaya et al., 2022). For example, research on the effects of elevated temperature on Symbiodiniaceae (31 ºC), found that *Cladocopium goreaui* had a higher proportion of lipids, including fatty acyls and phospholipids, increased in relative abundance compared against controls, compared to *Breviolum psygmophilum*. When temperatures were reduced however, (16 ºC), lipid profiles between these two Symbiodiniaceae species remained similar (Chapter 2; La Motta et al., 2024). For corals living at their environmental limits, this distinction may play a role in shaping future trajectories under climate change.

Here, to investigate the adaptive capacity of two sympatric coral species with different geographical distributions under high temperature fluctuations, we examined metabolic and photophysiological (photosystem II, F_v/F_m) responses of *P. aliciae* and *P. versipora* from temperate Sydney to acute temperature increases (+ 8 ºC of the summer maximum monthly mean temperature) and decreases (- 6 ºC of the winter minimum monthly mean) by utilising the Coral

Bleaching Automated Stress System (CBASS) (Voolstra et al., 2020). We analysed the lipid profiles of *P. aliciae* and *P. versipora* tissue and Symbiodiniaceae using the methanol/methyl-tert-butyl ether (MTBE) extraction protocol (Matyash et al., 2008) and Liquid chromatography-mass spectrometry (LC-MS) analysis to compare lipid composition and abundances between species, between coral and symbiont, and across temperatures. In doing so, we tested whether corals in high-latitude regions characterised by large temperature fluctuations have the capacity to withstand acute temperature stress and whether there is evidence of lipid profile remodelling at the different temperature exposures. Collectively this study will inform whether *P. aliciae* and *P. versipora* differ in their capacities to survive both acute heat and cold stress, and whether they use lipid remodelling to facilitate their survival. This information will contribute to our ability to predict how species with different distributions will fare in biogeographic transition zones under climate change and will potentially reveal lipid characteristics of corals that may contribute to survival at their environmental limits, providing knowledge that may support management and conservation efforts.

3.3.0 Methods and Materials

3.3.1 Coral Collection

The experiment was performed with two species of hard coral collected from temperate rocky reefs in Sydney representing different geographic distributions at the limits of their thermal tolerance: the subtropical NSW endemic, *Pocillopora aliciae* (Schmidt-Roach et al., 2013), at its poleward range limit and the more widely distributed temperate *Plesiastrea versipora* near the northern limit of its distribution (Juszkiewicz et al 2022). Coral collection of both species occurred concurrently and was undertaken at two timepoints during peak summer (April) and winter (August) of 2023. A total of five *P. aliciae* fragments (~10 cm) were collected by SCUBA diving (8–12 m depth) from the "Manly Coral Gardens" site at North Head, Manly, Sydney NSW (-33.799, 151.300) and five *P. versipora* fragments (~10 cm) were collected by snorkelling (<5 m depth) from Cobblers Beach, Mosman, NSW (-33.826, 151.264, Figure 3.1). All fragments were collected from the periphery of separate colonies using a hammer and chisel (McLachlan et al., 2021), placed in separate bags in nally bins and transported in native seawater to aquarium facilities at the Sydney Institute of Marine Science (SIMS; Sydney, NSW).

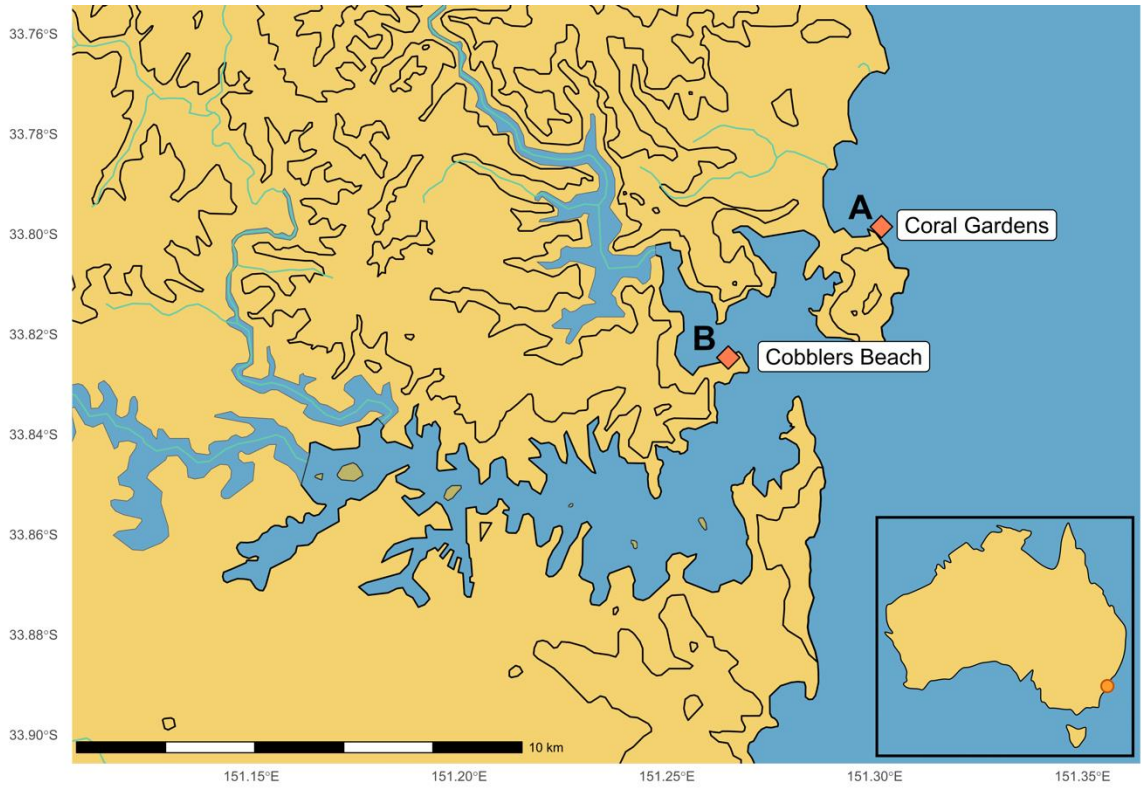


Figure 3.1. Sampling site map depicting the two sampling sites Manly Coral Gardens -33.799, 151.300 (**A**) and Cobblers Beach -33.826, 151.264 (**B**) in Sydney, NSW.

3.3.2 Temperature treatments

Temperature stress experimentation occurred utilising CBASS following assemblage and methodology adapted from Voolstra et al., (2020) (Figure 3.2) at SIMS in April (summer) and August (winter) 2023, using water provided via an inflow system from the Clifton Gardens Aquatic Reserve at a depth of 8 meters. Two separate experiments were run, using the CBASS protocol of 4 discrete temperatures replicated in two technical tank replicates. The CBASS protocol involves a 3 hour ramping period, a 3 hour hold, a 1 hour dark adapted return to control temperatures, and a 11 hour recovery period. The experimental temperatures were calculated utilising the Mean Monthly Maximum (summer) and Minimum (winter) temperatures from collection locations (-33.775, 151.325) between 2005 and 2023 (NOAA ERDDAP). The warmest yearly average was calculated to be 24 ºC, and the coldest 17 ºC, which were set as the control temperatures, with treatments at increasing/decreasing temperatures in increments of 3 ºC. We were unable to heat the inflowing water temperature beyond 32 ºC in the summer experiment, and so used a summer temperature range of 24 ºC, 27 ºC, 30 ºC, and 32 ºC (Figure

3.2). Similarly, we were unable to cool the inflowing water temperature below 10 ºC, and thus in winter we included a 20 ºC treatment to bridge the temperature gap between the winter and summer controls. This gave an actual temperature range in summer of 24 ºC (control), 27 ºC, 30 ºC and 32 ºC, and in winter from 20 ºC, 17 ºC (control), 14 ºC, and 11 ºC.

For each separate CBASS run, coral fragments from five distinct colonies per species were further fragmented into eight 3-4 cm nubbins so that each tank contained one fragment from each colony. Water flow rate into experiment tanks was 1 L/min. Lighting was controlled using Viperspectra LED lights and adjusted to photosynthetic active radiation of 60-80 µmol photons m^{-2} s^{-1}. HOBO Pendant Temperature Data Loggers were first validated using a thermometer and used to record the temperature of each tank every 10 s for the duration of the experiment. A small pump was placed in each tank to ensure water circulation. The start of the experiment was at 13:00 hr and ran until 09:00 hr the next day. At the end of the 18-hour period, all fragments were snap frozen in liquid nitrogen and stored at -80 ºC until extractions occurred. Temperature increases were controlled and maintained using 200W titanium aquarium heaters (Schego) and Inkbird ITC-208 digital temperature controllers (accurate to ±0.5 ºC) and decreases were controlled and maintained using a chiller system provided by SIMS (accurate to ±0.5 ºC).

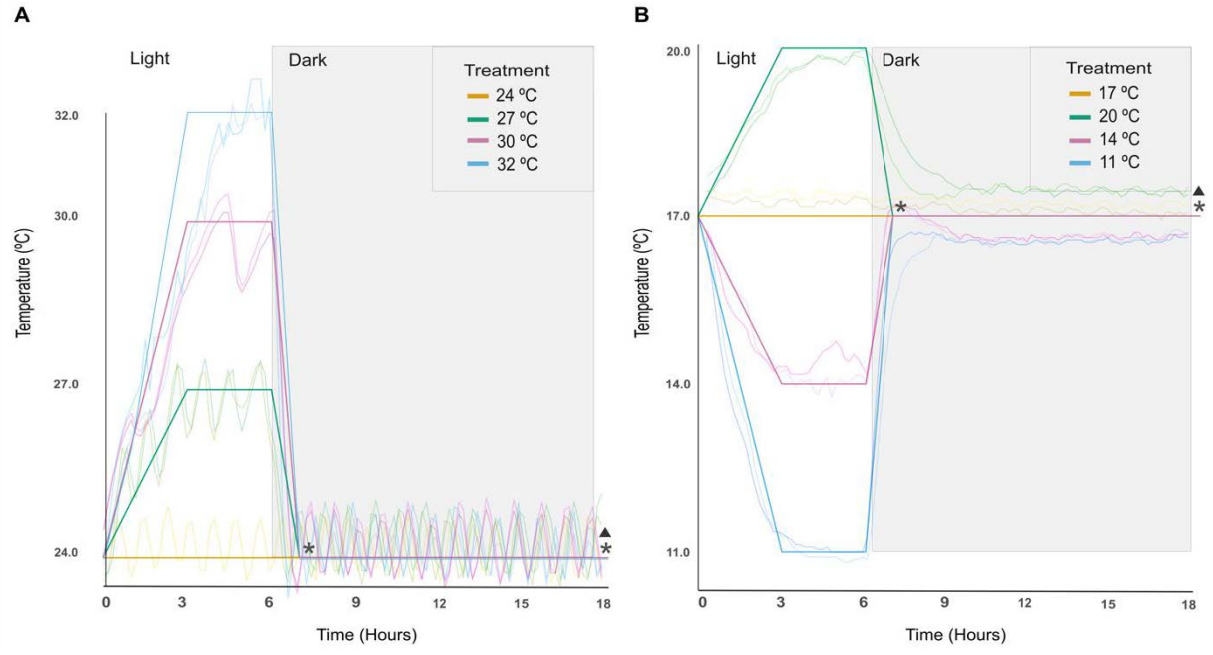


Figure 3.2. Temperature profiles depicting targeted (solid colour) and actual (transparent) temperature gradients within the CBASS for Heat Stress (A) and Cold Stress (B) experiments. Asterisks indicate timepoints of dark-adapted photosynthetic efficiency (Photosystem II) after one-hour dark adapted recovery, and after 11-hour overnight recovery; triangles indicate timepoints of coral sampling for lipidomics analysis.

3.3.3 Photophysiology

Dark acclimated photosynthetic efficiency of photosystem II (PSII, F_v/F_m) was recorded at two points throughout the experiment (Figure 3.2). All corals were dark adapted (PAR < 5 μmol photons m^{-2} s^{-1}) 15 min prior to measurements taken utilising a diving PAM (Pulse Amplitude Modulated; Walz, Effeltrich, Germany) fluorometer (settings: MI: 8, Gain: 4, DAMP: 2, SI: 11), conducting two PAM measurements for each fragment. A spacer was utilised (1 cm) to ensure equidistance between the coral tissue and PAM. Significant variations in F_v/F_m between temperatures, timepoints, and species were identified using PRIMER (v7.0). Data were normalised using Log+1 and used to create separate Bray-Curtis resemblance matrices, which were then employed in a Permutational Multivariate Analysis of Variance (PERMANOVA) (999-permutations) with pairwise testing and montecarlo corrections for small sample sizes (P_{mc}<0.05).

3.3.4 Lipid extraction and analysis via UHPLC-MS/MS

All extraction steps were performed at 4 ºC and using LC-MS grade glassware. Lipid extraction was based on the methanol/methyl-tert-butyl ether (MTBE) extraction and phase separation protocol for high-throughput lipidomics (Matyash et al., 2008). Corals were randomised and extracted from in groups of 10, with one blank in each run for both tissue and Symbiodiniaceae. Coral fragments were first rinsed with 2 mL of LC-grade ultra-pure water to remove excess salt and sediment before placement in a small zip-lock bag. 5 mL of LC-grade ultra-pure water was added to each bag, along with 50 µL of ethylenediaminetetraacetic acid (EDTA; 20 mg/mL LC-grade ultra-pure water) and 50 µL of butylated hydroxytoluene (BHT; 20 mg/mL LC-grade ultra-pure water) that function in protection of lipids from peroxidation and remove redundant ions from the sample (Metherel et al., 2013; Vazdar et al., 2023). Coral host tissue and Symbiodiniaceae cells were separated following the methods in Matthews et al., (2023). Briefly, the tissue was removed from corals using an air pick. 1mL of the homogenate was collected and stored at -20 ºC for later use in determining Symbiodiniaceae cell density relative to protein

content. The remaining homogenate was centrifuged at 3000 x *g* for 5 min at 4 ºC, the host supernatant collected, the Symbiodiniaceae pellet resuspended in 1mL of LC-grade ultra-pure water, and all samples were then vortexed for 30 s. This was repeated twice more, or until no symbiont pellet remained in the host fraction, and no tissue layer remained on the symbiont pellet. For each sample, the protein content was estimated using a Bradford assay (Bradford, 1976), and a volume equivalent to 75 µg of total protein content used for extraction. Each sample was resuspended up to 200 µL with LC-grade ultra-pure water.

Lipids were extracted following the methodology outlined in La Motta et al., (2024; Chapter 2), including the EquiSPLASH LIPIDOMIX mass spec standard (Avanti Polar Lipid 330707). Prior to analysis, samples were dried under a nitrogen stream, resuspended in 100 µL 2:1 isopropanol methanol (IPA:MeOH) and transferred to autosampler glass vials with a 125 µL glass insert. A quality control (QC) sample was created by combining 5 µL of each sample into a single tube and 100 µL transferred to an autosampler vial with a 125 µL glass insert. 5 µL injections of each sample was processed via Liquid Chromatography Mas-Spectrometry (Thermo Orbitrap LC-MS) in positive and negative ion mode. Each sample was run as per methodology developed by Violi (2022). Lipid spectral data was exported to .raw files and processed in MS-Dial (v5.2) for identification against the LipidMaps LipidFinder (v2) and Structure Database (LMSD). The identification of lipids was performed according to MS/MS profile fragmentation, with the following mass tolerance range: MS1 0.05, MS2 0.075. Any unmatched lipids and lipids with an identification score of <80% were removed. Peak areas for each identified lipid were aligned normalised to the peak area of the internal standards (EquiSPALSH), and data was exported to Microsoft Excel (v16.82) and each individual lipid blank corrected by subtracting the average concentration of the same lipid in the blank samples. Positive and negative data were then combined and arranged into individual lipid and lipid sub-class (Liebisch et al., 2020) Lipid relative abundance was generated by dividing each lipid's peak area by the sum of all lipid peak areas from each sample. Lipids were removed according to a relative standard deviation (RSD) threshold of 50% within each treatment, and when a lipid had a relative abundance ≤ 0 in more than 50% of sample replicates within a treatment. Samples in which more than 25% of lipids had ≤ 0 relative abundance were also removed (n = 1 from the heat-stressed symbiont 24 ˚C treatment, n = 1 from the cold-stressed symbiont 11 ºC treatment, n=1 from the 11 ºC treatment

in Symbiodiniaceae). One coral fragment from the 32 ºC treatment was not snap-frozen correctly and was subsequently removed (n = 1 from heat stress both symbiont and coral 32 ºC treatment). Lipid proportional abundance was generated by dividing the relative abundance of each lipid by the sum of the lipids from each sample and multiplying this by 100 to generate a percentage. This data was sorted into lipid-sub class, and categorical data to generate a stacked bar plot RStudio (2023.06.0 + 421). For categorical data, lipids were grouped into categories detailed by Liebisch et al. (2020) (Supplementary Table S3.1).

Multivariate analyses were undertaken using PRIMER (v7.0) to determine lipid trends between coral and associated Symbiodiniaceae species, and between temperature treatments. Data was normalised using Log(x+1) and used to create separate Bray-Curtis resemblance matrices. Lipids contributing to similarities and differences at 40% between temperature treatments amongst both coral and Symbiodiniaceae in summer (24 ºC) and winter (17 ºC) controls were identified using SIMPER (Similarity Percentage). PERMANOVA permutational MANOVA (999-permutations) with pairwise testing and montecarlo corrections (P_{mc}<0.05) were employed to determine significance between temperatures and species amongst both coral tissue and symbiodiniaceae. Lipid data was inputted into Metaboanalyst (v5.0) where a cube root transformation, and mean centred scaling occurred. Significance Analysis of Microarray (and Metabolites) (SAM) was performed to identify any individual lipids or lipid classes whose abundance differed significantly between treatments and species, determined by a false discovery rate (p_{adj} <0.05). To test for differences in the lipid profiles within each species across seasons, samples were grouped into proportional abundances of categorical lipids, and pairwise T-tests with Fischer's post hoc tests, using a false discovery rate (FDR) of < 0.05, was run on the tissue and Symbiodiniaceae of each species. The stacked bar plot on sub-class data was then used to present which lipids were driving changes in categorical proportional abundance. The relative abundance of unsaturated fatty acids (UFA) and saturated fatty acids (SFA) were summarized to obtain an UFA:SFA ratio and significance was determined using PERMANOVA permutational MANOVA (999-permutations) with pairwise testing and montecarlo corrections (P_{mc}<0.05).

3.3.5 Cell density and protein content analysis

250 µL of homogenate was taken from the 1mL removed prior to lipid extractions and centrifuged at 3200 rpm for 5 min at 4 ºC to separate host tissue and symbiont. A Bradford assay

was performed as per methodology above using 10 µL of tissue diluted 1:2 in ultra-pure water and run in triplicate. The symbiont pellet was resuspended in 250 µL of FSW for cell density calculations. Cell counts were undertaken by pipetting 10 µL of sample onto a haemocytometer and manually counting cells using a light microscope. This process was repeated until 10 technical replicates were obtained before data were entered into Microsoft Excel (v16.82), where average counts and relative standard deviation were calculated, and average cell density/mg protein were determined.

3.4.0 Results

3.4.1 Photophysiology

After the dark recovery period (1-hour return to control temperatures, Figure 3.2), the photosynthetic efficiency (F_v/F_m) of *P. aliciae* corals exposed to 32 ºC was 6% lower than controls (P_{mc}= 0.015, Supplementary Figure S3.1A, Supplementary Table S3.2). After the 11-hour recovery period, the F_v/F_m of all heat exposed *P. aliciae* returned to control levels (P_{mc} = 0.055, Supplementary Figure S3.1A, Supplementary Table S3.3). Under 11 ºC and 14 ºC, the F_v/F_m of *P. aliciae* was significantly reduced by 9% and 5%, respectively, compared to controls (P_{mc} =0.002, P_{mc} = 0.013), while the F_v/F_m of corals at 20 ºC were similar to controls (P_{mc} =0.533, Supplementary Figure S3.1C, Supplementary Table S3.4). After the 11-hour recovery period, F_v/F_m from the 11 ºC treatment within *P. aliciae* remained lowered compared to controls by an average of 5% (P_{mc} =0.021, Supplementary Figure S3.1C, Supplementary Table S3.5). Across the two seasonal experiments, F_v/F_m in *P. aliciae* did not seem to be altered, exhibiting similar photophysiological efficiency in both summer and winter (P_{mc}=0.096, Supplementary Figure S3.1).

No change in F_v/F_m was exhibited by *P. versipora* exposed to temperature increases compared to controls at either PAM sampling points (P_{mc}> 0.05, Supplementary Figure S3.1B, Supplementary Tables S3.6 and S3.7). However, under acute cold stress, the photosynthetic efficiency within *P. versipora* exposed to 11 ºC was reduced by 5% from controls after the 1-hour dark recovery period (P_{mc}= 0.01, Supplementary Figure S3.1D, Supplementary Table S3.8). All other cold-exposed corals maintained F_v/F_m levels similar to controls (P_{mc}= 0.63, Supplementary Figure S3.1D). After the 11-hour recovery period, *P. versipora* corals exposed to

11˚C maintained the lowest average F_v/F_m when compared to all other treatments, although no longer statistically significant (P_{mc}= 0.63, Supplementary Figure S3.1D, Supplementary table S3.9). Across the two seasonal sampling points, *P. versipora* exhibited higher photophysiological efficiency of photosystem II (F_v/F_m) in winter controls (17 ºC, P_{mc}<0.001, Supplementary Figure S3.1).

3.4.2 Symbiodiniaceae Cell Density

Symbiodiniaceae cell density remained stable across temperatures at the point of sampling. Following exposure to heat, cell density normalised to protein did not differ between temperatures in *P. aliciae* (P_{mc}=0.817, Supplementary Figure S3.2A), or in *P. versipora* (P_{mc}=0.075, Supplementary Figure S3.2A). Similar trends were observed in corals exposed to decreases in temperature, whereby at the time of sampling, there was no distinction in Symbiodiniaceae cell density across temperatures in either *P. aliciae* (P_{mc}=0.773, Supplementary Figure S3.2B), or *P. versipora* (P_{mc}=0.21, Supplementary Figure S3.2B).

3.4.3 Lipidomics

Coral samples were collected for lipidomics analysis following exposure to acute temperature stress followed by an 11-hour recovery period, as adapted from Voolstra et al., (2020). Heat exposure did not significantly alter the overall or individual lipid compositions within either *P. aliciae* coral tissues or associated Symbiodiniaceae cells (P_{mc}=0.375 and 0.729, respectively). Similarly, there were no significant shifts in overall lipid compositions in *P. versipora* coral tissues or associated Symbiodiniaceae cells following heat exposure (P_{mc}=0.433 and 0.483 respectively). However, one lipid, NAE 13:0 (N-acyl ethanolamine), significantly decreased in relative abundance in the Symbiodiniaceae cells of *P. versipora* exposed to 32 ºC, 30 ºC and 27 ˚C compared to the control (24 ºC, p_{adj} =0.047, Supplementary Table S3.10). Cold exposure did not significantly alter the overall or individual lipid profiles of either *P. aliciae* or *P. versipora* coral tissue (P_{mc}=0.567 and 0.767) or associated Symbiodiniaceae cells (P_{mc}=0.702 and 0.191, respectively). However, at the sub-class level, Acylsphingomyelin (ASM) significantly decreased in abundance in all treatments (11 ºC, 14 ºC and 20 ºC) compared against controls (p_{adj} <0.001) for *P. versipora* tissue.

While acute temperature exposure did not yield many changes in lipid profiles, comparing the control samples (summer controls 24 ºC, and winter controls 17 ºC) of each coral species across the seasons provided evidence of natural variation in lipid sub-class compositions between summer and winter (Figure 3.3, Supplementary Tables S3.11, S3.12). For *P. aliciae* tissue, the proportional abundance of all sterols (ST), oxidised lipids, and prenols, predominantly consisting of vitamin-A fatty esters (VAE), were higher during winter than summer (Figure 3.3A, Supplementary Figure S3.3A, Supplementary Table S3.13, ANOVA FDR <0.001). While the total proportion of ether-linked lipids and glycerophospholipids was not significantly different between summer and winter, *P. aliciae* tissue had a larger proportion of ether-linked phosphatidylcholine (EtherPC) during summer than winter, and ether-linked diacylglycerol (EtherDG) and phosphatidylserine (PS) were higher in winter than summer (Figure 3.3A, Supplementary Table S3.13). In Symbiodiniaceae associated with *P. aliciae,* ether-linked lipid abundance was larger in summer than winter (Figure 3.3A, Supplementary Table S3.14 ANOVA FDR<0.001). The composition of ether-lipids was also altered across seasons, with higher proportions of ether-linked phosphatidylethanolamine (EtherPE) and ether-linked Lysophosphatidylethanolamine (EtherLPE) in summer, and higher proportions of EtherDG in winter. Fatty acyls proportions were also higher in summer than winter in Symbiodiniaceae cells (Figure 3.3A, Supplementary Figure S3.3A, Supplementary Table S3.14, ANOVA FDR <0.001). The lipid classes sterols, prenols (predominantly VAE), glycerolipids and sphingolipids, were all higher in *P. aliciae* Symbiodiniaceae during winter *versus* summer (Figure 3.3A, Supplementary Figure S3.3A, Supplementary Table S3.14, ANOVA FDR 0.0027, <0.001 respectively). At the subclass level, within glycerolipids, both digalactosyldiacylglycerol (DGDG) and triacylglycerol estolides (TG-EST) had higher proportions during winter than summer, while proportions of monogalactosyldiacylglycerol (MGDG) abundance remained consistent between the two seasons (Figure 3.3A, Supplementary Table S3.14).

Similar trends in seasonal changes to lipid proportions were observed in *P. versipora*, including higher proportions of fatty acyls (specifically N-acyl ethanolamine (NAE)), sphingolipids (driven by ceramide non-hydroxyfatty acid-sphingosine (Cer-NS)) and glycerolipids in coral tissues in summer versus winter (Figure 3.3B, Supplementary Figure S3.3B, Supplementary

Table S3.15, ANOVA FDR <0.001, 0.0147, and 0.0015, respectively). Meanwhile, prenols (predominantly VAE, ANOVA FDR <0.001), and oxidised lipids (predominantly oxidised phosphatidylinositol (OxPI) and oxidised triacylglycerol (OxTG), ANOVA FDR <0.001) were higher in winter compared to summer (Figure 3.3B, Supplementary Table S3.15). Total ether-lipid proportions did not differ between seasons in the coral tissue; however, corals from summer had higher proportions of EtherPE and EtherTG compared to winter, while winter corals had higher proportions of EtherLPE than corals from summer (Figure 3.3B).

Of the lipids extracted from the Symbiodiniaceae of *P. versipora*, ether-linked lipids had higher proportions in summer than winter (Figure 3.3B, Supplementary Figure S3.3B, Supplementary Table S3.16, ANOVA FDR <0.001), driven by higher abundances of EtherPC and EtherLPE. Similar to the lipids extracted from Symbiodiniaceae of *P. aliciae*, larger proportions of EtherDG were detected during winter *versus* summer (Figure 3.3B). Additionally, glycerophospholipids had larger proportions in summer extracts (Figure 3.3B, Supplementary Figure S3.3B, Supplementary Table S3.16, ANOVA FDR<0.001), driven by increased abundances of lysophophatidylcholine (LPC) and lysophosphatidylethanolamine (LPE), although total PC proportion was greater in winter. Again, similar to Symbiodiniaceae from *P. aliciae*, the proportions of lipid classes prenols, sterols, glycerolipids (subclasses DGDG and TG-EST), and oxidised lipids, particularly subclass OxTG, were higher in *P. versipora* Symbiodiniaceae from winter *versus* summer (Figure 3.3B, Supplementary Figure S3.3B, Supplementary Table S3.16, ANOVA FDR <0.001).

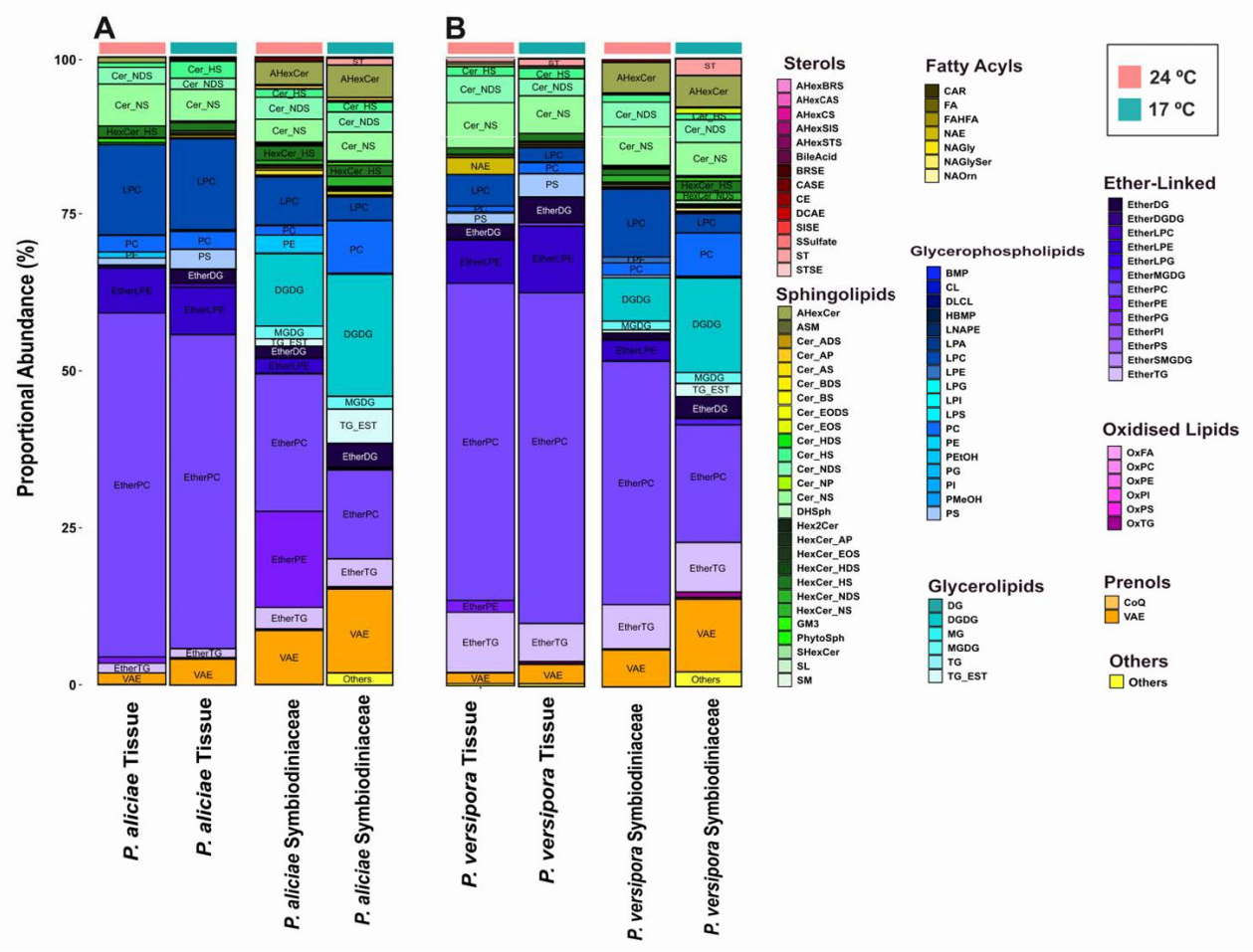


Figure 3.3: Proportional abundance of lipid sub-class data across control averages (Summer (24 ºC), and Winter (17 ºC) in *P. aliciae* host and Symbiodiniaceae **(A)** and *P. versipora* host and Symbiodiniaceae **(B).** N = 10, per species per season.

3.4.4 Unsaturated Fatty Acid (UFA) to Saturated Fatty Acid (SFA) ratio.

The unsaturated fatty acid to saturated fatty acid ratio (UFA:SFA) can inform the fluidity of biological membranes. We found the UFA:SFA ratio of both *P. aliciae* and *P. versipora* coral tissue or associated Symbiodiniaceae community was not impacted by acute heat exposure (Supplementary Table S3.17, P_{mc}>0.05). Following cold exposure, the UFA:SFA of *P. aliciae* coral tissue was not impacted by cold temperatures, but was lower in associated Symbiodiniaceae from the 11 ºC treatment compared to controls (P_{mc}=0.047, Supplementary Table S3.17). However, no significant change was detected in the UFA:SFA of *P. versipora* host tissue or associated Symbiodiniaceae (P_{mc}>0.05) following cold exposure.

3.5.0 Discussion

This study used a CBASS experimental design to test survivability and changes to fitness based on photobiology and lipidomics of coral tissue and Symbiodiniaceae cells from the subtropical coral *P. aliciae* collected from the poleward limit of its narrow distribution, and the temperate species *P. versipora* which has a wide high-latitude distribution. These species not only represent corals of varying geographical distribution, but also represent corals with different reproductive strategies, morphologies, associated symbionts, and microbiomes. These factors are all known to influence the lipid profile (Sikorskaya, 2023), so whilst impossible to directly compare the lipid profiles of these species, it was expected that they would respond differently to temperature stress. Corals were exposed to acute temperature changes reflecting large fluctuations possibly experienced during winter (20 – 11 ºC) and summer (24 – 32 ºC). Whilst temperature fluctuations are to be expected in temperate reefs like Sydney, the upper and lower temperatures chosen here exceed temperatures normally experienced in Sydney. This study represents the first known application of cold stress using a CBASS design, and on a temperate coral species. It is also the first application of coral lipidomics following the CBASS acute temperature stress exposure and recovery period procedure.

3.5.1 Photosynthetic Efficiency

Numerous CBASS applications on tropical coral species have demonstrated significant reductions in photophysiological efficiency under increased temperatures, allowing for the generation of ED50 models to determine at what specific temperature F_v/F_m values are 50% below controlled conditions (Evensen et al., 2022). All reductions in photophysiology within this study were less than 10% below baseline conditions, and mostly remained within 0.05 of control treatments, thus while we cannot reliably apply an ED50 model here, the trends observed do reflect the expected photophysiological declines under extreme heat (Iglesias-Prieto et al., 1992) and cold (Saxby et al., 2003). Klepac et al., (2024) warn against the use of F_v/F_m alone in determining the stress response of corals, and thus inclusion of broader metrics for future CBASS experimentation is advised and may enable increased understandings on the impacts of temperature-related stress on coral photobiology. To our knowledge, lipidomics has not similarly been applied to the CBASS framework and may provide further understanding of the

thermotolerance adaptive mechanisms that allow corals to survive under short-term temperature variation characteristic of thermal stress events.

Interestingly, the highest temperature (32 ºC) temporarily affected the photosynthetic efficiency of photosystem II (F_v/F_m) of the subtropical coral (*P. aliciae*), but not the temperate coral (*P. versipora*). In *P. aliciae*, F_v/F_m of corals at 32 ºC dropped 6% below controls (24 ºC) after the 1-hour dark recovery period and returned to control levels following 11-hours of recovery at control temperatures. However, *P. versipora* did not exhibit decreased photobiological function compared to controls under increased temperatures, as might be anticipated from a temperate coral. Indeed, *P. versipora* PSII was impacted in a previous study where temperatures were raised to 26 ºC; however, declines in F_v/F_m were only evident after a 6-day period (González-Pech et al., 2022). This suggests that *P. versipora* may be resilient to acute heat stress, but not if unfavourable conditions are prolonged or if temperatures continue to rise as predicted under climate change. Additionally, under controls in winter and summer experimentation, F_v/F_m of *P. versipora* was reduced at 24 ºC compared to 17 ºC. The unexpected difference in the photophysiological response of the corals is further supported by findings that encrusting corals may be more thermally tolerant when compared against branching corals (Loya et al., 2001; Qin et al., 2019). Indeed, previous studies comparing *P. versipora* and *Pocillopora damicornis* found the branching pocilloporid coral to be more affected by faster heating than *P. versipora* (Sahin et al., 2023). Furthermore, as Sydney, like other high-latitude temperate environments, is characterised by high seasonal fluctuations in temperature not similarly found in lower latitude or polar regions (Foster et al., 2014), the apparent acute temperature stress tolerance by both *P. aliciae* and *P. versipora* may be a mechanism for survival in highly-fluctuating environmental conditions, especially in the short-term. This tolerance to large temperature fluctuations is further supported by photophysiology evidence under cold stress. Both coral species had reduced photophysiological efficiency under extreme cold temperatures (11 ºC), however only *P. aliciae* photophysiology was impacted at 14 ºC. Additionally, the photophysiology of *P. aliciae* exposed to 11 ºC remained impaired even after the 11-hour recovery period. Corals under cold stress show more immediate effects to coral photophysiology, but a high recovery potential under longer term exposure (Roth et al., 2012). Further, it is plausible that *P. aliciae* photosynthetic efficiency may be more impacted by acute temperature stress, especially cold stress, due to

thermal conditions in Sydney being less optimal than in the more northerly core range of the species in the Solitary Islands region, although further research on *P. aliciae* photobiology is needed to test this. Despite this, *P. aliciae* exhibited no change in F_v/F_m in controls over the two seasonal sampling points, suggesting an adaptive capacity to temperature allowing for persistence in temperate Sydney.

3.5.2 Lipid Profiling

The minimal effects detected in the F_v/F_m data suggest the acute exposure to temperatures (11 ºC - 32 ºC) were within the physiological capacity of the corals and Symbiodiniaceae and did not elicit a stress response in *P. aliciae* nor *P. versipora*. This is further reflected in the lipid profiles of the corals and Symbiodiniaceae, which did not significantly shift in response to acute temperature exposures. Corals at higher latitudes are able to survive in areas characterised by colder temperatures and high seasonal as well as daily temperature variation (Moustafa et al., 2014) and have been found to show enhanced short-term performance, higher resilience to bleaching and increased recovery rates post stress compared to warmer regions (Cant et al., 2023; Riegl & Piller, 2003; Schoepf et al., 2015). As such, both *P. aliciae* and *P. versipora*, in occupying Sydney environments, may also have this capacity to withstand and recover from short-term stress, hence why no major metabolic change occurred even at temperatures well above, and below, seasonal averages. Further, previous metabolic profiling on *P. aliciae* and *P. versipora* revealed changes to the metabolic profile of *P. aliciae* under increased temperature stress, but lack of a metabolic response under the same stress in *P. versipora* (González-Pech et al., 2022). That experimental design consisted of a longer-term exposure to increased temperatures, and metabolomics sampling occurring at peak heat stress. Our study, however, aimed to assess lipid remodelling as an adaptive mechanism to changes in environmental conditions, rather than a direct stress response, hence lipidomic sampling was conducted following the recovery period. The lack of lipid remodelling under acute temperature variation suggests that short-term temperature fluctuations do not significantly affect lipid metabolism of subtropical *P. aliciae* at their poleward range limit, or in the temperate coral *P. versipora,* as previously hypothesised (Goyen et al., 2019). While a similar sampling protocol and CBASS design has been successfully used to detect changes in the genes, metabolites, and proteins of tropical corals under acute temperature stress (Savary et al., 2021; Voolstra et al., 2020), this is

the first application of CBASS to identify lipidomics changes. Sampling for lipidomics occurred at the end of the experiment, after an 11-hour post-stress recovery period. Previous studies on microalgae found variation to accumulation of lipids under environmental stress to occur from 4-hours of exposure (Cho & Shin, 2016). As such, this experimental design (i.e., the 3-hour duration of temperature treatment exposure) may not have been sufficient to significantly affect the lipid interactions or compositions in the coral holobionts. Furthermore, it is possible that the 11-hour recovery period allowed any potential lipid remodelling in response to external stimuli to return to pre-experimental abundances and composition. This highlights that the duration of stress treatment and recovery periods should be carefully considered in future studies evaluating homeoviscous adaptation in corals as a mechanism of survivability, particularly in regions where corals naturally exist in highly dynamic environments.

In *P. versipora* associated Symbiodiniaceae, one individual lipid, NAE 13:0 was found to be decreased in all corals exposed to increases in temperature (27 ºC, 30 ºC, and 32 ºC). While N-acyl ethanolamines (NAE's) are yet to be explored within microalgae, NAEs have been shown to be important molecules in signalling and regulatory pathways in plants and animals (Blancaflor et al., 2014; Coulon et al., 2012). Additionally, NAE is characterised as a lipid mediator, meaning these lipids are often produced through specific pathways in response to environmental stimuli (Coulon et al., 2012; Murakami, 2011). Changes to lipid stores and the metabolic profile have been linked to the breakdown of the coral-Symbiodiniaceae symbiosis (Nielsen & Petrou, 2023), including under increased temperatures (Strychar et al., 2004), and decreases in signalling molecules, such as NAE, could potentially be a precursor to symbiosis breakdown. Further, previous studies on Symbiodiniaceae found increases in N-acyl lipids (including NAE) in *Cladocopium goreaui* and *Breviolum psygmophilum* grown at 31 ºC compared to controls (26 ºC) (La Motta et al., 2024), suggesting the importance of NAE in resilience to heat stress. However, targeted future research is required to fully understand the role of the decrease in NAE 13:0 specifically under increased temperatures.

Under temperature decreases, there were no significant alterations to the lipid profiles or individual lipids between temperatures. However, when grouped into sub-class data, Acylsphingomyelin (ASM) was reduced in abundance in *P. versipora* tissue in each treatment

compared against controls. Sphingolipids are known to function within cellular membranes and cellular signalling in marine microalgae and are comprised of Spingoid bases as derivatives, including sphingomyelin (SM), ceramides (Cer) and sphingosines (Sph) (Li et al., 2017). Sphingomyelin (SM), a type of sphingolipid, has been shown to be a dominant sphingolipid within the plasma membrane (Slotte, 2013). ASM has not been well described, yet most likely performs a similar function within cellular membranes. The structure of ASM includes a fatty acid chain bonded to the hydroxyl group in sphingomyelin (Liebisch et al., 2020). The acyl chains of SM have an affinity for cholesterol (Engberg et al., 2020; Lönnfors et al., 2011), which performs an important function within cellular membranes under cold stress and increases permeability by preventing lipids from aggregating (Singer & Nicolson, 1972). Here, we found reductions in ASM under both cold treatments in *P. versipora* (11 ºC and 14 ºC), and the slight temperature increase treatment (20 ºC). However, with the minimal references of this interaction within algae, it is difficult to speculate why this change occurred. Interestingly, reductions in ASM abundances (20 ºC, 14 ºC and 11 ºC) coincided with increased unsaturated fatty acid to saturated fatty acid (UFA:SFA) ratios. While we did not detect significant differences in UFA:SFA ratios between treatments, UFA:SFA ratios were close to double in 11 ºC, 14 ºC and 20 ºC treatments compared to controls (17 ºC). This could suggest a diversion in membrane stabilisers from sphingolipids to unsaturated fatty acyls when temperatures are reduced, where increased abundances of unsaturated fatty acids have been linked to decreases in temperature as a mechanism to acclimate to temperature stress by maintaining viscosity and permeability of membranes (Holm et al., 2022; Lauritano et al., 2020; Sikorskaya et al., 2022). Furthermore, lipid unsaturation in *P. versipora* coral tissue and associated Symbiodiniaceae was significantly higher compared to *P. aliciae*, but especially throughout the cold-stress experiment, including controls representing the mean yearly minimum experienced in the Sydney Harbour area (17 ºC). Although we were not able to confirm trends in FA unsaturation with decreased temperatures, the higher proportion of UFA:SFA exhibited by *P. versipora*, especially in winter, may be a mechanism that allows the higher latitude distribution of this species (i.e., to Tasmania), whereas *P. aliciae* is at its poleward range limit.

There were significant differences in the lipid profiles of *P. aliciae* and *P. versipora* host and Symbiodiniaceae across a seasonal scale that could highlight lipid mechanisms contributing to coral survivability in this biogeographic transition zone. Seasonal metabolic remodelling within

corals has been previously recorded, especially in regard to storage lipids (TGs) and membrane lipids (FAs) (Imbs & Dang, 2021; Oku et al., 2003). Ether-linked lipids were the most proportionally abundant lipids across this experiment, especially within coral tissue, and had higher proportional abundances in the Symbiodiniaceae extracts from summer corals (Figure 3.4). Ether-linked lipids consist of a glycerolipid in which an alkyl chain is attached to the glycerol backbone via an ether bond (Dean & Lodhi, 2018). This class of glycerolipids has been determined to function in an antioxidant capacity by reducing reactive oxygen species (ROS) accumulation (Dean & Lodhi, 2018). ROS can accumulate as temperature increases (Amario et al., 2023) and decreases (Marangoni et al., 2021), with increased abundance of ROS eventually causing oxidative stress resulting in cell apoptosis (Su et al., 2019). Recent studies indicate that ROS could also be produced in the host cell, whereas previous notions indicated this presence was attributed to leakage of ROS from Symbiodiniaceae (Dungan et al., 2022; Nielsen & Petrou, 2023). The large proportions of ether-linked lipids in host tissue found here could present a mechanism that allows the reduction of ROS accumulation within coral tissue under temperature stress. Additionally, higher proportional abundances of ether-linked lipids in Symbiodiniaceae from winter corals, coincided with overall higher abundances of oxidised lipids within *P. versipora* Symbiodiniaceae (Figure 3.3B). This supports the notion that ether-linked lipids may be a mechanism in controlling ROS abundance, with the lower ether-linked lipid abundance observed within *P. versipora* Symbiodiniaceae exposed to cold stress potentially driving an increase in oxidised-lipids. Previous studies on Symbiodiniaceae, including *Cladocopium* and *Breviolum* species, have similarly observed reduced Ether-lipid abundance coinciding with increased abundance of oxidised lipids (La Motta et al., 2024). Despite this, it must be noted that there is the potential that the presence of ether-lipids may be due to translocation or contamination from the coral host, or from associated microbes, as there has been no documentation of ether-lipid production from Symbiodiniaceae (Sikorskaya, 2023). Evaluating Ether-lipid activity under peak stress would shed light on this hypothesis.

Both coral species and their associated Symbiodiniaceae exhibited potential lipid membrane remodelling that may be a mechanism for survival against higher seasonal variation in temperate reefs compared to the tropics. Cold stress is known to cause lipid packing within cellular membranes, increasing rigidity which may impact structure and cell signalling (Los & Murata, 2004; Wu et al., 2023). Larger proportions of VAE, a prenol lipid, and sterols (ST) were detected

in winter corals in both coral tissues and Symbiodiniaceae cells; both prenols and sterols are known to be essential for metabolism regulation and maintenance of homeostasis, increasing cell membrane permeability and maintaining cellular structure (Benveniste, 2004; Hayashi et al., 2014; Wollam & Antebi, 2011). In Symbiodiniaceae, VAE has previously been hypothesised to be involved in a pathway in which, under phosphorous limitation, membranes diverted from phospholipid to isoprenoid synthesis (La Motta et al., 2024). The higher proportion in winter corals detected here similarly suggests a mechanism in which membrane lipids are remodelled to prevent the destabilising of membranes under cooler temperatures.

Despite overall glycerophospholipid abundance remaining consistent between winter and summer in both species of coral and their Symbiodiniaceae, the composition of these lipids was found to differ. There was an overall higher proportion of PC in the host coral tissue and Symbiodiniaceae of both coral species in winter corals, which, in the case of *P. aliciae*, also coincided with lower proportions of PE (Figure 3.3A). This was similarly observed within the ether-linked version of these lipids in Symbiodiniaceae of *P. aliciae*. PE is not well described in algae, but is an abundant cellular membrane lipid in all eukaryotes, and increased abundance can decrease membrane permeability (Murzyn et al., 2005; Vance & Tasseva, 2013). Interestingly, the trend observed within *P. aliciae* has similarly been found in bacteria, with decreased PE abundance thought to counteract phospholipid packing with membranes, and increases in PC under colder temperatures evidence of lipid modulation in membrane adaption to temperature change (Chwastek et al., 2020; Wu et al., 2023). This mechanism, unique to *P. aliciae*, may be an adaptive strategy that allows this subtropical coral to tolerate cold conditions at the poleward limits of its distribution. Further, *P. aliciae* coral tissue exhibited a high abundance of LPC that did not differ between the seasons. LPC has been studied in microalgae for medicinal function, as it has been found to possess anti-inflammatory properties (Lauritano et al., 2020), but the specific function within microalgae has yet to be described. Lyso, as a prefix, denotes that the lipid (PC) has undergone hydrolysis, and lost one of its fatty acid chains (Wnorowski et al., 2021), and as such, may serve an altered function from PC. LPC has been identified as one of the main components of oxidised low-density lipoprotein (oxLDL) (Zhang et al., 2023), and can contribute to oxidative stress and eventual cell apoptosis when the antioxidative capacity of the cells becomes impaired (Hsieh, 2001), such as when under severe temperature stress. However, under controlled conditions as observed here, LPC may instead perform an important function

within cellular signalling, and potentially initiate an antioxidant response under external stimuli (Zingg et al., 2021). The higher proportions of LPC observed in *P. aliciae* coral tissue may be a mechanism that allows this subtropical coral to tolerate increased temperature stress.

Glycerolipids, mostly consisting of DGDG and MGDG, were more abundant within Symbiodiniaceae under winter controls (17 ºC) than in summer (24 ºC) (Figures 3.3, 3.4). DGDG and MGDG are important lipids within the thylakoid membrane, serving essential functions in photosynthesis (Boudière et al., 2014). Additionally increased proportions of DGDG and TG-EST were observed in the Symbiodiniaceae of both coral species under winter temperatures (17 ºC) compared to summer controls (24 ºC) (Figures 3.3, 3.4). Studies within plants have found that, under cold stress, increases in DGDG can be linked to increased cellular membrane permeability, and decreases in the MGDG:DGDG ratio in favour of higher DGDG abundance may be due to MGDG conversion into DGDG (Moellering et al., 2010; X. Zhao et al., 2021). In microalgae, glycerolipids play a major function in energy storage (Li-Beisson et al., 2019), with higher abundances of DGDG known to support photosynthetic function under external stimuli, such as temperature or nutrient stress (Kalisch et al., 2016). Photophysiology was most impacted by cold exposure in both corals; thus, higher proportions of glycerolipids under winter temperatures could suggest a mechanism in maintaining physiological function as temperatures continue to drop. This may thus be an adaptive strategy of Sydney corals allowing for survival in high-latitude environments characterised by varied light availability across a seasonal scale (Gattuso et al., 2006). TG-EST are essentially reservoirs of fatty acid ester of hydroxyl fatty acid (FAHFA), that function as lipid messengers within plants and mammals (Brejchova et al., 2021; Cudlman et al., 2023), as well as important lipids within the permeability barrier of membranes in bacteria (Wood, 2020). TG-EST similarly had higher proportions in Symbiodiniaceae from winter corals. The functions of FAHFA and TG-EST in algae remain unresolved but are thought to perform similar functions in membrane permeability.

3.5.3 Implications for CBASS experiments for temperate and subtropical corals

CBASS has proven a valuable tool for assessing the thermotolerance and photosynthetic efficiency of tropical corals under heat stress, and to elucidate metabolic and genetic markers of stress in corals (Evensen et al., 2023; Klepac et al., 2024; Voolstra et al., 2020). Here, we applied

the CBASS experimental on temperate and subtropical corals and adapted the profile to measure photosynthetic and physiological responses under temperature decreases, relevant to persistence in cooler high-latitude environments. Coral populations located at high latitudes are more resilient to short-term stress events, allowing for resilience to, and recovery from, significant daily and seasonal fluctuations in temperature (Cant et al., 2023; Foster et al., 2014; Riegl & Piller, 2003). As such, the CBASS experimental model may not be suitable, or may need to be altered (e.g., to expose corals to longer periods of stress and/or more extreme temperatures), if the intent is to derive an ED50 value for temperate corals. Furthermore, we conducted the first lipidomic sampling following the acute stress CBASS design. Lipidomics samples were collected following a recovery period rather than at peak stress in order to avoid detecting stress response-specific lipid profiles, and instead measure adaptive shifts in lipids. Prior evidence supports the capacity for *P. aliciae* and *P. versipora* to remodel their lipid profile in response to temperature stress (González-Pech et al., 2022; Goyen et al., 2019), however this was not observed within this study. It is therefore possible that the acute temperature exposure period was not sufficient to modify lipids, and if any had modified, it is possible the extended recovery period allowed for lipids to reset to control levels within the experimental period (Cho & Shin, 2016). Allowing for longer exposure to peak temperatures and/or sampling at an earlier timepoint (such as after the 1-hour dark recovery), might provide a more reliable assessment as to whether these corals can modify their lipid profiles in response to specific temperature fluctuations.

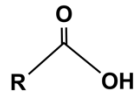
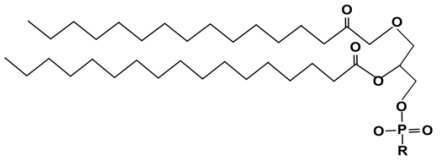
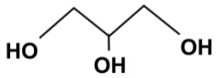
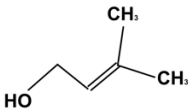
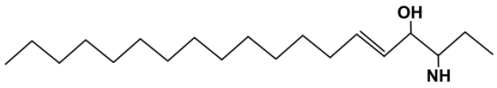
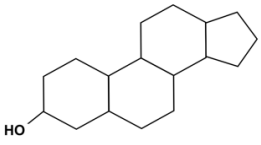
3.5.4 Conclusions

Despite minimal alterations to the lipid profile of *P. aliciae* and *P. versipora* across a temperature gradient, the modifications to lipidomic profiles of these corals between seasons highlight potential lipid mechanisms contributing to survival at the limits of their thermal distributions. Specifically, we observed a higher proportion of lipids with specific functions within cellular membranes (VAE, ST, PC) in both species of coral and their associated Symbiodiniaceae from winter samples. Meanwhile in summer corals, ether-linked lipid proportions were higher in the Symbiodiniaceae of both coral species, and lower proportions of ether-linked lipids in *P. versipora* Symbiodiniaceae from winter coinciding with increased

proportions of oxidised lipids, suggesting a link between higher ether-linked lipid proportion may control ROS accumulation. Finally, increased proportions of DGDG and TG-EST were also observed in Symbiodiniaceae under winter sampling, potentially enhancing stability in photosynthetic efficiency under lower light levels associated with winter conditions. Overall, these results suggest lipid remodelling between seasons that could allow for corals living in biogeographic transition zones in temperate reef environments to adapt to high short-term and seasonal variations in temperature.

3.6.0 Supporting Information

Table S3.1 Lipid molecular structural data for lipid categories

Lipid Category	Structure
Fatty acyls	
Glycerophospholipids	
Glycerolipids	
Prenol Lipids	
Sphingolipids	
Sterol Lipids	

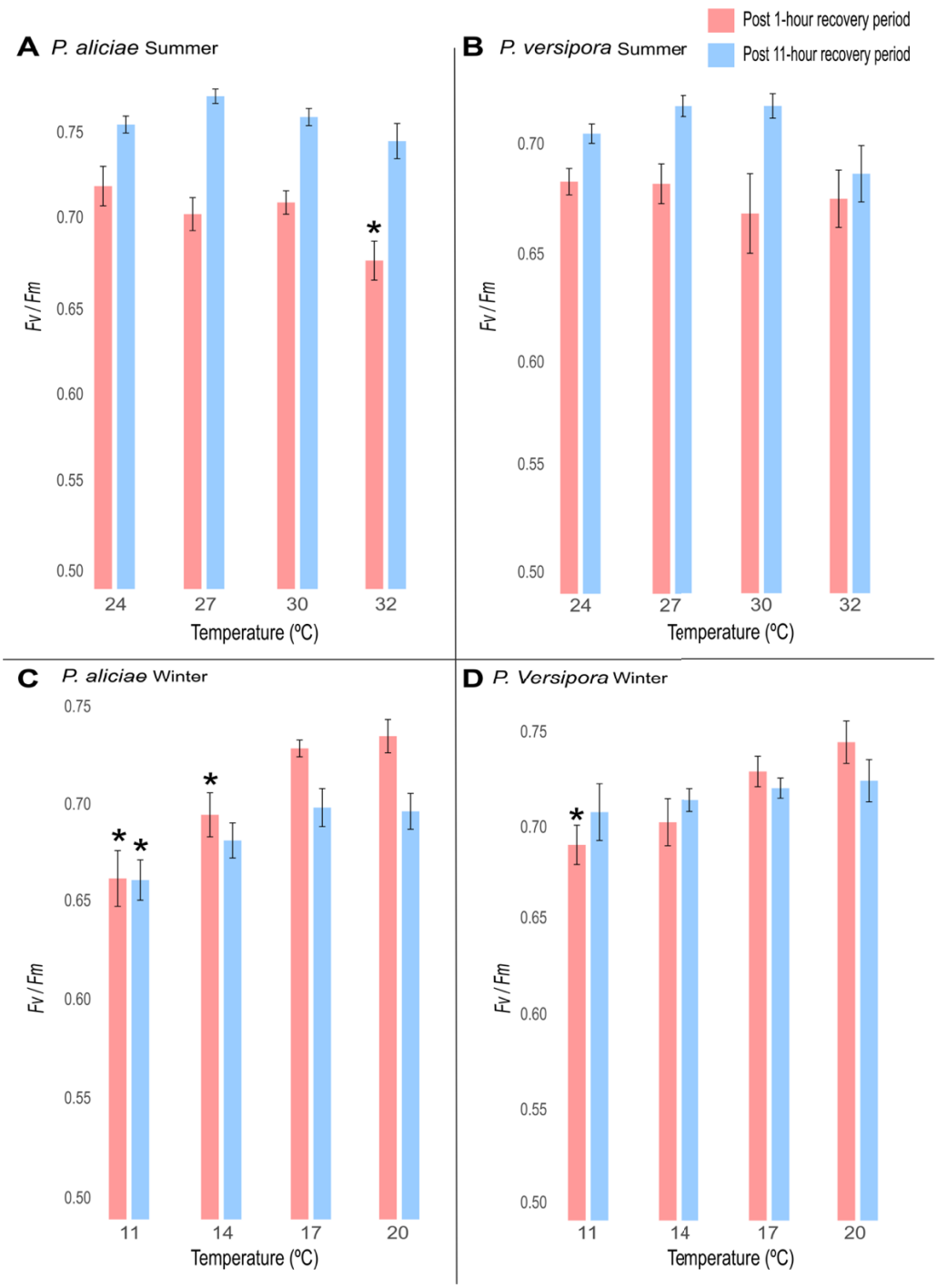


Figure S3.1 Photosynthetic efficiency (F_v/F_m, dimensionless) of *P. aliciae* (A) and *P. versipora* (B) in the summer experiment, and *P. aliciae* (C) and *P. versipora* (D) in the winter experiment after the 1-hour dark recovery period (red) and after the 11-hour recovery period (blue). PERMANOVA permutational MANOVA (PERMANOVA) (P_{mc}) significance shown using *(P_{mc}<0.05).

Table S3.2 Photosynthetic efficiency (F_v/F_m, dimensionless) of *P. aliciae* across temperatures in the summer experiment after the 1-hour dark recovery period, including percent change and pairwise testing (P_{mc}) significance from controls (24 ºC)

Temperature	F_v/F_m	% Change	pairwise (P_{mc})
24 ºC	0.719	-	-
27 ºC	0.703	-2.247	0.301
30 ºC	0.709	-1.315	0.524
32 ºC	0.676	-5.976	0.015

Table S3.3 Photosynthetic efficiency (F_v/F_m, dimensionless) of *P. aliciae* across temperatures in the summer experiment at the endpoint after an 11-hour recovery period, including percent change from controls (24 ºC) and pairwise testing (P_{mc})

Temperature	F_v/F_m	% Change	pseudo-f	pairwise (P_{mc})
24 ºC	0.75415	-	2.9768	0.054
27 ºC	0.7705	2.16800371		
30 ºC	0.7585	0.57680833		
32 ºC	0.7446	-1.2663263		

Table S3.4 Photosynthetic efficiency (F_v/F_m, dimensionless) of *P. aliciae* across temperatures in the winter experiment after the 1-hour dark recovery period, including percent change and pairwise testing (P_{mc}) significance from controls (17 ºC)

Temperature	F_v/F_m	% Change	pairwise (P_{mc})
20 ºC	0.734	0.859	0.002
17 ºC	0.728	-	-
14 ºC	0.694	-4.650	0.013
11 ºC	0.662	-9.108	0.533

Table S3.5 Photosynthetic efficiency (F_v/F_m, dimensionless) of *P. aliciae* across temperatures in the winter experiment at the endpoint after an 11-hour recovery period, including percent change and pairwise testing (P_{mc}) significance from controls (17 ºC)

Temperature	F_v/F_m	% Change	pairwise (P_{mc})
20 ºC	0.696	-0.272	0.893
17 ºC	0.698	-	-
14 ºC	0.681	-2.400	0.197
11 ºC	0.661	-5.295	0.021

Table S3.6 Photosynthetic efficiency (F_v/F_m, dimensionless) of *P. versipora* across temperatures in the summer experiment after the 1-hour dark recovery period, including percent change from controls (24 ºC) and pairwise testing (P_{mc})

Temperature	F_v/F_m	% Change	pseudo-f	pairwise (P_{mc})
24 ºC	0.684	-	0.38549	0.76
27 ºC	0.683	-0.146		
30 ºC	0.668	-2.216		
32 ºC	0.675	-1.192		

Table S3.7 Photosynthetic efficiency (F_v/F_m, dimensionless) of *P. versipora* across temperatures in the summer experiment at the endpoint after an 11-hour recovery period, including percent change from controls (24 ºC) and pairwise testing (P_{mc})

Temperature	F_v/F_m	% Change	pseudo-f	pairwise (P_{mc})	pairwise (P_{mc})
24 ºC	0.701	-	3.6876	0.018	-
27 ºC	0.719	2.603			0.072
30 ºC	0.719	2.611			0.1
32 ºC	0.687	-1.969			0.175

Table S3.8 Photosynthetic efficiency (F_v/F_m, dimensionless) of *P. versipora* across temperatures in the winter experiment at the endpoint after an 11-hour recovery period, including percent change and pairwise testing (P_{mc}) significance from controls (17 ºC)

Temperature	F_v/F_m	% Change	pairwise (P_{mc})
20 ºC	0.741	2.142	0.301
17 ºC	0.726	-	
14 ºC	0.699	-3.741	0.075
11 ºC	0.687	-5.394	0.01

Table S3.9 Photosynthetic efficiency (F_v/F_m, dimensionless) of *P. versipora* across temperatures in the winter experiment at the endpoint after an 11-hour recovery period, including percent change from controls (24 ºC) and pairwise testing (P_{mc})

Temperature	F_v/F_m	% Change	pseudo-f	pairwise (P_{mc})
20 ºC	0.721	0.551		
17 ºC	0.717	-	0.55113	0.63
14 ºC	0.711	-0.879		
11 ºC	0.704	-1.799		

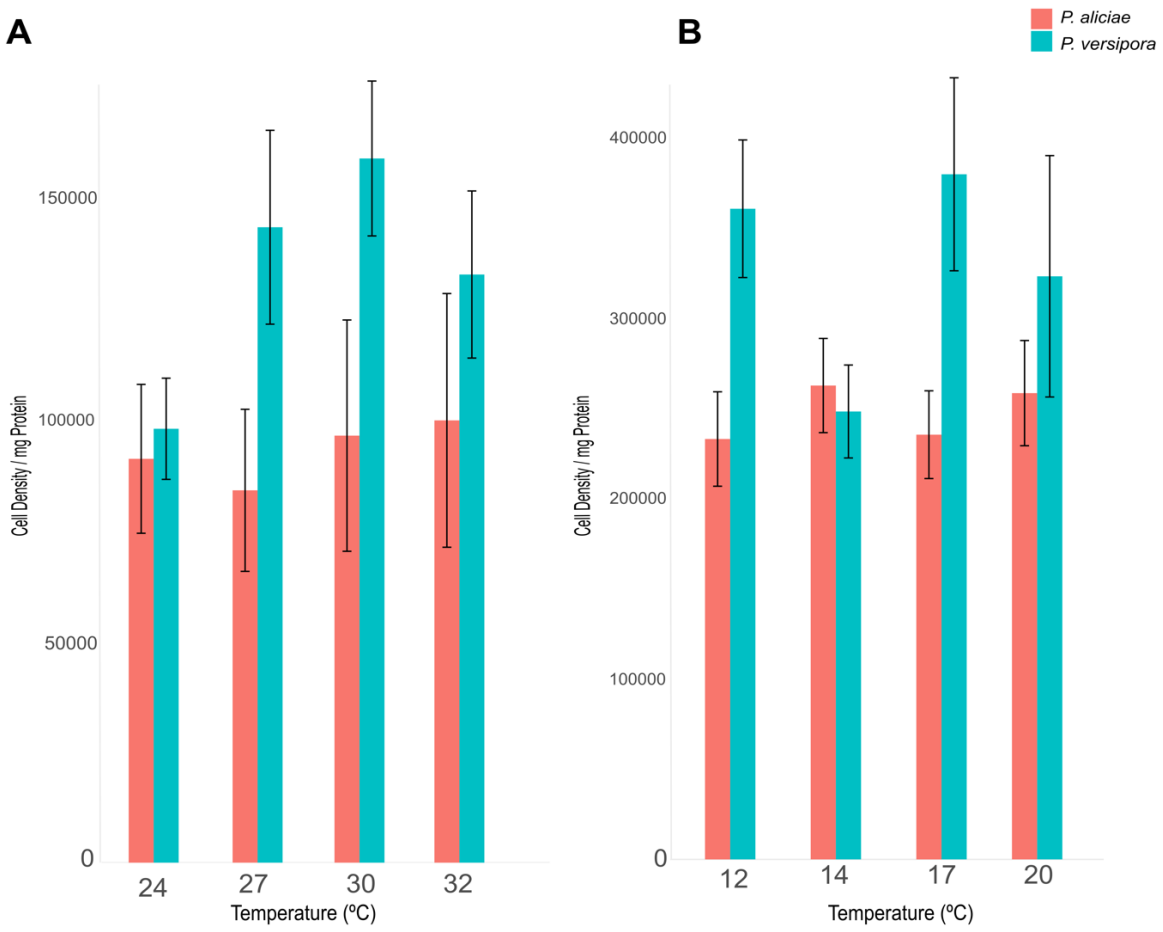


Figure S3.2 Symbiodiniaceae Cell Density / mg Protein of *P. aliciae* (Red) and *P. versipora* (Blue) in the summer experiment (A), and in the winter experiment (B)

Table S3.10: NAE 13:0 information and lipid structure

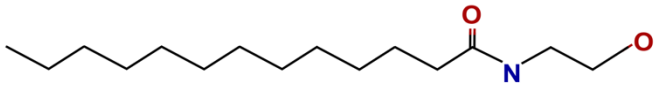
Name	**M/Z**	**Ionisation Mode**	**Structure**
NAE 13:0	258	[M+H]+	

Table S3.11: **SIMPER** Dissimilarity Analysis showing specific lipid driving separation between corals and Symbiodiniaceae in Summer and Winter controls (24 ºC, 17 ºC)

	Lipid	*P. aliciae* average abund	*P. versipora* average abund	Av diss.	Diss /sd	contrib%	cum%
Summer Symbiodiniaceae Av Dissim 59.91%	PC O-36:4	0.01	0.09	3.83	2.98	6.39	6.39
	PC O-35:8	0	0.04	2.1	3.71	3.51	9.9
	PE P-36:5	0.04	0	1.81	3.66	3.02	12.92
	LPC 15:0	0.03	0.05	1.33	1.36	2.22	15.13
	LPC 17:0	0.03	0.01	1.3	1.49	2.18	17.31
	PC O-38:6	0.03	0	1.3	2.61	2.18	19.49
	PE O-38:5	0.02	0	1.2	2.75	2	21.48
	PE O-40:6	0.02	0	1.19	1.06	1.98	23.46
Summer Tissue Av Dissim 60.62%	PC O-38:6	0.08	0	4.53	4.7	7.47	7.47
	PC O-36:4	0.03	0.11	4.51	2.71	7.43	14.91
	LPC 17:0	0.07	0.01	3.61	2.2	5.95	20.86
	PC O-34:3	0	0.04	1.83	3.38	3.01	23.87
	PC O-36:6	0.03	0	1.74	3.13	2.88	26.75
	LPE P-18:1	0.05	0.02	1.54	1.68	2.55	29.3
	PC O-38:5	0.03	0.01	1.51	3.66	2.48	31.78
	PC O-46:5	0.05	0.07	1.49	1.55	2.45	34.23
Winter Symbiodiniaceae Av Dissim 46.69%	PC O-38:5	0	0.04	1.94	1.94	4.15	4.15
	PC O-38:6	0.03	0	1.68	1.68	3.59	7.73
	PC 38:6	0.03	0.01	1.28	2.68	2.73	10.47
	PC O-36:5	0.02	0.05	1.27	1.76	2.72	13.19
	DGDG 36;8	0.04	0.02	1.1	1.81	2.36	15.54
	VAE 23:1	0.05	0.05	0.6	1.44	2.05	17.59
	TG 54:3;O3	0.02	0	0.93	1.59	1.99	19.57
	PC O-38:6	0	0.01	0.71	2.51	1.52	21.1
Winter Tissue Av Dissim 52.86%	PC O-38:6	0.12	0.01	6.28	3.78	11.89	11.89
	PC O-36:4	0.01	0.1	4.81	1.7	9.1	20.99
	TG 54:3;O3	0.1	0.12	3.65	1.36	6.9	27.89
	PC O-39:6	0.06	0	3.29	2.7	6.22	34.11
	PC O-36:5	0.07	0.12	2.69	2.17	5.08	39.19
	PC O-38:6	0	0.04	1.89	3.22	3.58	42.77
	LPE P-18:1	0.05	0.02	1.57	2.15	2.97	45.74
	PC O-36:6	0.03	0	1.54	3.16	2.92	48.66

Table S3.12: **SIMPER** Dissimilarity Analysis showing lipid sub-classes driving separation between corals and Symbiodiniaceae in Summer and Winter controls (24 ºC, 17 ºC)

	Species	*P. aliciae* av abund	*P. versipora* av abund	Av diss.	Diss /sd	contrib%	cum%
Summer Symbiodiniaceae Av Dissim 32.67%	EtherPE	0.17	0	9.01	6.68	27.56	27.56
	EtherPC	0.19	0.31	6.71	2.41	20.53	48.09
	LPC	0.09	0.09	2.09	1.35	6.41	54.5
	Cer-NS	0.04	0.08	2.02	2.61	6.19	60.69
	PE	0.04	0	1.94	2.6	5.93	66.62
Summer Tissue Av Dissim 23.69%	LPC	0.12	0.04	4.55	0.163	19.22	19.22
	EtherPC	0.35	0.35	4.06	1.28	17.15	36.37
	EtherTG	0.03	0.08	3.74	1.63	15.78	52.16
	NAE	0	0.03	1.69	1.81	7.13	59.28
	EtherLPE	0.06	0.07	1.36	1.12	5.74	65.02
Winter Symbiodiniaceae Av Dissim 17.29%	EtherPC	0.12	0.17	3.04	1.78	17.61	17.61
	DGDG	0.16	0.13	2.18	1.63	12.6	30.21
	TG-EST	0.05	0.02	1.5	2.19	8.67	38.88
	OxTG	0.03	0.01	1.39	1.56	8.03	46.91
	EtherTG	0.06	0.07	1.09	1.07	6.32	53.23
Winter Tissue Av Dissim 21.01%	EtherPC	0.38	0.33	0.62	1.11	29.49	29.49
	LPC	0.12	0.13	4.93	1.51	23.46	52.96
	EtherLPE	0.06	0.07	1.98	0.74	9.43	62.39
	EtherTG	0.02	0.04	1.5	2.48	2.48	69.53
	VAE	0.04	0.04	0.77	1.36	1.36	73.17

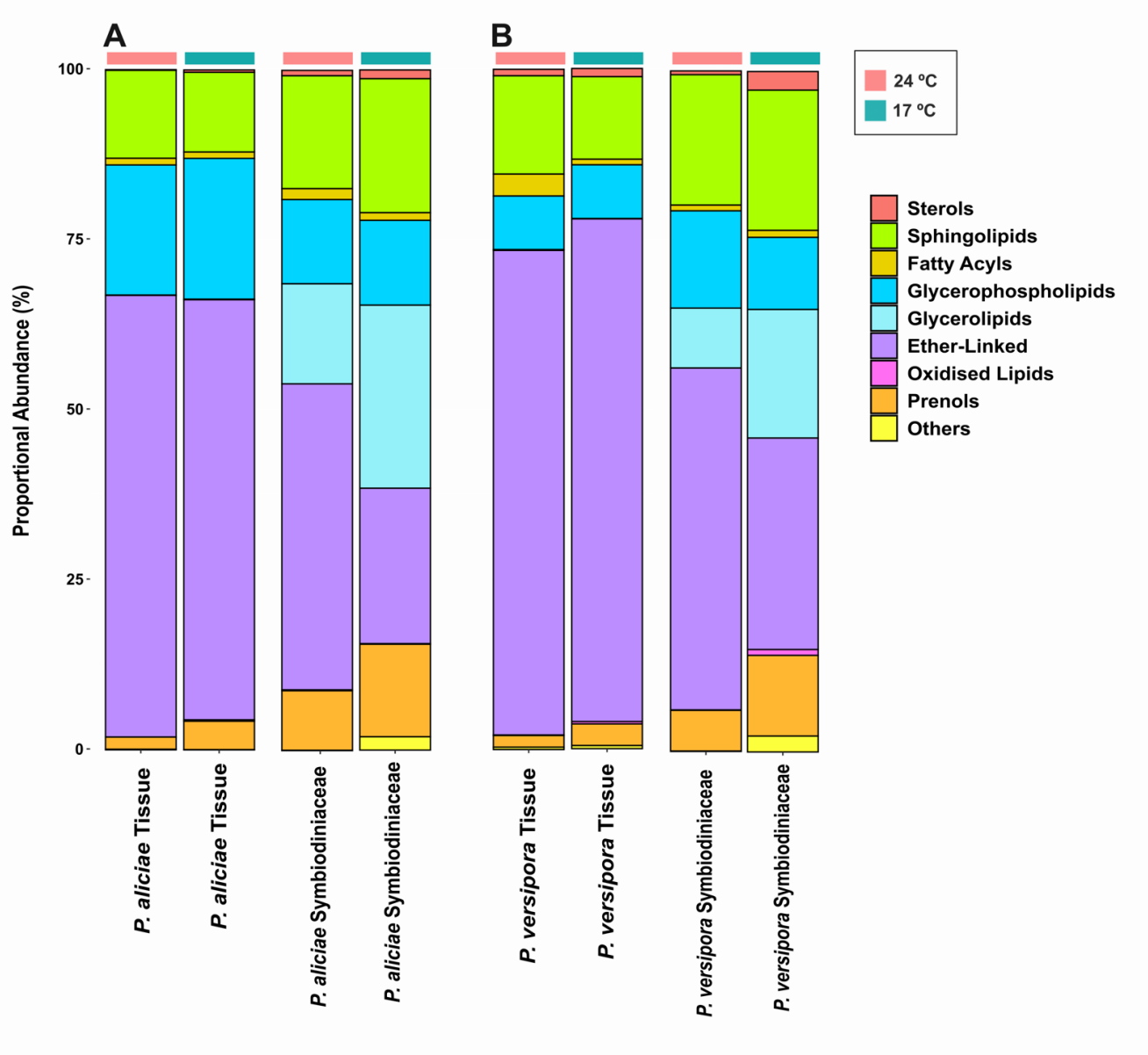


Figure S3.3: Proportional abundance of categorical grouped lipids across summer and winter controls (24 ºC and 17 ºC) in *Pocillopora aliciae* host and Symbiont **(A)** and *Plesiastrea versipora* host and symbiont **(B)** comparing sterols (red), sphingolipids (green), fatty acyls (gold), glycerophospholipids (blue), glycerolipids (aqua), ether-linked lipids (purple), oxidised lipids (pink), prenols (orange) and others (yellow).

Table S3.13: Variations in proportional abundance of lipid categories within *P. aliciae* coral tissue between Summer and Winter controls as determined by pairwise testing (FDR <0.05)

Category	t.stat	p.value	FDR
Sterols	-7.73	<0.001	<0.001
Prenols	-5.19	<0.001	<0.001
Oxidised Lipids	-21.74	<0.001	<0.001
Others	5.37	<0.001	<0.001
Glycerolipids	-2.66	0.212	0.0382

Table S3.14: Variations in proportional abundance of lipid categories within *P. aliciae* associated Symbiodiniaceae between Summer and Winter controls as determined by pairwise testing (FDR <0.05)

Category	t.stat	p.value	FDR
Sphingolipids	-6.2	<0.001	<0.001
Ether-Linked	12.61	<0.001	<0.001
Prenols	-6.19	<0.001	<0.001
Glycerolipids	-5.44	<0.001	<0.001
Others	-15.65	<0.001	<0.001
Sterols	-3.64	0.0021	0.0027
Fatty Acyls	4.81	<0.001	0.0013

Table S3.15: Variations in proportional abundance of lipid categories within *P. versipora* coral tissue between Summer and Winter controls as determined by pairwise testing (FDR <0.05)

Category	t.stat	p.value	FDR
Prenols	-6.85	<0.001	<0.001
Fatty Acyls	6.54	<0.001	<0.001
Oxidised Lipids	-6.09	<0.001	<0.001
Glycerolipids	3.81	0.0024	0.0049

Table S3.16: Variations in proportional abundance of lipid categories within *P. versipora* associated Symbiodiniaceae between Summer and Winter controls as determined by pairwise testing (FDR <0.05)

Category	t.stat	p.value	FDR
Oxidised Lipids	-18.59	<0.001	<0.001
Prenols	-11.44	<0.001	<0.001
Others	-14.47	<0.001	<0.001
Ether-Linked	8.69	<0.001	<0.001
Glycerolipids	-7.6	<0.001	<0.001
Sterols	-5.07	<0.001	<0.001
Glycerophospholipids	4.43	<0.001	0.0011
Fatty Acyls	-3.73	0.0016	0.0018

Table S3.17: The UFA:SFA ratio of P. aliciae and P. versipora coral tissue and Symbiodiniaceae across temperature treatments in summer and winter sampling points and significance determined by pairwise testing (P_{mc})

		Species	Temperature	SFA	UFA	UFA:SFA	Pairwise (P_{mc})
Summer	Symbiodiniaceae	*P. aliciae*	24 ºC	7.286E-09	1.154E-07	15.836	-
			27 ºC	5.056E-09	8.646E-08	17.099	0.612
			30 ºC	5.101E-09	8.060E-08	15.803	0.436
			32 ºC	5.452E-09	9.080E-08	16.653	0.454
		P. versipora	24 ºC	2.923E-09	6.088E-08	20.826	-
			27 ºC	3.509E-09	1.031E-07	29.373	0.578
			30 ºC	3.021E-09	9.129E-08	30.218	0.222
			32 ºC	5.749E-09	9.424E-08	16.393	0.242
	Tissue	*P. aliciae*	24 ºC	3.551E-09	1.196E-08	3.369	-
			27 ºC	2.185E-09	9.557E-09	4.374	0.219
			30 ºC	3.248E-09	1.600E-08	4.926	0.124
			32 ºC	2.528E-09	1.389E-08	5.496	0.100
		P. versipora	24 ºC	3.635E-09	4.109E-08	11.305	-
			27 ºC	1.627E-09	4.677E-08	28.747	0.655
			30 ºC	2.384E-09	1.060E-07	44.473	0.817
			32 ºC	2.680E-09	6.557E-08	24.467	0.205
Winter	Symbiodiniaceae	*P. aliciae*	11 ºC	8.158E-07	2.340E-06	2.868	**0.047**
			14 ºC	6.405E-07	2.088E-06	3.260	0.622
			17 ºC	8.046E-07	2.687E-06	3.339	-
			20 ºC	5.618E-07	1.983E-06	3.529	0.832
		P. versipora	11 ºC	4.457E-07	3.368E-06	7.558	0.745
			14 ºC	3.902E-07	3.445E-06	8.828	0.482
			17 ºC	4.241E-07	3.266E-06	7.701	-
			20 ºC	7.050E-07	4.667E-06	6.620	0.832
	Tissue	*P. aliciae*	11 ºC	5.881E-08	2.802E-07	4.765	0.880
			14 ºC	5.987E-08	2.741E-07	4.578	0.560
			17 ºC	4.983E-08	2.812E-07	5.643	-
			20 ºC	4.600E-08	2.942E-07	6.395	0.420
		P. versipora	11 ºC	1.530E-08	1.378E-06	90.089	0.382
			14 ºC	2.349E-08	1.772E-06	75.434	0.643
			17 ºC	4.123E-08	1.983E-06	48.092	-
			20 ºC	1.747E-08	1.658E-06	94.907	0.280

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3.0.0 Conflict of Interest

There are no conflicts of interest.

Chapter 4: General discussion and concluding remarks.

4.1 Summary

This thesis was developed in response to the hypothesis that lipid remodelling may be an adaptive mechanism adopted by corals and associated Symbiodiniaceae living at the extent of their thermal limits, allowing for survivability in response to external stimuli, such as temperature change and nutrient limitation (Rosset et al., 2019). With the effects of climate change driving marine organisms to extend their distribution and abundances poleward, identifying mechanisms that allow for resilience against stressors become fundamental in reef protection and management. Through this thesis, I have found evidence supporting lipid remodelling in Symbiodiniaceae under peak temperature and nutrient stress and identified key lipids within Symbiodiniaceae ranging from temperate to subtropical environments that may be biomarkers indicative of a stress response to external stimuli, such as nutrients and temperature (Chapter 2). I then utilised CBASS (Chapter 3) to investigate the adaptive capacity of *P. aliciae*, and *P. versipora*, representing coral species with different geographical distributions that overlap in the Sydney region, through examining metabolic and photophysiological responses under acute temperature increases/decrease (11 ºC – 32 ºC). In doing so, I have increased understanding of mechanisms that may enhance resilience of corals, like *P. aliciae*, that have increased in abundance in high-latitude areas representing the very limits of their environmental optima, as well as provide evidence of lipid remodelling across a seasonal scale. The knowledge obtained from this thesis can be used to help predict trajectories of corals with different distributions under climate change and inform further persistence of *P. aliciae* and *P. versipora* in high-latitude reef environments.

4.2 Lipid remodeling as an adaptive strategy

Algae have been utilised extensively in both industry and research due to high natural lipid content as well as the potential of these organisms to remodel lipid profiles to enhance abundances and target specific lipid production under altered culturing parameters (Hallenbeck et al., 2015; Khoo et al., 2023). This process, termed homeoviscous adaptation, has been found to be influenced by both temperature and nutrient variation (Ernst et al., 2016), and could be an

adaptive strategy of microorganisms in response to external stimuli (Cossins & Prosser, 1978). Although the lipid profile has been previously studied within both Symbiodiniaceae and coral, lipid remodelling as an adaptive strategy over a range of environmental parameters (nutrient limitation, temperature regimes) had not yet been studied. Lipids play an important functional role in cells, including structure, signalling, and repair (Horn & Jaiswal, 2019). As such, lipid remodelling in response to external stimuli, such as temperature (De Carvalho & Caramujo, 2018), nutrients (Martin et al., 2011) and acidification (Jin et al., 2021), is considered an adaptive strategy of marine microalgae to survive under these altered conditions. In Chapter 2, I utilised findings from targeted lipid remodelling within algae cultured for biofuel and medical use to predict how Symbiodiniaceae may respond to stress. In these studies, changes to nutrient concentrations and temperature regimes were found to result in increased abundances of storage lipids (TG, ST), as well as unsaturated fatty acids (Hu et al., 2008; Robertson et al., 2015). This was also found within my study, with increases in TG abundance found across all nutrient limited samples. However, I also found interesting trends in other, less described lipid groups, especially with roles in membrane function. In Chapter 3, I compared lipid profiles of two corals representing sympatric species with differing geographical distributions when under acute temperature stress, and over two seasonal timepoints representing the highest and lowest yearly temperature averages. Here, despite minimal alterations to lipid profiles across short-term temperature fluctuation I found evidence of lipid remodelling over a seasonal scale. Together, these results highlighted three specific potential lipid based adaptive mechanisms that may play a role in coral adaptation under altered environmental condition; 1. galactolipids, 2. oxidised lipids and 3. prenol lipids.

4.2.1 Galactolipid increases to support Symbiodiniaceae photophysiology.

Across both data chapters of this thesis, photophysiological efficiency of photosystem II (F_v/F_m) was not significantly impacted by temperature stress. In Chapter 2, I found no relationship between temperature and F_v/F_m across all Symbiodiniaceae species, which I theorised may be due to prioritising cellular function overgrowth and reproduction under increased, but sub-lethal temperatures. Although unexpected, this trend may be due to sub-lethal temperatures, as previous studies on Symbiodiniaceae (*Cladocopium* sp) similarly observed no profound change in F_v/F_m of symbionts cultured at 32 ºC within a 14-day period (Lawson et al., 2019). In Chapter

3, I observed minimal declines in photosynthetic efficiency (F_v/F_m) after severe acute temperature stress (11 ºC - 32 ºC). Here, F_v/F_m was more affected under temperature fluctuations in *P. aliciae* compared to *P. versipora* in both warmer and cooler temperature treatments, perhaps suggesting that *P. aliciae* photosynthetic efficiency may be more impacted by fluctuating temperatures due to Sydney being a less-optimal habitat for this species compared to core populations in the Solitary Islands. *P. versipora*, dominated by *Breviolum* sp. did not exhibit decreased photophysiological efficiency under acute temperate increases (32 ºC), however, F_v/F_m of controls in the summer experiment (24 ºC) were more reduced compared against winter (17 ºC), suggesting that increases in temperature, especially long term, may influence Symbiodiniaceae in the *P. versipora* holobiont. Additionally, F_v/F_m was impacted by decreased temperatures than increased temperatures across both *P. aliciae* and *P. versipora*. Coinciding with this were increased proportional abundance of the glycerolipid digalactosyldiacylglycerol (DGDG), as well as alterations to the monogalactosyldiacylglycerol:digalactosyldiacylglycerol (MGDG:DGDG) ratio favouring DGDG production in Symbiodiniaceae from both coral species over winter sampling (Figures 3.3, 3.4). MGDG and DGDG are important lipids within the thylakoid membrane and are thus important in maintain photosynthetic function (Ferrer-Ledo et al., 2023; Tian et al., 2022). Additionally, I found similar alterations to DGDG and MGDG in Chapter 2, specifically in *Cladocopium* and *Breviolum* spp. (dominant *in P. aliciae* and *P. versipora*, respectively). In this study, I found increased abundances of DGDG within cultures grown under phosphorus limitation, under which we also observed significant declines in photosynthetic efficiency. Previous studies on plants have noted increases in DGDG abundance leading to decreases in the MGDG:DGDG ratio as a mechanism to increase membrane stability and conserve photosynthetic function under stress (Awai et al., 2007; Ge et al., 2022). This notion supports findings, within increases in DGDG over winter a potential mechanism allowing for maintained thylakoid membrane stability and function under cold temperatures and decreased light availability in Symbiodiniaceae across both data chapters, with findings suggesting lipid remodelling within thylakoid membranes of Symbiodiniaceae can occur to maintain photosynthetic efficiency under altered external stimuli and over seasonal periods.

4.2.2 Oxidised Lipids and ROS

Symbiodiniaceae genera within Chapter 2 were chosen to represent those that are dominant in *P. aliciae* (*Cladocopium*) and *P. versipora* (*Breviolum*), as well as a known-thermotolerant genus, *Durusdinium* (Silverstein et al., 2017). In Chapter 2, bi-weekly cell physiology (size) analysis and density counts were undertaken to determine physiological effects altered conditions may have on the cell, and this then linked to metabolic changes observed. Based on the results from this study, *Breviolum psygmophilum* growth rate more affected by increased temperatures compared to *Cladocopium goreaui*, and *B. psygmophilum* was observed to have much lower instances of altered lipid composition and abundance in 31 ºC cultures compared against both *C. goreaui* spp., as well as *D. trenchii*. *Breviolum* sp. of Symbiodiniaceae are often associated with temperate corals and are considered to have relatively low tolerance to heat stress compared against other genera of Symbiodiniaceae (Karim et al., 2015), but despite this, *B. psygmophilum* was the only Symbiodiniaceae species within Chapter 2 that did not undergo rapid alterations to cell size, potentially indicative of oxidative stress and eventual cell apoptosis (Lima et al., 2022).

In Chapter 2, I found decreased prevalence of ether-linked lipids that coincided with increased of oxidised lipids under peak heat stress (31 ºC) in both *Cladocopium* and *Breviolum* Symbiodiniaceae, which was theorised to be linked to reactive oxygen species (ROS) accumulation, oxidative stress and the increased cellular size in *Cladocopium* cultures grown at 31 ºC (Su et al., 2019). Ether-linked lipids have not been researched within microalgae, but previous studies on mammalian cells have found ether bonding of lipids (predominantly glycerol and glycerophospholipids) to promote an antioxidant response within the cell (Dean & Lodhi, 2018). As such, ether-linked and oxidised lipids were of interest in Chapter 3, where proportional ether lipid abundance increased in Symbiodiniaceae over summer sampling. Additionally, within Chapter 3, I observed similar decreased proportions of ether-linked lipids coinciding with increased oxidised lipids in both *P. aliciae* and *P. versipora* Symbiodiniaceae under winter sampling. *P. versipora* has been found dominated by *Breviolum* sp., and *P. aliciae Cladocopium* sp. (González-Pech et al., 2022), so similar trends observed across both Chapters 2 and 3 do support potential lipid remodeling as a mechanism in Symbiodiniaceae to control ROS accumulation and prevent oxidative damage under temperature variation. Nevertheless, the symbiont community of each coral across both sampling points have not yet been analysed, so it is possible that changes over a seasonal scale within associated Symbiodiniaceae may be due to

symbiont shuffling (Cunning et al., 2015). Although this response found in Chapter 3 cannot be attributed to acute temperature stress, these findings support a link between ether-linked and oxidised lipids, and these lipids should be further examined within Symbiodiniaceae as potential biomarkers indicating a lipid-response under environmental change.

4.2.3 Prenol lipids (VAE)

Despite no evidence of Symbiodiniaceae remodeling across temperatures, I did find potential remodeling over a seasonal scale (Chapter 3). When comparing lipid profiles of *P. aliciae* and *P. versipora* coral host and Symbiodiniaceae across controls from each seasonal experiment (17 ºC, 24 ºC), I found proportional abundance of lipid groups differed significantly between summer and winter, particularly of lipids that function within cellular membranes, such as phospholipids, sterols, and prenols, across a seasonal scale. Under decreased temperatures, membrane lipids become more rigid and risk peroxidation (Alonso et al., 1997; Los & Murata, 2004). Lipid membrane remodelling in both Symbiodiniaceae and coral over a seasonal scale suggest an adaptive capacity of corals living in marginal environments and a mechanism for survival over high seasonal variation. Phosphatidylcholine (PC) is the most abundant lipid within cellular membranes in marine microalgae (Han et al., 2017) and exhibited higher proportional abundances in both coral and Symbiodiniaceae in winter (Chapter 3). Similarly, PC was one of the notable lipids found undergo membrane lipid remodelling under nutrient limitation (Chapter 2). I found, when phosphorous became limited, all Symbiodiniaceae species were able divert from phospholipid to isoprenoid (vitamin-A fatty acid (VAE)) synthesis in cellular membranes, a process previously described in the fungus *Saccharomyces cerevisiae* (Jordan et al., 2019; Wadhwa et al., 2018). This allowed for cellular structure to remain stable despite limited phosphorus availability.

Membrane phospholipids also exhibited changes in abundances (Chapter 3), as glycerophospholipids, including both PC and VAE, increased in proportion across both coral and associated Symbiodiniaceae in winter controls (17 ºC) compared to summer controls (24 ºC). Increased PC as temperatures decrease is thought to be a mechanism allowing for survival under cold conditions in plants (Adhikari et al., 2022), and could potentially perform similar functions in algae. Additionally, in Chapter 3, larger proportions of VAE and sterols were observed in

coral tissue under winter controls. VAE has not been well described, however, as a prenol lipid, it is thought to function in cellular membranes (Jordan et al., 2019). Sterols, specifically cholesterol, also perform specific functions in cellular membranes, with increased abundance observed in both coral and Symbiodiniaceae under winter controls (17 ºC) (Chapter 3), supporting seasonal lipid remodelling to increased membrane permeability as temperatures decrease (Lönnfors et al., 2011). These lipids may also be important biomarkers in studying remodelling in coral holobionts as potential mechanisms for maintaining cellular structure despite variations in environmental condition, as well as indicators of Symbiodiniaceae with adaptive capability against temperature decreases. Further, the degree of saturation of fatty acids has also been theorised to function within membrane remodelling under changes in temperature (Zhao et al., 2020). In Chapter 2, increased saturation of fatty acids was found at high temperatures (31 ºC), but also at low temperature (16 ºC) which did not follow hypotheses that colder temperatures induce higher unsaturation of fatty acids in algae (Holm et al., 2022). Although, similarity in Chapter 3 trends in the unsaturated fatty acid, saturated fatty acid ratio (UFA:SFA) did not follow expected unsaturation under decreased temperatures, in coral tissue, *P. versipora* fatty acids were more unsaturated in winter compared to summer, whereas *P. aliciae* coral fatty acid unsaturation remained consistent between the seasons. Future studies should aim to analyse the UFA:SFA ratio in corals under peak temperature stress to determine a pattern across temperature gradients, as well as target prenol lipid (specifically VAE) synthesis within cellular membranes to gain an enhanced understanding of the functional importance of this lipid in cell membranes under alterations to external stimuli (including both temperature and nutrient concentration).

4.3 The persistence of corals at their thermal limits

This thesis collectively revealed evidence of several key lipids that mediate the stress response of coral and Symbiodiniaceae under altered environmental condition, that may be important in allowing corals to survive in high-latitude reef environments. While some tropical coral species have migrated poleward to subtropical regions in Australia (Baird et al., 2012), and overseas (Precht & Aronson, 2004; Yamano et al., 2011) due to warming ocean temperatures (Hastings et al., 2020), mounting evidence shows abundance increases of subtropical corals close to their lower thermal limits in temperate regions (Booth & Sear, 2018; Fifer et al., 2022), with fossilised

records also noting similar trends under past warming events (Couce et al., 2013; Price et al., 2019). Sydney is situated within a biogeographic transition zone, and as such is characterised by overlap of temperate and subtropical species (Sommer et al., 2014). Despite occupying different distributions, the two corals examined within this study, *Pocillopora aliciae* and *Plesiastrea versipora*, are sympatric within Sydney. *P. aliciae* is a small-range subtropical endemic, while *P. versipora* is a widely distributed temperate/subtropical coral (Juszkiewicz et al., 2022; Schmidt-Roach et al., 2013). Initial documentation of individual *P. aliciae* colonies, then thought to be *Pocillopora damicornis,* in Sydney dates back to 1955 (Wells, 1955). *P. aliciae* was recognized a species in 2013 (Schmidt-Roach et al., 2013) and has increased in benthic coverage in the Sydney region over recent years (Booth & Sear, 2018). The presence of *P. aliciae* within Sydney has also been linked with increased tropical and subtropical organism interaction (Booth & Sear, 2018; O'Connell et al., 2023), including a small crab, *Trapezia* spp. (pers. obs., Figure 4.1), which forms an obligate symbiotic relationship with pocilloporid corals (Canizales-Flores et al., 2021). The findings from this thesis can aid in predicting future trajectories of *P. aliciae*, and associated organisms, in Sydney and potentially further south, especially as oceanic warming continues.

Figure 4.1: *Trapezia* crab found within *P. aliciae* in Sydney (-33.799, 151.300)

By comparing two coral species of differing geographical distributions that co-occur in Sydney, as well as their associated Symbiodiniaceae, I gained an insight into the adaptive mechanisms of corals contributing to survive at both warm and cold-range boundaries (Figure 4.2). In studying thermal tolerance and metabolic response of these sympatric species with differing distributions, the findings of this thesis help identify the lipid-based mechanisms that govern persistence of

corals at high latitudes and inform future trajectories of poleward coral expansion under a changing climate. However, specific lipid function, and in some cases, the function of some lipid sub-classes has yet to be described within microalgae. As such, many functions of lipids suggested within this thesis are speculative, based primarily on studies within medical and nutritional research. This highlights a significant knowledge gap that future studies must address to develop upon understandings of metabolic function, and the functional significance of lipids that drive coral and Symbiodiniaceae response to stress in marginal environments.

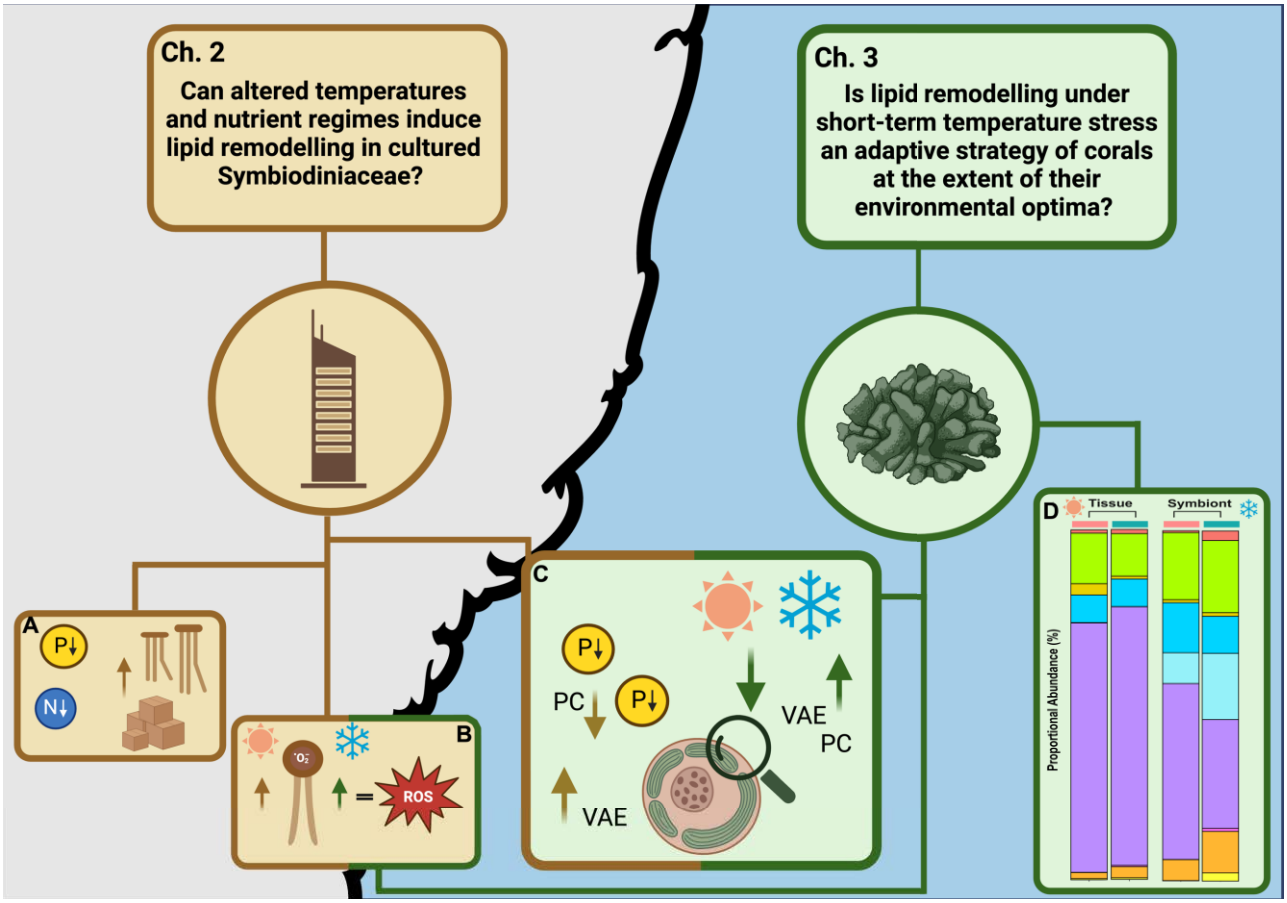

Figure 4.2: Visual illustration of the main findings from this thesis, including lipid remodeling within Symbiodiniaceae under various temperatures and nutrient reductions (Chapter 2) and seasonal lipid remodeling across *P. aliciae* and *P. versipora* host and associated Symbiodiniaceae (Chapter 3). This shows the evidence of increased TG abundance under nutrient-limitation (A), alterations to prenol and phospholipid membranes across temperature and nutrient regimes (C) and seasonal lipid remodeling in both coral tissue and Symbiodiniaceae (D) as potential lipid-based adaptive strategies in corals, as well as increased abundances of oxidized lipids indicating ROS accumulation (B) as potential biomarkers of oxidative stress. Created with BioRender.com

4.4 Challenges and future considerations

The findings of this thesis highlight potential homeoviscous adaptation of Symbiodiniaceae representing genera found in *P. aliciae* and *P. versipora* under sub-lethal temperature stress (Chapter 2), and that baseline compositions in both tissue and Symbiodiniaceae alter slightly in different seasons (Chapter 3). For corals living at the extent of their thermal limits, lipid remodeling may be an important mechanism for survival, yet within Chapter 3, analyzing both coral host and Symbiodiniaceae *in hospite* revealed no trends supporting lipid remodeling across temperatures under both cold and heat stress. However, experimental design within Chapter 3 may have limited the capacity to determine coral and Symbiodiniaceae lipid remodeling under various short-term temperatures reflecting increased and decreased cold stress. In Chapter 2, I analysed the lipid profile of Symbiodiniaceae under peak stress, and after a 14-day experimental duration. The aim of this study was to determine whether Symbiodiniaceae can adapt to sublethal environmental shifts through lipid remodeling and compare findings between species with different geographical distributions (i.e., species that would be found in temperate corals compared to generalist species found in subtropical and tropical corals). The experimental design thus supported this aim, providing evidence of remodeling in Symbiodiniaceae under peak stress, but also noting differences in lipid remodeling, as well as growth rate and changes in cellular size under prolonged exposure to stress. In Chapter 3, the aim was to reveal lipid characteristics allowing the sympatric corals with differing geographical distributions, *P. aliciae* and *P. versipora*, to survive at their lower thermal limits in Sydney. Corals that abide in temperate regions are often resilient to fluctuations in temperature and show remarkable recovery rates post stress events. To then observe whether these corals utilise lipid remodeling as a mechanism that enables survivability in highly varied areas, I implemented a short-term temperature stress experiment (CBASS), under both temperature increases and decreases (Chapter 3). I followed methodology as per Voolstra et al., (2020), and undertook lipid sampling after an overnight (11-hour) recovery period. CBASS has, to date, only been performed in heat-stress experimentation, and only using corals from tropical environments (Alderdice et al., 2022; Klepac et al., 2024; Voolstra et al., 2020), which are known to be sensitive to elevated temperature (Heron et al., 2016). Despite the CBASS experimental design perhaps not being the optimal choice for determining thermotolerance within temperate systems, or for lipidomics in determining the

effects of acute temperature stress (Chapter 3), this study does provide essential knowledge on the specific lipids that can help drive resilience of *P. aliciae* and *P. versipora* under temperature stress, identifying potential lipid biomarkers and lipid compositions that may aid in survivability under altered environmental condition.

Additionally, the manipulation of Symbiodiniaceae and coral microbes in order to increase coral thermotolerance, thereby creating increased resilience in corals under climate change has gained traction as a methodology to preserve coral reefs (Doering et al., 2021; Guibert, 2024; Van Oppen et al., 2011). This thesis provides valuable knowledge of the potential mechanisms inducing these variations in Symbiodiniaceae thermotolerance and could prove informative within this area of research in selecting for traits that best allow for survivability under a changing climate. Moreover, recent studies have shown shifts in the polar component of the metabolome (such as sugars and amino acids) may be useful diagnostic markers indicating a stress response in corals, and may also be involved in resilience and longer term survival under increased temperatures (Farag et al., 2018; Henry et al., 2021). Although studying remodelling of the full metabolomic coverage was beyond the scope of this thesis, future studies would benefit from a more comprehensive metabolomic approach. Doing so would allow for a more holistic understanding of metabolomic remodelling as an adaptive response to stressors associated with climate change in temperate corals, and further contribute to coral conservation under a changing climate.

4.5 Conclusions

This thesis set out to identify adaptive traits of *P. aliciae* and *P. versipora*, representing corals enduring at the extent of their geographical distributions and allowing for survival in high-latitude reef environments, characterised by lower temperatures and high seasonal (as well as daily) variation. Through research reported in Chapter 2, I found altered environmental conditions (nutrient (N, P) limitations and temperature increases/decreases (16 ºC - 31 ºC)) affected cell physiology and photophysiology, and employed lipid remodelling in Symbiodiniaceae (genera *Durusdinium*, *Cladocopium* and *Breviolum*) as a potential adaptive strategy to environmental change. Similarly, in Chapter 3 I reported minimal evidence of physiological, photophysiological and metabolic signs of stress in two Sydney corals, *P. aliciae*

and *P. versipora*, representing a subtropical coral at its poleward range limit and a temperate coral with a broad distribution, but potential seasonal lipid remodelling over two sampling periods representing yearly maximum (summer) and yearly minimum (winter) temperatures. Overall, lipid remodelling within *P. aliciae* and associated Symbiodiniaceae (*Cladocopium* sp) may be an important mechanism allowing for survival in highly fluctuating, high-latitude reef environments at the extent of their poleward distribution. Across both experiments, I found evidence supporting the altered composition of lipids that function in cell membranes in both coral host and associated Symbiodiniaceae, that may allow for longer term survivability under altered environmental conditions. I identify specific lipids and lipid groups that could be targeted in future studies as potential biomarkers that may indicate a cellular stress response and provide evidence of lipid remodelling over a seasonal scale that may be a strategy for *P. aliciae* to adapt to highly varied reef environments. Overall, this thesis provides valuable information that, when expanded upon, may be able to help predict the future trajectory of *P. aliciae* in Sydney, as well as *P. versipora* and other temperate Sydney corals as climate change intensifies. I show that lipid-remodelling in Symbiodiniaceae and in coral tissue could potentially be an essential adaptive mechanism in the survivability of *P. aliciae* and *P. versipora* under stress-conditions and should be further examined in temperate ecosystems.

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