ResearchOnline@JCU

JAMES COOK UNIVERSITY AUSTRALIA

This file is part of the following work:

Morris, Luke Aaron (2021) ***The role of nutrients in coral bleaching.*** **PhD Thesis, James Cook University.**

Access to this file is available from:

https://doi.org/10.25903/4ab0%2Dj554

Copyright © 2021 Luke Aaron Morris.

The author has certified to JCU that they have made a reasonable effort to gain permission and acknowledge the owners of any third party copyright material included in this document. If you believe that this is not the case, please email researchonline@jcu.edu.au

The role of nutrients in coral bleaching

Thesis submitted by:

Luke Aaron Morris

For the degree of Doctor of Philosophy in Marine Biology

College of Science and Engineering

James Cook University

November 2021

Acknowledgements

I acknowledge the Bandjin, Bindal, Djiru, Gimuy Yidinji, Girramay, Gulnay, Gurabana Gunggandji, Guru Gulu Gunggandji, Lower Coastal Yidinji, Mamu, Manbarra, Mandingalbai Yidinji, Nywaigi, Warrgamay and Wulgurukaba people as the Traditional Owners of the land and sea country on which this research was conducted.

I would like to thank my team of supervisors: Line Bay, Neal Cantin, David Bourne and Kate Quigley. I was incredibly lucky to find myself being persuaded by you, Line, to come work at AIMS via Skype call in early 2017. Since then, you have mentored and encouraged me so generously and patiently through many challenges (scientific and otherwise) which at times seemed insurmountable. I really can't imagine that I would have gotten to this point of submitting this thesis without you – thank you beyond words. Neal, I am really glad that I got the chance to work with you partway into my candidature. It really benefitted me personally and in my work by having you there to challenge my crazy theories and encouraging to me reality check my work. David, thanks for always so generously supporting me and your numerous students – your wisdom in what a PhD should constitute from an "outside AIMS" perspective and timely feedback really helped guide me over the years. Kate, thanks for answering my highly complicated and technical questions regarding analysis, and always being able to empathise with me as a recent PhD yourself. Overall, it has just really been great to work with you all and contribute to such an important and broad research project at AIMS.

I would also like thank my collaborators who I had the pleasure of working with during my PhD: Christian Voolstra, Dave Baker, Mark Baird, and Zach Fuller. Chris, it was really an honour to hear such enthusiasm from you regarding my work, and a dream to write a literature review about nutrients and corals with your input. Dave, without you I would never have discovered some wonderful insights in coral bleaching. Thank you for being so enthusiastic in collaborating and to and your team (Kim-Sum Leung, Jane Wong and Inga Conte-Jerpe) for all the advice and facilitating the remote analysis. Mark, thanks for always being so interested in hearing about nutrients and corals on the phone, it was so great to help inform your models and place them within the prevailing theories of bleaching. Zach, thanks for inviting me to be

one part of such a big collaboration and publishing such a big paper – which I admit I don't think I will ever understand.

There are too many staff at AIMS to thank here, but without them all my work would not have been possible. Special thanks to Vero and Jo for always being so patient with me, helping me out with coral collections and sampling (and anything else I needed, really), and teaching me how work in the lab efficiently and at scale. Big thanks to the SeaSim team for supporting two long, hard, and stressful experiments (and listening to me moan about cleaning tanks), and basically replumbing half of the facility just for me. Thanks to Analytical Services team for facilitating priority measurements of my 1000s of water samples. And thanks to Brett, Flo and Muhsen for going out of their way to help with lab queries, and especially in facilitating my return to the lab last year to conduct the analysis of my final experiment. Finally, thanks to all AIMS@JCU staff for kindly and generously supporting me to complete this thesis.

Thanks to my fellow AIMS@JCU students and the many placement students that were integral to my laboratory and experimental work. This includes those tirelessly drove me to AIMS and back every day, those who enjoyed making fun of my not so posh British accent and "cynical" nature, and those who assumed I was disqualified from, rather than incapable of, driving. Special thanks to Felicity for helping me settle in to living in Townsville and putting up with living with me for years! And I am so truly indebted to an army of volunteers who were not just delightful but also made endless months of scrubbings tanks, long sampling days, and processing 1000s of samples possible. It was really a pleasure to work with you all.

Finally, thanks to my family back home on the other side of the world. Thanks to my auntie Hazel for financially supporting me in getting me here in the first place, and for learning how to Skype for me. Thanks, and love to my Nans who are no longer with me but helped shape who I am today – it is a real shame that you didn't get to see this. Thanks to my parents and siblings who put up with me moving to Australia for almost five years, and for being particularly special in never questioning my choices or what I what I wanted to do with my life. Finally, thanks to Alice for being, supporting and putting up with me through this crazy time in our lives, first via a super long-distance relationship and then joining me in this Australian adventure.

Statement of the Contribution of Others

Nature of support	Contribution	Contributors
Intellectual support	Supervision	Line K. Bay (AIMS) David G. Bourne (JCU) Neal E. Cantin (AIMS) Kate M. Quigley (AIMS)
	Editorial assistance	Line K. Bay (AIMS) David G. Bourne (JCU) Neal E. Cantin (AIMS) Kate M. Quigley (AIMS) Christian R. Voolstra (UKON)
	Methodological advice	David M. Baker (HKU) Florita Flores (AIMS) Jane C. Y. Wong (HKU) Josephine Nielsen (JCU) Inga E. Conte-Jerpe (HKU) Lesa Peplow (AIMS) Sara Bell (AIMS) Veronique Mocellin (AIMS)
	Statistical advice	Murray Logan (AIMS)
Data collection	Coral sampling fieldwork	Andrea Severati (AIMS) Carine Lefevre (AIMS) Carlos Alvarez Roa (AIMS) Carly Randall (AIMS) Christopher Brunner (JCU) Craig Steinberg (AIMS) Daniel Roberts Eric Matson (AIMS) Grace Frank (AIMS) Grant Milton (AIMS)

Data collection (continued)	Coral sampling fieldwork (continued)	Frederieke Kroon (AIMS) Hannah Epstein (JCU) Heidi Luter (AIMS) Ian Miller Jose Montalvo-Proaño (AIMS) Josephine Nielsen (JCU) Katharina Fabricius (AIMS) Kristen King (JCU) Lena Despringhere Line Bay (AIMS) Lonidas Koukoumaftsis (AIMS) Matthew-James Bennett Michael McWilliam (JCU) Michael Natt Mikayla Huizer Muhammad Abdul Wahab (AIMS) Neal Cantin (AIMS) Nora Sadoun Oscar Ward Sam Noonan (AIMS) Stephanie Di Perna (JCU) Ramona Brunner (JCU) Veronique Mocellin (AIMS) Wade Pemberton
	Experimental infrastructure	AIMS National Sea Simulator and staff: Craig Humphrey Andrea Severati Brett Andersen Grant Milton Justin Hochen Tom Barker

Data collection (continued)	Experimental maintenance	Alice Webster Charlie Nicholas Darren Anderson Eanna McAtamney Gina Matthews Hugo Kiff Lynn Wang Rachael Collins Romain Py Sebastien Comarmond
	Experimental sampling	Alice Webster Charlie Nicholas Gina Matthews Hugo Kiff Josephine Nielsen (JCU) Rachael Collins Veronique Mocellin (AIMS)
	Laboratory infrastructure	AIMS and staff: Brett Baillie Carlos Alvarez Roa Florita Flores Jason Doyle Muhsen Aljada
	Laboratory assistance	Charlie Nicholas Eanna McAtamney Gina Matthews Josephine Nielsen (JCU) Romain Py Sebastien Comarmond Veronique Mocellin (AIMS)

Data collection (continued)	Stable Isotope Analysis	Stable Isotope Laboratory (HKU) David M. Baker Kit-Sum Leung
	Water quality analysis	AIMS Analytical Services Andrew Warnes Jane Wu Won Natalie Giofre Natasha Cameron Neale Johnston
	Sequencing	Ramaciotti Centre for Genomics (UNSW)
	Bioinformatic analysis	SymPortal: Benjamin C. C. Hume (UKON)
	Logistical support	AIMS Stores Lahnie Clarke Peter Davern Simon Fleming Tania Stevens Pia Wimmer Veronique Mocellin Wendy Hiles-Stewart
	Graphic design	Hillary Smith (JCU)
Financial Support	Stipend and tuition fee support	AIMS@JCU Australian Government James Cook University College of Science and Engineering, James Cook University
	Research funding	AIMS@JCU Australian Institute of Marine Science Australian Government's National Environmental Science Programme Tropical Water Quality Hub

Financial Support (continued)	Research funding (continued)	College of Science and Engineering, James Cook University Centre for Tropical Water and Aquatic Ecosystem Research, James Cook University

Authorship declaration for published chapters

Chapter	Reference	Contributions of each author	Consent for republication in thesis
1	Morris LA, Voolstra CR, Quigley KM, Bourne DG, Bay LK (2019) Nutrient availability and metabolism affect the stability of coral-Symbiodiniaceae symbioses Trends Microbiol 27:678-689 doi:10.1016/j.tim.2019.03.004	Luke Morris conceptualised the study with Line Bay and David Bourne. Luke Morris wrote and revised all drafts with editorial input from Christian Voolstra, Kate Quigley, David Bourne and Line Bay.	Christian Voolstra Signature: Content has been removed for privacy reasons Kate Quigley Signature: Content has been removed for privacy reasons David Bourne Signature: Content has been removed for privacy reasons Line Bay Signature: Content has been removed for privacy reasons

Every reasonable effort has been made to gain permission and acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

General Abstract

Coral reefs are built by Scleractinian corals, whose association with intracellular algae supports high levels of biodiversity and important ecosystem services. The partnership between corals and their intracellular algal symbionts (family Symbiodiniaceae), supplies the nutrition critical for tissue and skeletal growth in tropical oligotrophic oceans but makes them sensitive to environmental change driven by anthropogenic stressors. This is realised on Australia's Great Barrier Reef (GBR), where corals are exposed to mass bleaching during recurrent marine heatwaves and additional cumulative stress from poor inshore water quality. Coral bleaching is currently understood to result from photo-oxidative stress, but emerging hypothetical and empirical studies demonstrate that the destabilisation of nutrient exchange, resulting from nutrient enrichment and/or heat stress, may also be central to the breakdown of the coral-algal symbiosis. The aim of this thesis is to provide a timely investigation into the impacts of nutrient availability and metabolism on the bleaching of corals within the environmental context of the GBR, using the common coral model *Acropora millepora*.

Chapter 1 assesses the evidence of nutritional mechanisms of bleaching, demonstrating how the modulation of carbon translocation from symbiont to host is mediated by the forms and ratios of environmental nutrients. It is hypothesised that impairment of autotrophic nutrient provision from the symbionts shifts the symbiotic relationship to one that is detrimental to the host, which manifests as bleaching. This nutritional model of bleaching is further discussed in the context of coral heat tolerance, and how this is affected by acclimatisation and the genetic adaptations of distinct coral-algal partnerships.

Chapter 2 surveys bleaching and recovery (through chlorophyll content) and the composition of the algal symbiont communities (next-generation sequencing) in *A. millepora* sampled from 12 central GBR reefs spanning latitudinal and cross-shelf gradients. Levels of bleaching and recovery were similar across the cross-shelf sampling gradient throughout the central GBR in 2017, meaning that inshore impacts of nutrient and sediment exposure did not enhance the coral bleaching response under severe levels of heat stress (≥ 5 °C-weeks).

Bleaching and recovery was also largely unrelated to the algal symbiont communities, which remained stable in their composition of *Cladocopium* type profiles over time in corals sampled from most of the studied reefs. Spatial gradients were important drivers of algal symbiont community structure, with distinct *Cladocopium* types separated by long-term average temperatures, and a more diverse range of *Cladocopium* and *Dursudinium* type profiles present on a subset of inshore reefs. Notably, an unprecedented shift from *Durusdinium* D2 to *Cladocopium* C21 was observed on one inshore reef, contradicting prior observations that *Durusdinium* symbionts confer bleaching resilience. This chapter demonstrates that severe levels of heat stress (≥ 5 °C-weeks), rather than local variation in the coral symbiont communities or water quality conditions, were the predominant driver of coral bleaching severity in the central GBR during a natural marine heatwave.

Chapter 3 exposes *A. millepora* sourced from inshore and mid-shelf reefs to ecologically relevant levels of nutrient enrichment (nitrate and/or phosphate) during a simulated heat stress event (31.5 °C) in a controlled aquarium experiment. Prior to heat stress, there was little impact of prior shelf position or nutrient exposure on coral physiology. At elevated temperatures, corals bleached regardless of nutrient availability, though mid-shelf corals were clearly more susceptible to bleaching (chlorophyll loss) and damage to photosynthesis ($^{\Phi}$PSII, F_v/F_m, respirometry) compared to their inshore counterparts. These patterns of bleaching were likely explained by the thermal history of corals, which led to higher levels of accumulated heat stress for the mid-shelf corals, at 3.9 °C-weeks compared to 2.9 °C-weeks for inshore corals under the single experimental heating profile. Cross-shelf differences were found in the *Cladocopium* communities hosted by corals which may have also contributed to this differential temperature tolerance. This experiment confirms that the levels and duration of nutrient enrichment which occurs on the inshore GBR during summer does not impact bleaching susceptibility of *A. millepora* during mild to moderate heat stress.

Chapter 4 investigates the influence of the coral nutrient metabolism in thermal bleaching and mortality, using *A. millepora* from the cross-shelf reefs as in Chapter 3 along with similar nutrient (nitrate and/or phosphate) and ramp and hold temperature (31.5 °C) treatments. Instantaneous nutrient cycling ($^{13}C/^{15}N$ labelling) was applied alongside measurements of the nutrient storage (C/N and host protein contents) after simultaneous nutrient and heat stress

exposure, and again after a period of "recovery" at ambient temperature (26.0 °C). The initial suppression of $^{13}C/^{15}N$ fixation and translocation was most severe within mid-shelf corals, corresponding with their increased levels of bleaching. Following heat stress, cross-shelf differences in the metabolic response continued with loss of carbon and nitrogen observed in both the host and algal symbionts, along with severe loss of host proteins, continued bleaching, and moderate levels of mortality. Inshore corals, by contrast, remained relatively stable after heat stress. The heat stress response of *A. millepora* was therefore consistent with the initial reversal of coral nutrient cycling from protein anabolism to catabolism, but later diverged with coral starvation and mortality primarily limited to the temperature sensitive mid-shelf corals. This study extends the photo-oxidative theory of thermal bleaching, to show that nutritional destabilisation is present from the early stages of bleaching through to the starvation and mortality of corals following severe heat stress.

The **General Discussion** provides a comprehensive synthesis of the role of nutrients in the thermal bleaching of a GBR coral. Within the ecological context of the GBR, management to reduce inshore levels of nutrient pollution will have many known benefits for coral health and reef resilience. However, it appears unlikely to protect corals from mass bleaching and mortality under severe heat stress. Overall, this thesis presents comprehensive evidence for a cascade of nutritional destabilisation within the coral-algal symbiosis under heat stress, which is likely to explain the patterns of mass bleaching and mortality observed during recent pantropical marine heatwaves.

Table of Contents

List of Tables

List of Figures

List of Boxes

General Introduction

Coral reefs

Tropical coral reefs are marine ecosystems which sustain global hotspots of marine biodiversity (Fisher et al. 2015; Roberts et al. 2002) and productivity (Brandl et al. 2019; de Goeij et al. 2013; Gove et al. 2016) in otherwise sparse oligotrophic oceans. As a result, healthy coral reefs underpin essential ecosystem goods and services (Woodhead et al. 2019), including fisheries (Newton et al. 2007), coastal protection (Ferrario et al. 2014) and tourism (Spalding et al. 2017) which, together, are valued at an estimated 2.7 trillion USD per year (International Coral Reef Initiative et al. 2021). Despite their significance, coral reefs are suffering acute degradation due to anthropogenic stress (Eddy et al. 2021; Hughes et al. 2017a), primarily through the mass bleaching and mortality of reefs corals owing to global climate change (Hughes et al. 2018a; Lough et al. 2018). These acute impacts of heat stress are superimposed on local pressures including overfishing and nutrient pollution which subsequently impede reef recovery and promote the transition of reefs to novel and degraded states (Hughes et al. 2017a). Building a comprehensive and nuanced understanding of the combined and interacting impacts of these global and local drivers (Hughes et al. 2017a) is critical for informing targeted management actions to stem coral reef decline (Anthony et al. 2017).

Coral-algal symbiosis: the strength and weakness of coral reefs

Shallow-water corals, which build the structural foundations of coral reefs, are the amalgamation of a cnidarian host in symbiosis with intracellular algae (Symbiodiniaceae (LaJeunesse et al. 2018)) and other associated microbiota (Bourne et al. 2016). The coral-algal symbiosis has evolved highly efficient autotrophic nutrient assimilation, exchange, and retention mechanisms (Ferrier-Pagès et al. 2016; Muscatine and Porter 1977; Rädecker et al. 2015). In healthy corals that live in oligotrophic environments, the coral host environment constrains inorganic nitrogen supply to the algal symbionts (Falkowski et al. 1993; Rädecker et al. 2021). In turn, these symbionts efficiently fix inorganic nutrients through

photosynthates and translocate these to the host (Muscatine et al. 1984). The constant supply of nutrients enables the calcifying growth of corals and their production of the aragonite structures which underpin the development of coral reef ecosystems (Tambutté et al. 2011).

Ironically, the highly optimised nature of the coral-algal symbiosis (Muscatine and Porter 1977) may also underpin its susceptibility to anthropogenic stress, leading to coral bleaching (Morris et al. 2019; Rädecker et al. 2021). Bleaching is the loss of algal symbionts and/or photosynthetic pigments, and therefore their autotrophic function, from the coral host tissues (Hoegh-Guldberg 1999). After bleaching corals must consequently rely on heterotrophic feeding (Grottoli et al. 2006), or their own nutrient stores (Rädecker et al. 2021), to survive. Recent evidence suggests that nutritional destabilisation between the host and symbiont occurs during the early stages of bleaching, following exposure to elevated temperatures (Baker et al. 2018; Rädecker et al. 2021) and/or nutrient stress (see (Morris et al. 2019) for review). This nutritional destabilisation is thought to lead into a later photo-oxidative stress response (Weis 2008) and contribute to the mass bleaching of corals seen on coral reefs (Hughes et al. 2018a). However, further experimental evidence is required to fully characterise the cascade of nutritional processes (Morris et al. 2019; Rädecker et al. 2015), and potential effects on bleaching and mortality observed following marine heat waves (Hughes et al. 2018a).

Unique coral host and algal symbiont combinations possess different metabolic and genetic traits and hence vary in their resilience to thermal bleaching (Baird et al. 2009; Putnam et al. 2017; Suggett et al. 2017). For example, stress tolerant *Durusdinium* algal symbionts can confer increasing resistance of corals to nutritional destabilisation (Baker et al. 2013), bleaching (Berkelmans and van Oppen 2006; Fuller et al. 2020) and mortality (Baker 2001; Bay et al. 2016) under heat stress. In contrast *Cladocopium* generally perform best under non-stressful conditions (Cantin et al. 2009; Jones and Berkelmans 2010; Pernice et al. 2015). At the same time, acclimatisation to marginal conditions (e.g. poor water quality and elevated temperature) can increase the heat tolerance of corals (Kenkel et al. 2013a) potentially by priming coral-algal nutrient cycling against destabilisation (Dixon et al. 2020; Gibbin et al. 2018). A study in the coral model Aiptasia suggests that host and symbiont co-adaptation contributes the variation in metabolic traits observed in corals (Rädecker et al. 2018). Overall,

mechanisms of coral adaptation and acclimatisation which are known to impact heat tolerance seem consistent with those mediating the stability of nutrient cycling within the symbiosis, but this remains to be explicitly tested.

Nutrient and heat stress on the Great Barrier Reef

Australia's Great Barrier Reef (GBR) represents a key location to study the combined impacts of nutrient pollution and climate change, due to its current and historic exposure to both stressors. The GBR has suffered five mass coral bleaching events in a little over two decades (1998, 2002, 2016, 2017 and 2020), with the three most recent events occurring in rapid succession (Hughes et al. 2021) due to short-term marine heatwaves superimposed on a century-long trend of ocean warming (0.8 °C Lough et al. (2018)). This recurrent bleaching has contributed to half of all coral colonies on the GBR being lost to mortality over a similar timespan (1995-2017) (Dietzel et al. 2020). Despite the potential for corals to recover in the absence of disturbance (Australian Institute of Marine Science 2021) further declines are forecasted if climate change is allowed to continue unabated under current emissions trajectories (Ortiz et al. 2018; Wolff et al. 2018). Therefore, conventional, and novel management strategies are now required to ensure the best future for the GBR in the era of ocean warming (Anthony et al. 2017; Hughes et al. 2017a).

European settlement of the Queensland coastline in the late 1800s led to significant changes to land use in the catchments adjacent to the GBR (Lewis et al. 2021). It is estimated that, since then, nutrient inputs into the GBR lagoon have increased 5.7- and 8.9-fold for nitrogen and phosphorus respectively (Kroon et al. 2012), creating a strong inshore to mid-shelf gradient of water quality (Waterhouse et al. 2021) relating to the proximity of reefs to major rivers and their exposure to flood plume impacts. These inputs have physiological consequences for the coral-algal symbiosis (Shantz and Burkepile 2014) through the aforementioned destabilisation of internal nutrient cycling (Ezzat et al. 2015; Morris et al. 2019). Nutrient pollution is likely to persist in the GBR lagoon for years to decades after its initial influx (Brodie et al. 2012), and has thus far resulted in chronically degraded inshore reefs dominated by stress-tolerant corals (De'ath and Fabricius 2010; Fabricius et al. 2005).

Given the impacts of nutrient pollution on coral physiology and ecology it is unsurprising that inshore reefs with degraded water quality feature reduced coral abundance and biodiversity (De'ath and Fabricius 2010; Fabricius et al. 2005).

Concerted efforts are being made to manage GBR water quality through the Reef 2050 Water Quality Improvement Plan 2017 - 2022 (Commonwealth of Australia and State of Queensland 2018). Modelling studies show that improving water quality can play a key role in building the resilience of the GBR, given predictions that nutrient-rich riverine flows lowered bleaching thresholds for inshore corals by ≥ 1 °C during 1998 and 2002 (Wooldridge and Done 2009), and inhibit the recovery of coral populations following acute stress like bleaching (MacNeil et al. 2019; Ortiz et al. 2018; Wolff et al. 2018). If improvements to water quality can enhance coral heat tolerance as proposed (Wooldridge 2009b; Wooldridge et al. 2011), then local water quality management has the potential to prolong the persistence of reef-building corals on the GBR, especially when combined with strong actions to curb ocean warming (Logan et al. 2021).

The relationships between nutrient enrichment and thermal bleaching remain hard to detect on reefs (Lesser 2021) and severe levels of heat stress have overwhelmed signals of nutrient-mediated bleaching in French Polynesia (Donovan et al. 2020) and the GBR (Hughes et al. 2017b). Despite the clear importance of experimental studies in understanding nutrient impacts on coral beaching (reviewed by (Morris et al. 2019)), only one such study investigating nutrient impacts on GBR coral heat tolerance has been published to date (Fabricius et al. 2013). Uncertainty therefore remains with respect to the effectiveness that local nutrient and sediment management strategies can have mitigating the effects of ocean warming related heat stress events (Lesser 2021). Investigations into the physiological and metabolic consequences of combined nutrient and heat stress are critical to unravel the extent to which nutrient enrichment may exacerbate mass coral bleaching on the GBR.

Mechanistic interactions between nutrient and heat stress

Nutrient impacts on coral heat tolerance are complex and dependent on the concentrations, forms, and stoichiometry of available nutrients (Morris et al. 2019). Identified mechanisms which underpin the role of nutrients in modulating coral heat tolerance include: 1) disruption to cellular growth and maintenance processes if over-enrichment of one key nutrient drives limitation of another (Rodriguez-Casariego et al. 2018; Wiedenmann et al. 2013), 2) modulation of energetic stress in the host where available nutrients stimulate or inhibit carbon fixation and translocation (Ezzat et al. 2015) and, 3) the impacts of nutrient enrichment in generating or abating oxidative and nitrosative stress (Fernandes de Barros Marangoni et al. 2020). Laboratory experiments in which corals are exposed to varying nutrient levels, generally show that nutrients provided in balanced stoichiometry do not increase bleaching sensitivity (Dobson et al. 2021; Ezzat et al. 2016b; Hoadley et al. 2016; Tanaka et al. 2014). In contrast, bleaching can be alleviated by ammonium (Béraud et al. 2013; Ezzat et al. 2019; Fernandes de Barros Marangoni et al. 2020) or exacerbated by an oversupply of nitrate relative to phosphate (Fernandes de Barros Marangoni et al. 2020; Wiedenmann et al. 2013).

Drawing comparisons between laboratory enrichments and the natural environment is challenging, as nutrient levels that are tightly controlled in aquaria do not necessarily represent conditions on natural reefs (Lesser 2021), where nutrients are often rapidly assimilated and transformed by plankton (D'Angelo and Wiedenmann 2014). Recent work on coral reefs in Mo'orea, French Polynesia, do however associate nutrient enrichment with coral bleaching. In these cases, existing nitrogen levels (Donovan et al. 2020) or experimental nitrate enrichment (Burkepile et al. 2019) were found to increase coral bleaching prevalence, severity, duration and/or resultant mortality, but only during mild heat stress events. Together, these results suggest that there is mechanistic evidence that nutrient pollution can exacerbate coral bleaching, however no studies to date have comprehensively linked exogenous nutrient enrichment to a destabilisation of internal nutrient cycling and exacerbation of bleaching under heat stress.

Thesis overview

The overarching goal of this thesis is therefore to investigate the role of nutrients in coral bleaching at the organismal level, using physiological, metabolic, and molecular analyses. The study specifically investigates the response of the common model GBR reef-building coral *A. millepora* subjected to combined nutrient and heat stress.

Chapter 1, published in the journal *Trends in Microbiology* (Morris et al. 2019), undertakes a comprehensive review and synthesis of the known experimental evidence implicating nutritional mechanisms in the process of coral bleaching. The main hypothesises considered are whether nutrient enrichment uniformly exacerbates bleaching or, if nutrient and heat stress interactions are more nuanced by influencing symbiont growth, density and/or function. These hypotheses are then applied to explain how nutritional mechanisms may mediate coral bleaching across the heterogenous environmental and genetic seascape of natural reefs.

Chapter 2 examines the evidence for local water quality mediating coral bleaching by sampling *A. millepora* during a natural bleaching event. The spatial patterns of the physiological bleaching response and algal symbiont communities in *A. millepora* are investigated during the 2017 bleaching event, across six inshore and six mid-shelf reefs in the central GBR. Corals were sampled across a latitudinal gradient and thus exposed long-term to distinct environmental conditions, including water quality and temperature. These gradients have the potential to profoundly shape the distribution of algal symbiont species and therefore impact patterns of coral bleaching and recovery. Results from this chapter further documents cross-shelf patterns in coral physiology and indirectly informs the extent to which water quality management can be used to mitigate coral bleaching during severe marine heatwaves.

Chapter 3 investigates how proximate nutrient exposure and the local adaptation of GBR corals modulates their thermal bleaching response. *A. millepora* from inshore and mid-shelf reefs (with distinct water quality and thermal regimes) were collected and exposed to GBR-

relevant levels of nutrient enrichment (nitrate and/or phosphate), followed by simulated heat stress (31.5 °C) in highly controlled aquarium experiment. Physiological responses and changes in the algal symbiont communities were followed over the course of the experiment, identifying how anthropogenic nutrient ratios impacts the heat tolerance of corals among shelf positions.

Chapter 4 directly tests the hypothesis that nutritional destabilisation leads to the bleaching and mortality of corals under heat stress. Building upon the experimental design of Chapter 3, *A. millepora* corals from the same inshore and mid-shelf reefs were exposed to similar GBR-relevant nutrient and heat stress conditions (nitrate and/or phosphate enrichment at 31.5 °C) followed by a "recovery" period at a non-stressful temperature (26.0 °C). This experiment focused on using short- and long-term measures of the coral-algal nutrient metabolism ($^{13}C/^{15}N$ stable isotope labelling, coral-algal C/N content and host proteins), to characterise the full cascade of nutritional destabilisation underpinning heterogeneity in the response of corals to heat stress.

The General Discussion provides a comprehensive synthesis of the insights on the nutritional mechanisms of coral bleaching and mortality delivered by this thesis. It also and provides suggested avenues for future research into how nutrient availability and metabolism underpin the stability of the coral-algal symbiosis. While there was a lack of evidence for impacts of water quality impacts on the bleaching of a common GBR coral during a mass event, a cascade of nutritional destabilisation was found to occur within the coral-algal symbiosis during bleaching which manifests as the starvation and mortality of corals under severe heat stress. The nutritional insights provided by this thesis can be applied to advance the understanding of mass bleaching and mortality events on corals reefs and efforts to mitigate to impacts of ocean warming through local management approaches.

Chapter 1: Nutrient Availability and Metabolism Affect the Stability of Coral-Symbiodiniaceae Symbioses

This chapter is published as:
Morris LA, Voolstra CR, Quigley KM, Bourne DG, Bay LK (2019) Nutrient availability and metabolism affect the stability of coral-Symbiodiniaceae symbioses Trends Microbiol 27:678-689 doi:10.1016/j.tim.2019.03.004

Highlights

- Mass coral bleaching is occurring at an unprecedented rate due to anthropogenic ocean warming and represents the greatest threat to coral reef ecosystems globally.
- Coral bleaching has been predominantly attributed to photo-oxidative stress under elevated temperature and light, but recent experiments have unveiled nutritional mechanisms that can regulate bleaching.
- Bleaching may result when the coral-Symbiodiniaceae symbiosis shifts from a mutualistic to a parasitic relationship under thermal stress.
- Nutrient availability, specifically the forms and ratios of nutrients such as nitrogen and phosphorus, mediates symbiont parasitism.
- Stable metabolic compatibility between the coral host and algal symbiont can ameliorate bleaching and increase resilience to environmental stress.

Abstract

Coral reefs rely upon the highly optimised coral-Symbiodiniaceae symbiosis, making them sensitive to environmental change and susceptible to anthropogenic stress. Coral bleaching is predominantly attributed to photo-oxidative stress, yet nutrient availability and metabolism underpin the stability of symbioses. Recent studies link symbiont proliferation under nutrient enrichment to bleaching, however the interactions between nutrients and symbiotic stability are nuanced. Here, we demonstrate how bleaching is regulated by the

forms and ratios of available nutrients and their impacts on autotrophic carbon metabolism, rather than algal symbiont growth. By extension, historical nutrient conditions mediate host-symbiont compatibility and bleaching tolerance over proximate and evolutionary timescales. Renewed investigations of the coral nutrient metabolism are required to truly elucidate the cellular mechanisms leading to coral bleaching.

Coral reefs under anthropogenic stress

Coral reef ecosystems are hotspots of biodiversity and productivity, which provide vital and extensive ecosystem services (Crossland et al. 1991; Fisher et al. 2015; Moberg and Folke 1999). However, these values of coral reefs are under threat due to global mass bleaching events triggered by ocean warming (Hughes et al. 2018a). Coral bleaching is a stress response to elevated heat and light levels, where corals lose their algal symbionts (Symbiodiniaceae) (Hoegh-Guldberg 1999; LaJeunesse et al. 2018). Corals acquire most of their energy through photosynthates translocated by the algal symbionts (Muscatine and Porter 1977) and the loss of this energy source for long periods can result in starvation and mortality (Hoegh-Guldberg 1999). Bleaching mortality can result in reductions in coral cover, species, and genetic diversity, leading to ecosystem changes away from a coral-dominated state, which impedes ecosystem resilience (Graham et al. 2015; Hughes et al. 2018b). Although some reefs remain resilient and there exists potential to adapt to warming oceans through natural means (Matz et al. 2018) and human interventions (Anthony et al. 2017), strong reductions in anthropogenic carbon emissions are ultimately required to ensure the persistence of coral reefs.

Coral reefs are also impacted by local stressors, which reduce water quality and have the potential to interact with warming to increase coral bleaching susceptibility (D'Angelo and Wiedenmann 2014). Changes in land use adjacent to reefs result in primary nutrient enrichment that can be further altered through biological and physical processes (D'Angelo and Wiedenmann 2014). Organisms across a range of trophic levels can secondarily modify the nutrient environment (D'Angelo and Wiedenmann 2014; Rädecker et al. 2015) and localised fishing results in the removal of significant nutrient subsidies from reefs (Allgeier et

al. 2017). Climate change also influences marine biogeochemistry at a global scale, where increased storm activity intensifies enrichment events through riverine flux and water column mixing (D'Angelo and Wiedenmann 2014; Knutson et al. 2010; Sinha et al. 2017). In contrast, ocean warming increases water column stratification which reduces nutrient availability (Behrenfeld et al. 2006; D'Angelo and Wiedenmann 2014). Synergistically, global and local drivers and subsequent biological processes not only impact nutrient levels on coral reefs but also change the forms and ratios of nutrients, making nutrient limitation possible (D'Angelo and Wiedenmann 2014). Recent experiments suggest that nutrient limitation, rather than nutrient enrichment *per se*, lowers the temperature at which coral bleaching occurs (Courtial et al. 2018; Ezzat et al. 2019; Wiedenmann et al. 2013).

This review will therefore discuss and synthesise the direct impacts of external nutrient availability on the health of tropical scleractinian corals and demonstrate how this, together with internal nutrient metabolism, underpins the thermal tolerance of the coral holobiont.

Maintenance and breakdown of the coral-Symbiodiniaceae symbiosis

The nutritional interactions between corals and their algal symbionts permit the existence of coral reefs in oligotrophic waters (Muscatine and Porter 1977). Tight nutrient recycling within the symbiosis provides the algal symbionts with respiratory CO_2 and nitrogenous waste products and in exchange the coral host receives photosynthetically fixed carbon (Davy et al. 2012). Additionally, the algal symbionts efficiently assimilate dissolved inorganic nitrogen and phosphorus into the holobiont (Ferrier-Pagès et al. 2016; Rädecker et al. 2015). Corals also acquire nutrients through heterotrophic feeding (Houlbrèque and Ferrier-Pagès 2009) and their microbiome through translocation and digestion (Bourne et al. 2016). The relative modes of nutrient acquisition depend on the individual capabilities of each holobiont member, for example nitrogen fixation by diazotrophs may compensate for limited inorganic or heterotrophic nitrogen uptake (Bednarz et al. 2017; Pogoreutz et al. 2017b) and heterotrophy may compensate for reduced autotrophic capabilities (Houlbrèque and Ferrier-Pagès 2009). Metabolic compatibility between individual coral hosts and their algal symbiont communities likely underpins holobiont performance and their tolerance to environmental

stress (Rädecker et al. 2018; Suggett et al. 2017). But when corals bleach, they are depleted of their major nutrient source and their chances of recovery are partly determined by their ability to restore algal symbiont autotrophy or compensate through heterotrophic nutrient acquisition (Grottoli et al. 2006; Levas et al. 2018; Tremblay et al. 2016). Additionally, nutrient acquisition and loss through the microbiome changes when corals bleach (Bednarz et al. 2017; Cardini et al. 2016; Littman et al. 2011; Pogoreutz et al. 2017a; Pootakham et al. 2018). While corals use heterotrophically-acquired nutrients to help maintain and recover their algal symbiont populations (Lyndby et al. 2020; Tremblay et al. 2016), the relative contribution of inorganic nutrient sources to coral autotrophic recovery are not well understood.

At the cellular level, the contemporary and widely accepted understanding of coral bleaching is one triggered by temperature and light-induced photodamage to the algal symbionts, leading to oxidative stress in both partners (Weis 2008). However, recent studies have shown coral bleaching in the absence of heat, light and/or oxidative stress (Nielsen et al. 2018; Pogoreutz et al. 2017a; Rosset et al. 2015; Rosset et al. 2017; Tolleter et al. 2013; Wiedenmann et al. 2013) highlighting the existence of alternative pathways to coral bleaching (Box 1). Importantly, there is now mounting evidence for the role of nutritional mechanisms in the response of corals to thermal stress and the initiation of bleaching (Baker et al. 2013; Baker et al. 2018; Ezzat et al. 2016a; Gibbin et al. 2018; Krueger et al. 2018; Krueger et al. 2017; Pogoreutz et al. 2017b; Rosset et al. 2017; Wiedenmann et al. 2013). Therefore, the internal nutrient metabolism and external nutrient environment should be considered, in addition to photo-oxidative stress, when predicting the response of corals to thermal stress.

Box 1.1 Coral bleaching in the absence of photo-oxidative stress

Coral bleaching is contemporarily understood to result from photo-oxidative damage to both corals hosts and their algal symbionts (Weis 2008). More specifically, increased temperatures render the algal symbionts susceptible to incoming light, resulting in photodamage and the production of reactive oxygen species (ROS) that can cause cellular damage to both host and symbiont tissues (Weis 2008). However, recent studies have shown that coral bleaching can also occur without the characteristic photo-oxidative stress response (Nielsen et al. 2018; Pogoreutz et al. 2017a; Rosset et al. 2017; Tolleter et al. 2013).

Tolleter *et al.* (Tolleter et al. 2013) observed that thermal bleaching of corals and the coral model Aiptasia can occur in the dark, independently of ROS. Bleaching was similar in nature to control organisms (kept under light), demonstrating that high temperatures can directly damage the photosystems of the algal symbionts (Tolleter et al. 2013). Nielsen *et al.* (Nielsen et al. 2018) later found that bleaching independent of ROS can also occur under light. Although ROS were produced in the symbiont, they were not released to the host tissues and no attributable physiological effects were detected in host or symbiont (Nielsen et al. 2018), corroborating field observations that coral superoxide production is unrelated to bleaching status (Diaz et al. 2016). Furthermore, corals can expel healthy symbiont populations during thermal stress (Bhagooli and Hidaka 2004; Hill and Ralph 2007; Ralph et al. 2001; Ralph et al. 2005), highlighting that bleaching does not require photo-oxidative stress and could instead result from a need to eject dividing symbionts (Baghdasarian and Muscatine 2000).

Coral bleaching can also occur solely from the disruption of coral nutrient metabolism (Pogoreutz et al. 2017a; Rosset et al. 2015; Rosset et al. 2017; Wiedenmann et al. 2013). Corals kept under phosphate limitation can only sustain minimal symbiont communities, with corresponding reductions in host biomass (Rosset et al. 2015; Rosset et al. 2017; Wiedenmann et al. 2013). Furthermore, increasing the severity of phosphate limitation by increasing N:P ratios (nitrate enrichment) results in moderate photodamage (Rosset et al. 2017; Wiedenmann et al. 2013). This pathway to bleaching can also originate internally, for example when N:P ratios are skewed by increased microbial nitrogen fixation within the coral holobiont (Pogoreutz et al. 2017a).

It is important to note that these examples are not mutually exclusive to the extensively-characterised photo-oxidative mechanisms of bleaching (Weis 2008). Rather, they point to nutritional mechanisms of bleaching which exacerbate a later photo-oxidative response under heat and/or light stress (Pogoreutz et al. 2017a; Wiedenmann et al. 2013; Wooldridge 2009a).

The role of nutrients in the early stages of coral bleaching has long been hypothesised (Yonge and Nicholls 1931), but it was Wooldridge (2009a) who first posited that temperature increases could shift the algal symbiont populations from mutualism to parasitism. At elevated temperatures, the relative contribution of the symbionts to the carbon metabolism of the symbiosis is hypothesised to decrease (Wooldridge 2009a), due to reduced photosynthate translocation and/or increased host metabolism. Additionally, the symbiont's heat/light protection mechanisms, including super-quenching, could halt carbon fixation without oxidative stress (Slavov et al. 2016). Under these scenarios, the coral host is forced to catabolise its own carbon reserves to maintain CO_2 and adenosine triphosphate (ATP) delivery for symbiont photosynthesis. Once these stores diminish, photosynthetic dysfunction ensues and triggers coral bleaching through the photo-oxidative pathway (Jones et al. 1998; Slavov et al. 2016; Weis 2008; Wooldridge 2009a). Carbon limitation has often been reported in cnidarian-Symbiodiniaceae symbioses (Goiran et al. 1996; Herfort et al. 2008; Marubini et al. 2008; Muscatine et al. 1989b; Rädecker et al. 2017; Tansik et al. 2017; Tremblay et al. 2013; Weis 1993) and the carbon limitation model of bleaching is supported by recent empirical studies (Baker et al. 2018; Ezzat et al. 2016a; Kenkel and Bay 2018; Krueger et al. 2018; Krueger et al. 2017; Lyndby et al. 2020; Slavov et al. 2016; Tremblay et al. 2016) and extends the photo-oxidative theory. Furthermore, external nutrient availability may also mediate coral bleaching susceptibility through these mechanisms (Ezzat et al. 2015; Ezzat et al. 2016a; Lyndby et al. 2020; Rosset et al. 2017; Tremblay et al. 2016; Wiedenmann et al. 2013). Therefore, the nutritional status of corals, driven by external conditions and internal metabolism, can have a profound influence on bleaching susceptibility.

The impacts of nutrient availability on coral health and thermal tolerance

The direct impacts of nutrient enrichment on coral holobiont physiology (Figure 1.1) were initially controversial since coral reefs exist in a wide range of nutrient environments and experimental studies failed to yield consistent results (Szmant 2002). However, recent laboratory studies have clearly linked declines in coral holobiont health to specific nutrient sources and the ratios they occur in (D'Angelo and Wiedenmann 2014; Houlbrèque and Ferrier-Pagès 2009; Shantz and Burkepile 2014). These studies have primarily focused on

three dissolved inorganic nutrient forms present in reef waters (ammonium, nitrate, and phosphate) (D'Angelo and Wiedenmann 2014) and organic nutrients in the form of particulate food (Houlbrèque and Ferrier-Pagès 2009). Ammonium (NH_4^+) is derived from metabolic processes of the coral host (Rädecker et al. 2015) and other reef organisms (Allgeier et al. 2017) and is the preferred inorganic nitrogen source of the algal symbionts (Grover et al. 2003). In contrast, nitrate (NO_3^-) produced from anthropogenic sources is less favoured (Grover et al. 2003), perhaps because its utilisation diverts electrons away from photosynthesis (Ezzat et al. 2015). Phosphate (PO_4^{3-}) is supplied through a mixture of natural and anthropogenic sources (Ferrier-Pagès et al. 2016).

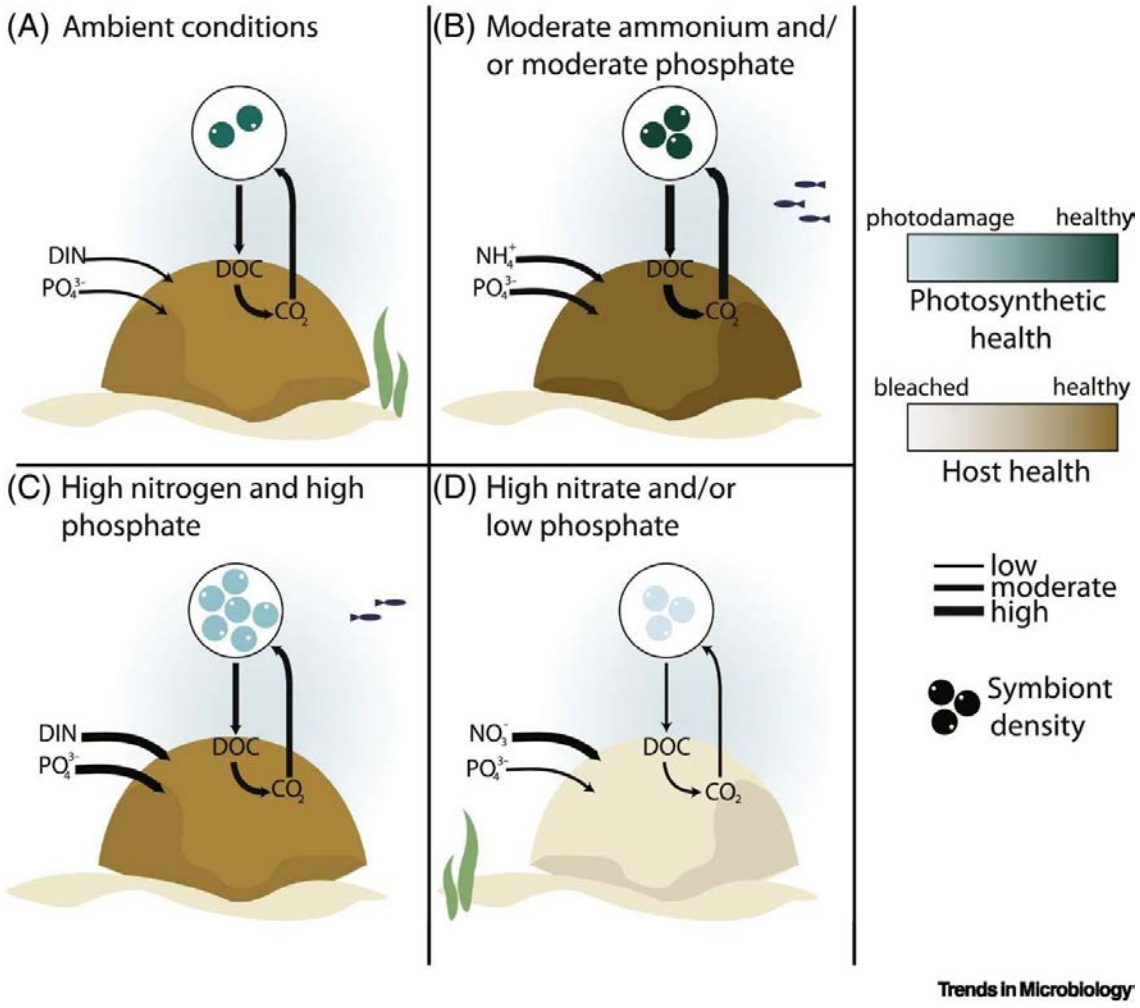

Figure 1.1 Carbon metabolism and health of the coral-Symbiodiniaceae endosymbiosis under different inorganic nutrient scenarios. (A) Oligotrophic conditions constrain Symbiodiniaceae growth which stimulates carbon translocation to the host (Ezzat et al. 2015). The

translocation of photosynthates as dissolved organic carbon (DOC) facilitates the reverse translocation of inorganic carbon (CO_2) and is hypothesised to be integral to the stability of the symbiosis (Wooldridge 2009a). (B) Moderate enrichments of ammonium (NH_4^+) and/or phosphate (PO_4^{3-}) can increase Symbiodiniaceae abundance and promote photosynthetic health, enhancing total carbon translocation and coral health (Ezzat et al. 2015; Ezzat et al. 2016a). (C) High dissolved inorganic nitrogen (DIN) and phosphate enrichments stimulate rapid Symbiodiniaceae population growth, resulting in competition for resources, which lowers photosynthetic performance and carbon translocation per cell (Ezzat et al. 2015). (D) Nitrate (NO_3^-) enrichment and/or phosphate limitation can damage photosynthesis which greatly reduces total carbon translocation and coral health (Ezzat et al. 2015; Rosset et al. 2017; Wiedenmann et al. 2013). Nutrient conditions which weaken the symbiosis (c, d) can be expected to act synergistically with thermal stress to further reduce translocation and induce bleaching (Cunning et al. 2017a; Tremblay et al. 2016; Wiedenmann et al. 2013). Conversely, nutrient conditions which strengthen the symbiosis (b) may ameliorate reductions in translocation to increase coral thermal tolerance (Béraud et al. 2013; Ezzat et

Particulate food and moderate levels of ammonium and phosphate tend to benefit coral holobiont health (Figure 1.1) and increase thermal tolerance (Béraud et al. 2013; Ezzat et al. 2016a; Ezzat et al. 2019; Houlbrèque and Ferrier-Pagès 2009; Shantz and Burkepile 2014), whereas nitrate negatively impacts the coral holobiont (Figure 1.1) and reduces thermal tolerance unless accompanied by phosphorus (D'Angelo and Wiedenmann 2014; Rosset et al. 2017; Shantz and Burkepile 2014; Wiedenmann et al. 2013). Corals with larger algal symbiont populations due to enrichments of nitrogen and phosphorus are usually healthy (Shantz and Burkepile 2014), despite some evidence that dense populations can become parasitic (Figure 1.1) (Anthony et al. 2009; Ezzat et al. 2015; Hoogenboom et al. 2010) and reduce coral thermal tolerance (Cunning and Baker 2013; Cunning et al. 2015b; Cunning et al. 2017b). Therefore, the effects of nutrient enrichment on the coral holobiont are mixed, but negative impacts are largely attributed to increased N:P ratios (D'Angelo and Wiedenmann 2014; Shantz and Burkepile 2014).

Symbiodiniaceae growth rates and coral thermal tolerance

Wooldridge (2013) expanded the carbon limitation model of coral bleaching, suggesting that growing algal symbiont populations under nutrient and thermal stress become parasitic and induces bleaching. Elevated temperatures can directly stimulate cell division (through increased metabolism) and the reductions in algal symbiont density during bleaching may free up resources, including inorganic nitrogen, for the remaining population to grow (Baker et al. 2018; Hoegh-Guldberg and Smith 1989; Levas et al. 2018; Rädecker et al. 2015). At the same time, the dividing algal symbiont populations may retain more photosynthates for their own growth (Baker et al. 2018; Wooldridge 2013), which could prevent the host from mediating nitrogen provision to the symbionts (Cui et al. 2019; Rädecker et al. 2015). However, this hypothesis contradicts the finding that ammonium enlarges algal symbiont populations whilst also increasing thermal tolerance, whereas nitrate increases bleaching susceptibility without prior enlargement of symbiont density (Figure 1.1) (Shantz and Burkepile 2014). It is important to note that nitrogen enrichments may not result in increased algal symbiont cell division *per se* (Hoegh-Guldberg and Smith 1989; Muscatine et al. 1989a), but rather changes to the stable density of symbiont populations (Shantz and Burkepile 2014). Therefore, coral holobionts could simply reach a new and healthy equilibrium that balances nutrient availability, symbiont growth and carbon translocation under nutrient enrichment.

The opposing impacts of ammonium and nitrate on coral thermal tolerance are better explained by their relative effects on holobiont carbon metabolism and oxidative stress. Ammonium stimulates photosynthesis and allows the algal symbionts to maintain photoprotective pigmentation and carbon translocation to the host under thermal stress (Figure 1.1) (Béraud et al. 2013; Ezzat et al. 2015; Ezzat et al. 2019). In contrast, increased nitrate assimilation, due to external enrichment and/or thermal stress encourages symbiont parasitism (Figure 1.1), where the algal symbionts pass the energetic costs of nitrate utilisation onto the coral host (Baker et al. 2018; Ezzat et al. 2015; Tanaka et al. 2017). Therefore, nitrogen enrichment mediates coral bleaching through the carbon limitation model. However, this is realised through direct impacts on photosynthesis rather than symbiont growth.

Symbiodiniaceae density and coral thermal tolerance

An extension to the carbon limitation bleaching hypothesis is that very dense algal symbiont populations become parasitic due to intercellular competition and thereby reduce coral thermal tolerance (Cunning et al. 2017a; Wooldridge 2020). In this case, excess nutrients which enlarge algal symbiont populations to levels at which other resources (e.g. CO_2 and light availability) limit photosynthetic output are linked to parasitism (Wooldridge 2020). Some studies correlate algal symbiont density with coral bleaching susceptibility under ambient nutrient conditions (Cunning and Baker 2013; Cunning et al. 2015b; Cunning et al. 2017b; Kenkel and Bay 2018), citing increased oxidative stress. However, this is contradicted by others which suggest that coral holobiont genetic identity is more important, or that self-shading within dense algal symbiont populations protects against photo-oxidative damage (Bay et al. 2016; Scheufen et al. 2017b). The impact of symbiont abundance on coral bleaching susceptibility therefore remains difficult to resolve.

The impacts of algal symbiont abundance on coral carbon metabolism may better explain the observed impacts on thermal tolerance. Symbiont densities above an optimal range (~1-3 x 10^6 cells cm^{-2} depending on coral host species) have the potential to reduce carbon availability within the coral holobiont (Anthony et al. 2009; Hoogenboom et al. 2010; Wooldridge 2020). Balanced nutrient enrichments (nitrogen plus phosphorus) lead to the highest algal symbiont densities but can also reduce carbon translocation per symbiont cell (Figure 1.1) (Ezzat et al. 2015; Shantz and Burkepile 2014). However, total carbon translocation to the host is unaffected (Ezzat et al. 2015; Kenkel and Bay 2018; Tanaka et al. 2017), indicating that dense populations remain mutualistic. In general, studies which combine nitrate and phosphate enrichments with elevated temperature suggest that balanced nutrient enrichments have little impact on coral thermal tolerance (Courtial et al. 2018; Ezzat et al. 2016b; Hoadley et al. 2016; Tanaka et al. 2014; Wiedenmann et al. 2013), although exceptions can occur (Hall et al. 2018; Vega Thurber et al. 2014). Conversely, corals that are depleted in both nutrients take on a relatively bleached appearance (Rosset et al. 2015; Rosset et al. 2017; Wiedenmann et al. 2013) and reduced thermal tolerance (Courtial et al. 2018; Ezzat et al. 2019). Based on these studies, there is equivocal evidence that dense algal symbiont populations increase the

susceptibility of corals to thermal stress. No single study thus far has simultaneously linked coral carbon metabolism and thermal tolerance with intraspecific variation in symbiont population size, and/or exposure to balanced nutrient enrichment. This leaves major gaps in our understanding of how algal symbiont density relates to thermal tolerance and should be a focus for future research.

Phosphorus stabilises the coral-Symbiodiniaceae symbiosis

Phosphorus has beneficial impacts on coral growth (Shantz and Burkepile 2014) and is integral to the stability of the coral holobiont (Ferrier-Pagès et al. 2016). Without an adequate supply of phosphate, coral holobionts that are enriched with nitrate can suffer reduced health (Rosset et al. 2017; Wiedenmann et al. 2013) and impaired carbon metabolism (Figure 1.1) (Ezzat et al. 2015; Tanaka et al. 2017); negatively impacting their thermal tolerance (Ezzat et al. 2016b; Wiedenmann et al. 2013). However, corals also require a baseline supply of phosphate (regardless of nitrogen levels) to maintain autotrophy and thermal tolerance (Ezzat et al. 2016a; Rosset et al. 2017). A lack of phosphorus limits the synthesis and maintenance of crucial molecules for cellular growth, including phospholipids (Wiedenmann et al. 2013) and DNA (Rodriguez-Casariego et al. 2018), which could therefore inhibit cell division in both coral hosts and the algal symbionts (Ferrier-Pagès et al. 2016; Wang et al. 2013).

The negative impacts of phosphate limitation (relative to nitrate) on the coral holobiont have been mechanistically linked to the substitution of phospholipids with sulpholipids, which compromises the stability of algal symbionts' photosynthetic membranes and renders them susceptible to heat and light stress (Rosset et al. 2017; Wiedenmann et al. 2013). Furthermore, there is emerging evidence that high N:P ratios inhibit DNA repair in corals during thermal stress (Rodriguez-Casariego et al. 2018). Under the high N:P condition, severe competition for phosphorus can occur where the algal symbionts become parasitic, retain nutrients (Li et al. 2016; Rosset et al. 2017) and potentially sequester ATP from their hosts (Lin et al. 2015; Luo et al. 2017). In response, the coral host may digest its' symbiont population to recuperate lost nutrients (Tanaka et al. 2018; Thomas and Palumbi 2017). Shifts

towards phosphorus limitation of the coral holobiont have the potential to severely compromise the stability of the coral-Symbiodiniaceae symbiosis, leading to carbon limitation, photo-oxidative stress, and an increased susceptibility to coral bleaching.

Heterotrophic feeding mediates inorganic nutrient availability

Heterotrophic feeding is known to improve the health of corals under ambient conditions (Houlbrèque and Ferrier-Pagès 2009) and when faced with thermal stress (Lyndby et al. 2020; Tremblay et al. 2016). Heterotrophic food sources contain carbon, in addition to providing nitrogen and phosphorus (Houlbrèque and Ferrier-Pagès 2009), which prevents carbon limitation under thermal stress and enhances coral resistance to bleaching (Lyndby et al. 2020; Tremblay et al. 2016; Wooldridge 2014). However, heterotrophic feeding is not always beneficial, particularly under stressful inorganic nutrient conditions (Ezzat et al. 2019; Ezzat et al. 2016b; Rosset et al. 2015) or when food is of poor quality (Conlan et al. 2018; Fabricius et al. 2013; Lim et al. 2017; Pogoreutz et al. 2017a; Tagliafico et al. 2018; Tagliafico et al. 2017). When coral holobionts experience stressful inorganic nutrient conditions, heterotrophic feeding can exacerbate the nutrient imbalance (Ezzat et al. 2016b; Ferrier-Pagès et al. 2018; Rosset et al. 2015) or alternatively, heterotrophic nutrient assimilation may decrease (Ezzat et al. 2019). Changes to the nutritional composition of food may also have negative implications for the coral holobiont, particularly when combined with thermal stress (Conlan et al. 2018; Fabricius et al. 2013; Lim et al. 2017; Tagliafico et al. 2018; Tagliafico et al. 2017). These findings are particularly relevant given that corals on productive nearshore reefs rely more on heterotrophic feeding (Fox et al. 2018), due to increased organic nutrient availability and/or nutrient stress (Ezzat et al. 2019; Ezzat et al. 2016b; Rosset et al. 2015; Wiedenmann and D'Angelo 2018). Overall, heterotrophic feeding under oligotrophic conditions provides benefits to the coral holobiont, but stressful inorganic nutrient environments may decrease food quality or negatively interact with heterotrophy to impact coral health and thermal tolerance.

Nutrient availability and the coral microbiome

The coral microbiome (specifically bacteria and archaea) has been implicated in the cycling of essential nutrients within the coral holobiont and may therefore play a role in the nutrient metabolism of corals and influence their responses to environmental stress (Bourne et al. 2016). However, the microbial metabolic pathways integrated with the coral host and their algal symbionts remain poorly characterised (Ferrier-Pagès et al. 2016; Rädecker et al. 2015). Nitrogen fixation is perhaps the best characterised microbial metabolic function that supports the coral-Symbiodiniaceae symbiosis (Rädecker et al. 2015). Corals harbour a diverse community of diazotrophs (Benavides et al. 2017) and nitrogen fixation has been observed in a range of coral species (Cardini et al. 2015; Pogoreutz et al. 2017b) to potentially provide nitrogen to both the host and algal symbiont tissues (Bednarz et al. 2017). Importantly, nitrogen fixation is influenced by environmental conditions (Cardini et al. 2015; Cardini et al. 2016; Pogoreutz et al. 2017a; Santos et al. 2014) and has been suggested to both support and hinder the stability of the coral-Symbiodiniaceae symbiosis (Rädecker et al. 2015). Nitrogen fixation is known to increase in warm summer conditions, when both external nutrient availability and algal symbiont populations are low, and putatively allows the coral holobiont to maintain its productivity through these stressful conditions (Bednarz et al. 2018; Cai et al. 2018; Cardini et al. 2015; Cardini et al. 2016) much like ammonium enrichment (Béraud et al. 2013; Ezzat et al. 2015; Ezzat et al. 2019). However, other studies have linked elevated nitrogen fixation to thermal stress and bleaching (Cardini et al. 2016; Pogoreutz et al. 2017a; Santos et al. 2014). The activity of nitrogen fixers in corals is known to be stimulated by dissolved organic carbon (DOC) enrichment, which increases N:P ratios within the coral holobiont and can lead to bleaching through carbon limitation and photo-oxidative stress (Pogoreutz et al. 2017a). In the same way, summer conditions on coral reefs coupled with increased nitrogen fixation could represent a 'perfect storm' for coral bleaching, where the algal symbionts become overwhelmed by a combination of high temperatures, elevated irradiance, and increased N:P ratios. Furthermore, diazotrophs may act as an additional sink of carbon and bypass the host's ability to restrict external nitrogen supply to the algal symbionts, thereby enhancing the likelihood of coral bleaching. Microbial nitrogen fixation therefore represents a highly dynamic pathway by which other coral holobiont members can

acquire nitrogen, however, the consequences of this can differ depending on the environmental context.

Changes to environmental nutrient conditions may impact the coral microbiome, manifesting as microbial dysbiosis (Zaneveld et al. 2016) and resulting in coral disease (Vega Thurber et al. 2014). For example, field and laboratory studies have linked nutrient enrichment to coral disease, where enrichments of ammonium, nitrate and phosphate induced and/or enhanced a range of coral diseases including black band disease (Voss and Richardson 2006), yellow band disease (Bruno et al. 2003), and dark spot syndrome (Vega Thurber et al. 2014). In addition, these combined nutrient enrichments triggered the production of herpes-like viruses in *Porites compressa* (Vega Thurber et al. 2008), while both DOC and nutrient enrichments were shown to shift the microbiome towards a pathogenic state (Vega Thurber et al. 2009). However, other studies have shown that the coral microbiome structure does not consistently respond to nitrate and/or phosphate enrichment (Wang et al. 2018), nor to DOC or urea enrichment (Pogoreutz et al. 2018). These latter experiments suggest that coral microbiomes can either shift into unique and random dysbiotic states (Wang et al. 2018), or remain inflexible (Pogoreutz et al. 2018) under nutrient stress. Therefore, although nutrient enrichment can be broadly associated with impacting coral health and increasing disease, relationships with specific nutrient forms are unclear and should be a focus for future study.

Nutrient availability and coral bleaching recovery

It is well established that particulate nutrients ingested by the coral host promote recovery following thermal bleaching (Grottoli et al. 2006; Levas et al. 2018; Tremblay et al. 2016). However, information regarding the comparative impacts of inorganic nutrients is lacking. Heterotrophic feeding can promote recovery from bleaching by alleviating coral carbon limitation (Tremblay et al. 2016; Wooldridge 2014), whereas bio-energetic modelling suggests that inorganic nitrogen enrichment has an opposite, detrimental effect (Cunning et al. 2017a).

Empirical studies testing inorganic nutrient impacts on corals following thermal stress are few in number, but nitrate enrichment can either trigger the rapid growth of photodamaged algal symbionts (Chumun et al. 2013; Ezzat et al. 2016b) or prolong coral bleaching and increase mortality (Wang et al. 2018). Both responses are well explained by a bio-energetic model (Cunning et al. 2017a). Firstly, dense algal symbiont communities can form naturally in corals post-bleaching, owing to a sudden increase in resources per symbiont cell (Cunning et al. 2017a; Cunning et al. 2015b; Levas et al. 2018). Secondly, the growing algal symbiont population retains photosynthates, delaying recovery of the coral host (Cunning et al. 2017a; Wooldridge 2013). External nitrogen enrichment could therefore be expected to prolong the carbon-limited status of a bleached coral holobiont and increase the risk of mortality through starvation. However, to date, the impacts of inorganic nutrient availability on bleaching recovery have only been tested in experiments that enrich corals throughout thermal stress (Chumun et al. 2013; Ezzat et al. 2016b; Wang et al. 2018). To fully understand the nutritional processes involved in coral bleaching recovery, future experiments should apply nutrient manipulations following thermal bleaching, rather than before, to explicitly separate pre- and post-bleaching nutrient impacts.

Inorganic nutrient metabolism underpins coral stress tolerance

Evolutionary theory predicts that the stability of nutritional symbioses is controlled by finely balanced conflict mediation between partners (Bronstein 2009). The same theory can be applied to the coral holobiont, where selection favours symbiont cells which retain photosynthates for their own growth, yet the coral host requires symbionts to translocate photosynthates or else they are eliminated (Blackstone and Golladay 2018). Genetic and phenotypic variation underpins the environmental stress tolerance of coral holobionts and may manifest in processes such as the control of inorganic nutrient metabolism, which mediates whether it is the coral host or the algal symbionts that benefits from autotrophic carbon fixation (Baker et al. 2018; Blackstone and Golladay 2018; Rädecker et al. 2015; Suggett et al. 2017).

The processes that regulate the supply of nutrients to the algal symbionts and the subsequent translocation of carbon are still poorly understood (Davy et al. 2012; Rädecker et al. 2015). However, some coral hosts can actively decrease the N:P ratio of nutrients supplied to the algal symbionts when at risk of bleaching (Ezzat et al. 2016a; Ezzat et al. 2019; Godinot et al. 2011; Pogoreutz et al. 2017a). Combined transcriptome-metabolome analyses in *Exaiptasia diaphana* anemones, common name Aiptasia, also suggests that coral hosts may use photosynthates to sequester their own ammonium wastes into amino acids (Cui et al. 2019). Both these actions may represent host-derived mechanisms that act to maintain the algal symbionts in a nitrogen-limited state and in turn prevent carbon limitation of the holobiont. However, the algal symbionts have evolved to counteract nitrogen limitation (Aranda et al. 2016), rendering corals susceptible to symbiont parasitism under nitrate enrichment and thermal stress (Baker et al. 2018; Ezzat et al. 2015; Tanaka et al. 2017). Despite this, interspecific differences in the nutrient acquisition and utilisation of the symbionts offer a potential avenue to holobiont stress tolerance (Aranda et al. 2016; Suggett et al. 2017).

Metabolic compatibility between coral and Symbiodiniaceae lineages

Coral holobionts containing high-performance 'generalist' algal symbionts (e.g. *Cladocopium goreaui*) often outperform more 'specialised' stress-tolerant symbionts (e.g. *Durusdinium trenchii*), in terms of key traits such as photosynthesis and host growth (Cantin et al. 2009; Jones and Berkelmans 2012; Little et al. 2004; Mieog et al. 2009). This can be attributed to the greater fixation and translocation of inorganic carbon and nitrogen by generalist types (Baker et al. 2013; Cantin et al. 2009; Jones and Berkelmans 2011; Pernice et al. 2015). However, these trends are reversed at elevated temperatures where stress-tolerant types outperform generalists (Cunning et al. 2015a; Cunning et al. 2017b; Jones and Berkelmans 2010). These observations may be related to differences in nutrient metabolism, where thermally tolerant types upregulate their nitrate intake to maintain carbon translocation (Baker et al. 2013). Hence, thermally tolerant algal symbiont species may convey resistance to nutrient and/or thermal stress, whereas corals hosting other algal species become carbon-limited (Baker et al. 2018; Ezzat et al. 2015; Krueger et al. 2018; Krueger et al. 2017; Lyndby et al. 2020; Tanaka et al. 2017; Tremblay et al. 2016). Furthermore, increased nitrogen

availability per symbiont following bleaching can promote recovery of corals with stress-tolerant types (Levas et al. 2018). It is possible that thermally tolerant species direct additional nitrogen towards carbon translocation, as their growth rates are low and stable across environmental conditions compared to other species (Klueter et al. 2017), which may proliferate and subsequently reduce overall holobiont health (Pogoreutz et al. 2018). Therefore, the identity of the algal symbionts appears to alter holobiont tolerance to nutrient and thermal stress through differences in inorganic nutrient metabolism, although this remains to be fully determined.

Evidence from Aiptasia suggests that both host and algal symbiont identity mediate inorganic nutrient metabolism and thermal tolerance. The photosynthetic response of Aiptasia to thermal stress depends on both the host strain and algal symbiont genus: where heterologous ("novel") holobionts outperform those that are homologous ("normal") at elevated temperatures (Goulet et al. 2005). Similar host-symbiont interactions can occur in corals, where host species impacts the relative thermal tolerance of holobionts containing different, yet homologous, algal symbiont genera (Abrego et al. 2008). At the nutritional level, carbon fixation and nitrogen assimilation by the algal symbionts in Aiptasia has been shown to depend solely on symbiont identity, whereas host benefits through carbon translocation are co-dependent on host genotype (Rädecker et al. 2018). In this case, the algal symbionts retained a constant amount of fixed nutrients regardless of their host (Rädecker et al. 2018). In general, homologous holobionts outperform heterologous holobionts as evidenced by reduced carbon translocation (Matthews et al. 2017; Matthews et al. 2018; Rädecker et al. 2018; Starzak et al. 2014) and growth (Gabay et al. 2018) in heterologous symbioses. Furthermore, novel symbionts may sustain themselves by manipulating host nitrogen cycling (Matthews et al. 2018). Although the later observations appear to contradict earlier findings (Goulet et al. 2005), it remains to be seen how nutrient and thermal stress impact host-symbiont interactions in nutrient metabolism.

Phenotypic plasticity in nutrient metabolism

The ability of the coral holobiont to activate mechanisms which maintain and enhance metabolic compatibility may be partly determined by adaptation or acclimation to their environment. For example, algal symbionts in warm-acclimated coral holobionts assimilate less nitrogen and translocate more carbon than their ambient-acclimated counterparts upon exposure to acute thermal stress (Gibbin et al. 2018). Although both sets of coral holobionts were physiologically unaffected by thermal stress in this experiment (Gibbin et al. 2018), other studies have found that acclimation to elevated and variable temperatures prior to acute thermal stress reduces the severity of coral bleaching (Ainsworth et al. 2016; Middlebrook et al. 2008; Safaie et al. 2018). Therefore, as part of, or in addition to observed genetic mechanisms (Barshis et al. 2013), temperature acclimation may help coral hosts to resist bleaching, by invoking metabolic processes which act to maintain their symbionts in a mutualistic state.

Similarly, prior exposure to different water quality environments may provide another avenue for acclimation. Inshore reefs, despite increased exposure to elevated and variable nutrient and thermal stress compared to those offshore, are often resistant to bleaching (Gintert et al. 2018; Guest et al. 2016; Morgan et al. 2017; van Woesik et al. 2012). In the Florida Keys, the growth of inshore corals is reduced upon transplantation to new off- or along-shore environments, suggesting that their thermal tolerance is linked to fine-scale nutritional specialisation (Kenkel et al. 2015; Kenkel et al. 2013a; Kenkel and Matz 2016). In contrast, the evolved thermal tolerance of corals in oligotrophic regions, such as the Red Sea, may be highly sensitive to minor increases in nutrient availability (Fine et al. 2013; Hall et al. 2018). Combined, these results suggest that the historical nutrient conditions (over proximate and evolutionarily timescales) can prime coral hosts for the nutritional disruption that occurs during acute stress.

Concluding remarks

The coral bleaching process is currently mainly understood through photo-oxidative pathways, though recent evidence indicates that nutritional mechanisms are involved. The coral-Symbiodiniaceae relationship is primarily a trophic mutualism, therefore the stability of this symbiosis is dependent on the balance and exchange of nutrients in response to environmental conditions. In this review, an integration of novel experimental evidence has been used to show how nutrient availability and metabolism can mediate coral bleaching with and without photo-oxidative stress. Nutrient availability has previously been postulated to influence bleaching susceptibility through increasing symbiont growth rates, however here we demonstrate that bleaching is better attributed to changes to autotrophic carbon metabolism, which depend on nutrient form and ratio. Furthermore, historical nutrient conditions may influence host-symbiont metabolic capability and therefore bleaching susceptibility. Future experiments should determine how nutrient and temperature conditions alter the metabolic co-operation and stability of distinct coral-Symbiodiniaceae combinations (see Outstanding Questions). An added focus should also be placed on understanding how inorganic nutrients mediate the re-establishment of the coral-Symbiodiniaceae symbiosis following bleaching. Nutrient metabolism within the coral holobiont still remains poorly characterised beyond the identification of putative nutritional pathways and therefore the specific metabolic pathways which destabilise the symbiosis should be elucidated by manipulating the genes which encode enzymes and transporters involved in nutrient cycling. To truly understand the cellular mechanisms leading to coral bleaching, a renewed focus must be placed upon the nutrient metabolism of the coral holobiont.

Outstanding questions

- How do nutrient and thermal stress interact to affect algal symbiont parasitism and coral holobiont function? How does this impact coral bleaching susceptibility? Are the impacts related to algal symbiont density?

- Do nutrient conditions that increase coral bleaching susceptibility, specifically high N:P ratios, also impact recovery from bleaching? If so, do these facilitate or inhibit coral recovery?
- Do natural nutrient subsidies and anthropogenic nutrient pollution influence coral thermal tolerance in similar or different ways? Are the impacts the same even when the nutrients take the same form (e.g. nitrate from runoff versus natural remineralisation)?
- To what extent does the metabolic compatibility of the coral hosts and their algal symbionts contribute to their thermal tolerance? Are compatible symbioses susceptible to nutrient stress?
- Do stress high-performance generalist symbioses and specialised stress-tolerant symbioses have common metabolic responses to stress? Can nutrient stress be unequivocally diagnosed through specific metabolic biomarkers?
- Does coral acclimation to environmental stress occur through the reconfiguration of internal nutrient metabolism? Can acclimation to nutrient stress also increase resistance to thermal stress, and *vice versa*?
- What are the outstanding mechanisms in which nutrient availability and metabolism mediate coral holobiont health and stress tolerance?

Chapter 2: Algal symbiont communities, but not bleaching severity and recovery of *Acropora millepora*, vary across water quality and temperature gradients on the Great Barrier Reef

Abstract

Australia's Great Barrier Reefs (GBR) is declining due to multiple anthropogenic stressors, including the cumulative impacts of riverine nutrient and sediment efflux combined with mass coral bleaching events which have increased in frequency, severity, and spatial extent. Laboratory and field experiments demonstrate that ocean warming and inshore water quality degradation can interact to enhance coral bleaching severity and reduce recovery. However, links between local-scale stressors (e.g. water quality stressors including nutrient and sediment pollution) and coral bleaching remain hard to detect, especially during severe marine heatwaves (≥ 5 °C-weeks). Colonies of the common reef-building coral *Acropora millepora* (n = 460) were sampled from paired inshore and mid-shelf reefs spanning 260 km of the central GBR, first during the peak of the 2017 bleaching event (March/April) and then six months later (September). These samples were analysed for their photosynthetic pigmentation (chlorophyll) levels and/or ITS2 profiling of their algal symbiont communities, which are known to mediate bleaching tolerance. Both cross-shelf comparisons and those among individual reefs showed that chlorophyll levels (normalised to host protein) were spatially homogenous across the central GBR, both during and after the 2017 bleaching event, but pigment recovery rates following bleaching were greater across inshore reefs. *Cladocopium* C3 was the most common algal symbiont for *A. millepora*, with two C3 type profiles (C3/50c and C3/C3k) dominating most reefs during and following the bleaching event. Within *Cladocopium* C3, C3/C50c was replaced at the cooler, southernmost mid-shelf reefs by C3/C3k. Across the inshore reefs, *A. millepora* hosted either C3/C50c-dominated algal symbiont communities or a more diverse consortia of *Cladocopium* (e.g. C1, C21) and *Durusdinium* (D1 or D2) type profiles, which changed following the bleaching event. Most notably, *A. millepora* at the North Barnard Islands were conspicuous in a shift from

Durusdinium D2 during bleaching to *Cladocopium* C21 six months later. This survey of *A. millepora* indicates that local environment conditions structure the algal symbiont communities, but not the severity of bleaching and recovery of corals during severe heat stress events.

Introduction

Corals reefs are experiencing major declines due to ocean warming (Lough et al. 2018) that triggers mass coral bleaching and mortality events (Hughes et al. 2018a). The world's largest living structure, Australia's Great Barrier Reef (GBR), has now suffered from mass coral bleaching on five occasions (1998, 2002, 2016, 2017, 2020), including three recent events within five years (Hughes et al. 2021). Coral bleaching is a cellular process by which corals lose their algal symbionts (Weis 2008), typically in response to anthropogenic ocean warming (Hoegh-Guldberg 1999). Healthy corals derive substantial nutrition from the autotrophic algal symbionts (Conti-Jerpe et al. 2020; Muscatine and Porter 1977). Under heat stress, this nutrient source declines (Baker et al. 2018; Rädecker et al. 2021) and bleached corals face reduced fitness and potential mortality unless they compensate through alternative nutrient sources (e.g. particulate food) or recover their symbiont communities (Grottoli et al. 2006; Tremblay et al. 2016). As a result of repeated mass bleaching, coral abundance decreased by 50 % on the GBR from 1995-2017, with the steep declines in taxonomic diversity (Dietzel et al. 2020; Hughes et al. 2018b). This decline of coral on the GBR is predicted to continue unless abated by measures to rapidly address anthropogenic pressures and build reef resilience (Condie et al. 2021; Wolff et al. 2018).

Coral reefs are also exposed to anthropogenic pressures at local scales including degraded water quality. This occurs primarily through nutrient and sediment inputs and has the potential to alter ecosystem functioning (Fabricius 2005). The GBR has experienced > 5-fold increases in anthropogenic nutrient and sediment loading since European colonisation in the mid-1800s (Kroon et al. 2012), owing to the wholesale transformation of adjacent land primarily for agricultural purposes (Lewis et al. 2021). These water quality inputs are closely monitored through annual *in situ* sampling for inshore reefs (Waterhouse et al. 2021)

alongside satellite observations (Álvarez-Romero et al. 2013) and modelling (Baird et al. 2020) of the entire ecosystem. Degraded inshore GBR water quality has been associated with reduced coral abundance and/or diversity (Fabricius et al. 2005). Across the wider GBR, reduced water quality lowers coral resilience following acute disturbances including coral bleaching (Mellin et al. 2019; Ortiz et al. 2018), a trend which is predicted to continue over the next 30 years (MacNeil et al. 2019; Wolff et al. 2018). These ecological impacts of water quality stress occur through several mechanisms which include indirect pressures such as competition from other benthic organisms (e.g. macroalgae, sponges) which benefit from poor water quality, and direct weakening of fundamental aspects of coral fitness including growth and reproduction (D'Angelo and Wiedenmann 2014; Fabricius 2005).

Ocean warming and water quality can interactively impact the spatial extent and severity of mass coral bleaching events (D'Angelo and Wiedenmann 2014; Zweifler et al. 2021). On the GBR, nutrient enrichment was associated with the spatial distribution of thermal stress and coral bleaching during the 1998 bleaching event (Wooldridge et al. 2015) where the most severe bleaching occurred on inshore reefs (Berkelmans et al. 2004; Berkelmans and Oliver 1999). In contrast, little relationship between water quality and bleaching was found in 2016 where heat stress was comparatively high and homogenous across both inshore and mid-shelf reefs (Hughes et al. 2017b). The mechanisms by which water quality and bleaching interactions may occur include nutrient enrichment (Donovan et al. 2020; Morris et al. 2019) and high turbidity from suspended sediments (Morris et al. 2019; Sully and van Woesik 2020). The contradictory relationships of bleaching severity with water quality gradients on the GBR through past bleaching events may reflect differences in the spatial footprint of heat stress over the different bleaching years (Hughes et al. 2021). It may also be reflective of the varying levels of heat exposure which can overwhelm local nutrient impacts during strong marine heat waves (Donovan et al. 2020).

The coral-algal partnership exhibits genetic variation at both the host and symbiont level and can dictate bleaching outcomes under heat stress (Cleves et al. 2020; Suggett et al. 2017). For the common reef-building coral *Acropora millepora*, a recent genome-wide association study (GWAS) showed little spatial structure in the host genetics contributing to bleaching tolerance during 2017 in the central GBR (Fuller et al. 2020). However, corals also host genetically

diverse algal symbionts within and across multiple genera with different ecological niches (LaJeunesse et al. 2018). Early work using Sanger sequencing and electrophoresis methods on the ITS1 region of DNA showed that the algal symbionts hosted by GBR *A. millepora* 1) are ecologically differentiated by temperature and water quality (Cooper et al. 2011; van Oppen et al. 2005; van Oppen et al. 2001), 2) may (Bay et al. 2016; Jones et al. 2008) or may not (van Oppen et al. 2005) change over the course of bleaching events, and 3) that *Durusdinium* symbionts can increase bleaching tolerance by 1 °C or more, relative to *Cladocopium* (Berkelmans and van Oppen 2006). The GWAS study of *A. millepora* indeed found that accounting for proportion of *Durusdinium* symbionts hosted by corals significantly improved prediction of bleaching (Fuller et al. 2020). Modern and dedicated methods now exist to resolve inter- and intra-genomic variation in the algal symbiont communities and simultaneously profile dominant and low abundance symbionts (Hume et al. 2019; Quigley et al. 2014). Utilisation of these methods in studies going forward would build upon previous analyses of environmental and bleaching related patterns in the distribution of *A. millepora*-associated algal symbionts quality (Cooper et al. 2011; Jones et al. 2008; van Oppen et al. 2005; van Oppen et al. 2001). Such studies are now warranted given that it now known that low abundance symbionts (Bay et al. 2016) and intra-genomic variation (Swain et al. 2017) may contribute significantly to the heat tolerance of corals.

To assess the spatial patterns of coral bleaching severity, 460 *Acropora millepora* samples were collected at the peak of thermal stress in 2017 during the GBR summer bleaching event (March/April) and six months later at the start of September. The study region spanned almost 250 km of the central GBR and included six pairs of inshore and mid-shelf reefs. Bleaching severity was assessed through the levels of photosynthetic pigmentation and genetic profiling of the algal symbionts through ITS2-amplicon sequencing. It was hypothesised that poor water quality on inshore reefs would increase the diversity of the coral-associated algal symbiont communities, while also increasing the severity of coral bleaching and delaying coral recovery, relative to corals inhabiting mid-shelf reefs.

Methods

Coral collection

Samples from a total of 460 *A. millepora* colonies were collected from 12 reefs in the central GBR during peak heat stress in March/April 2017 and six months later in September 2017 (Figure 2.1, Table S2.1). Six reefs were located inshore and impacted by riverine outflow (Waterhouse et al. 2021) and the remaining six were mid-shelf reefs primarily influenced by oceanic waters rather than terrestrial inputs (Lough et al. 2015). The latitudinal gradient between Townsville and Cairns was equally represented across the shelf, with paired inshore and mid-shelf reefs proportionally spaced in latitude. For each *A. millepora* colony, one or more branches were sampled after being haphazardly selected from the reef flat under permit (GBRMPA G16/38488.1). The samples were brought to the surface in individual zip-lock bags within 5 min of collection and fixed in liquid nitrogen before long-term storage at -75 °C.

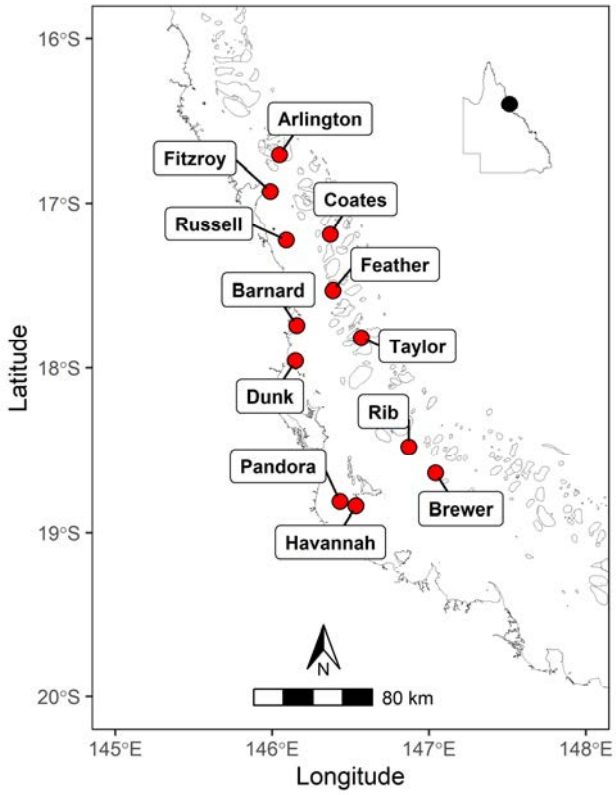

Figure 2.1 Map of sampling reefs for *A. millepora* colonies in the central Greater Barrier Reef. Inset map shows the study region relative to the landmass of Queensland, Australia.

Most reefs were initially sampled in March 2017 prior to the intensification of severe tropical cyclone (TC) Debbie. However, sampling at John Brewer Reef was delayed until the start of April 2017 due to the crossing of this weather system 260 km to the south. Collections were made on a different sample of colonies at the same locations in September 2017. September samples from Feather Reef thawed in transit and were not analysed.

Coral tissue preparation

Coral processing for chlorophyll and protein analysis were conducted under dim light. First the base of each sample was removed and preserved in 100 % ethanol for DNA analysis. Remaining coral tissues were separated from the skeleton with pressured air into ~10 ml of cold 0.04 µM ultra-filtered seawater (FSW) and homogenised for 30 s (Bio-Gen PRO200, PRO Scientific, USA). A 1 ml aliquot was then taken, centrifuged (1,500 x g, 3 min, 4 °C) and the resulting supernatant was discarded leaving the algal symbiont pellet. The remaining coral homogenate was also centrifuged (1,500 x g, 3 min, 4 °C), and the resulting host supernatant was aliquoted in triplicate of 500 µl into deep-well plates. Processed samples were then returned to -75 °C for further analysis.

Chlorophyll and protein content

For chlorophyll content, the algal symbiont pellet was suspended in 700 µl of pre-chilled 95% (v/v) ethanol and sonicated (3 min, 50 % power) in an ice bath attached to an ultrasound generator (Sonic Power MU-600, Mirae Ultrasonic, South Korea). The samples were then resuspended, incubated on ice (20 min), centrifuged (10,000 x g, 5 min, 4 °C) and aliquoted in triplicate in 200 µl into clear 96-well microplates. Absorbance at 632, 649 and 665 nm was measured on a microplate reader (Synergy H4, BioTek Instruments, USA) set to 25 °C. The absorbance values were corrected against blanks and total chlorophyll was calculated using established equations (Ritchie 2008).

For coral host protein analysis, NaOH was added to each sample well to reach a concentration of 0.5 M. The samples were resuspended, sonicated (5 min), incubated (1 hr, 90 °C) and centrifuged (1,500 x g, 10 min, 25 °C). The supernatant was then used to measure coral host

protein in triplicate using the manufacturer's microplate protocol for the DC Protein Assay (Bio-Rad Laboratories, USA). Sample protein concentrations were calculated against a standard curve (50-750 $\mu g.ml^{-1}$) of Protein Standard II (Bio-Rad Laboratories, USA). Where a sample measured with ≥ 10 % coefficient of variation or outside of the standard curve range, it was repeated (after dilution if necessary). The protein concentrations were used to normalise total chlorophyll as a measure of coral bleaching, which may reflect the photosynthetic potential of the algal symbionts relative to host biomass (Cunning and Baker 2014). Previous analysis using a subset of samples from March/April (Fuller et al. 2020) showed that total chlorophyll correlated strongly with algal symbiont density (Spearman's ρ = 0.80).

Chlorophyll data analysis

Total chlorophyll data were analysed using Bayesian generalised linear mixed models (BGLMMs) in R 4.0.4 (R Core Team 2021) and brms 2.15.0 (Bürkner 2017; Bürkner 2018) via rstan 2.26.1 (Carpenter et al. 2017). Initial BGLMMs examined the two-way interaction between shelf-position and sampling period with reef as the random effect. All models were run with default priors, 5,000 iterations (2,000 warmup), three Markov chains and a thinning rate of two. Diagnostics were performed in brms to check autocorrelation, chain mixing, convergence, and effective sample size and residuals were simulated from the observed data and compared to the model residuals using DHARMa 0.4.0 (Hartig 2021). Model fitting was repeated to test the effects on alternative probability distributions, random effects, and variance structures on the model fits. Model candidates were compared using loo 2.4.1 (Vehtari et al. 2017; Yao et al. 2018) to select the optimal model. Following this, the same methods were used to examine the two-way interaction between reef and sampling period. Feather Reef was excluded from the reef level model due to the missing September samples.

Both the shelf position and reef level models were fit to a student's distribution. The shelf position model incorporated the random intercepts of reef, whereas the reef model allowed for heterogenous variances across each reef and sampling period combination. Finally, the model results were summarised and Tukey's pairwise comparisons were extracted using

emmeans 1.5.5.1 (Lenth 2021). Statistical inferences were based on 95% Bayesian credibility intervals (95% CIs) for the modelled posterior high density median effects.

DNA extraction, ITS2 amplicon sequencing and data analysis

Coral DNA was extracted from the ethanol-stored subsamples using the manufacturer's protocol of the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek, USA) with modifications. Surface material was removed from each sample, weighed to 20 ± 2.5 mg (EX224, OHAUS, USA) and combined with TL buffer, OB Protease, and 0.5 mm sterile glass beads. The sample solution was homogenised three times at 4 $m.s^{-1}$ for 30 s (FastPrep-24 5G, MP Biomedicals, USA) and incubated (30 rpm, ~16 hrs, 55 °C; Model 777, SciGene, USA). The remaining steps followed the manufacturer's protocol before eluting DNA into 50 µl of elution buffer.

The ITS2 gene marker associated with the coral algal symbionts was amplified using polymerase chain reactions (PCRs). PCRs consisted of 1X AmpliTaq Gold 360 Master Mix (Thermo Fisher Scientific, USA), 0.4 µM each of forward and reverse primers (Quigley et al. 2014) incorporating Illumina sequencing adapters (Sigma-Aldrich, Australia), 50 ng DNA template and PCR-grade water in reaction volumes of 25 µl. PCR conditions were 95 °C for 7 min, followed by 31 cycles of 95 °C for 30 s, 59 °C for 30s and 72 °C for 30 s, with a final extension of 72 °C for 7 min. ITS2 amplicons were sent for bead clean-up, library preparation and sequencing on the MiSeq v3 2 x 300 bp system (Illumina, USA) at the Ramaciotti Centre for Genomics (UNSW Sydney, Australia).

Demultiplexed forward and reverse reads were submitted for remote analysis using SymPortal (Hume et al. 2019). SymPortal utilises inter- and intra-genomic information in the ITS2 reads to assign defining intragenomic variants (DIVs) which represent unique ITS2 sequences. Patterns within these sequences are then used to define ITS2-type profiles which putatively represent distinct algal symbiont species (Hume et al. 2019). The 376 samples returned 11,877,508 raw contigs (mean ± s.d. 31,589 ± 5,949 per sample), and 7,415,419 (19,722 ± 4,413 per sample) remained after minimum entropy decomposition (MED) which is comparable to other studies which leverage SymPortal (Damjanovic et al. 2019; Damjanovic et al. 2020; Gardner et al. 2019; Howells et al. 2020; Smith et al. 2020).

Prior to analysis, 270 DIVs (out of 515) each comprising < 0.002 % of total post-MED reads were manually removed, and ITS2-type profile data were normalised to relative abundance. All subsequent manipulations (e.g. rarefaction) and analyses (e.g. PERMANOVA) were performed using vegan 2.5.7 (Oksanen et al. 2020) unless otherwise specified. Trimmed DIVs were rarefied to the lowest sample sequencing depth (1,228 reads). Shannon's (*H'*) and Simpson's (1-*D*) and indices of alpha diversity were calculated for the DIVs and modelled at the reef level as described for chlorophyl. Both metrics were fit to a student's distribution with variances allowed to vary for each reef. Equivalent models for the impacts of shelf-position were also tested but not considered further due to the high variability among reefs.

For beta diversity analyses, permutational multivariate analyses of variance (PERMANOVA) was applied using the adonis2 function on Bray-Curtis dissimilarities of the DIV and ITS2-type profile data with 9999 permutations of residuals. The PERMANOVAs were fit to test the interactive impacts of sampling period at the spatial levels of shelf-position and reef. The sampling design was unbalanced, meaning that the ordering of the variables within the models had potential to influence the results. Therefore, multiple PERMANOVAs were fit to isolate the interactive (marginal) effect, before shuffling the order of variables to test the individual (main) effect of one variable while controlling for the other.

The imbalanced designs also meant that the PERMANOVAs were susceptible to homogeneity in group dispersions (Anderson and Walsh 2013). These were tested using PERMDISP (betadisper function). As the betadisper function does not support model interactions, each variable (e.g. shelf-position or reef) was first subset to control for the impacts of the other variable (sampling period) and vice versa. This same subset strategy was also used to test for post-hoc PERMANOVA and PERMDISP for changes over time at the reef level. Finally, the beta diversity of DIVs was visualised with non-metric multidimensional scaling (nMDS).

Characterisation of central GBR heat stress in 2017

Sea surface temperature data were taken from the National Oceanographic and Atmospheric Administration (NOAA) Coral Reef Watch CoralTemp Version 3.1 (Liu et al. 2014) which is a 5

km resolution satellite product. These data included the maximum monthly mean (MMM) climatology for each reef site and the derived degree heating week (DHW) metric of accumulated heat stress which incorporates the severity and duration of thermal anomalies ≥ 1 °C above the MMM integrated over a 12-week window (Liu et al. 2003). For this study, two DHW values were taken for each reef site: the DHW value at the time of sampling in March/April, and the maximum DHW value if different.

Results

Accumulated heat stress on the Central GBR a during the 2017 autumn marine heatwave

All reefs sampled experienced severe levels of accumulated heat stress (≥ 5 °C-weeks) during the 2017 marine heatwave across the central GBR (Table S2.1). Heat stress was imposed upon a slight cross-shelf difference in the long-term climatology (MMM; mean ± s.d. 28.85 ± 0.16 °C inshore and 28.63 ± 0.14 °C mid-shelf), and respectively ranged from 5.81 to 9.54 and 5.21 to 9.31 °C-weeks for inshore and mid-shelf reefs up to the initial sampling timepoint. Additional heat stress accumulation resulted in respective maximum DHW ranges from 7.38 to 10.67 and 5.72 to 11.00 °C-weeks for inshore and mid-shelf reefs prior to the winter recovery phase. Respective initial and maximum DHW averages (mean ± s.d.) were 7.47 ± 1.63 and 9.05 ± 1.31 °C-weeks for the inshore reefs and 6.88 ± 1.48 and 8.26 ± 1.77 °C-weeks for the mid-shelf reefs. The high variability of DHW exposure among the shelf positions meant that a trend of increasing heat stress at lower latitudes for both inshore and mid-shelf reefs (Table S2.1) prevailed over any the cross-shelf differences.

Physiological response of coral during and after 2017 heat stress event

Total chlorophyll levels in *A. millepora* (R^2 = 0.3680, 95 % CIs 0.3142-0.4222) did not vary statistically between the sampled inshore and mid-shelf reefs both during and after the bleaching event (Figure 2.2; Figure S2.1). Regardless, corals from inshore reefs tended to have slightly lower chlorophyll content (6.95 $\mu g.mg^{-1}$ protein, 4.36-9.71) during bleaching compared to the mid-shelf (8.53 $\mu g.mg^{-1}$ protein, 5.94-11.4), a trend which reversed six

months later (inshore 21.0 µg.mg^{-1} protein, 18.3-23.8; mid-shelf 17.9 µg.mg^{-1} protein, 15.2-20.7). More notably, the increase of chlorophyll between March/April and September (Figure S2.1) was statistically higher for the inshore corals (+14.0 µg.mg^{-1} protein, 12.5-15.6) relative to mid-shelf corals (+9.4 µg.mg^{-1} protein, 7.8-11.3).

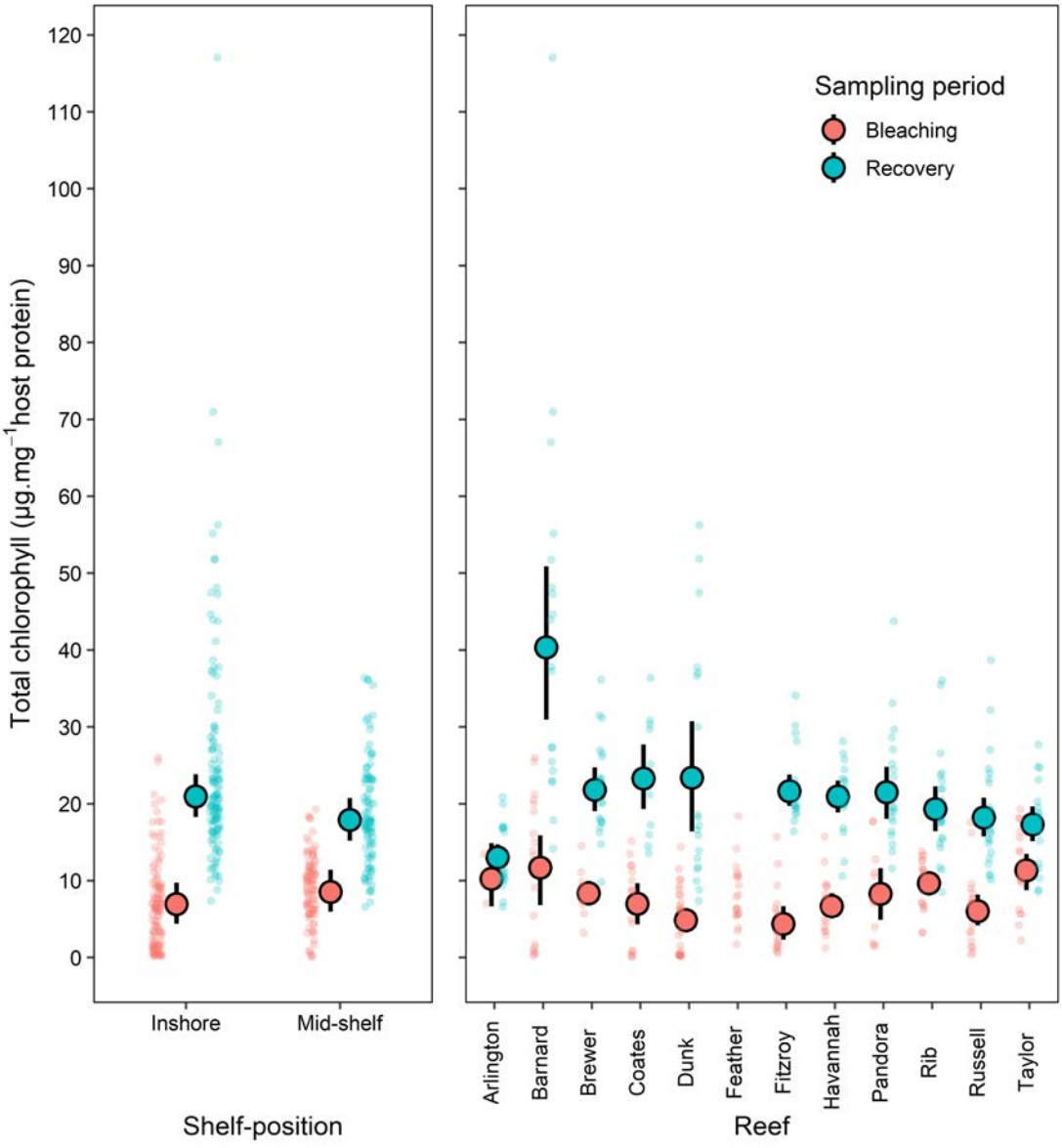

Figure 2.2 Total chlorophyll concentration (µg.mg^{-1} host protein) by shelf-position and individual reef during the peak of bleaching in March/April 2017 and following six months of recovery in September 2017. Coloured points show median model simulations with 95% Cis and coloured dots show individual samples.

At the reef level, chlorophyll was statistically indistinguishable within each sampling point across most inshore and mid-shelf reefs (R^2 = 0.5375, 0.4353-0.6165; Figure 2.2; Figure S2.1). Two key trends, however, were that corals at Arlington Reef did not increase their chlorophyll following bleaching (Figure S2.1) and that those at North Barnard experienced a large increase in chlorophyll levels with high variability (Figure 2.2; Figure S2.1). These trends meant that six months after bleaching, corals at Arlington Reef had less chlorophyll, and corals at the North Barnard Islands had more chlorophyll, compared to all other reefs (Figure 2.2).

Algal symbiont community dynamics

Cladocopium were the most abundant algal symbionts identified after trimming and rarefaction, with 210 *Cladocopium* DIVs and 24 *Cladocopium* ITS2-type profiles making up 91.8 % of total DIVs (Figure 2.3). This was followed by 31 *Durusdinium* DIVs and six *Durusdinium* type profiles comprising 8.1 % of DIVs and the remaining 0.1 % of DIVs spread across four *Symbiodinium* DIVs and one *Symbiodinium* type profile (Figure 2.3).

Corals across all mid-shelf reefs almost exclusively associated with two *Cladocopium* type profiles: C3/C50c-C3b-C50a-C21-C3gj-C21.12 and C3/C3k-C29-C21ab-C3b-C50a-C3gj-C21.12 (Figure 2.3). The former C3/C50c profile was most abundant at the four northern-most mid-shelf reefs whilst *A. millepora* at the two southernmost reefs primarily hosted the C3/C3k profile. Half of the inshore reefs predominantly shared this C3/C50c profile with the mid-shelf reefs, but limited proportions of *Symbiodinium* A1, *Cladocopium* C1, C3/C3k, C3/C50c/C50a, C3/C50c/C50a/C21 and *Durusdinium* D1ak and D2 were also present (Figure 2.3).

The remaining inshore reefs in the middle of the study region hosted more variable diversity of type profiles characterised by *Cladocopium* C1, C3, C2 and *Durusdinium* D1 and D2 (Figure 2.3). Corals sampled from Pandora Reef were characterised mainly by two type profiles: C3/C50c/C50a-C3b-C21-C3gj and D1-D1u-D2-D4-D2.2 which were common across multiple samples in dominant-to-background proportions. Additional *Cladocopium* profiles (characterised by DIVs from C1, C3/C50c or C3/C50c/C50a/C21 DIVs) were also found in low abundance. Dunk Island was co-dominated by *Cladocopium* C1-C1b-C1c-C42.2-C1bh-C1br-

C1cb-C3 and *Durusdinium* D1-D1u-D2-D4-D, with additional types also present (C1, C3, C21, D1 or D2). Corals at the North Barnard Islands were distinguished by their dominance of the *Durusdinium* D2-D1ak-D1-D2.2-D2h-D4 profile in March, with lower abundances of *Cladocopium* profiles (C1, C1/C3, C1/C15/C3/C42.2, C3/C50c/C50a/C21 or C21). Six months later, the *Durusdinium* profile was still present in many samples but C21-C3-C21aq had higher relative abundance as the most dominant profile, with further *Cladocopium* types also present (characterised by C1 or C21).

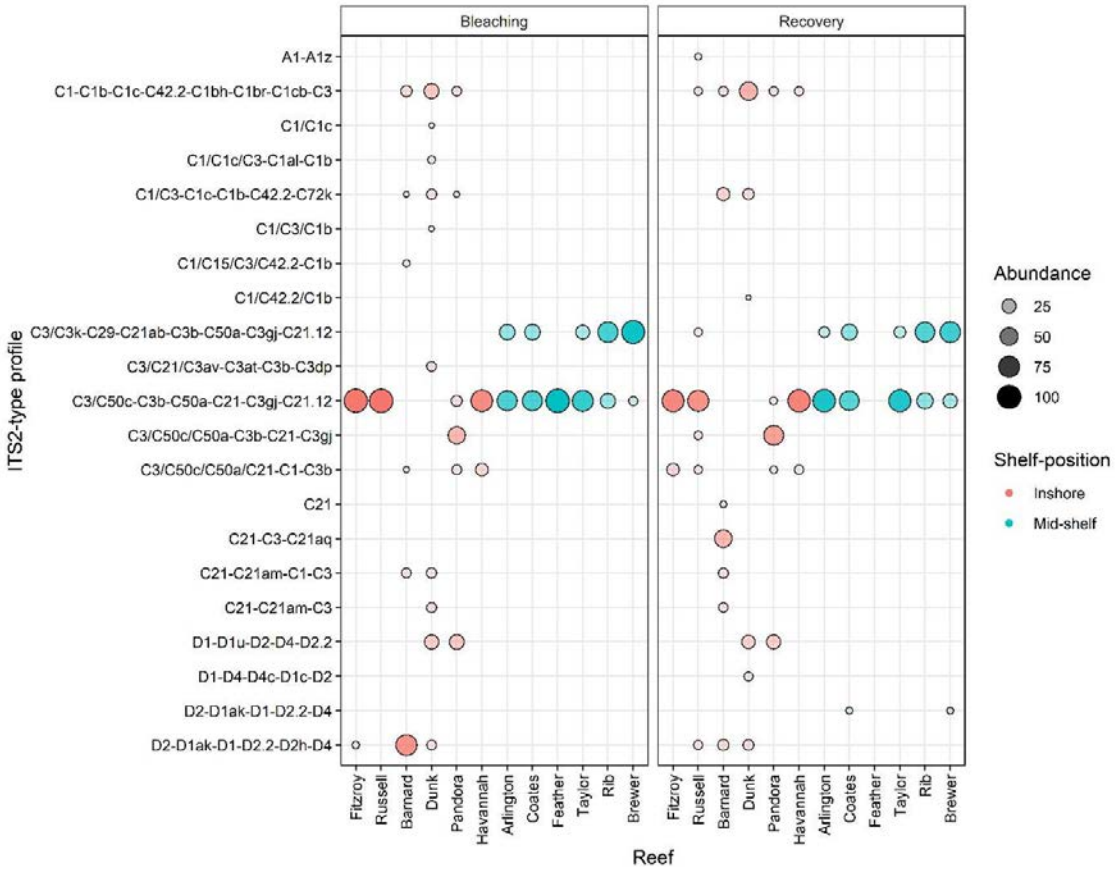

Figure 2.3 Proportion normalised ITS2-type profiles of *A. millepora* at each reef (inshore = red, mid-shelf = blue) during the peak of bleaching in March/April and following six months of recovery in September. The size and opacity of points represent the mean percentage of each type of profile within each reef population. Only type profiles with an abundance known to influence coral bleaching resilience (> 0.3 % within a population) are shown (Bay et al. 2016).

The alpha diversity of DIVs varied among reefs, with the North Barnard Islands being the only site where alpha diversity declined between the peak of bleaching and September (Figure S2.2). During both bleaching and recovery, alpha diversity tended to be lower at the inshore reefs of the North Barnard and Dunk Islands compared to all other reefs (Figure S2.2). Significant variation in DIV-level alpha diversity across the other inshore reefs, and the mid-shelf reefs, was relatively low or absent (Figure S2.2).

PERMANOVA results showed that differences in the relative abundance and beta diversity of algal symbiont DIVs ($p < 0.0001$, $R^2 = 0.1202$; Figure 2.4) and type profiles ($p < 0.0001$, $R^2 = 0.0805$; Figure 2.3) varied cross-shelf but not over time (DIVs $p = 0.4433$, $R^2 = 0.0021$; profiles $p = 0.4640$, $R^2 = 0.0021$) or interactively (DIVs $p = 0.5222$, $R^2 = 0.0018$; profiles $p = 0.2425$, $R^2 = 0.0031$). At the reef level (Figure 2.4), there were significant interactions between reef and sampling period (DIVs $p < 0.0001$, $R^2 = 0.0418$; profiles $p = 0.0034$, $R^2 = 0.0271$) with reef identity explaining the greatest variation (DIVs $p < 0.0001$, $R^2 = 0.5074$; $p < 0.0001$, $R^2 = 0.4417$) relative to the insignificant impacts of time (DIVs $p = 0.1459$, $R^2 = 0.0021$; $p = 0.2062$, $R^2 = 0.0021$). Due to the unbalanced design, the PERMANOVAs were confounded by unequal dispersion across the spatial factors (DIVs $p < 0.0001$; profiles for shelf-position $p = 0.0174$ and reef $p < 0.0001$) but not over time (DIVs $p = 0.4334$; profiles $p = 0.4957$).

Post-hoc analyses of beta diversity per reef revealed that corals on all mid-shelf reefs and the two southernmost inshore reefs maintained their algal symbiont community abundance, diversity, and dispersion of DIVs and type profiles during bleaching and recovery. In contrast, the four northernmost inshore reefs showed signs of change in their DIV abundance and diversity over time (Figure 2.4): Fitzroy Island ($p = 0.0508$, $R^2 = 0.0580$, marginally insignificant); Russell Island ($p = 0.0391$, $R^2 = 0.0454$); North Barnard Islands ($p < 0.0001$, $R^2 = 0.3081$); Dunk Island ($p = 0.0651$, $R^2 = 0.0613$, marginally insignificant). For the type profiles, the changes over time were limited to Russell Island ($p = 0.0141$, $R^2 = 0.0650$) and the North Barnard Islands ($p = 0.0008$, $R^2 = 0.2195$). Changes at Fitzroy Island ($p = 0.1121$, $R^2 = 0.1016$) and Dunk Island ($p = 0.2166$, $R^2 = 0.0333$) were insignificant. Corresponding changes in dispersion over time were limited to Russell Island (DIVs $p = 0.0467$; profiles $p = 0.0149$).

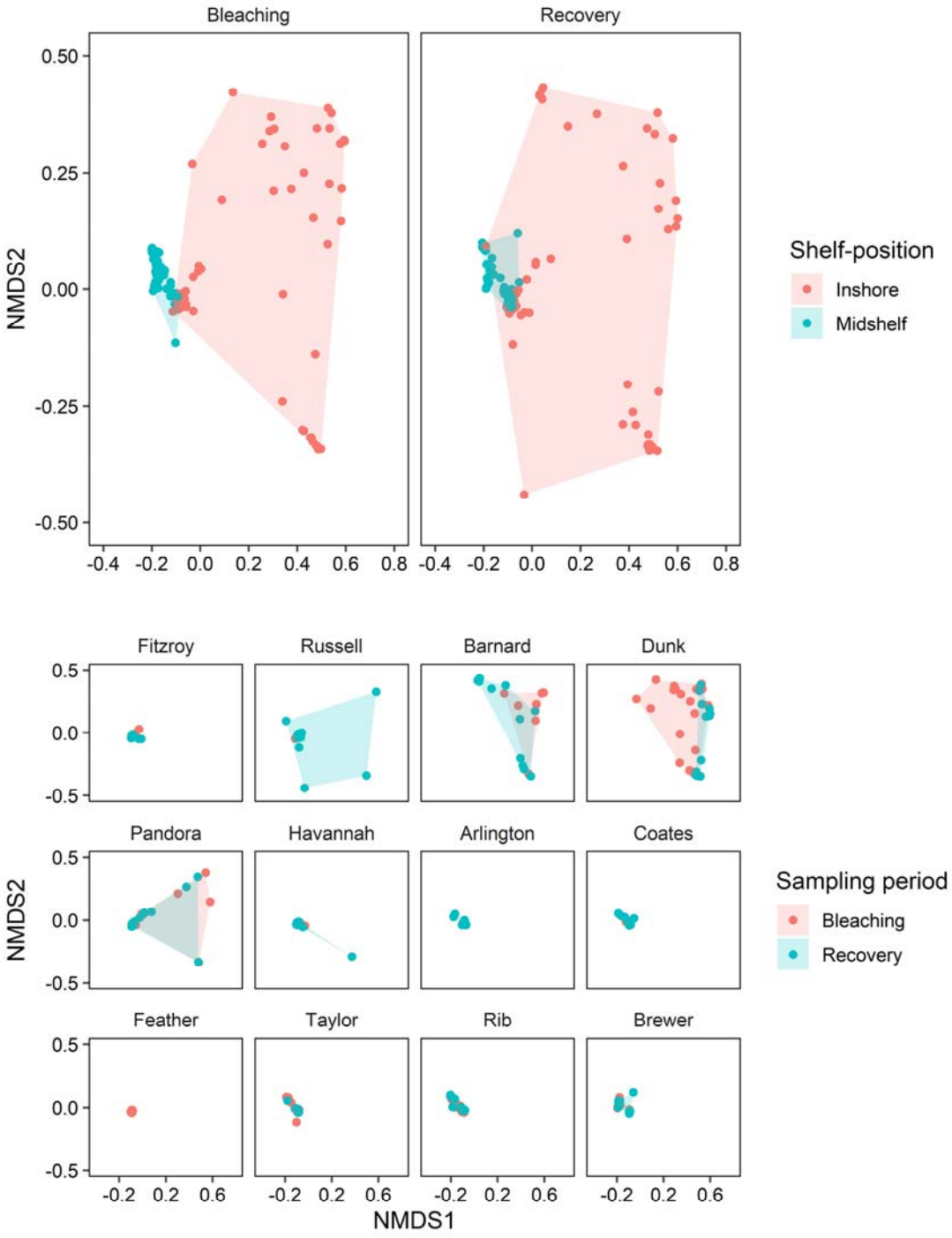

Figure 2.4 nMDS plots of inshore and mid-shelf algal symbiont DIVs from Bray-Curtis dissimilarities by shelf-position and individual reef during the peak of bleaching in March/April 2017 and following six months of recovery in September 2017.

Discussion

The main finding of this field survey of *A. millepora* on the central GBR was that their algal symbiont communities were largely stable following the 2017 bleaching event, and instead structured by cross-shelf and latitudinal gradients. Corals on most inshore and mid-shelf reefs hosted one of two *Cladocopium* type profiles depending on their thermal history, whereas a subset of inshore reefs hosted more variable *Cladocopium* and *Durusdinium* communities. The severity of coral bleaching and extent of recovery (in terms of chlorophyll pigmentation) were largely consistent across the spatial gradients likely owing the exposure of all studied reefs to severe levels of heat stress (≥ 5 °C-weeks). Inshore corals had a much greater increase in chlorophyll content (49%) relative to mid-shelf corals between March/April and September. Therefore, local water quality conditions and algal symbiont community composition did not influence the susceptibility and resilience of *A. millepora* to bleaching in the central GBR during the severe marine heatwave of 2017.

Cross-shelf patterns of bleaching and recovery

The 2017 marine heatwave represents an ideal opportunity to assess cross-shelf gradients in how chronic stressors (e.g. water quality) impact the bleaching severity of corals, due to the homogenous distribution of heat stress between the shelf positions (inshore and mid-shelf) on the central GBR (Table S2.1). It has been proposed that inshore conditions of degraded water quality can reduce coral heat tolerance and thus increase the risk of coral bleaching and mortality on the GBR (Fabricius et al. 2013; Fernandes de Barros Marangoni et al. 2020; Morris et al. 2019; Wiedenmann et al. 2013). This finding is supported by aquarium (Burkepile et al. 2019; Wang et al. 2018) and field (Hughes et al. 2017b) experiments conducted on corals from reefs other than the GBR. Despite this, the observations of 2017 GBR bleaching event presented here agree with ecological surveys conducted during the preceding 2016 event, which found that local stressors such as water quality did not influence the relationship between accumulated heat stress and the bleaching severity of corals under natural conditions (Donovan et al. 2020). It therefore appears likely that during recent mass coral

bleaching events on the GBR were driven primarily by accumulated heat stress (generally ≥ 4 °C-weeks), and not impacted by local water quality conditions at these levels.

The greater rate of increased chlorophyll concentrations for inshore *A. millepora* relative to those on the mid-shelf following the bleaching event is suggestive of a larger increase in algal symbiont density and/or pigment content (Fuller et al. 2020). This can increase the autotrophic potential of corals (Cunning and Baker 2014) relative to the biomass of the coral host, but within the context of the current study does not necessarily indicate the increase fitness of inshore corals relative to those mid-shelf. It is possible that the pre-bleaching pigmentation of corals may have differed cross-shelf prior to the 2017 bleaching event, as inshore *A. millepora* in the central GBR are known to be highly pigmented compared to their mid-shelf counterparts under non-bleaching conditions (Fabricius 2006). Environmental reasons behind this inshore elevation of symbiont densities on inshore reefs bleaching may include inorganic nutrient enrichment (Shantz and Burkepile 2014; Waterhouse et al. 2021) and/or increased heterotrophic feeding (Anthony 2000; Conlan et al. 2018; Fox et al. 2018) alongside photo-acclimation to low-light, turbid conditions (DiPerna et al. 2018; Jones et al. 2020).

The greater increase in chlorophyll content in colonies throughout the inshore reefs may additionally, or alternatively, reflect a concurrent loss of host protein as observed for *A. millepora* during thermal acclimation (Nielsen et al. 2020), heat stress (Hoadley et al. 2015; Kochman et al. 2021; Rosic et al. 2020) and bleaching (Chapter 4; (Bourne et al. 2008)). It must be noted that high algal symbiont densities in corals following bleaching (Cunning et al. 2015b; Kemp et al. 2014; Levas et al. 2018), especially those exposed to nutrient enrichment (Chumun et al. 2013; Ezzat et al. 2016b), may engage in “selfish” behaviour through which they retain photosynthates for their own growth, leading to prolonged stress for the coral host (Baker et al. 2018; Cunning et al. 2017a; Rädecker et al. 2021). Overall, the broad physiological consequences of accelerated increases to inshore coral pigmentation following bleaching, relative to mid-shelf corals, are not clear.

Algal symbionts of *A. millepora* in the central GBR during bleaching and recovery

This study significantly builds upon existing surveys of algal symbiont diversity in *A. millepora* on the GBR (Cooper et al. 2011; van Oppen et al. 2005; van Oppen et al. 2001) by incorporating modern next-generation sequencing (Quigley et al. 2014) and bioinformatic methods (Hume et al. 2019) to analyse algal symbiont diversity with higher sensitivity. This approach revealed that the algal symbionts communities of *A. millepora* were stable following a severe bleaching event on across most of the studied reefs in the central GBR. The exception to this rule was a subset of inshore reefs (North Barnard, Dunk and Pandora), which showed changes in their diverse consortia of *Cladocopium* (C1, C1/C3, C3/C50c/C50a, C3/C50c/C50a/C21 and/or C21) and *Durusdinium* (D1 and/or D2) type profiles between the peak of heat stress and the following six months. But on most reefs, including all mid-shelf reefs, there were high levels of fidelity between *A. millepora* and *Cladocopium* C3/C50c and C3/C3k type profiles with an increasing dominance C3/C50c at warmer locations (those more northernly or inshore). This lack of diversity therefore highlights that changes in the algal symbionts of *A. millepora*, through symbiont shuffling and/or selective mortality, may be limited in the central GBR.

Algal symbiont dynamics through bleaching

The stability in beta diversity of the C3-dominated algal symbiont communities on most of the sampled reefs, and their persistence through bleaching, is notable when considering the Anna Karenina Principle (AKP; Zaneveld et al. (2017)). AKP dictates that host organisms experiencing dysbiotic reactions to stress vary more in their microbial communities than those who remain healthy (Zaneveld et al. 2017). This trend has previously been identified in the bacterial communities of multiple coral species (Zaneveld et al. 2016) and the algal symbiont communities of GBR *Acropora tenuis* (Quigley et al. 2017b) and *A. millepora* (Howe-Kerr et al. 2020) under multiple stressors or in the wild. In the latter study, *A. millepora* colonies the were stress tolerant had low beta diversity of ITS2 ASVs, relative those that were stress sensitive (Howe-Kerr et al. 2020).

It is notable that previous inferences of heat sensitivity in *Cladocopium* C3 were partly built upon a sole observation of symbiont shuffling from *Cladocopium* to *Durusdinium* in *A.*

millepora at inshore Keppel Islands of the southern GBR (Bay et al. 2016; Jones et al. 2008). The current study contradicts this finding of symbiont shuffling by showing complete persistence of C3/C3k or C3/C50c in most communities following the 2017 bleaching event, agreeing with other studies which show continuous C3-dominance of *A. millepora* over time and through bleaching events (Howells et al. 2013; Stat et al. 2009; van Oppen et al. 2005). However, it must be noted that the SymPortal approach (Zaneveld et al. 2016) used here is limited in identifying low abundance symbionts (Howe-Kerr et al. 2020; Quigley et al. 2019), meaning that changes in the background communities of the algal symbionts remain possible. Overall, the low and persistent levels of beta diversity observed here indicate that *Cladocopium* C3 are high fidelity partners for *A. millepora* in the central GBR even after exposure to severe levels of heat stress.

Although many of the inshore reefs showed minor changes to their algal symbiont communities between the peak of heat stress (March) and following recovery (September), there was a conspicuous shift from *Durusdinium* D2 to *Cladocopium* C21 type profiles at the North Barnard Islands. This contradicts the general perception that *Durusdinium* improve heat tolerance of *A. millepora* (and corals in general) relative to *Cladocopium* (Berkelmans and van Oppen 2006) as well as a prior observations in *A. millepora* from the inshore central GBR whereby a shift from *Cladocopium* to *Durusdinium* conferred resilience following bleaching (Bay et al. 2016; Jones et al. 2008).

The unprecedented shift from *Durusdinium* to *Cladocopium* is somewhat analogous to the selective mortality of *Platygyra ryukyuensis* colonies hosting *Durusdinium* after exposure to extreme heat stress (> 20 °C-weeks) in Kiritimati (Howells et al. 2013; Stat et al. 2009; van Oppen et al. 2005). There, *Durusdinium*-dominated corals were most common at anthropogenically disturbed reefs whereas those at less disturbed reefs hosted *Cladocopium* (Bay et al. 2016; Berkelmans and van Oppen 2006; Cooper et al. 2011; Jones et al. 2008; van Oppen et al. 2005; van Oppen et al. 2001), consistent with the relative distribution of these symbionts on the GBR (Bay et al. 2016; Berkelmans and van Oppen 2006; Cooper et al. 2011; Jones et al. 2008; van Oppen et al. 2005; van Oppen et al. 2001). However, *Cladocopium*-hosting corals in Kiritimati subsequently survived the prolonged heat stress by shuffling towards *Durusdinium* (Cooper et al. 2011; Tonk et al. 2013; Ulstrup and van Oppen 2003; van

Oppen et al. 2001). Overall, although shifts in the algal symbiont communities between *Cladocopium* and *Durusdinium* can occur on coral reefs, questions remain over their consistency and ability to confer bleaching resilience over large spatial scales as often hoped (Logan et al. 2021).

Spatial patterns in the algal symbiont communities

High variation in the *Cladocopium* and *Durusdinium* communities associating with *A. millepora* on the central inshore reefs may reflect of local water quality conditions. *Durusdinium*, which were common components of these more variable algal symbiont communities, are generally known to be restricted to corals living under challenging inshore conditions of elevated temperatures, high nutrients, turbidity, and/or low light (Bay et al. 2016; Jones et al. 2008). Other common symbionts which were restricted to the inshore reefs included *Cladocopium* C1 and C21 types, which likewise favour inshore conditions (Claar et al. 2020). *Durusdinium* is known to confer higher heat tolerance to *A. millepora* compared to the more widely distributed *Cladocopium* C3 (Berkelmans and van Oppen 2006). Similarly, *Cladocopium* C1 can outperform even *Durusdinium* at elevated temperatures in juveniles *Acropora tenuis* (Claar et al. 2020), and *Cladocopium* C21 is known to outperform other *Cladocopium* symbionts in terms of heat tolerance (Dove et al. 2006; Swain et al. 2017). Regardless of these prior observations, neither *Durusdinium* nor *Cladocopium* C1 appeared to confer greater bleaching tolerance (in terms of chlorophyll loss) to *A. millepora* on these inshore reefs under the observed levels of severe accumulated heat stress. *Cladocopium* C21 was however associated with high levels chlorophyll pigmentation (during September at the North Barnards Islands) consistent with a previous observation from the southern GBR (Dove et al. 2006). But, as explained prior for inshore corals in general, hosting *Cladocopium* C21 may come with energetic trade-offs (Xu et al. 2020) and it is unclear whether it constitutes a resilient (*sensu* Bay et al. (2016); Jones et al. (2008)) or opportunistic (*sensu* Baker et al. (2018); Cunning et al. (2017a)) symbiont for *A. millepora*. Overall, the occurrence of distinct and diverse *Cladocopium* and *Durusdinium* type profiles on this subset of inshore reefs are more likely linked to the influence of local genetic or environmental factors (e.g. water quality) rather than their provision of heat tolerance to *A. millepora*.

The widespread presence of the C3/C3k type profile across the mid-shelf reefs, and C3/C50c type profiles across mid-shelf and most of the inshore reefs, is consistent with previous surveys highlighting the C3 and/or C3k DIVs as abundant partners for *A. millepora* and their congeners in the central GBR (Alderdice et al. 2021; Camp et al. 2019; Chan et al. 2019; Epstein et al. 2019; Howe-Kerr et al. 2020; Howells et al. 2013; LaJeunesse et al. 2004; Nielsen et al. 2020; Ulstrup and van Oppen 2003; van Oppen et al. 2005; van Oppen et al. 2001). *Cladocopium* C3 tends to associate with sun exposed *Acropora* colonies or those living in clear waters overlying carbonate sediments (Bay et al. 2016; Jones et al. 2008). Although the C3 DIV is heat sensitive (Berkelmans and van Oppen 2006), substantial variation in heat tolerance exists within *Cladocopium* (Swain et al. 2017) which may be resolved using other intragenomic approaches (Howe-Kerr et al. 2020; Quigley et al. 2019) in addition to those used here (Hume et al. 2019). Consistent with this, the relative distribution of C3/C3k and C3/C50c appear related to the long-term thermal history of corals, as the C3/C3k type is most abundant at the two southernmost mid-shelf reefs in our study are cooler (mean MMM = 28.49 °C) relative to the more northernly reefs (MMM ≥ 28.65 °C). The C3k DIV is also largely absent from warmer, inshore reefs (MMM ≥ 28.77 °C), where *A. millepora* possess higher bleaching thresholds (Chapters 3 and 4). Overall, the spatial distribution of the *Cladocopium* C3/C50c and C3/C3k profiles may be driven by variation in long-term thermal history (MMM) of *A. millepora* across the central GBR, however further investigations are warranted which consider a wider range of thermal (e.g. temperature range and variability) and alternative (e.g. light levels, wave exposure) parameters.

Conclusion

The recent GBR bleaching events are currently understood through large-scale ecological surveys, which have led to the conclusion that patterns of coral bleaching and mortality are almost exclusively driven by accumulated heat stress. The current survey of *A. millepora* presented here builds on these observations to show that total chlorophyll levels (relative to host protein) at the colony-level were not spatially influenced by inshore water quality exposure during the peak of severe heat stress (≥ 5 °C-weeks), or six months later, following a recovery period. These observations add to the evidence that local stressors, including

water quality, do little to influence the severity of coral bleaching at moderate to high levels of heat exposure. In contrast, the *Cladocopium* and *Durusdinium* communities hosted by *A. millepora* appeared to be primarily distributed according to chronic environmental conditions (long-term temperature and water quality) rather than acute heat stress. Corals at most reefs associated with one of two *Cladocopium* types depending on temperature, but a wider range of *Cladocopium* and *Durusdinium* types were additionally present at a subset of inshore reefs. At one of these inshore reefs, an unprecedented shift occurred from *Durusdinium* D2 during the peak of heat stress to *Cladocopium* C21 six months later. However, *A. millepora* at most reefs exhibited high levels of fidelity through the bleaching event, and algal symbiont communities did not appear to strongly relate to patterns of bleaching and mortality. The association of *A. millepora* with a range of *Cladocopium* and *Durusdinium* symbiont warrants further studies into their effects on coral host fitness under changing environmental conditions.

Chapter 3: Great Barrier Reef-relevant nutrient enrichment does not impact *Acropora millepora* bleaching under moderate heat stress

Abstract

Coral reef ecosystems are facing multiple threats from global and local stressors. While ocean warming is regarded as the main trigger of coral bleaching, local pressures including nutrient enrichment are thought to influence the severity of bleaching during heat stress events. The links between nutrient availability and bleaching patterns on Australia's Great Barrier Reef (GBR) are potentially confounded by the local adaptation of corals to large cross-shelf environmental gradients in temperature and water quality. To assess the relative impacts of short-term nutrient exposure and environmental history on GBR coral heat tolerance, *Acropora millepora* was collected from inshore and mid-shelf reefs on the GBR and subject to ecologically relevant levels of nitrate and/or phosphate enrichment (N, P, NP) followed by heat stress (31.5 °C). Prior to heat stress, there was little impact of any experimental nutrient scenario on coral physiology. After temperatures were elevated, all corals bleached regardless of nutrient availability, however chlorophyll loss was greatest in the mid-shelf corals (73 to 78 % mid-shelf vs 52 to 60 % inshore). At the same time, while inshore corals largely maintained photosynthetic performance under heat stress (12 and 11 % decreases in $^{\Phi}$PSII and F_v/F_m; unchanged photosynthetic rates), mid-shelf corals experienced greater levels of photoinhibition (23 % $^{\Phi}$PSII; 30 % F_v/F_m) and reduced net photosynthesis (by 40 %). These cross-shelf differences in heat tolerance were matched by differences in the algal symbiont communities: inshore corals primarily hosted two similar *ITS2*-type profiles whereas 75 % of mid-shelf corals contained unique *ITS2*-type profiles and a greater diversity of intragenomic variants. Overall, the cross-shelf differences in coral heat tolerance overwhelmed any impacts of nutrient availability, and were largely driven by the local adaptation of inshore corals to their higher maximum monthly mean temperature, resulting in differential exposure to accumulated heat stress (2.9 °C-weeks inshore vs 3.9 °C-weeks mid-shelf) by the end of the experiment.

Introduction

Alarming declines in coral reef ecosystems are being reported globally due to increasing sea temperatures which trigger mass coral bleaching events (Hughes et al. 2018a; Lough et al. 2018). Coral bleaching is a stress response whereby corals lose their symbiotic algae (Hoegh-Guldberg 1999; LaJeunesse et al. 2018), which are required to meet their nutritional needs and enable the growth of coral reef ecosystems in oligotrophic waters (Muscatine and Porter 1977). Without the recovery of the algal symbionts or the provisioning of alternative nutrient sources, bleached corals will likely suffer mortality (Grottoli et al. 2006; Tremblay et al. 2016). Rapid restrictions to global carbon emissions are required to secure the best future for corals reefs (van Hooidonk et al. 2016) and buy time for coral communities to adapt to warming oceans (Logan et al. 2021; Matz et al. 2018). However, even if global warming is limited to 1.5 °C in line with the Paris Agreement, local interventions including the management of nutrient pollution will be necessary to promote reef resilience to climate change (Anthony et al. 2017; D'Angelo and Wiedenmann 2014; Donovan et al. 2020; Donovan et al. 2021).

Coral bleaching is traditionally understood to result from photo-oxidative stress in response to elevated temperatures and irradiance (Lesser 1997). Heat stress renders the algal symbiont susceptible to photo-damage, and reactive oxygen and nitrogen species (ROS and RNS) are rapidly produced at levels which exceed the corals antioxidant and anti-nitrosative capabilities (Weis 2008). However, emerging studies are now challenging the photo-oxidative dogma of bleaching by placing destabilisation of environment-coral-algal nutrient exchange at the forefront of the coral heat stress response, as reviewed by Morris et al. (2019). This nutritional impairment of the coral-algal symbiosis may be caused by heat stress alone (Rädecker et al. 2021) or in concert with external nutrient enrichment (Baker et al. 2018) when triggering the later photo-oxidative response. These timely and important findings raise the possibility that local actions to manage nutrient pollution (D'Angelo and Wiedenmann 2014) may partially protect coral reefs from bleaching under climate change.

The coral-algal symbiosis is thought to perform optimally under oligotrophic conditions which restrict the growth of symbiont communities and stimulate the translocation of

photosynthates their hosts (Falkowski et al. 1993). Local nutrient availability may therefore perturb the symbiosis dynamics with strong implications for coral physiology (Morris et al. 2019; Shantz and Burkepile 2014) although a range of (sometimes contradictory) nutrient effects on corals have been previously observed (Fabricius 2005). More recent studies have clarified the impacts of nutrient availability on corals, by linking specific nutrient forms and their ratios to distinct coral health outcomes (D'Angelo and Wiedenmann 2014; Shantz and Burkepile 2014). Damaging forms of anthropogenic nitrogen combined with an undersupply for beneficial phosphorus are now linked to the metabolic breakdown of the coral-algal symbiosis (Morris et al. 2019). This nutrient-mediated bleaching can occur at sub-bleaching temperatures if nutrient ratios are extremely skewed (211 N:P, Rosset et al. (2017)) or when more realistic enrichments (17-43 N:P) are applied under heat stress (Fernandes de Barros Marangoni et al. 2020; Wiedenmann et al. 2013). It is now clear that the response of corals to nutrient pollution is more nuanced than originally anticipated and specifically dependent on the exact nature of available nutrients, rather than the simply the magnitude of nutrient enrichment (Morris et al. 2019).

Although most corals are typically sensitive to anthropogenic stressors, some coral populations live in extreme environments (Camp et al. 2018). For example, corals of the Persian/Arabian Gulf persist under extreme temperatures by hosting unique algal symbionts (Hume et al. 2015) and producing antioxidants in response to high salinity (D'Angelo et al. 2015; Ochsenkühn et al. 2017). Similar processes may exist on inshore reefs of the GBR where anthropogenic stress has winnowed reef communities down to stress-tolerant species (Fabricius et al. 2005). This idea of stress-tolerant inshore communities is supported by coral communities in the Florida Keys, where inshore corals are more heat tolerant than their offshore conspecifics (Kenkel et al. 2013a; Kenkel and Matz 2016), which likely results from their exposure to stressful temperature and water quality conditions (Kenkel et al. 2015). However, trade-offs in desirable physiological traits (e.g. high growth and heat tolerance) can arise when these stress tolerant corals are removed from their natural environments (D'Angelo et al. 2015; Kenkel et al. 2015). Therefore, locally adapted corals and their symbionts may be paradoxically dependent on these marginal conditions to thrive, and management actions to improve inshore water quality could have unexpected consequences.

The idea that inshore corals and their symbionts are locally adapted and resistant to marginal conditions (and conversely that those on the mid-shelf are susceptible) can be applied to understand patterns of bleaching sensitivity along the GBR. Previous research shows that GBR corals and their symbionts from warmer inshore environments are tolerant of higher temperatures (Dixon et al. 2015; Howells et al. 2011; Quigley et al. 2020). Local adaptations of the coral-algal symbiosis to other environmental conditions on the GBR, such as water quality, are comparatively understudied. However, there is evidence from GBR corals that genetic variation in the host (Jin et al. 2019) and symbiont (Cooper et al. 2011; Quigley et al. 2017a) are structured by water quality and temperature, with potential implications for coral heat tolerance (Berkelmans and van Oppen 2006; Jin et al. 2016). This local adaptation has the potential to mask the hypothesised impacts of nutrient enrichment on the bleaching of GBR corals (Wooldridge 2020) and may help to explain why water quality did not appear to influence the 2016 GBR bleaching event (Hughes et al. 2017b).

Despite decades of research into the impacts of nutrient enrichment on GBR corals (Koop et al. 2001) the question over whether water quality increases the impacts of heat stress events remains highly debated (Hughes et al. 2017b; Wooldridge 2020). Potential factors which complicate the relationship between water quality and bleaching outcomes include 1) the differential impacts of different nutrients sources on coral physiology (Shantz and Burkepile 2014) and heat tolerance (Morris et al. 2019), 2) along- and across-shelf gradients in nutrient availability (Waterhouse et al. 2021) and 3) the local adaptation of corals their environment (Jin et al. 2019). To tease apart these three factors and test their impacts on heat stress, corals from inshore and mid-shelf regions of the GBR were exposed environmentally relevant levels of nitrate and phosphate enrichment in a full-factorial design. The corals were then subject to simulated heat stress and sampling for changes in coral physiological traits (chlorophyll, protein, photosynthesis) alongside the genetic composition of their algal symbiont communities. By accounting for the confounding impacts of environmental history and short-term nutrient stress, it is shown, that local thermal adaptation, rather than nutrient availability, is the main driver of bleaching in the GBR populations of *A. millepora* studied here.

Methods

Coral collection

A. millepora was collected from four reefs in the Burdekin region of the central GBR (Figure 3.1): Falcon Island Reef (18°46'S, 146°32'E; FAL), Havannah Island Reef (18°50'S, 146°32'E; HAV), Hopkinson Reef (18°33'S, 147°12'E; HOP) and John Brewer Reef (18°38'S, 147°03'E; JBR). Coral collections were carried out 5th-9th June 2018 under permit (GBRMPA GB12/35236.1). At each reef 12 partial colonies separated by ≥ 5 m distance (to ensure genetic diversity) were collected from approximately 4 m depth. Corals from Falcon and Havannah Reefs were classified as inshore corals and those from Hopkinson and John Brewer Reefs were mid-shelf corals. Inshore reefs are influenced by riverine outflow and therefore corals from these reefs have a history of exposure to more stressful conditions (relative to their mid-shelf counterparts), including nutrient and sediment pollution as well as elevated and more variable temperature (Jin et al. 2016; Waterhouse et al. 2021). In contrast, mid-shelf reefs are primarily influenced by oceanic water and fleetingly exposed to riverine inputs during major flood events (Lough et al. 2015).

Following collection, the coral colonies were kept under shaded flow-through seawater conditions until arrival at the outdoor aquarium facilities of the National Sea Simulator (SeaSim) at the Australian Institute of Marine Science (AIMS) on 10th June 2018. At AIMS, they remained shaded in flow-through filtered seawater (FSW) at 24.2 °C (the collection temperature). Corals were fed daily with freshly hatched *Artemia* at a concentration of approximately 0.5 nauplii.ml^{-1}. Shortly after arrival, each colony was cut into 16 experimental nubbins of approximately 2 cm height using a diamond band saw (AquaSaw XL, Grphyon, USA). Cut nubbins were affixed to aragonite plugs using cyanoacrylate glue and held in racks of 48 plugs under the same conditions as before.

Three weeks after fragmentation, the corals were transferred to an indoor experimental room containing 16 individual 48 L tanks. Each tank was supplied with FSW at a rate of 48 L.hr^{-1}, contained an internal circulation pump (Turbelle nanostream 6015, TUNZE, Germany), and

was situated within an individual flow-through water jacket for additional temperature control. Corals were initially supplied with 4.32 mol.m^{-2}.d^{-1} of light (peak 150 µmol.m^{-2}.s^{-1}; Sol, Aqua Illumination, USA) which was doubled to 8.64 mol.m^{-2}.d^{-1} (peak 300 µmol.m^{-2}.s^{-1}) over nine days, and at the same time the temperature was increased from 24.2 °C to 26 °C (0.2 °C.d^{-1}). Corals were then kept under these conditions for four weeks prior to the experiment and feeding with *Artemia* was maintained.

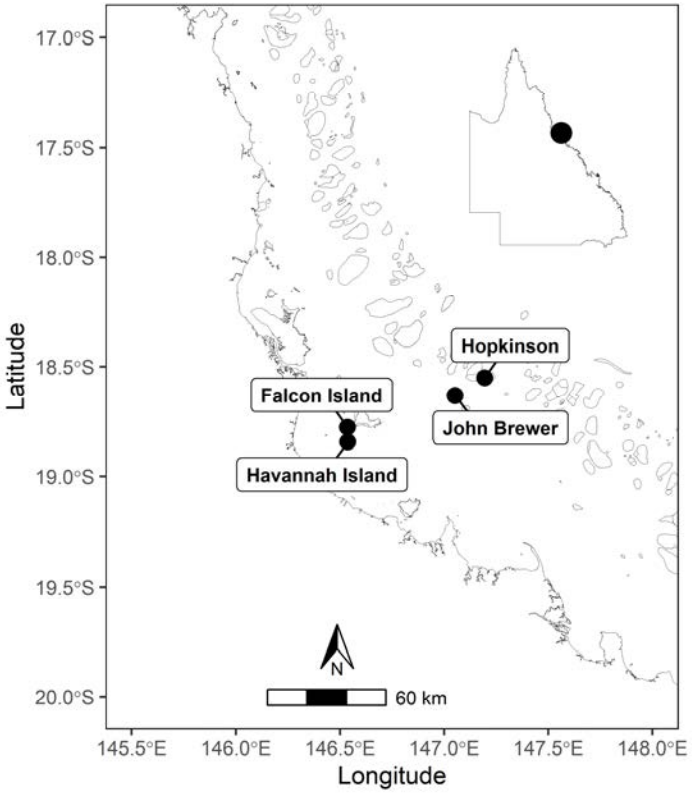

Figure 3.1 Map of the inshore and mid-shelf collection sites for experimental *A. millepora* colonies in the central Greater Barrier Reef. Inset map shows the position relative to the landmass of Queensland, Australia.

Nutrient enrichment

The experiment was initiated (Day 1, 13/08/18) by exposing corals to four nutrient conditions: individual nitrate (N) and phosphate (P) enrichment, combined enrichment (NP) and a control condition (C). Heterotrophic feeding, as already described, was also maintained throughout the experiment. Nutrient levels were nominally increased by 2 µM nitrate and/or 0.25 µM phosphate by continuously dosing concentrated stock solutions (replenished twice weekly)

of sodium nitrate (1 mM; Sigma-Aldrich, USA) and sodium phosphate monobasic (125 µM; Sigma-Aldrich, USA) in FSW using a peristaltic pump (IPC 8, Ismatec, Germany), and each nutrient condition was replicated across four tanks. These levels of nutrient enrichment supplied (Table S3.1) were based on those found in secondary water types of river plumes experienced in the study region (Álvarez-Romero et al. 2013; Waterhouse et al. 2021). Inorganic nutrient availability was monitored up to three times per week by taking 0.45 µM-filtered (Minisart NML, Sartorius, Germany) triplicate water samples from each tank. Inorganic nutrients were determined spectrophotometrically (Ryle et al. 1981) using an auto-analyser (AA3 HR, SEAL Analytical, UK). To minimise biological nutrient uptake by non-coral organisms, tanks were emptied and cleaned thoroughly twice per week. The nutrient dosing and cleaning regime was effective at providing enrichment of the relevant inorganic nutrients in each treatment, despite natural variability in the nutrient content of the inshore FSW supplied to the experiment (Table S3.1; Figure S3.1).

Acute heat stress

After six weeks of nutrient enrichment (Day 44, 25/09/18) the temperature in all experimental tanks was raised by 1 °C.d^{-1} until 30 °C (reached on Day 48, 29/09/18). Thereafter, ramping to the final temperature of 31.5 °C (reached on Day 62, 13/10/18) was achieved by repeated holding of the temperature for five days, followed by repeated increases of 0.5 °C (Figure 3.2). The temperature of each tank was measured weekly (376 Datalogger RTD Thermometer, CENTER Technology, Taiwan) during the nutrient exposure and daily during heat stress. The final heat exposure of six days at 31.5 °C (Day 67, 18/10/18) amounted to mild-to-moderate accumulated heat stress with the degree heating weeks metric (DHW) (Liu et al. 2003). The inshore and mid-shelf corals were exposed up to 2.86 ± 0.30 and 3.86 ± 0.33 DHW respectively (Figure 3.2) using Havannah and John Brewer Reefs as reference sites and taking the maximum monthly mean (MMM) from the NOAA Coral Reef Watch 5 km satellite product version 3.1. This level of heat stress was chosen to induce bleaching without significant mortality (Fordyce et al. 2019).

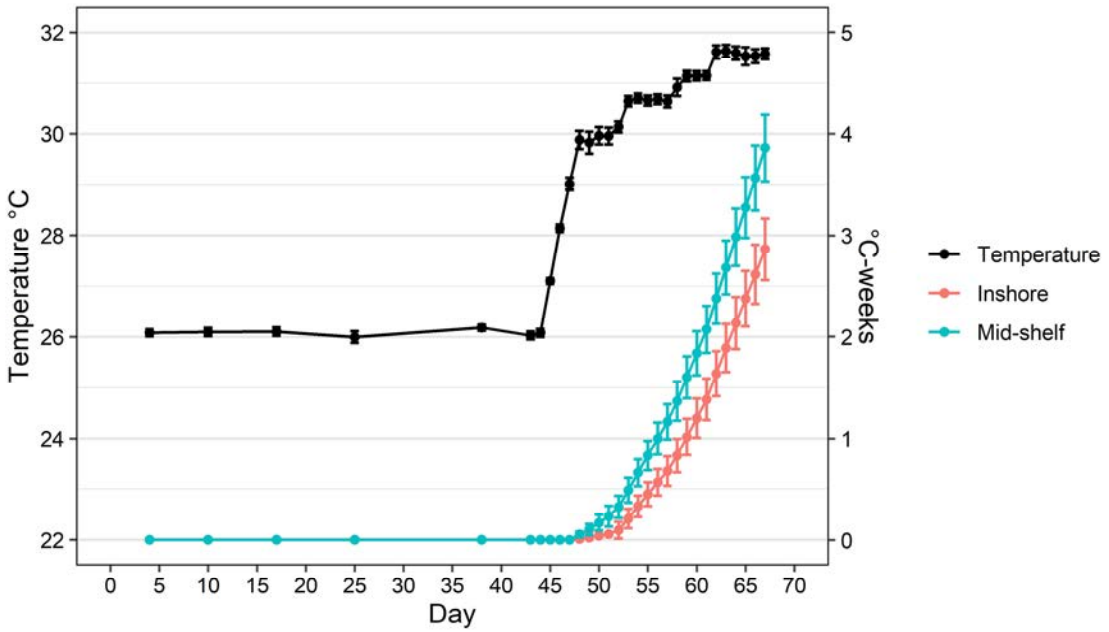

Figure 3.2 Experimental temperature (°C) and accumulated heat stress (°C-weeks) profiles. Inshore heat stress is calculated relative to Havannah Island and mid-shelf heat stress relative to John Brewer Reef. Points and error bars represent the mean ± s.d. for replicate tanks.

Experimental and sampling design

Within nutrient treatments, each tank contained four randomly placed nubbins from 12 coral colonies, with 48 nubbins in total, and six colonies per shelf position, in each tank. This meant that each replicate tank within a nutrient treatment contained a unique group of colonies. These colony groupings were replicated exactly for all nutrient treatments.

Corals were sampled at two timepoints: 1) following one month of nutrient exposure at 26 °C (Days 31-32, 12/09/18-13/09/18) and 2) and after 9.5 weeks of nutrient exposure and four days at the peak temperature of 31.5 °C (Days 66-67, 17/10/18-18/10/18). At these timepoints coral respirometry measurements were taken before samples were fixed in liquid nitrogen and stored at -75 °C for *ITS2* sequencing and analyses of chlorophyll and protein. At each timepoint, four nubbins each from sixteen unique colonies were sampled from each nutrient treatment. However, respirometry measurements and subsequent analyses were only conducted on one nubbin per colony within each treatment, with the remainder kept as spares. Nubbins were sampled evenly from the replicate tanks, meaning that 16 nubbins per

tank, representing four genotypes (two per shelf position) per tank, were removed from at each sampling point. The same genotypes were sampled across the nutrient treatments at each timepoint, however different sets of 16 colonies were sampled between timepoints.

Repeated measures sampling of photosynthetic efficiency was carried out between to the two timepoints described above (Days 39-65, 20/09/18-16/10/18). These measurements were performed on all nubbins remaining in the experiment after the first sampling timepoint. This meant that within each nutrient treatment, four nubbins were measured for each of the 32 unique colonies (eight colonies per tank) that remained. This design was replicated exactly across nutrient treatments, except where individual nubbins died.

The surface area and volume of each coral nubbin were calculated using modifications of a previous method (Nielsen et al. 2020), to respectively enable the standardisation of physiological metrics and volume correction of respirometry measurements. Digital callipers were used to take multiple perpendicular diameter measurements along each nubbin, which were then averaged, in addition a single measurement of nubbin height. The lateral surface area and volume of each nubbin were then calculated assuming that they were cylindrical ($\pi r^2 h$; $2\pi rh$).

Coral respirometry

To measure light photosynthesis and dark respiration, nubbins were transferred to 600 ml transparent acrylic cylinders filled and sealed completely with treatment-specific seawater (TSW). Within each cylinder, one coral nubbin was mounted above a magnetic stir bar which was rotated with a magnetic pulley system (Strahl et al. 2019). Incubations were carried out within an opaque flow-through water bath maintained at experimental temperatures. For light incubations, corals were exposed to the same peak lighting conditions of the experiment (300 $\mu mol.m^{-2}.s^{-1}$) for approximately 90 minutes. Following this, oxygen concentrations were measured using a hand-held dissolved oxygen meter (HQ30D with Intellical LDO101 probe, Hach, USA). For the dark incubations, cylinders were resealed using TSW and the procedure was repeated exactly in the dark. Control incubations without corals were also performed and used to correct coral measurements for background metabolic activity. Gross photosynthesis

rates were estimated by adding net photosynthesis rates and respiration rates for each coral. As a result of light-dependent effects on respiration, gross photosynthesis rates were likely be underestimates (Schrameyer et al. 2014).

Coral tissue preparation, chlorophyll, and protein analysis

Coral tissue preparation and chlorophyll and protein content analysis were carried out as described previously (Chapter 2), but with a reduced volume (~8 ml) of ultra-filtered seawater in the air blasting step.

Photosynthetic efficiency

Pulse-amplitude-modulation fluorometry (MINI-PAM, Walz, Germany) was used as non-invasive measurement of coral bleaching (Warner et al. 1999) during the application of heat stress. Repeated measurements were taken using a 5.5 mm fibre optic (Walz, Germany) spaced ~2 mm from the coral surface with the following settings: measuring light = 4; Gain = 1; Saturating intensity = 8; and Saturating width = 0.8. Effective quantum yield of PSII ($^{\Phi}$PSII) was measured in the middle of the light cycle (starting Days 39-40, 20/09/18-21/09/18) and maximum quantum yield of PSII (F_v/F_m) was measured after one-hour of dark adaptation (starting Day 43, 24/09/18), following the conclusion of the daily photoperiod. After these initial measurements $^{\Phi}$PSII was measure daily (Day 43 onwards) and F_v/F_m was measured every other night (Day 48, 29/09/18 onwards).

DNA extraction and ITS2 amplicon sequencing

Coral DNA extraction was carried out as described previously (Chapter 2) and polymerase chain reactions (PCRs) were performed using modifications to the previous method (Chapter 2) in terms of the PCR recipe (+1 mM $MgCl_2$, 5 µl DNA template and 30 µl final volume) and cycling conditions (initial denaturation extended to 10 min, cycle number reduced to 30, annealing temperature reduced to 56 °C for a longer period of 60 s). ITS2 amplicons were sent to the Ramaciotti Centre for Genomics (UNSW Sydney, Australia) for bead clean-up, library preparation and sequencing on the MiSeq v3 2 x 300 bp platform (Illumina, USA).

Statistical design for physiological data analysis

For analysis of chlorophyll, protein, and respirometry data, analyses were carried out using the methods detailed in Chapter 2. Bayesian generalised linear mixed models (BGLMMs) were fit (using brms) to examine the three-way interaction between nutrients, shelf position, and temperature. After the model checking and selection procedures (Chapter 2), the optimal models were gaussian in distribution with a random intercept in all instances, except for respiration which was fit to a student's distribution. Model results were interpreted as detailed previously (Chapter 2).

Photosynthetic efficiency (F_v/F_m and $^{\Phi}$PSII) data were analysed using piecewise linear mixed models fit in the "glmmTMB" 1.0.21 package (Brooks et al. 2017) for the interaction of nutrients and shelf position over a time series of heat stress. Random effects of tank, coral colony and coral nubbins were also included. Models were manually optimised regarding the position of knots, random effects, and variance structures. Models were diagnosed with DHARMa and compared using second order Akaike Information Criterion via MuMIn package 1.43.17 (Bartoń 2013). For F_v/F_m, a single knot was placed at 62 days, the first slope allowed to vary across tanks, and the second slope allowed to vary across genotypes and nubbins. An Ornstein–Uhlenbeck (OU) variance structure was also fitted to control autocorrelation of colonies and nubbins over time. For $^{\Phi}$PSII, knots were placed at 51 and 62 days with the third slope allowed to vary across all random factors, and the second slope allowed to vary across colonies and nubbins, and OU variance modelled for all random effects over time. Post-hoc comparisons on the final selected models were made using emmeans and statistical inferences were based on estimated means and p-values.

ITS2 amplicon sequence analysis

Fastq files were submitted to SymPortal (Hume et al. 2019) for bioinformatics analysis as detailed in Chapter 2. The 128 analysed samples returned 7,937,097 raw sequencing reads or an average (mean ± s.d.) of 62,009 ± 11,688 reads per sample, and 3,715,883 reads or 29,030 ± 8,193 reads per sample remained after minimum entropy decomposition (MED).

For the analysis of DIVs and *ITS2*-type profiles, DIVs present in only one sample or comprising < 0.005 % of total post-MED reads were manually removed, and *ITS2*-type profile data were normalised to relative abundance. Further analyses were based in the vegan package as detailed in Chapter 2, with rarefaction to 12,249 reads, and alpha diversity (Shannon's *H'* and Simpson's 1-*D*) was again analysed using BGLMMs as described above for the physiological data. *H'* and 1-*D* were fitted to gaussian and student's distributions respectively, with a random intercepts structure. PERMANOVAs were carried out to examine the three-way interaction between nutrients, shelf position and temperature, but due to the balanced experimental design, PERMANOVA was robust to the impacts of unequal variances (Anderson and Walsh 2013) and therefore PERMDISP testing was not carried out.

Results

Physiological response of coral-algal symbiosis to nutrient and heat stress

The dark- (F_v/F_m) and light- ($^{\Phi}$PSII) adapted photosynthetic efficiencies of *A. millepora* were tracked throughout heat stress until corals inshore and mid-shelf were exposed to 2.38 ± 0.27 (mean ± s.d.) and 3.28 ± 0.30 °C-weeks, respectively (Day 65). Piece-wise linear mixed models (F_v/F_m R^2 = 0.9998; $^{\Phi}$PSII R^2 = 0.9641) showed that photoinhibition of corals under heat stress was strongly related to shelf-position and seldom impacted by short-term nutrient exposure (Figure 3.3). Therefore, unless stated, the following results represent cross-shelf pairwise comparisons with collapsed nutrient treatments.

Initially, photosynthetic yields were slightly but significantly higher for mid-shelf corals compared to their inshore counterparts ($p < 0.05$ for F_v/F_m and $^{\Phi}$PSII). This changed on days 62 and 63 respectively for F_v/F_m and $^{\Phi}$PSII, when modelled estimates showed that yields were equivalent between inshore and mid-shelf corals (F_v/F_m p = 0.1466; $^{\Phi}$PSII p = 0.8460). After day 62, the rate of photoinhibition rapidly accelerated for mid-shelf corals ($p < 0.0001$ for change in F_v/F_m and $^{\Phi}$PSII slopes) but remained unchanged for the inshore corals (F_v/F_m p = 0.0900; $^{\Phi}$PSII p = 0.2186). Notably, the increased rate of mid-shelf coral F_v/F_m decline was

partially mitigated by phosphate enrichment (vs control $p = 0.0019$; vs N $p = 0.0081$; vs NP $p < 0.0001$). Beyond day 64, photosynthetic yields for mid-shelf corals were significantly lower than their inshore counterparts ($p < 0.05$ F_v/F_m and $^{\Phi}$PSII). Ultimately, on day 65, modelled estimates of photosynthetic yields for mid-shelf corals had declined to 0.523 (95 % CIs 0.488-0.558) F_v/F_m and 0.549 (0.518-0.580) $^{\Phi}$PSII, representing declines of 0.219 F_v/F_m and 0.162 below peak levels. Inshore corals were only half as much affected (-0.080 F_v/F_m and -0.087 $^{\Phi}$PSII), reaching minimum photosynthetic efficiency levels of 0.628 (0.593-0.662) F_v/F_m and 0.600 (0.569-0.631) $^{\Phi}$PSII.

Modelled estimates of total chlorophyll content ($R^2 = 0.8916$, 95 % CIs 0.8612-0.9125), gross ($R^2 = 0.4534$, 0.3156-0.5638) and net ($R^2 = 0.5171$, 0.3819-0.6147) photosynthesis of inshore and mid-shelf corals (Figure 3.4), respectively exposed to up a maximum of 2.86 ± 0.30 and 3.86 ± 0.33 °C-weeks (Days 66-67), closely followed the prior observations of photosynthetic efficiency whereby mid-shelf corals were most severely impacted by heat stress. Prior to heat stress (Days 31-32), these physiological metrics were largely unimpacted by nutrient treatment or shelf-position, with estimated values of 18.48 $\mu g.cm^{-2}$ (95 % CIs 17.07-19.77) for chlorophyll (Figure S3.2), 3.21 $\mu mol\ O_2.h^{-1}.cm^{-2}$ (2.88-3.56) for gross photosynthesis (Figure S3.3) and 2.65 $\mu mol\ O_2.h^{-1}.cm^{-2}$ (2.36-2.94) for net photosynthesis (Figure S3.4). However, within the mid-shelf corals, chlorophyll content tended to be slightly elevated in the nutrient-enriched treatments (N: 19.2 $\mu g.cm^{-2}$, 16.7-21.7; P: 19.6 $\mu g.cm^{-2}$, 17.1-22.2; NP: 20.0 $\mu g.cm^{-2}$, 17.4-22.5) compared to the control condition (16.6 $\mu g.cm^{-2}$, 14.3-19.2).

Following heat stress, total chlorophyll levels decreased for all coral regardless of experimental treatment, falling by 56 % to 7.97 $\mu g.cm^{-2}$ (6.12-9.70) for the inshore corals and by 76 % to 4.46 $\mu g.cm^{-2}$ (2.58-6.21) for the mid-shelf corals. However, within the control nutrient treatment, chlorophyll levels remained statistically indifferent between the inshore (6.66 $\mu g.cm^{-2}$, 4.20-9.04) and mid-shelf corals (4.46 $\mu g.cm^{-2}$, 2.18-6.97) after heat stress. In contrast, mid-shelf corals in all nutrient-enriched treatments showed reduced levels of chlorophyll compared to their inshore counterparts (Figure S3.2). Nutrient additions, regardless of their form or ratio, therefore led to cross-shelf differences in the coral bleaching response as measured through chlorophyll.

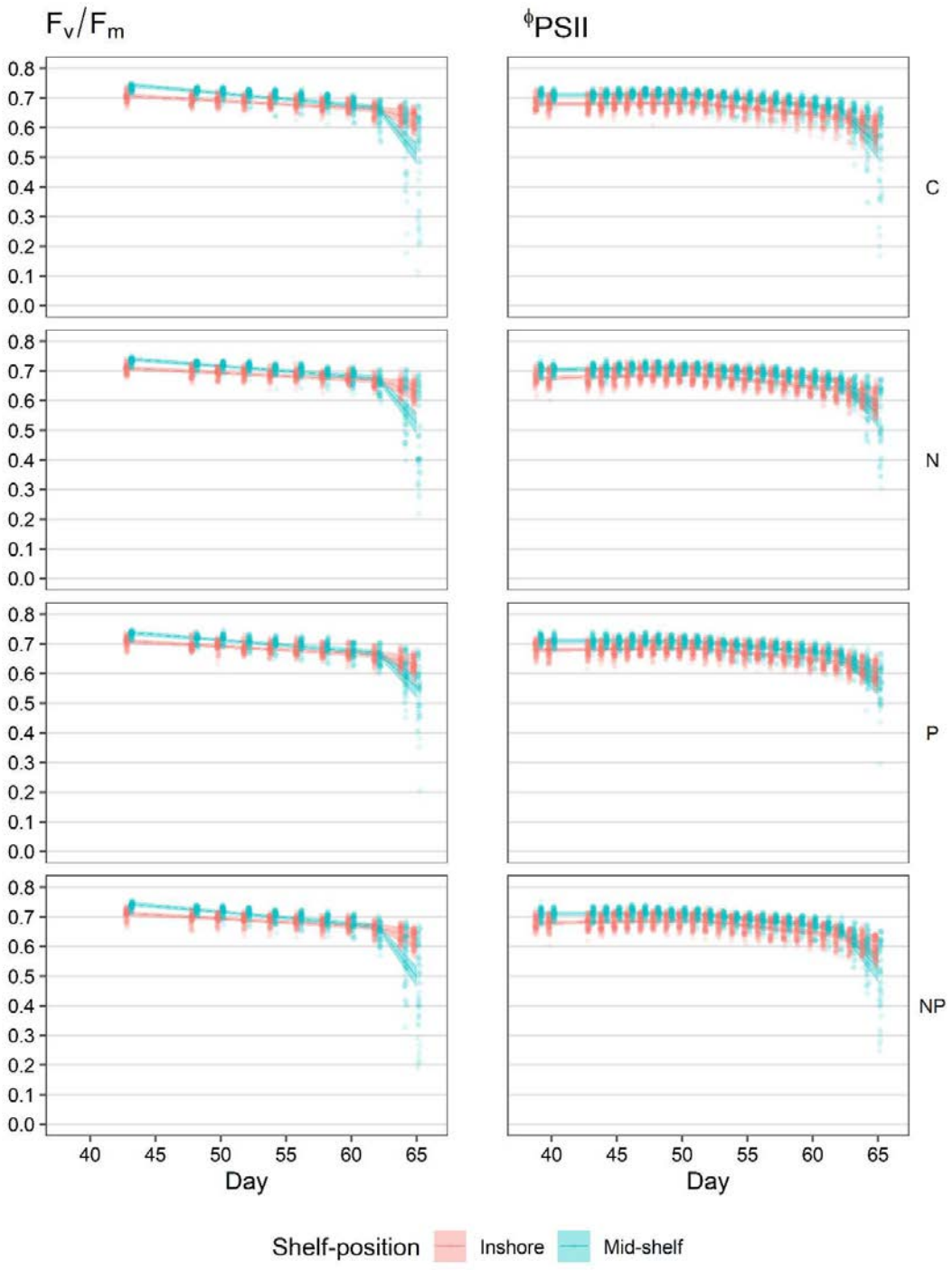

Figure 3.3 Dark- (F_v/F_m) and light- ($^{\Phi}$PSII) adapted photosynthetic efficiency according to nutrient exposure (top to bottom), shelf-position and increasing exposure to heat stress over time. Trendlines represent piecewise model estimates surrounded by 95 % confidence intervals and raw data points coloured according to shelf-position.

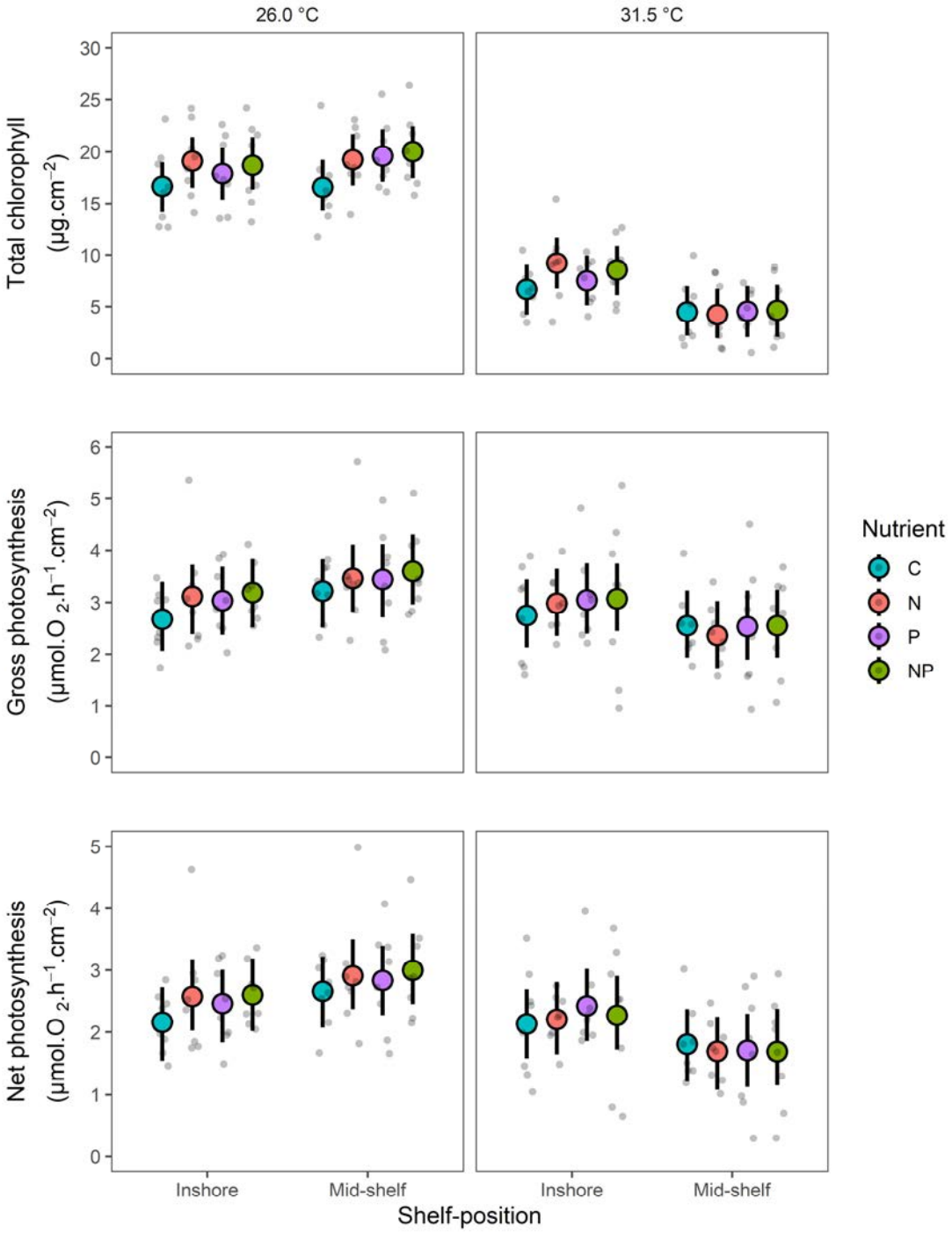

Figure 3.4 Total chlorophyll concentration (µg.cm^{-2}), gross and net photosynthesis (µmol O_2.h^{-1}.cm^{-2}) of inshore and mid-shelf corals, according to temperature (left vs right) and nutrient treatment (colour). Coloured points and error bars show median model simulations with 95% CIs. Grey dots show the raw data points.

Cross-shelf differences in the coral heat stress response were also found in their photosynthetic rates. Although these remained unchanged for the inshore corals (Figure S3.4), net photosynthetic rates of mid-shelf corals declined by 40 % following heat stress, to 1.72 µmol $O_2.h^{-1}.cm^{-2}$ (1.33-2.13). Gross photosynthesis of mid-shelf corals also declined across temperatures (Figure S3.3), but only in the nutrient-enriched treatments (by 32, 26 and 29 % respectively for N, P and NP-treated corals).

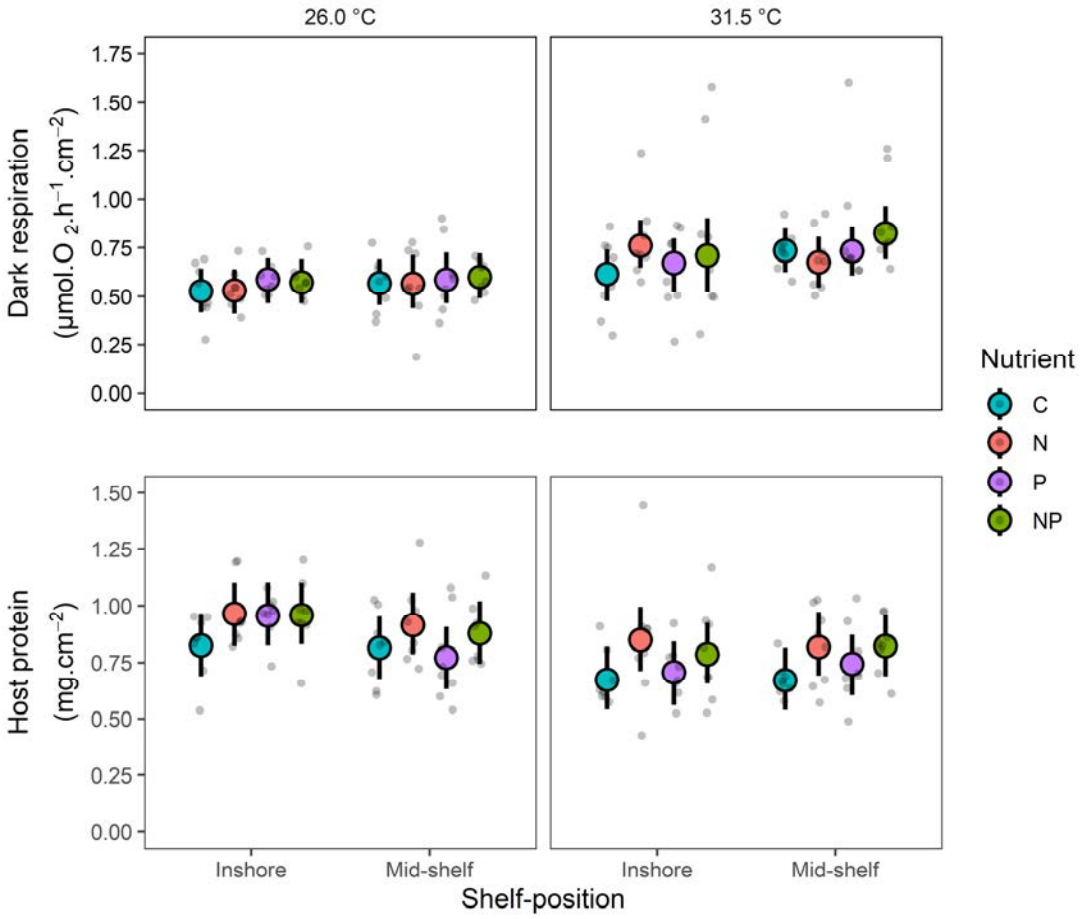

Figure 3.5 Dark respiration (µmol.h^{-1}.cm^{-2}) and host protein content (mg.cm^{-2}) of inshore and mid-shelf corals, according to temperature (left vs right) and nutrient treatment (colour). Coloured points and error bars show median model simulations with 95% CIs. Grey dots show the raw data points.

Modelled patterns (Figure 3.5) of experimental impacts on coral dark respiration (R^2 = 0.3037, 0.1673-0.4645) and host protein content (R^2 = 0.5518, 0.4237-0.6419) were less clear than

the symbiont-related metrics of bleaching. Prior to heat stress, corals had equivalent respiration rates across the experiment (0.57 µmol.h^{-1}.cm^{-2}, 0.51-0.62). After heat stress, respiration rates increased by 43 % for the N-treated inshore corals and by 29 and 37 % respectively for the control and NP-treated mid-shelf corals (Figure S3.5). Regarding host protein content, levels in the inshore P-enriched corals (0.96 mg.cm^{-2}, 0.82-1.10) were higher than their mid-shelf counterparts (0.77 mg.cm^{-2}, 0.63-0.91) prior to heat stress (Figure S3.6). Within the inshore corals, protein declined due to heat stress in all but the N-enriched treatment (by 18, 26 and 18 % respectively for C, P and NP corals), and, after heat stress, inshore N-enriched corals retained higher protein (0.85 mg.cm^{-2}, 0.71-0.99) compared to the control (0.67 mg.cm^{-2}, 0.54-0.82).

Algal symbiont communities

After trimming and rarefaction, 72 *Cladocopium* DIVs made up 99.9 % of the total with the remainder shared across six *Durusdinium* DIVs. Model estimates (Figure S3.7) of Shannon's (*H'*; R^2 = 0.7674, 0.7078-0.8103) and Simpson's (1-*D*; R^2 = 0.6780, 0.5952-0.7376) diversity indices of algal symbiont DIVs were higher for the mid-shelf corals (*H'* 2.15, 2.03-2.29; 1-*D* 0.804, 0.779-0.829) compared to the inshore corals (*H'* 1.79, 1.67-1.92; 1-*D* 0.755, 0.730-0.7779) prior to heat stress, but were equal after heat stress, except for *H'* in the NP treatment (Figure S3.8; Figure S3.9). PERMANOVA revealed significant differences in the community structure (Figure S3.10) when accounting for changes in relative symbiont abundance and composition in terms of algal symbiont DIVs due to the impacts of temperature ($p = 0.0158$, R^2 = 0.0197), shelf position ($p < 0.0001$, R^2 = 0.5614) and their interaction ($p = 0.0153$, R^2 = 0.0193). Nutrient treatment did not influence the community structure of DIVs on its own ($p = 0.9837$, R^2 = 0.0013), or by interacting with shelf position and/or temperature.

Ten *ITS2*-type profiles were identified across all samples (Figure 3.6). These were significantly impacted by shelf position (PERMANOVA $p < 0.0001$, R^2 = 0.2639), rather than nutrients of temperature. The nine most abundant type profiles were *Cladocopium* with the remaining profile being *Durusdinium*. Three dominant *ITS2*-type profiles made up 95.0 % of normalised profiles: C3k/C50a-C3-C3ba-C50f-C50q-C3dq-C3a (33.5 %), C50c/C50a/C3-C50f-C3b-C50 (32.8 %) and C50c/C50a/C3/C1-C50f-C3b-C50u (28.80 %). For the inshore corals, 54.7 % of

normalised profiles were C50c/C50a/C3-C50f-C3b-C50u and 43.8 % were C50c/C50a/C3/C1-C50f-C3b-C50u. In contrast, C3k/C50a-C3-C3ba-C50f-C50q-C3dq-C3a comprised of 66.9 % of normalised profiles for the mid-shelf corals, followed by 13.9 % C50c/C50a/C3/C1-C50f-C3b-C50u and 10.8 % C50c/C50a/C3-C50f-C3b-C50u. The remaining 8.4 % were made up of six other profiles. Overall, 75.3 % of mid-shelf normalised profiles were unique to the mid-shelf corals.

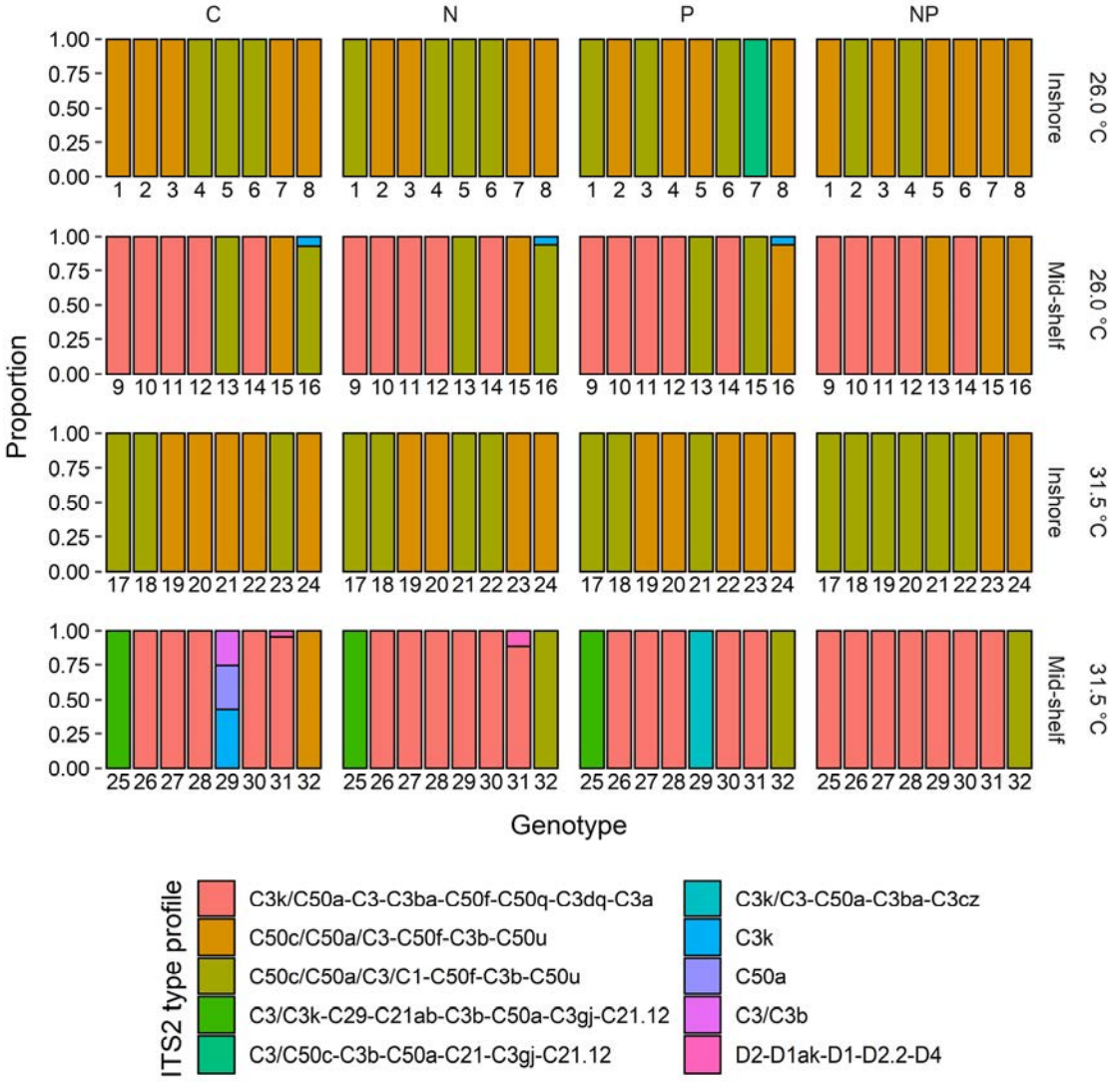

Figure 3.6 Proportion normalised ITS2-type profiles for individual samples within each experimental treatment. Genotype shows the unique coral colonies used in the experiment.

Discussion

My study of the coral *A. millepora* sourced from inshore and mid-shelf reefs revealed that there were minimal impacts of nutrient dosing or shelf position on the measured physiological traits prior to heat stress. However, corals in all nutrient treatments bleached after exposure to heat stress up to 31.5 °C. The stress response was most severe for the mid-shelf corals likely due to increased levels of accumulated heat stress (DHW) they experienced at this temperature. Inshore and mid-shelf corals also hosted distinct algal symbiont communities which may partially explain these differences in absolute temperature tolerance. In contrast, short-term nutrient enrichment had comparatively minor impacts on coral heat tolerance. It is therefore unlikely that local actions to manage nutrient pollution will provide tangible benefits in mitigating the initial intensity of coral bleaching during future marine heatwaves on the GBR.

Coral heat stress response driven by cross-shelf environmental history

The primary finding was that the response of corals to heat stress was strongly determined by cross-shelf differences in environmental history. Prior to heat stress, all photo-physiological metrics were equal between inshore and mid-shelf corals, excluding minor differences in photosynthetic efficiency. After heat stress, mid-shelf corals bleached more strongly as measured through total relative declines in chlorophyll content compared to their inshore conspecifics. This trend was closely followed the observations of greater declines in photosynthetic efficiency and photosynthetic rates for the mid-shelf corals after heat stress, while inshore corals were comparatively unaffected.

The cross-shelf patterns of bleaching and photoinhibition can be explained by the differences in environmental conditions between source reef sites. Relevant conditions include average temperatures and temperature variability (Safaie et al. 2018) and water quality impacts including nutrients and sediments (D'Angelo and Wiedenmann 2014; Fabricius et al. 2013). Inshore corals from the Palm Islands region of the GBR have a ~0.35 °C greater upper thermal limit (MMM) compared to the mid-shelf collection sites, greater annual temperature

variability (Jin et al. 2016), and more frequent exposure to nutrient and sediment loading (Waterhouse et al. 2021). As temperature ramping profiles were applied equally to the same maximum value (31.5°C), for the same duration, mid-shelf corals experienced ~1 °C-week greater accumulated heat stress by the end of the experiment, compared to their inshore counterparts. These differences in historical heat stress exposure were likely responsible for the differential bleaching responses observed here and agree with field observations of previous heat stress events, where °C-weeks were positively related to the probability of severe coral bleaching in the central GBR (Hughes et al. 2021).

Cross-shelf gradients in coral heat tolerance could also be partially explained by phenotypic differences prior to bleaching. Poor water quality conditions, including high nutrients and turbidity, are typically associated with algal symbiont growth within corals (Fabricius 2006; Shantz and Burkepile 2014). Dense algal symbiont communities are hypothetically linked to an increased production of ROS (Cunning and Baker 2013) and reductions to the flow of autotrophic carbon from the symbionts to the host (Ezzat et al. 2016b; Wooldridge 2020). Both factors are implicated in the cellular mechanisms of coral bleaching (Morris et al. 2019; Rädecker et al. 2021). However, there were no cross-shelf differences in relevant physiological traits prior to heat stress: chlorophyll content (a proxy for algal symbiont density in *A. millepora* as shown in Fuller et al. (2020)) and rates of oxygen metabolism (related to carbon fixation and translocation in branching coral by Ezzat et al. (2016b)) were equivalent between the inshore and mid-shelf corals. Therefore, phenotypic differences resulting from historical water quality exposure seem unlikely to explain to strong cross-shelf differences in experimental bleaching.

Limited impacts of nutrient enrichment on coral physiology and heat tolerance

The nutrient enrichment treatments were designed to be reflective of the secondary water type which occurs during the wet season in the inshore areas of the GBR (Devlin et al. 2012). The duration of the experiment (9.5 weeks) matched well with the ~9-week exposure of the inshore collection sites to secondary waters during the summer preceding the experiment (Gruber et al. 2019). In these waters, mean nitrate levels of 3.3 µM were higher than the experimental treatment (~2.4 µM), although the experimental phosphate enrichments (~0.32

µM) were double those of secondary waters (0.15 µM) (Gruber et al. 2019). The experimental treatments were also similar with previous work in terms of nutrient exposure (Table S1) and led to strong variation in nitrate to phosphate ratios (from 2.3 to 21.8) which may be expected to influence coral physiology before and during heat stress (Morris et al. 2019). Despite this, the nutrient effects were subtle: a slight (15-20 %) increase in chlorophyll in mid-shelf corals prior to heat stress, alongside increases in the cross-shelf differences of bleaching (mid-shelf 33 % lower than inshore in control, 41-54 % nutrient-enriched) and reductions to mid-shelf gross photosynthesis (21 % control, 26-32 % nutrient-enriched) after heat stress. Regardless, the final levels of bleaching and photo-physiological parameters were consistent within the inshore and mid-shelf corals across all nutrient conditions.

Previous nutrient and heat stress experiments have demonstrated strong impacts of nutrient form and ratio in dictating the coral bleaching response (Morris et al. 2019). Among these, nitrate enrichment has been mechanistically identified to reduce coral heat tolerance through mechanisms including photo-oxidative damage (Fernandes de Barros Marangoni et al. 2020; Higuchi et al. 2015; Wiedenmann et al. 2013) and the impairment of carbon exchange within the symbiosis (Baker et al. 2018; Ezzat et al. 2015). The equivalent impacts of phosphate enrichment are comparatively unknown but can contrast with nitrate by promoting coral autotrophic function during heat stress (Ezzat et al. 2016a). When the two nutrient sources are applied together, they do not usually act synergistically with heat stress, but can instead uniformly increase the algal symbiont density across temperatures (Dobson et al. 2021; Ezzat et al. 2016b; Hall et al. 2018; Tanaka et al. 2014). Despite major differences in the nitrate to phosphate ratios of the experimental treatments applied here, slight and uniform increases in chlorophyll were observed across all nutrient-enriched (N, P, NP) treatments, matching the typical response of corals to balanced nutrient enrichment (Morris et al. 2019). Although this increase in pigmentation could be expected to negatively impact heat tolerance (Fuller et al. 2020) as discussed earlier, increased bleaching under nutrient enrichment did not occur in the current experiment.

The relative lack of nutrient impacts on coral heat tolerance observed here may have been due to differences in the experimental design compared to previous studies (Table S1). Similar null results have been reported prior, likely due to factors including short exposure duration

(Faxneld et al. 2010), confounding impacts of biological nutrient uptake (Fabricius et al. 2013), pulsing of nutrients (Dobson et al. 2021; Hoadley et al. 2016) and the use of sub-bleaching temperatures (Rice et al. 2019). None of these factors were relevant to the current study, which was long-term in comparison to most previous experiments (Table S1) and effective in providing continuous nutrient enrichment alongside a coral bleaching response. Many other previous experiments which linked nutrient enrichment with coral bleaching often provided treatments which were not realistic in their duration and/or magnitude (Chumun et al. 2013; Higuchi et al. 2015; Nordemar et al. 2003; Schlöder and D'Croz 2004; Thummasan et al. 2021; Wiedenmann et al. 2013) when considering the environmental context of the GBR (Waterhouse et al. 2021) which provided the foundation for this experiment.

It is notable that the highest N:P ratio applied here (~22) is identical to that of inshore, secondary GBR waters preceding the experiment (Gruber et al. 2019) and has been previously associated with healthy coral-algal symbioses (Rosset et al. 2017; Wiedenmann et al. 2013). In the latter case, high absolute phosphate availability of ~0.3 µM was suggested to buffer corals against elevated nitrate levels of ~6.5 µM (Rosset et al. 2017). The current experiment expands on these previous observations to show that that 0.11 µM phosphate is sufficient to avoid stress in GBR corals at similarly elevated N:P ratios.

Biotic and abiotic factors which were fixed across this experiment may have also dictated the coral response to nutrient and heat stress. Most studies which produced significant results using nutrient treatments similar to the current experiment (Blanckaert et al. 2021; Ezzat et al. 2016a; Ezzat et al. 2016b; Fernandes de Barros Marangoni et al. 2020; Hall et al. 2018; Tanaka et al. 2014) were carried out on corals sourced from the Red Sea (Table S1), which have been identified as particularly susceptible to nutrient enrichment due to their naivety to eutrophic conditions (DeCarlo et al. 2020; Fine et al. 2019; Hall et al. 2018). In contrast, the GBR corals used for this study are frequently exposed to inorganic nutrients during summer either through inshore river runoff or mid-shelf upwelling (Waterhouse et al. 2021) and may have adapted to cope with these conditions (Morris et al. 2019). Furthermore, branching corals tend to cease nitrate assimilation and accelerate phosphate uptake under elevated temperatures (Ezzat et al. 2016a; Godinot et al. 2011), in an apparent effort to combat high internal N:P ratios which exacerbate bleaching (Pogoreutz et al. 2017a; Wiedenmann et al.

2013). Potentially, branching *A. millepora* in this study also modified its nutrient uptake under heat stress, which would explain why strong effects of nutrient enrichment were not observed in the bleaching response of this species. Finally, fixed experimental conditions, such as levels of ultraviolet radiation which were not measured here (Blanckaert et al. 2021) and the provision of coral were particulate food (Ezzat et al. 2016b) may have also acted to dampen the response of corals to inorganic nutrients. Therefore, further studies of nutrient impacts on GBR coral bleaching are warranted to detect potential interacting impacts of factors such as coral species, light and food availability.

Cross-shelf genetic drivers of heat tolerance overwhelm short-term nutrient impacts

The cross-shelf patterns in coral heat tolerance may have been related to genetic composition of the algal symbiont communities which they host. Bleaching resistance is known to be closely linked to the algal symbiont genera hosted by *A. millepora* (Berkelmans and van Oppen 2006; Fuller et al. 2020). However, *Durusdinium* spp., which is typically associated with coral heat tolerance (Berkelmans and van Oppen 2006; Fuller et al. 2020), was almost absent from corals in this experiment. Instead, all corals hosted *Cladocopium* spp. which are known to be highly diverse (LaJeunesse et al. 2018) and importantly, highly variable in their heat tolerance (Beltrán et al. 2021; Swain et al. 2017). Heat sensitive mid-shelf corals primarily hosted *ITS2* type profiles containing C3k or C3 as the dominant DIV. In contrast, the inshore corals were dominated by two similar type profiles sharing the C50c DIV. The C50c-led type profiles putatively conferred heat tolerance to inshore *A. millepora* in an adaptive process, since cross-shelf gradients in temperature and water quality are known to shape the distribution of in the algal symbionts of *A. millepora* on the GBR (Cooper et al. 2011; Quigley et al. 2017a). Overall, cross-shelf environmental gradients have likely driven the differences in the *Cladocopium* communities observed within the corals collected for this experiment and putatively explain the differential bleaching responses of inshore and mid-shelf corals.

The algal symbiont communities hosted by corals can also confer different nutritional traits (Baker et al. 2013; Wong et al. 2021) in addition to heat tolerance. This includes differences in nitrogen affinity (Wong et al. 2021), and therefore changing nutrient levels may be expected to structure the algal symbiont communities through symbiont "shuffling" (Baker

2003; Morris et al. 2019). Very little information is available on the impacts of inorganic nutrients on algal symbiont community dynamics, although a release from inorganic nutrient limitation in corals following bleaching (Levas et al. 2018) may facilitate symbiont shuffling and the rapid recovery of the symbiont populations (Grottoli et al. 2014). My experiment represents the first next-generation sequencing analysis of how the algal symbiont communities of corals in response to inorganic nutrient enrichment. However, very little evidence was found to suggest that symbiont community dynamics were substantially impacted by nutrient availability.

Although not studied here, genetic adaptations of the coral host can contribute to coral heat tolerance in addition to those in the symbiont population. A combination of host and symbiont genetic variation has been identified to explain the bleaching responses of *A. millepora* within same study region (Fuller et al. 2020) and in the Florida Keys, inshore coral hosts exposed to more stressful water quality and temperature conditions have been identified as more heat tolerant than their offshore counterparts (Kenkel et al. 2013a; Kenkel and Matz 2016). On the inshore GBR, foraminifera-microalgal symbioses are naturally resistant to nutrient and heat stress (Prazeres et al. 2017; Prazeres et al. 2016) and genetic markers of heat tolerance in *A. millepora* have been associated with nitrate enrichment and temperature variability (Jin et al. 2019; Jin et al. 2016). Therefore, local adaptation of the coral host, in addition to the variability of host associations with heat tolerant symbionts, is an additional factor which could contribute to variations in heat tolerance and should be a focus for further study.

This experiment demonstrates only limited evidence for nutrient impacts on coral heat tolerance. When heat tolerant corals are removed from their natural environmental conditions, trade-offs in their physiology (Kenkel et al. 2015) and bleaching resistance (D'Angelo et al. 2015; Ochsenkühn et al. 2017) may occur. Mechanisms behind these trade-offs include a reliance on other environmental conditions (e.g. salinity) to produce heat tolerant traits (D'Angelo et al. 2015; Ochsenkühn et al. 2017) and the potential energetic costs involved in mounting plastic responses to novel temperature and water quality environments (Kenkel et al. 2015; Kenkel and Matz 2016). Subtle interactions of nutrients were apparent in this experiment, where nutrient-enriched treatments relevant to inshore reefs (Waterhouse

et al. 2021) increased mid-shelf chlorophyll content before heat stress and magnified cross-shelf differences in bleaching and reductions in mid-shelf gross photosynthesis following heat stress. These trends may be explained by the dependence of mid-shelf corals on oligotrophic conditions for optimal stress tolerance, similar to corals from the Red Sea (DeCarlo et al. 2020; Fine et al. 2019; Hall et al. 2018) and hypothesised for the GBR, where nutrient upwelling events may contribute to coral bleaching (Berkelmans et al. 2010; DeCarlo and Harrison 2019). Conversely, inshore corals may perform best when exposed to a baseline level of nutrient enrichment (Jin et al. 2019; Morris et al. 2019). Regardless, these minor nutrient affects were not sufficient to substantially impact the coral bleaching as key metrics (chlorophyll, photosynthesis) were constant across the nutrient treatments after heat stress, in both inshore and mid-shelf corals

Conclusion

The influence of chronic water quality on the bleaching severity of GBR corals under acute heat stress has long been debated. To address this, corals from inshore and mid-shelf reefs were exposed to multiple nutrient conditions and heat stress in the under laboratory. Results from this showed that coral bleaching primarily responded to cross-shelf differences in historical thermal acclimation (MMM), rather than experimental nutrient enrichment. Inshore corals were therefore more tolerant to elevated temperatures than mid-shelf corals, and these cross-shelf patterns of bleaching may be partially explained by differences in the genetic composition of the algal symbiont communities. While the current study suggests that nutrient management is unlikely to prevent bleaching under heat stress, it does not preclude the potential benefits of managing water quality to promote the recovery of corals (Donovan et al. 2021) and coral reefs (MacNeil et al. 2019) following mass bleaching events. Instead, strong and rapid action to both curb ocean warming, in combination with local management approaches to build reef resilience and enhance recovery, will be required to deliver better outcomes for the GBR under climate change (Knowlton et al. 2021).

Chapter 4: Thermal bleaching leads to protein catabolism in the coral *Acropora millepora*

Abstract

Evidence has demonstrated that coral bleaching predominately occurs through photo-oxidative mechanisms, though recent studies indicate it can also be mediated by nutrient cycling and regional water quality. In particular, the reversal of coral nitrogen cycling from protein anabolism towards catabolism may encourage "selfish" growth of the algal symbionts and trigger their expulsion from the coral host. To investigate whether coral nutrient cycling underpins coral heat tolerance, coral fragments of the coral *Acropora millepora* collected from different thermal and water quality regimes were exposed to heat stress and nutrient enrichment (nitrate and/or phosphate). Physiological metrics of bleaching (chlorophyll, photosynthetic efficiency) were combined with ITS2 amplicon sequencing of the algal symbiont communities alongside short- ($^{13}C/^{15}N$ cycling) and long-term (protein, C/N content) assays to explore the coral-algal nutrient metabolism. Results demonstrate that baseline nutrient cycling in the corals was reversed towards protein catabolism during peak heat stress as identified through suppression of exogenous inorganic $^{13}C/^{15}N$ assimilation and translocation in corals, regardless of their environmental history or nutrient exposure. However, initial bleaching and disruption to $^{13}C/^{15}N$ was most severe for more heat-sensitive mid-shelf corals leading to divergent outcomes once heat stress was removed: mid-shelf corals continued to bleach and lost substantial portions of their nutrient stores (carbon, nitrogen, and host protein contents) leading to mortality while inshore corals remained comparatively stable following the peak of heat stress. These cross-shelf patterns of nutrient cycling and starvation following heat stress reveal that nutritional co-operation underpins the thermal bleaching resilience of a reef-building coral.

Introduction

The highly efficient nutrient exchange between corals and their algal symbionts (LaJeunesse et al. 2018) provides the foundation for thriving coral reef ecosystems in tropical waters

(Muscatine and Porter 1977). However, anthropogenic threats, principally recent ocean warming (Lough et al. 2018), superimposed on local threats including nutrient pollution (D'Angelo and Wiedenmann 2014), result in a rapid and mass decline in coral reefs through coral bleaching (Eddy et al. 2021; Hughes et al. 2018a). Coral bleaching, the loss of a corals' algal symbionts (Hoegh-Guldberg 1999), has long been understood to occur through light- and heat-induced oxidative stress mechanisms (Weis 2008). However, subsequent hypothetical (Morris et al. 2019; Wooldridge 2009a) and empirical research (Baker et al. 2018; Rädecker et al. 2021) now highlights how the destabilisation of coral-algal nutrient cycling could be fundamental in triggering the process of coral bleaching.

Rädecker et al. (2021) recently showed through measurements of nutrient flux and gene expression, that the coral *Stylophora pistillata* catabolises amino acids to meet the increased costs of living at elevated temperatures. A side effect of this is that the ammonium released from amino acids stimulates growth in the algal symbionts, causing them to "selfishly" sequester carbon and positively feedback on the escalating energetic stress of the host (Baker et al. 2018; Rädecker et al. 2021). Heat stress therefore triggers a complete reversal in the regular processes of algal carbon translocation and host amino acid synthesis required for the initiation and maintenance of cnidarian-Symbiodiniaceae symbioses (Cui et al. 2019; Xiang et al. 2020). This dysbiosis in the coral-algal nutrient metabolism is therefore implicated in the subsequent bleaching response (Rädecker et al. 2021) and potentially contributes to the large-scale decline of coral reefs following marine heat waves (Hughes et al. 2018b).

The breakdown of the coral-algal nutrient metabolism under heat stress may be modulated by local factors including environmental nutrients (Morris et al. 2019), which can be altered through anthropogenic activities including fishing (Allgeier et al. 2016) and pollution (D'Angelo and Wiedenmann 2014). Particularly strong perturbations to the nutrient environment through these drivers are most likely to occur on inshore reefs adjacent to human populations which are exposed to flood plumes following major weather events (Cinner et al. 2018; Waterhouse et al. 2021). Natural nutrient sources, such as ammonium and phosphate released by fish populations (Meyer et al. 1983), are crucial in alleviating photo-oxidative stress and the risk of the algal symbionts withholding photosynthates (Béraud et al. 2013; Ezzat et al. 2015; Ezzat et al. 2016a; Fernandes de Barros Marangoni et

al. 2020). In contrast, anthropogenic nitrate enrichment exacerbates oxidative stress in corals under heat stress and actively encourages selfish growth in the algal symbiont communities (Baker et al. 2018; Fernandes de Barros Marangoni et al. 2020; Wiedenmann et al. 2013). Overall, it is emerging that nutrient availability can moderate coral bleaching by acting on the processes of nutrient cycling and oxidative stress as previously hypothesised (Morris et al. 2019; Wooldridge 2009a).

Despite facing increased temperatures, corals may become resilient to heat stress after previous exposure to higher temperatures (Ainsworth et al. 2016; Hughes et al. 2019), such as those which occur on the inshore GBR (Berkelmans and van Oppen 2006; Cantin et al. 2021; Jin et al. 2016). Warm acclimation can increase bleaching tolerance and host protein content in *Acropora* corals (Ainsworth et al. 2016; Bellantuono et al. 2012; Middlebrook et al. 2008; Yu et al. 2020) and may go further to modify the nutrient dynamics of the coral-algal relationship (Morris et al. 2019). For example, Gibbin et al. (2018) showed how algal symbionts in warm acclimated corals had reduced nitrate assimilation but increased rates of carbon translocation under sub-bleaching heat stress. Host protein content was also elevated compared to unacclimated controls (Gibbin et al. 2018). Further evidence for this effect can be observed from a recent transcriptomic meta-analysis (Dixon et al. 2020), which found that low intensity stress caused *Acropora* corals to downregulate genes involved in protein degradation. These results suggest that the maintenance of algal carbon translocation and host protein synthesis is a frontloading response to avoid the nutritional breakdown and bleaching that can occur during heat stress (Morris et al. 2019; Rädecker et al. 2021).

The co-adaptation of hosts and their symbionts may mediate nutrient exchange, and by extension coral bleaching (Morris et al. 2019). It has been demonstrated in the cnidarian model Aiptasia (Matthews et al. 2017; Matthews et al. 2018; Rädecker et al. 2018; Sproles et al. 2020; Starzak et al. 2020; Starzak et al. 2014) and corals themselves (Baker et al. 2013; Cantin et al. 2009; Pernice et al. 2015) that genetically distinct host-symbiont combinations vary in their degree of metabolic co-operation in terms of carbon and nitrogen exchange. For instance, *Durusdinium* symbionts are most common to *A. millepora* on the inshore GBR due to higher temperatures and degraded water quality in these environments (Chapter 2; Cooper et al. (2011)). *Durusdinium* are often touted for their superior bleaching tolerance relative to

Cladocopium (Bay et al. 2016; Berkelmans and van Oppen 2006; Fuller et al. 2020; Jones et al. 2008), yet they can struggle to establish and maintain optimal nutritional relations with their host in the absence of stress (Baker et al. 2013; Cantin et al. 2009; Matthews et al. 2017; Matthews et al. 2018; Pernice et al. 2015; Sproles et al. 2020). The benefits of associating with *Durusdinium* only manifest under heat stress where they increase or maintain C/N fixation and translocation in contrast to the adversely affected *Cladocopium* (Baker et al. 2013). Algal symbiont identity is therefore central to understanding how nutrient availability and metabolism relate to bleaching risk, but few studies have examined this relationship to date (Morris et al. 2019).

To examine how disruption to nutrient cycling may underpin coral bleaching resilience, corals from inshore and mid-shelf reefs, with distinct algal symbiont communities and relative temperature tolerance (Chapter 3), were exposed to heat stress along with nutrient conditions (nitrate and/or phosphate) which are known to alleviate (phosphate) or exacerbate (nitrate) bleaching. Traditional bleaching metrics (chlorophyll, photosynthetic efficiency) and ITS2 amplicon sequencing to characterise diversity in the algal symbiont communities were used alongside records of short-term, stable isotope enrichment ($^{13}C/^{15}N$) and long-term indicators of metabolic tissue storage (proteins, C/N content), during the peak of heat stress, to quantify how nutritional destabilisation relates to coral thermal tolerance. The corals were then returned to ambient temperature and the sampling was repeated to examine post-bleaching changes to their physiological and metabolic condition. The study revealed that the extent to which the coral-algal metabolism is destabilised is strongly determined by historical environmental conditions, rather than exposure to short-term nutrient stress. Signs of a metabolic switch towards protein catabolism were observed early in the bleaching process (through $^{13}C/^{15}N$ flux) for both inshore and mid-shelf corals, however severe loss of nutrient stores (protein, C/N content) occurred primarily for mid-shelf corals and led to the initiation of their mortality.

Methods

Coral collection

Partial colonies of *A. millepora* were collected following the methods outlined in Chapter 3, on the 23rd and 24th June 2019 at the same inshore and mid-shelf reef sites (permit GBRMPA GB12/35236.1). On 25th June 2019, the coral colonies were transferred to outdoor aquarium facilities of the National Sea Simulator at the Australian Institute of Marine Science, where they were kept at the temperature recorded at the time of collection (23.3 °C) and fed daily (Chapter 3) in shaded flow-through filtered seawater (FSW). Corals were fragmented shortly after collection (Chapter 3) and following this FSW temperatures were raised to 24.7 °C over seven days. Fourteen days after fragmentation, corals were transferred to an indoor experimental room containing 12 individual 48 L tanks (outfitted as described in Chapter 3). Corals were supplied initially with 4.32 $mol.m^{-2}.d^{-1}$ of light (peak 150 $\mu mol.m^{-2}.s^{-1}$) at 24.7 °C, which were raised to 8.64 $mol.m^{-2}.d^{-1}$ (peak 300 $\mu mol.m^{-2}.s^{-1}$) and 26 °C over seven days. Feeding was maintained in the experimental room where corals were held under these conditions for a further six weeks.

Nutrient enrichment

The experiment was initiated by exposing corals to nutrient treatments (as per Chapter 3; +2 µM nitrate and/or +0.25 µM phosphate, and a control condition), with each nutrient condition (N, P, NP, C) replicated across three tanks. Nutrient conditions were maintained through repeated measurements and cleaning three times per week (Figure S4.1; Chapter 3).

Acute heat stress

Heat stress was initiated simultaneously with nutrient enrichment (Figure S4.2). All temperature adjustments were made at a rate of 1 $°C.d^{-1}$ unless stated. Initially, the temperature across all tanks was raised from 26 °C to 30 °C (starting Day 0, 25/08/19) and held for five days. Following this, it was decreased to 28 °C (starting Day 8, 02/09/19), held

for eight days, and returned to 30 °C (starting Day 17, 11/09/19). Thereafter, the temperature was increased by 0.5 °C every fifth day until 31.5 °C (reached on Day 34, 28/09/19), held for six days and finally returned to 26.0 °C (starting Day 39, 03/10/19). The experiment ran for an additional four weeks at 26.0 °C (until Day 74, 07/11/19) to assess coral recovery dynamics. Temperatures were continuously monitored in each tank using in-water probes calibrated against a mercury thermometer.

The heat exposure of corals during the experiment caused mild-to-moderate heat stress as measured using the NOAA Coral Reef Watch degree heating week (DHW) methodology (Liu et al. 2003) calculated following Chapter 3. Inshore and mid-shelf corals were exposed to a maximum of 3.08 and 4.52 °C-weeks respectively (Figure S4.2), a level of heat stress designed to induce bleaching without high levels of coral mortality (Fordyce et al. 2019). The temperature profile, which exposed corals to temperatures above their maximum monthly mean (MMM) before allowing them a brief respite prior to the main period of DHW accumulation, to simulate typical warming patterns of heat stress in the central GBR (Ainsworth et al. 2016).

Experimental and sampling design

One nubbin from each of the 48 collected colonies was placed randomly within each tank such that each coral colony was represented thrice per nutrient condition. Discrete sampling of corals occurred at two points: 1) during the initial stages of bleaching after five weeks exposure to nutrient enrichment and three days exposure to 31.5 °C (Day 37, 01/10/19), and 2) one month later, after continued nutrient enrichment and four weeks at 26 °C (Day 72, 05/11/19) to allow examination of longer-term responses following the peak of heat stress. These sampling periods occurred over three consecutive days due to time constraints. At each timepoint $^{13}C/^{15}N$ incubations were carried out before samples were preserved for later analyses of physiology, stable isotopes, and algal symbiont genetics. One nubbin from each colony was sampled once from each nutrient condition at each timepoint. In total, 24 colonies per shelf position and nutrient combination were sampled each time, with the sampling effort evenly distributed across tanks except in cases where coral nubbins suffered mortality.

All coral nubbins were monitored using non-invasive time series measurements of photosynthetic efficiency (see Chapter 3), beginning four days prior to the experiment (Day -3, 22/08/19). Measurements of $^{\Phi}$PSII and F_v/F_m were taken three times per week throughout the experiment, with $^{\Phi}$PSII measured in the middle of the photoperiod and F_v/F_m measurements beginning after 45 minutes of dark adaptation.

Stable isotope incubations

Corals were incubated using respirometry equipment (described in Chapter 3) to measure their autotrophic carbon and nitrogen metabolism. All incubations were carried out using FSW from control tanks (CFSW) enriched with 1.2 mM sodium bicarbonate-^{13}C (ISOTEC, USA) and 3.5 µM sodium nitrate-^{15}N (Sigma-Aldrich, USA). Incubations lasted for approximately 130 minutes at the same temperature as the aquarium tanks and under peak experimental irradiance. Afterwards, corals were placed in unlabelled CFSW for 10 minutes under dim light to dissociate unassimilated ^{13}C/^{15}N, rinsed in Milli-Q water and fixed in liquid nitrogen before storage at -75 °C. Nitrate measurements of incubated CFSW (including controls without corals) confirmed the effective addition and repletion of ^{15}N in each incubation chamber (data not shown).

Coral tissue preparation

Coral tissues were processed according to previous protocol (Chapter 2) but with modifications to improve sample yield and purity for DNA and stable isotope analyses. Briefly, samples were removed from storage and air-blasted into approximately 8 ml Milli-Q water into 15 ml tubes and homogenised for 10 s at 22,500 rpm (SilentCrusher M, Heidolph, Germany). Both the airgun and homogeniser were decontaminated sequentially between samples in 10 % bleach, Milli-Q water, 100 % ethanol and Milli-Q water. The homogenate was centrifuged (150 x g, 5 min, 4 °C) and the supernatant poured into a new 15 ml tubes before repeated centrifugation. This supernatant was aliquoted in triplicate 100 µl into clear 96-well microplates which were aluminium-sealed. The microplates and remaining supernatant were stored at -75 °C for host protein and stable isotope analysis, respectively.

The algal symbiont pellets remaining in the original 15 ml tubes were resuspended in 850 µl Milli-Q water (final volume ~1 ml) and transferred to 1.5 ml tubes. From the suspension, 20 µl was aliquoted in duplicate for DNA extraction and 140 µl aliquoted into new 1.5 ml tubes for chlorophyll analysis. Remaining cells were designated for stable isotope analysis. All 1.5 ml tubes were then centrifuged (1,000 x g, 5 min, 4 °C) and the supernatants discarded before -75 °C storage.

Chlorophyll, host protein and surface area

Measurements of chlorophyll content, host protein content and skeletal surface area followed previous methods (Chapters 2 and 3). For the protein assays, samples with low protein concentrations (below the standard range) were repeated with double the amount of sample material (10 µl) and to compensate for this 5 µl Milli-Q water was added to each standard well.

Stable isotope analysis

Frozen coral host and algal symbiont samples designated for stable isotope analysis were directly lyophilised and exported under permit (CITES PWS2021-AU-000077-000002) to the Stable Isotope Laboratory at the University of Hong Kong. The samples were first weighed into pre-tared tin capsules and then combusted in an elemental analyser (EA3028, EuroVector, Italy) coupled in continuous flow to a stable isotope ratio mass spectrometer (Perspective, Nu Instruments, UK) for measurements of % C/N and $\delta^{13}C/\delta^{15}N$. Samples were normalised to an internal standard (acetanilide #1, Indiana University, USA) with repeated measurements yielding results (mean ± standard deviation: -29.50 ± 0.48 ‰ $\delta^{13}C$, 1.16 ± 0.41 δ15N, 70.80 ± 2.09 % C, 10.36 ± 0.20 % N) that were close to certified reference values (-29.53 ± 0.01 ‰ $\delta^{13}C$, 1.18 ± 0.02 ‰ δ15N) and expressed relative to Vienna Pee Dee Belemnite and atmospheric N_2, respectively. Samples with inadequate mass after processing were either excluded from further analysis or mixed with standard to facilitate measurement.

Physiology and stable isotope data analysis

Statistical analyses for discrete physiological (chlorophyll, protein) and stable isotope (% C, % N, C:N, $\delta^{13}C$, $\delta^{15}N$) data were performed as previously (Chapters 2 and 3), but with variance across treatment factors modelled where appropriate. All Bayesian (generalised) linear mixed models were fit with a fixed effects structure of the interactions between nutrient condition, sampling period and shelf position and a random intercepts structure for tank and coral colony. Further details on model design are given in Table S4.1.

For the continuous time-series' of photosynthetic efficiency ($^{\Phi}$PSII and F_v/F_m), gaussian location scale additive mixed models were fit using the mgcv package 1.8-34 (Wood et al. 2016). Since additive models in mgcv do not allow for linear interactions (e.g. nutrient by shelf-position) by continuous smoothing of the third factor (e.g. day), nutrient and shelf-position were collapsed into a one-way "treatment" factor (e.g. inshore_C, mid-shelf_C, inshore_N, etc). Models were fitted such that they allowed the treatment factor to vary over time, and random factors of tank, coral colony, and replicate coral nubbin were considered. Model fitting was repeated to test for the impacts of different random effects structures and the number of knots for each smoother, until the optimal model was identified using the "k.check" function from mgcv and the "appraise" function from gratia package 0.6.0 (https://cran.r-project.org/package=gratia). The final selected models were checked for autocorrelation using the "acf_plot" function in itsadug package 2.4 (van Rij J et al. 2020).

For both parameters, a model with fixed effect of treatment was fitted with a thin-plate regression spline (tprs) (Wood 2003) for measuring day by treatment, smooth factor interactions (fs) for day by each individual coral nubbin with 3 knots and a variance structure for day by treatment. Random factors of tank and coral colony were excluded as they did little to improve the models and were computationally impractical to include alongside coral nubbin. For $^{\Phi}$PSII, the tprs had 14 knots and the variance smooth 5 knots. For F_v/F_m, the number of knots was 17 and 8, respectively. For the day effect within each treatment, pairwise comparisons were made with emmeans as previously described (Chapter 2). For the treatment effect within each measuring day emmeans was also used, but planned comparisons were made with false discovery rate corrected p-values (Benjamini and Yekutieli

2001) to compare nutrient effects within each shelf-position, and the shelf-position effects within each nutrient treatment.

DNA extraction, ITS2 amplicon sequencing and analysis

DNA was extracted from one 20 µl algal symbiont aliquot per coral nubbin using modifications of an established protocol (Wilson et al. 2002). Glass beads (G1152, Sigma-Aldrich, USA) were added to each symbiont pellet with 730 µl homogenisation buffer plus 0.04 $mg.ml^{-1}$ Proteinase K. The sample solution was then homogenised three times at 4 $m.s^{-1}$ for 30 s (FastPrep-24 5G, MP Biomedicals, USA) and incubated (1 h, 65 °C). Afterwards, 187.5 µl KOAc (final concentration 1 M) was added to each sample which was then vortexed and incubated on ice (30 min). Following this, samples were centrifuged (13,000 x g, 15 min) and 800 µl of the resulting supernatant was transferred to a new 1.5 ml tube with 640 µl isopropanol. Each tube was inverted slowly and rested (15 min, room temperature) before a second centrifugation (20,238 x g, 15 min), after which the supernatant was carefully discarded and replaced with 150 µl of 70 % ethanol. The samples were again inverted slowly and centrifuged (20,238 x g, 5 min). The supernatant was carefully removed, and the resulting DNA pellet left to dry. Finally, 40 µl DNA-grade water was added and the extracted DNA stored at -20 °C for PCR.

PCRs were performed following conditions (Chapter 2) and constituents (Chapter 3) outlined previously but with slight modifications: Reactions were made to 25 µl final volume with 2 µl DNA template and conditions included an annealing temperature of 56 °C and 35 cycles. Half of each reaction was then diluted with an equal volume of PCR-grade water and sent for amplicon sequencing at the Ramaciotti Centre for Genomics (UNSW Sydney, Australia).

ITS2 sequences were submitted to SymPortal (Hume et al. 2019) as detailed previously (Chapter 2). The 374 samples submitted for analysis yielded 16,777,488 raw reads (mean ± s.d. 44,860 ± 15,0477 per sample) and 7,413,036 reads (19,821 ± 9,436 per sample) after minimum entropy composition (MED). Samples with < 1,000 post-MED reads were manually removed along with DIVs comprising < 0.005 % of total post-MED reads, and ITS2-type profiles were normalised to relative abundance. Further manipulations and analyses of alpha and beta

diversity were carried out as described previously (Chapters 2 and 3), with the trimmed DIVs randomly rarefied to 1,479 reads. Shannon's (*H'*) and Simpson's (1-*D*) diversity indices were modelled with BLGMMs described in the previous section. For beta diversity, multiple PERMANOVAs were fit to isolate interactive and individual treatment effects as the experimental design was imbalanced, and PERMDISP and post-hoc testing was carried out on subset data (see Chapter 2).

Results

Physiological response of coral to nutrient and heat stress

Measurements of light- ($^{\Phi}$PSII) and dark- (F_v/F_m) adapted photosynthetic efficiency revealed increased photoinhibition in mid-shelf corals compared to their inshore counterparts during exposure to maximum accumulated heat stress levels (Day 41) of 4.52 and 3.08 °C-weeks, respectively (Figure 4.1). Initially, mid-shelf corals had significantly higher $^{\Phi}$PSII and F_v/F_m relative to the inshore corals ($p < 0.0001$), but this trend switched between 31 to 36 days (corresponding to 1.16-2.19 °C-weeks inshore and 2.08-3.37 °C-weeks mid-shelf), depending on nutrient treatment and metric. Photosynthetic yields of inshore and mid-shelf corals continued to decline until approximately the point at which heat stress ceased to accumulate (Day 40-45 depending on treatment and metric). Following reductions in temperature (Day 47 onwards), $^{\Phi}$PSII recovery of inshore corals was strong and consistently significant between measuring days ($p < 0.05$), but F_v/F_m recovery was relatively limited. Recovery in mid-shelf corals was comparatively limited for either metric. In terms of nutrient effects, inshore corals under control conditions had significantly higher ($p < 0.01$) photosynthetic yields than any nutrient-enriched treatment (N, P, NP), and yields of nitrate-enriched inshore corals were significantly higher ($p < 0.05$) than in either phosphate-enriched treatment (P, NP) through much of the post-heat stress period. Post-heat stress trends were similar for the mid-shelf corals as those in phosphate-enriched treatments (P, NP) suffered significantly larger ($p < 0.05$) declines to $^{\Phi}$PSII compared with those without (C, N). For F_v/F_m, the most notable trend was that mid-shelf phosphate-enriched (P) corals had significantly higher ($p < 0.05$) levels of

photoinhibition for much of the post-bleaching period compared to the other treatments (C, N, NP).

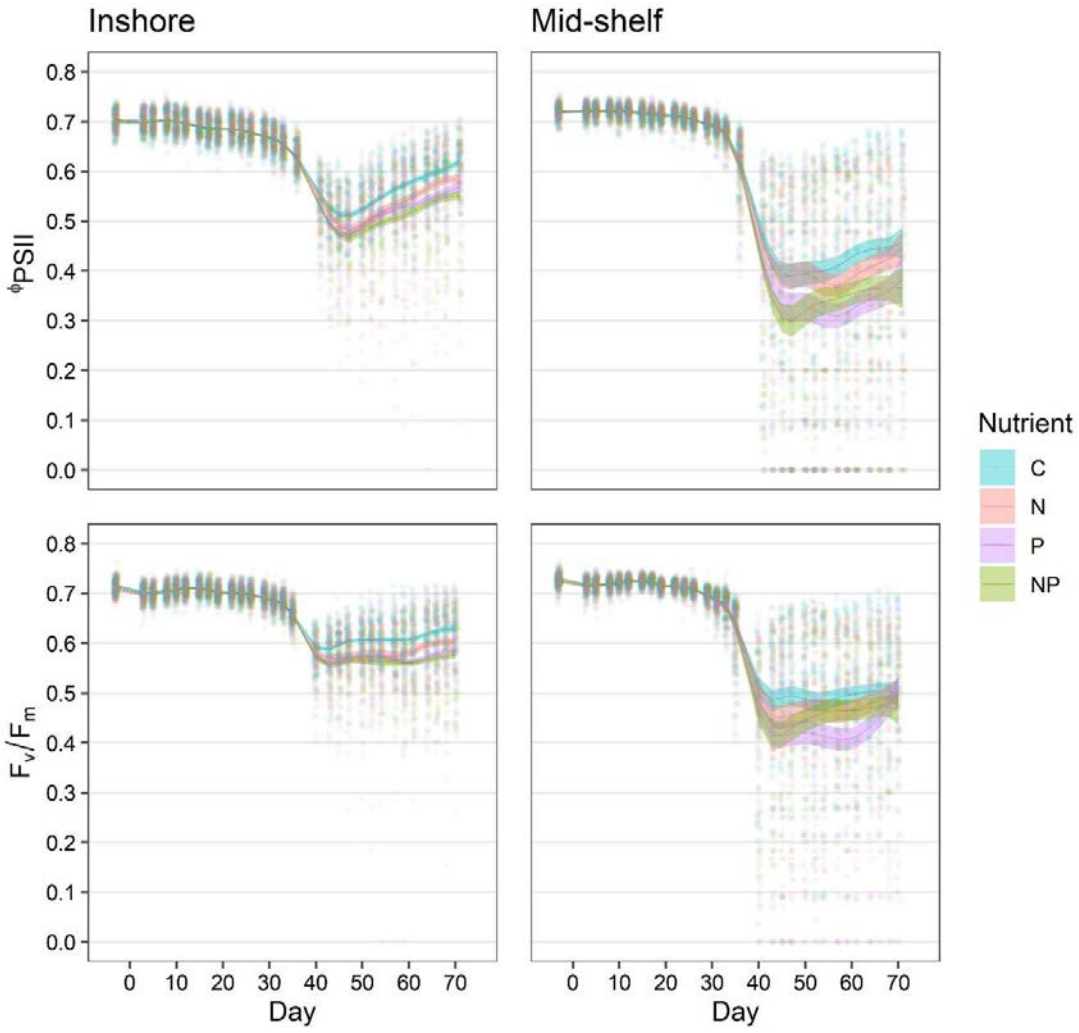

Figure 4.1 Light- ($^{\Phi}$PSII; top row) and dark- (F_v/F_m; bottom row) adapted photosynthetic efficiency according to shelf-position (columns) and nutrient exposure (colours). The gap in measurements just prior to day 40 indicates the first experimental sampling point, just prior to peak accumulated heat stress, and the second sampling point started following the final measurements. Trendlines represent piecewise model estimates surrounded by 95 % confidence intervals and raw data points.

Coral bleaching, as measured through changes in chlorophyll content, was consistently most severe in mid-shelf corals relative to inshore corals (Figure 4.2). Bleaching occurred equally

within the inshore and mid-shelf corals across the nutrient treatments. The BGLMM-estimated chlorophyll of inshore corals (6.34 µg.cm^{-2}, 95 % CIs 4.45-8.46) was more than double that of mid-shelf corals (2.59 µg.cm^{-2}, 1.87-3.50) during the peak of heat stress (Days 38-40), and sixfold higher after heat stress (Days 72-74; inshore 4.86 µg.cm^{-2}; 3.23-6.50; mid-shelf 0.81 µg.cm^{-2}, 0.54-1.11). It was evident that all corals tended to lose pigmentation after the peak of heat stress (Figure S4.3), except for the inshore control corals, however, the chlorophyll content of inshore and mid-shelf corals was largely unaffected by nutrients within experimental timepoint (Figure S4.3).

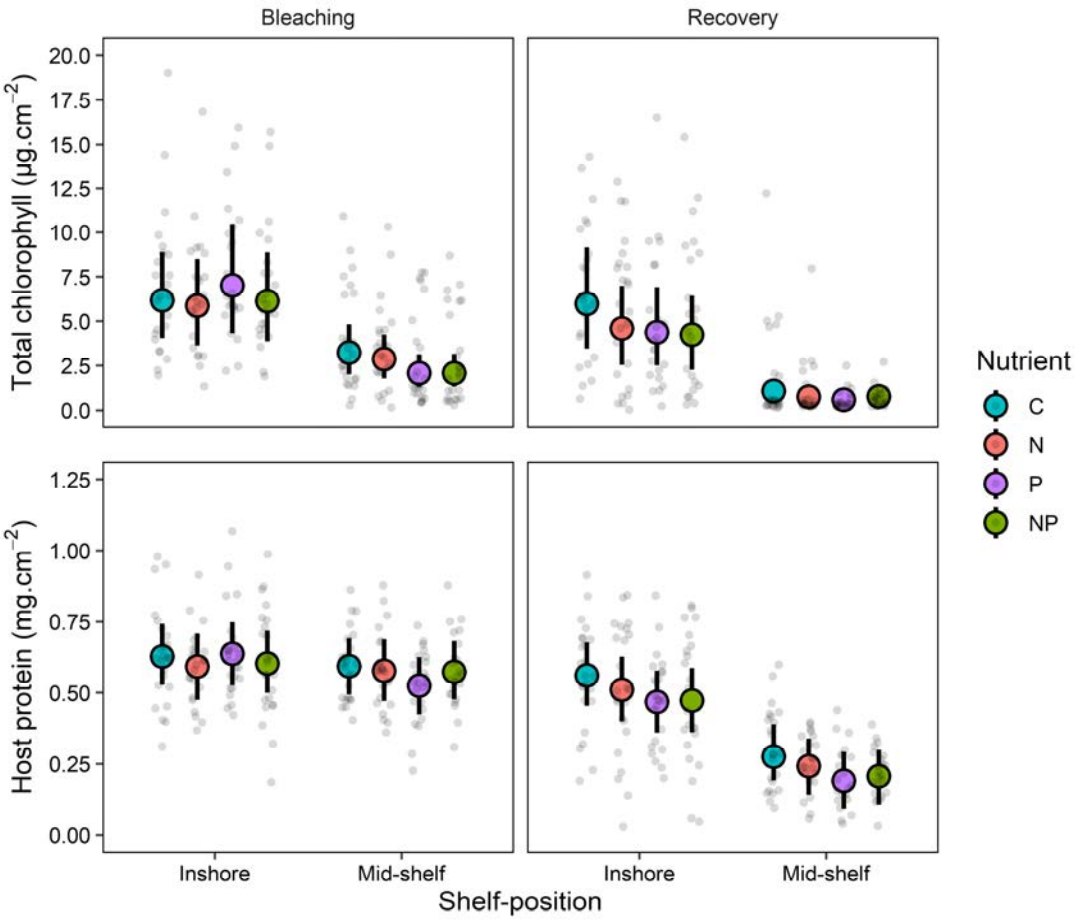

Figure 4.2 Total chlorophyll concentration (µg.cm^{-2}) and host protein content (mg.cm^{-2}) of inshore and mid-shelf corals, according to sampling period (left vs right) and nutrient treatment (colour). Coloured points and error bars show median model simulations with 95% CIs. Grey dots show the raw data points.

Concurrent with continued bleaching after heat stress, mid-shelf coral hosts lost 59.6 % of their protein over time regardless of nutrient condition (Figure 4.2). To a lesser extent, inshore corals also lost protein (-18.3 %), although most of this loss was attributed to the phosphate-enriched treatments (P: -26.6 %; NP: -21.6 %) as opposed to statistically marginal losses in the other treatments (C: -10.6 %; N: -13.8 %). These trends meant that inshore and mid-shelf corals went from having statistically equivalent respective protein levels of 0.62 $mg.cm^{-2}$ (0.55-0.67) and 0.57 $mg.cm^{-2}$ (0.41-0.62) during heat stress (apart from in the P treatment; Figure S4.4), to inshore corals having more than double the protein content of their mid-shelf counterparts after heat stress (inshore 0.50 $mg.cm^{-2}$, 0.44-0.56; mid-shelf 0.23 $mg.cm^{-2}$; 0.18-0.28). Like chlorophyll measurements, the protein content of inshore and mid-shelf corals was constant across the nutrient treatments at each timepoint (Figure S4.4).

The mortality rates of coral nubbins throughout the experiment were low to moderate, following the moderate heat stress exposure of 3.08 and 4.52 °C-weeks for inshore and mid-shelf corals respectively. Prior to the heat stress sampling period, only a single nubbin from each shelf-position had died (0.3 % of all nubbins). Between the peak of heat stress and the end of the experiment, 3.6 % of remaining inshore corals and 19.8 % of mid-shelf corals died. Zero deaths were observed for inshore corals under control nutrient conditions, mirroring the reduced mortality rates of control mid-shelf corals (8.3 %) relative to those that were nutrient-enriched (N: 22.9 %; P: 27.1 %; NP: 20.8 %).

Coral and algal symbiont nutrient metabolism under nutrient and heat stress

Trends in the percentage carbon and nitrogen content of the algal symbionts (Figure 4.3) closely mirrored those for chlorophyll content. Mid-shelf symbionts experienced greater carbon and nitrogen loss from 41.5 (39.4-43.5) to 29.4 % C (27.0-32.1) and 7.63 (7.22-8.03) to 5.89 % N (5.30-6.54) over time (Figure S4.5; Figure S4.6), compared to those inshore which had smaller declines from 47.7 (46.0-49.3) to 43.6 % C (39.4-43.5) and 8.81 (8.49-9.08) to 7.64 % N (7.26-8.04). Like chlorophyll, reductions to inshore symbiont % C were largely restricted to the nutrient-enriched (N, P, NP) treatments, but there was little evidence of nutrient impacts when controlling for the other factors (Figure S4.5). For the % N content of inshore symbionts during bleaching, those in the N-enriched (N, NP) treatments tended to have

slightly elevated % N contents (Figure S4.6). Overall, inshore symbionts tended to have greater % C and % N than mid-shelf symbionts both during and after heat stress (Figure S4.5; Figure S4.6).

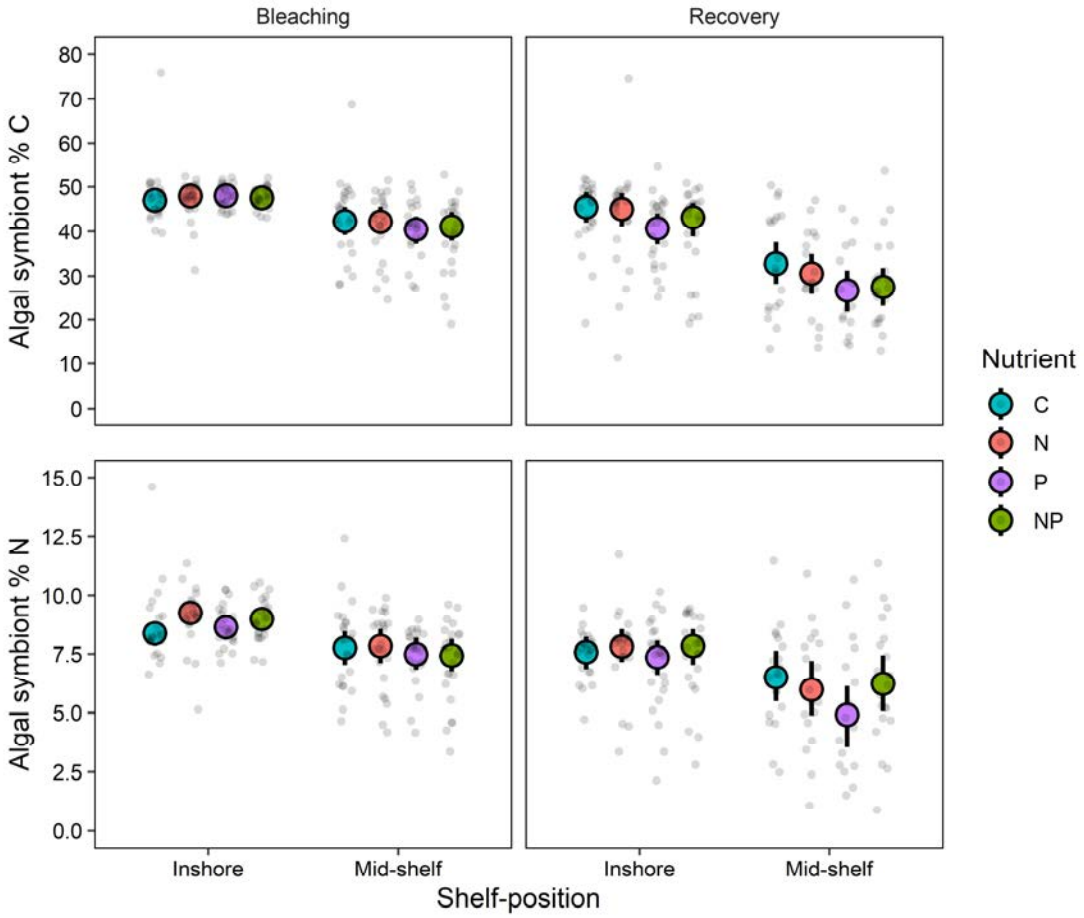

Figure 4.3 Algal symbiont percentage carbon and nitrogen contents within inshore and mid-shelf corals, according to sampling period (left vs right) and nutrient treatment (colour). Coloured points and error bars show median model simulations with 95% CIs. Grey dots show the raw data points.

Declines to the carbon and nitrogen contents of the coral host tissues (Figure 4.4) over time were comparable to host protein content. Consistent with other metrics, mid-shelf coral host tissues experienced greater carbon and nitrogen loss following heat stress (Figure S4.7; Figure S4.8), going from 22.6 (21.2-24.1) to 15.1 % C (13.5-16.5) and 3.65 (3.44-3.86) to 2.45 % N (2.21-2.63). Inshore corals retained more of these key nutrients, declining from 25.2 (23.4-

26.6) to 21.0 (19.4-22.4) % C and 3.92 (3.73-4.15) to 3.29 (3.10-3.50) % N. Accordingly, inshore corals had greater carbon and nitrogen content than their mid-shelf counterparts after heat stress, whereas during heat stress, cross-shelf differences were only apparent in the P-enriched treatments (P, NP; Figure S4.7; Figure S4.8). However, within the inshore and mid-shelf corals individually, there was no sign of nutrient impacts during and after heat stress (Figure S4.7; Figure S4.8).

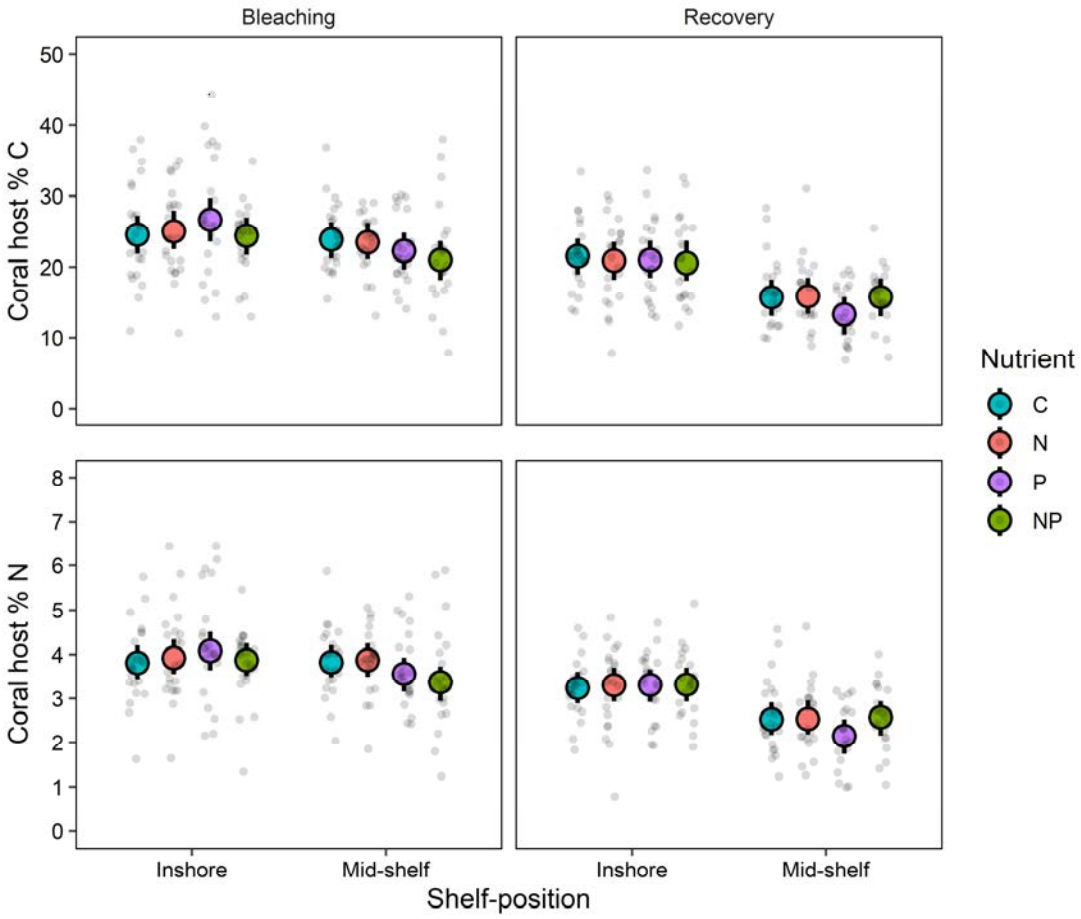

Figure 4.4 Host percentage carbon and nitrogen contents of inshore and mid-shelf corals, according to sampling period (left vs right) and nutrient treatment (colour). Coloured points and error bars show median model simulations with 95% CIs. Grey dots show the raw data points.

Losses of carbon and nitrogen from the algal symbiont and host tissues (Figure S4.9) largely occurred in fixed proportions since C:N ratios were relatively unimpacted by the experimental

treatments (Figure S4.10, Figure S4.11). During heat stress, inshore algal symbionts in the N-enriched (N, NP) treatments had slightly lower C:N ratios than those that were unenriched (C, P), and this trend was also reflected in the host tissues to a lesser extent. Other differences included inshore control corals and symbionts having slightly higher C:N than mid-shelf control treatment after heat stress, and a slight increase for inshore symbiont C:N in the control and nitrate treatments over time. Global estimated means for C:N were 5.46 (5.31-5.59) for the algal symbionts and 6.31 (6.20-6.40) for the coral host.

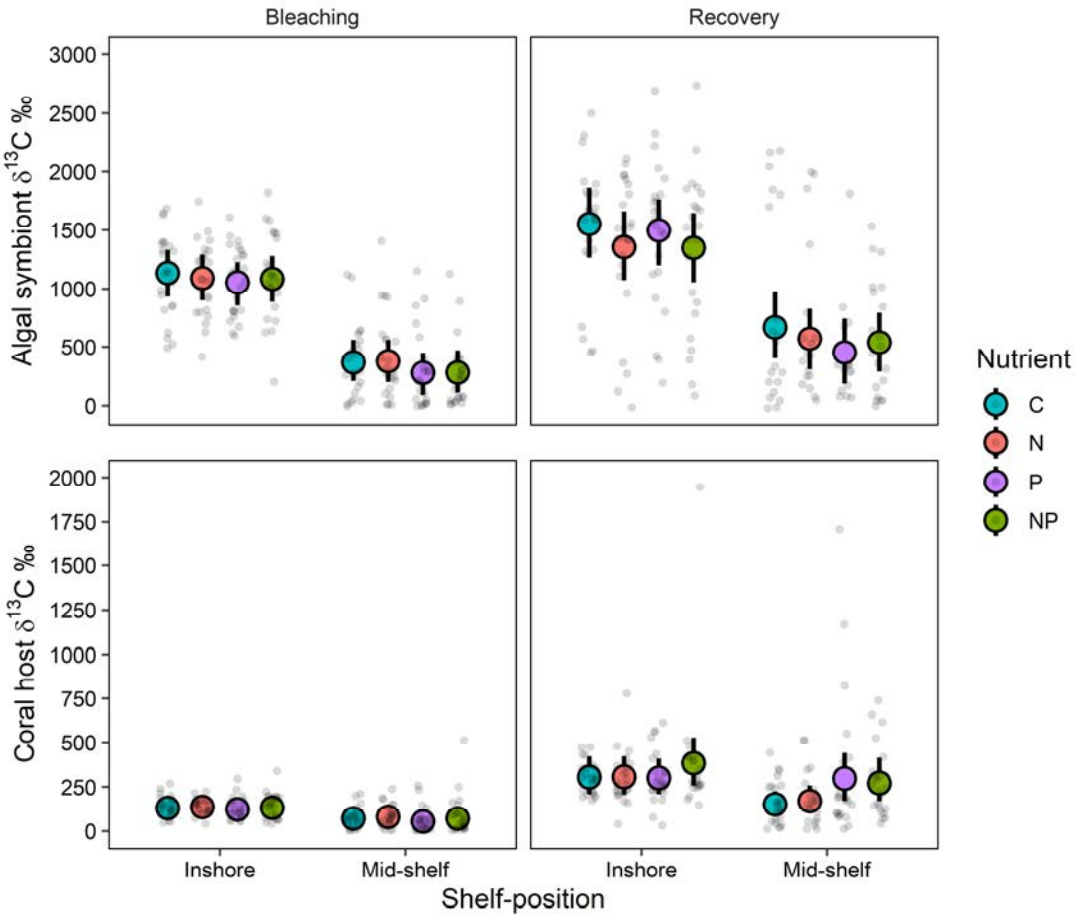

Figure 4.5 Algal symbiont and host $H^{13}CO_3^-$ enrichment within inshore and mid-shelf corals, according to sampling period (left vs right) and nutrient treatment (colour). Coloured points and error bars show median model simulations with 95% CIs. Grey dots show the raw data points.

Inorganic carbon ($H^{13}CO_3^-$) incorporation by the algal symbionts (Figure 4.5) tended to increase between the peak of heat stress and the end of the experiment for both inshore and mid-shelf corals (Figure S4.12). Carbon-13 enrichment was 1,092 ‰ $\delta^{13}C$ (961-1,232) for the inshore symbionts during heat stress before later increasing to 1,439 ‰ $\delta^{13}C$ (1,258-1,613). Respective values for the mid-shelf symbionts, which were lower than the inshore symbionts both during and after heat stress, were 323 ‰ $\delta^{13}C$ (193-465) and 554 ‰ $\delta^{13}C$ (396-747).

Like patterns in the algal symbionts, host ^{13}C enrichment (Figure 4.5) increased from 131 ‰ $\delta^{13}C$ (105-159) to 326 ‰ $\delta^{13}C$ (253-400) over time for the inshore corals (Figure S4.13). For the mid-shelf host tissues, enrichment was initially 71 ‰ $\delta^{13}C$ (49-95) and therefore lower than the inshore host (Figure S4.13). After heat stress, mid-shelf host enrichment increased to 226 ‰ $\delta^{13}C$ (169-297) with estimates taken across the nutrient treatments. At this timepoint, mid-shelf host ^{13}C enrichment were equivalent to inshore samples when phosphate was supplied (P: 297 ‰ $\delta^{13}C$, 170-439; NP: 270 ‰ $\delta^{13}C$; 169-411). In the other treatments, mid-shelf ^{13}C enrichment tended to be lower (mid-shelf C: 151 ‰ $\delta^{13}C$, 95-225; N: 171 ‰ $\delta^{13}C$, 101-258) than the phosphate enriched treatments and the corresponding inshore samples (Figure S4.13).

Nitrate ($^{15}NO_3^-$) assimilation by the algal symbiont (Figure 4.6) definitively increased as the corals were released heat stress (Figure S4.14). Nitrogen-15 enrichment during heat stress was low at 68.0 ‰ $\delta^{15}N$ (61.3-75.8) and 52.7 ‰ $\delta^{15}N$ (47.3-58.7) respectively for the inshore and mid-shelf algal symbionts. After heat stress, ^{15}N enrichment tended to remain higher for inshore symbionts compared to those from mid-shelf corals (Figure S4.14), however there was variability within and across the nutrient treatments. Typically, symbionts with a history of exposure to nitrate enrichment had depressed nitrate assimilation during the short-term incubations after bleaching, a feature that was particularly apparent for the inshore control corals (Figure S4.14).

During heat stress, low symbiont nitrate assimilation meant that ^{15}N enrichment in the host (Figure 4.6) was minimal at 23.0 ‰ $\delta^{15}N$ for inshore (19.0-27.4) and 13.9 ‰ $\delta^{15}N$ (11.7-16.3) for mid-shelf corals. After heat stress, inshore host ^{15}N enrichment increased (Figure S4.15), particularly for the control corals which tended to have higher ^{15}N than the nutrient-enriched

(N, P, NP) treatments. In contrast, mid-shelf coral tissues in the P-enriched (N, NP) treatments increased the most after heat stress and tended to have higher enrichment than the other treatments (C, N). These contrasting trends indicated that for host ^{15}N enrichment after heat stress, inshore corals were greater than their mid-shelf conspecifics in the control and nitrate treatments (C, N) but tended to be less enriched when phosphate was added (P, NP).

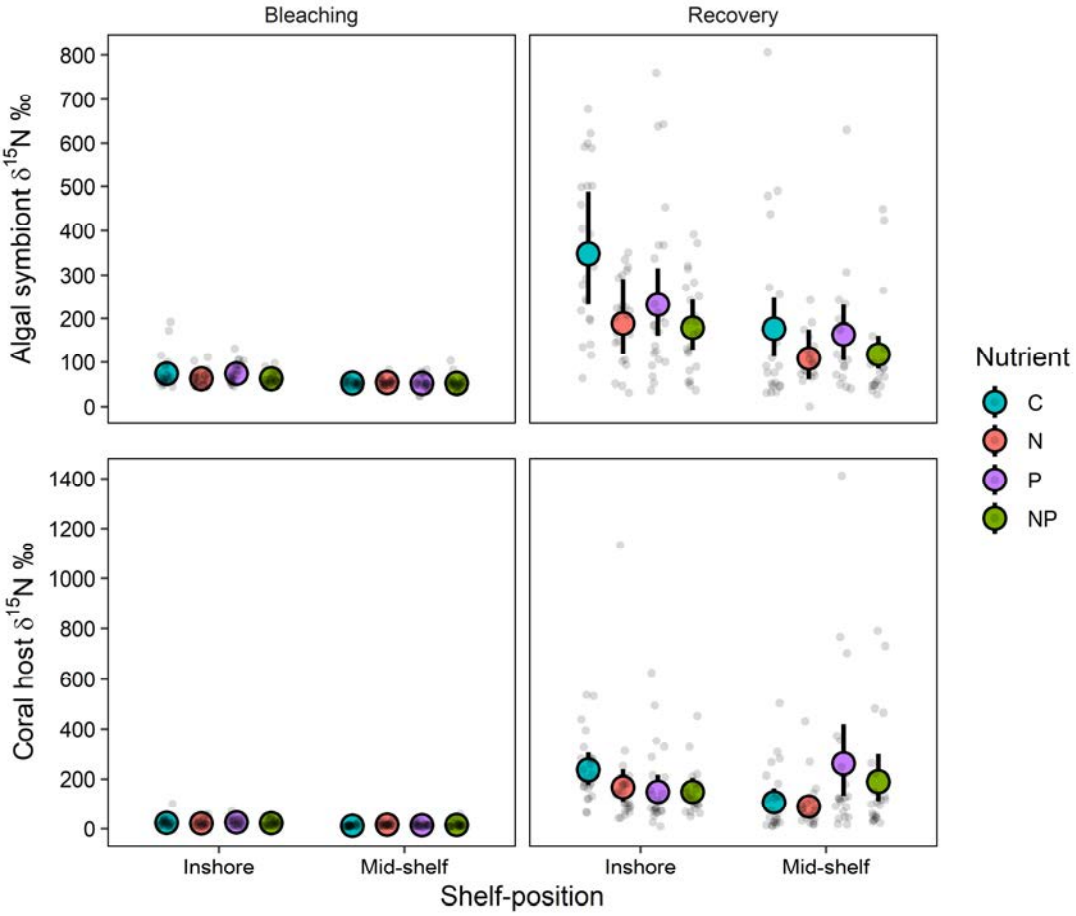

Figure 4.6 Algal symbiont and host $^{15}NO_3^-$ enrichment within inshore and mid-shelf corals, according to sampling period (left vs right) and nutrient treatment (colour). Coloured points and error bars show median model simulations with 95% CIs. Grey dots show the raw data points.

Algal symbiont communities

After trimming and rarefaction, 279 *Cladocopium* DIVs and 35 ITS2-type profiles represented virtually all sequences (> 99.9 %) with the remainder being a single *Symbiodinium* DIV and type profile (Figure 4.7).

Figure 4.7 Proportion normalised ITS2-type profiles of inshore and mid-shelf *A. millepora* subject to nutrient stress during and following the peak of a simulated heat stress event. The colour of points denotes the nutrient treatments, and their size represents the mean

percentage of each type of profile within each treatment population. Type profiles are sorted (top to bottom) based on their relative abundance within the entire experiment, and only type profiles with an abundance known to influence coral bleaching resilience (> 0.3 % within a population) are shown (Bay et al. 2016).

ITS2-type profiles which differentiated inshore and mid-shelf corals were the dominance of C50c/C50a/C3-C50f-C3b-C50u-C3ad type profile (73.8 % of type profiles), followed by the C3/C50a/C50c-C3b-C21-C3fk-C21.12-C3.10 (9.1 %) and C50c/C50a/C3/C1-C50f-C3b-C50u (7.5 %) profiles in the inshore samples, which were scarce by comparison in mid-shelf corals (6.7, 1.7 and 0.6 %, respectively). Mid-shelf corals instead hosted a greater diversity of ITS2-type profiles which, during heat stress, included C3k/C50a/C3-C3ba-C50f-C50q-C3dq-C3a (32.4 %), C3k/C3-C50a-C29-C21ab-C3b (16.9 %), C50a/C50c/C3/C3k-C3b-C50f-C21 (16.8 %), C50c/C50a/C3-C50f-C3b-C50u-C3ad (the dominant inshore profile; 10.1 %), C1/C3k-C1b-C3-C1c-C42.2-C1br-C1bh (5.6 %) and C1/C50c/C50a/C3-C1b-C1c-C42.2-C1bh-C1br-C1cb (5.5 %). After heat stress, the relative abundance of these type profiles changed, mostly decreasing, to 11.2, 9.0, 13.5, 3.4, 11.1 and 1.1 %, respectively. At the same time, several new type profiles became compositionally important including: C3k/C3/C50a (13.5 %), C50c/C50a/C3 (7.3 %) and C3k/C50a (5.1 %).

Treatment effects on alpha diversity (Shannon's *H'* and Simpson's 1-*D*; Figure S4.16) of DIVs were minimal with the only clear trend being a tendency for phosphate-enriched (P) inshore corals to have slightly reduced *H'* and 1-*D* during heat stress (Figure S4.17; Figure S4.18) in addition to a decrease in *H'* for phosphate-enriched (P) mid-shelf corals over time (Figure S4.17).

PERMANOVA analyses demonstrated significant differences in algal symbiont community structure based on DIV (Figure S4.19) and ITS2-type profile abundance and composition (Figure 4.7). An interaction between shelf-position and temperature was found for both DIVs ($p = 0.0176$, $R^2 = 0.0068$) and ITS2-type profiles ($p = 0.0106$, $R^2 = 0.0066$). Most of this variance was explained by shelf-position (DIVs $p < 0.0001$, $R^2 = 0.2666$; profiles $p < 0.0001$, $R^2 = 0.1762$) rather than temperature (DIVs $p = 0.0109$, $R^2 = 0.0079$; profiles $p = 0.0025$, $R^2 = 0.0083$). A small nutrient effect was also observed for type profiles ($p = 0.0457$, $R^2 = 0.0108$) but not for

DIVs observed ($p = 0.7835$, $R^2 = 0.0039$). Due to limited mortality of some fragments and sequencing constraints, the PERMANOVAs were potentially confounded by differences in dispersion of DIVs and type profiles between shelf-locations ($p < 0.0001$) but not across temperatures (DIVs $p = 0.1890$; profiles $p = 0.1654$).

Post-hoc testing showed that community structure of DIVs and type profiles varied between shelf-positions during (DIVs $p < 0.0001$, $R^2 = 0.3557$; profiles $p < 0.0001$, $R^2 = 0.2074$) and after heat stress ($p < 0.0001$, $R^2 = 0.2115$; $p < 0.0001$, $R^2 = 0.1628$). Differences in dispersion were also found between shelf-positions at each timepoint (all $p < 0.0001$). Changes in community structure over time were limited to the mid-shelf corals (DIVs $p = 0.0024$, $R^2 = 0.0239$; profiles $p = 0.0002$, $R^2 = 0.0244$) which also differed in dispersion (DIVs $p < 0.0001$; profiles $p = 0.0069$). The inshore corals, therefore remained constant in their symbiont community structure (DIVs $p = 0.4520$, $R^2 = 0.0045$; profiles $p = 0.3667$, $R^2 = 0.0053$). Post-hoc comparisons across nutrient treatments failed to locate the apparent nutrient effect regarding ITS2-type profiles.

Discussion

Coral bleaching response strongly driven by environmental history

The differential bleaching responses of inshore and mid-shelf corals following heat stress may be explained by their prior thermal history (Hughes et al. 2019; Safaie et al. 2018), the distinct algal symbionts that they host (Berkelmans and van Oppen 2006; Fuller et al. 2020) or local co-adaptation of both partners. This result confirms findings from an experiment undertaken a year earlier which exposed the same coral species from the same reefs to a similar nutrient and temperature challenge (Chapter 3) showing coral sensitivity was relatively unimpacted by short-term nutrient enrichment, though strong cross-shelf differences exist in the levels of bleaching and photosynthetic disfunction experienced after heat stress. This inshore coral thermal tolerance is a function of their approximately 0.35 °C greater maximum monthly mean temperature (MMM). This may have been influenced further by cross-shelf water quality gradients (Waterhouse et al. 2021), but there was little impact of short-term exposure to ecologically relevant nutrient enrichment in the bleaching response, apart from a trend of

nutrient-enriched (especially phosphate-enriched) corals suffering slightly elevated photoinhibition relative to controls. As found previously (Chapter 3), inshore corals hosted a limited range of algal symbiont type profiles, characterised by the C50c DIV, whereas mid-shelf corals were more varied and associated with type profiles dominated by C3k. This study also builds on previous results to show that the cross-shelf bleaching trends persist even after heat stress is alleviated. While inshore corals were able to largely maintain their levels of pigmentation following heat stress and showed signs of a release from photoinhibition, mid-shelf corals suffered further as demonstrated by almost total chlorophyll loss and continued photoinhibition during the experimental recovery period, coupled with changes to the community structure of their algal symbiont communities. These contrasting impacts of coral bleaching were matched by minimal mortality of inshore corals relative to the moderate mortality of their mid-shelf counterparts.

Reversal of the coral-algal nitrogen cycling during heat stress

In healthy cnidarian-algae symbioses, the host limits nitrogen availability to the symbionts by sequestering ammonium and photosynthates into amino acids through the glutamine synthetase (GS)/glutamate synthetase (GOGAT) cycle (Cui et al. 2019; Wang and Douglas 1999; Xiang et al. 2020). However, with increasing levels of heat stress and bleaching, there is now widespread transcriptomic, proteomic and metabolomic evidence that the GS/GOGAT cycle is supressed and instead the host initiates protein and amino acid degradation pathways, including glutamate dehydrogenase (GDH), which catalyses ammonium and α-ketoglutarate release from glutamate (Avila-Magaña et al. 2021; Cui et al. 2019; Davies et al. 2016; Dixon et al. 2020; Hillyer et al. 2016a; Li et al. 2021; Maor-Landaw et al. 2014; Oakley et al. 2016; Petrou et al. 2018; Petrou et al. 2021; Rädecker et al. 2021; Rouan et al. ; Seneca and Palumbi 2015; Traylor-Knowles et al. 2017; Wang and Douglas 1998; Williams et al. 2021; Xiang et al. 2020; Zhang et al. 2021). The cross-shelf patterns of coral-algal $^{13}C/^{15}N$ cycling observed in the current study likely reflects coral nitrogen cycling being reversed from ammonium sequestration to ammonium release under heat stress, proportional with levels of initial coral bleaching.

During the peak of heat stress, algal $H^{13}CO_3^-$ fixation and the translocation of organic ^{13}C to the host were supressed in the highly bleached mid-shelf corals relative to the more temperature-tolerant inshore corals. Concurrent levels of $^{15}NO_3^-$ assimilation by the algal symbionts were extremely low, especially within mid-shelf corals. This latter inhibition of exogenous nitrate assimilation is common for coral-associated algal symbionts under elevated temperatures (Ezzat et al. 2016a; Godinot et al. 2011; Rädecker et al. 2021) and highly suggestive of their saturation in ammonium, derived from the breakdown of host proteins (Badgley et al. 2005; Grover et al. 2003; Rädecker et al. 2021). The low translocation of photosynthates from symbiont to host during initial heat stress and bleaching can therefore be explained through 1) stimulation of symbiont growth after uncontrolled access to ammonium (Baker et al. 2018; Ferrier-Pagès et al. 2001; Muscatine et al. 1998; Rädecker et al. 2021) and 2) the thermal impairment of photosynthetic carbon fixation (Baker et al. 2013; Ezzat et al. 2016a; Hughes et al. 2010; Tremblay et al. 2016).

The catabolism of proteins by the coral host to compensate for an inadequate supply of autotrophic carbon is supported by previous observations of protein loss from corals during heat stress and/or bleaching (Aichelman et al. 2021; Anderson et al. 2019; Bourne et al. 2008; Hoadley et al. 2015; Kochman et al. 2021; Muller et al. 2021; Nielsen et al. 2020; Rodrigues and Grottoli 2007; Rosic et al. 2020; van der Zande et al. 2020). Organic carbon substrates released from these proteins may be converted to glucose via gluconeogenesis or utilised in the citric acid cycle to help corals cope with energetic stress. This former process is demonstrated by previous studies which found upregulation of key transcripts and metabolites involved in gluconeogenesis in cnidarians subject to heat stress and bleaching (Ganot et al. 2011; Hillyer et al. 2016b; Kenkel et al. 2013b; Lee et al. 2018).

Inshore and mid-shelf corals exhibited divergent metabolic responses after heat stress was alleviated. Inshore corals largely avoided further bleaching, losses to their nutrient stores or mortality. In contrast, mid-shelf corals lost more than two thirds of their remaining chlorophyll, over half their host protein and substantial portions of their total carbon and nitrogen content. This starvation of mid-shelf corals likely contributed to the observed levels of mortality (19.8 %) and occurred despite corals having the oppoto buffer their losses and survive through the provision of particulate food (Conlan et al. 2018; Connolly et al. 2012;

Tremblay et al. 2016). Carbon-to-nitrogen ratios in the host and symbiont tissues were largely consistent over time and among treatments, indicating that carbon and nitrogen were lost in relatively fixed proportions around 6 C:N. This suggests that nitrogen-rich compounds, including proteins, accounted for a substantial portion of the material lost from the coral host, and is supported by previous studies showing that protein is one of the primary macromolecules lost from corals under elevated temperatures (Kochman et al. 2021; Nielsen et al. 2020). In the symbionts, the proportional C/N losses may have resulted from the degradation of carbon-rich photosynthetic pigments (peridinin and chlorophyll) along with their associated antennae proteins (Takahashi et al. 2008). This integration of short- and long-term measures of coral-algal nutrient cycling, show that the reversal of nitrogen cycling towards ammonium release (Rädecker et al. 2021) is a common response of corals to elevated temperature regardless of their heat tolerance. However, the initial destabilisation of nutrient cycling does not necessarily translate to net losses of host carbon, nitrogen, and proteins unless the nutritional disruption is particularly severe and prolonged, as was in the case for the mid-shelf corals which began to perish. Therefore, while measures of nutrient flux capture the ubiquitous response of corals to heat stress, bleaching resilience is more clearly reflected by long-term biomarkers of the coral host fitness including protein content.

Restoration of nutrient cycling after a return to ambient temperature

Regardless of prior bleaching and protein loss, there were signs of recovery in the nutrient cycling of both inshore and mid-shelf corals at the end of the experiment. The assimilation and translocation of $H^{13}CO_3^-$ and $^{15}NO_3^-$ had increased from the peak of heat stress in essentially all corals regardless of nutrient treatment or shelf-position, indicating a release from ammonium saturation of the symbionts and protein degradation in the host. However, fixation of both nutrients, but especially ^{13}C, into the mid-shelf algal symbionts tended to remain subdued compared to those in inshore corals. This trend is contrary to the expectation that the low-density populations of algal symbionts in the mid-shelf corals (indicated by low chlorophyll) would be subject to a release from resource limitation in terms of light and nutrients (Enríquez et al. 2005; Krueger et al. 2020; Scheufen et al. 2017a; Wangpraseurt et al. 2017; Wangpraseurt et al. 2012; Wangpraseurt et al. 2016) and therefore be highly productive on a per cell basis. The lower performance of the mid-shelf symbionts therefore

highlights potential lag effects of photoinhibition and/or protein catabolism during coral recovery.

In contrast to $^{13}C/^{15}N$ enrichment in the symbionts, the patterns of organic carbon and nitrogen translocation to the host were partly determined by nutrient exposure. Increases in photosynthate translocation from the algal symbionts to the host tended to be higher in mid-shelf corals in treatments where phosphate was supplied (P, NP) and therefore independent of nitrate availability. Unexpectedly, the same nutrient treatments appeared to be those most adversely photo-inhibited by heat stress initially. This trend meant that translocation from phosphate-replete mid-shelf symbionts tended to outperform those lacking phosphate (C, N) and matched symbionts of inshore corals. Phosphate supply is known to be essential for maintaining photosynthetic carbon fixation and translocation under heat stress (Ezzat et al. 2016a; Wiedenmann et al. 2013) and may have been used to buffer the co-occurring excess of host-derived ammonium (Ezzat et al. 2016a; Godinot et al. 2011; Rädecker et al. 2021). However, algal symbionts in corals (including *Acropora*) from the GBR are naturally phosphorus limited (mean C:N:P 603:69:1, Blanckaert et al. (2020)) relative to the Redfield ratio (106:16:1), suggesting that this is their natural condition (see also Krueger et al. (2020)), and previous studies showing deleterious impacts of phosphorus limitation in corals applied severe treatments that may not be ecologically relevant (Lesser 2021; Rosset et al. 2017; Wiedenmann et al. 2013). These potential benefits of phosphate availability were not apparent for inshore corals, potentially due to their relative heat tolerance (and therefore inactivation of heat stress processes) or differences in their symbiont communities that were not amenable to additional phosphate. Furthermore, phosphate limitation did not appear to prevent initial bleaching or the consequent starvation and mortality of mid-shelf corals. Overall, impacts of phosphorus are limited to stimulating the resumption of autotrophic condition only in recovering mid-shelf corals that have experienced the greatest degree of stress. Although the vitality of phosphorus for bleached corals warrants further study, ecologically realistic phosphate subsidies do not appear to alleviate the initial mechanisms of heat stress.

Explaining patterns of nutrient cycling under heat stress

The short-term exposure of corals to changes in nitrate and phosphate availability did not impact on the initial coral bleaching responses or any subsequent changes to nutrient stores. Impacts on nutrient fluxes were mostly limited to recovery in mid-shelf corals. The lack of strong nutrient impacts throughout the experiment is contrary to previous studies showing that nitrate enrichment exacerbates coral bleaching (Fernandes de Barros Marangoni et al. 2020; Higuchi et al. 2015; Rosset et al. 2017; Wiedenmann et al. 2013) which suggest that carbon stifles translocation from algal symbionts due to the energetic costs of its utilisation (Baker et al. 2018; Ezzat et al. 2015). Interacting cryptic factors (e.g. ultraviolet radiation shown by Blanckaert et al. (2021)) or those to do with the application of nutrient dosing may explain the lack of nutrient response (see Chapter 3 for extensive discussion). Ammonium released from proteins during and after heat stress potentially inhibited the uptake of nitrate and its consequent impacts (Godinot et al. 2011; Grover et al. 2003; Rädecker et al. 2021). However, Rädecker et al. (2021) demonstrates that symbiont assimilation of exogenous ammonium and nitrate is still possible even during net release from the coral. In the current experiment, slight trends towards lower C:N ratios and $^{15}NO_3^-$ assimilation of the nitrate-exposed (N, NP) inshore algal symbionts during heat stress are potentially indicative of their nitrate satiation (Grover et al. 2003). Regardless, nutrient-related differences were low and experiment-wide C:N ratios of the symbionts were low (< 6), relative to symbionts in wild GBR corals (Blanckaert et al. 2020). Potentially the baseline nutrient supply (CSFW + *Artemia*) in the experiment kept corals in a state of nitrogen sufficiency, which would explain the lack of nitrate impacts.

Adaptation of corals to their natural environment may explain the cross-shelf patterns of bleaching and nutritional breakdown measured in this study. Previous studies show that corals maintain higher protein levels under heat stress if they are acclimated to warm conditions (Gibbin et al. 2018; Middlebrook et al. 2008). This may occur through a frontloading downregulation of host protein degradation under low intensity stress (Dixon et al. 2020), evidenced by reduced nitrate assimilation and increased carbon translocation in algal symbionts following warm acclimation (Gibbin et al. 2018). Potentially, inshore corals, given their history of exposure to warmer, more variable temperature conditions and

increased nutrient loading (Jin et al. 2019; Waterhouse et al. 2021) used these frontloading mechanisms to buffer their nutrient cycling from heat stress (Morris et al. 2019). Aside from acclimation, genetic co-adaptation of the host and symbiont may have mediated coral nutrient cycling (Morris et al. 2019; Rädecker et al. 2018). A study of *A. millepora* on the GBR during the 2017 bleaching event found that genes involved in heat tolerance were not spatially structured across a cross-shelf gradient (Fuller et al. 2020). However, algal symbiont genus was a strong contributor in explaining the severity of bleaching, with *Durusdinium* being more tolerant than *Cladocopium* (Fuller et al. 2020). These same cross-genus patterns of heat tolerance were also found in the response of coral nutrient cycling to heat stress in juvenile *Acropora tenuis* (Baker et al. 2013). In the current study, distinct *Cladocopium* DIVs and ITS2-type profiles were detected within inshore and mid-shelf corals. Inshore corals were dominated by C50c/C50a/C3-characterised type profiles (e.g., C50c/C50a/C3-C50f-C3b-C50u-C3ad) whereas mid-shelf corals primarily associated with type profiles involving the C3k DIV (C3k/C50a/C3-C3ba-C50f-C50q-C3dq-C3a, C3k/C3-C50a-C29-C21ab-C3b and C50a/C50c/C3/C3k-C3b-C50f-C21). Potentially, algal symbionts containing the C3k DIV possess unique traits related to nutrient acquisition and translocation (Suggett et al. 2017; Wong et al. 2021) which make them particularly "selfish" under heat stress (Baker et al. 2013; Baker et al. 2018) and contribute to the heat sensitivity of mid-shelf corals, relative to inshore corals hosting C50c/C50a/C3 symbionts.

Conclusion

This study of coral-algal nutrient cycling demonstrates a full cascade of nutritional destabilisation leading to coral bleaching and mortality. Heat-stressed corals experienced a shift from protein anabolism to protein catabolism regardless of their environmental history and short-term nutrient exposure. However, whether the coral ultimately sheds its nutrient reserves and risks mortality is strongly determined by initial levels of bleaching and metabolic disruption. Regardless, after heat stress is alleviated, there are signs that the coral-algal nutrient cycling begins to return to normal, especially if surviving corals are heat tolerant in the first place or aided by a supply of phosphorus. Overall, the hypothesis that the breakdown of fundamental nutrient exchange between corals and their algal symbionts underpins their

stability (Morris et al. 2019) has been strengthened by this study, which links key adaptation and acclimation mechanisms that regulate patterns of coral bleaching and mortality to the reversal of coral-algal nitrogen cycling and corresponding losses to the coral and algal nutrient reserves.

General discussion

This thesis investigated the role of nutrients in coral bleaching using the common GBR coral *A. millepora* sourced from inshore and mid-shelf reefs. Despite prior hypotheses and laboratory studies demonstrating a strong role of nutrient enrichment in modulating coral bleaching, results here demonstrated that the bleaching tolerance of *A. millepora* under mild to moderate heat stress was largely unaltered in both the field and in laboratory conditions under ecologically relevant nutrient conditions. Instead, differences in the thermal thresholds for bleaching and mortality were found to relate to the historical exposure to summer maximum temperatures and water quality regimes, and their associations with algal symbiont communities. Importantly, the cross-shelf variation in coral heat tolerance was consistent with an observed cascade of destabilisation in the carbon and nitrogen cycling of the coral-algal symbiosis, which was ubiquitous in corals but only manifested as starvation and mortality in the severely heat stressed mid-shelf corals. These novel insights into the roles of nutrient availability and metabolism in the thermal stability of coral-algal symbioses advance the understanding of the underlying mechanisms behind mass coral bleaching and mortality events.

Nutrient destabilisation hypothesis of coral bleaching

Chapter 1 synthesised the existing evidence for the involvement of nutritional mechanisms in coral bleaching. The established dogma of coral bleaching is centred around heat- and light-induced damage to the photosynthetic machinery of the symbionts, leading to photo-oxidative stress and the loss of the symbionts from the coral tissues (Weis 2008). However, recent studies demonstrate thermal bleaching without photo-oxidative stress (Diaz et al. 2016; Nielsen et al. 2018), and bleaching due to nutrient stress (Pogoreutz et al. 2017a; Rosset et al. 2017), highlighting alternative pathways to bleaching. Chapter 1 therefore builds on the existing hypothesis (Pogoreutz et al. 2017a; Rosset et al. 2017) and emerging studies (Wooldridge 2009a) to show how the impairment of coral nutrient cycling may be central to the breakdown of the coral-algal symbiosis. It was shown how nutrient conditions that enhance coral heat tolerance (ammonium and/or phosphate) are also those which enhance

carbon translocation from algal symbiont to host (Baker et al. 2018; Tremblay et al. 2016). Conversely, nitrate assimilation by symbionts possesses energetic costs which are imposed on the host (Baker et al. 2018; Ezzat et al. 2015), leading to enhanced bleaching susceptibility (Rosset et al. 2017; Wiedenmann et al. 2013). Further to this, it was hypothesised that the nutrient exchange characteristics between genetically distinct coral-algal partnerships (Béraud et al. 2013; Ezzat et al. 2016a; Wiedenmann et al. 2013) or acclimation (Gibbin et al. 2018) may mediate the tolerance of corals to bleaching under heat stress. Chapter 1 sets the scene for the data chapters of the thesis which investigate how nutrient stress and the environmental history interact with heat stress to impact bleaching tolerance, and the mechanisms by which this occurs using the model coral species *A. millepora*.

Spatial profiling of algal symbiont physiology and genetics during bleaching

Chapter 2 examined spatial patterns of *A. millepora* bleaching and recovery, together with the dynamics of the algal symbiont communities of corals sampled from the central GBR in 2017 following a mass coral bleaching event. Accumulated heat exposure was severe across the study region and likely explained the observed homogeneity in coral bleaching and recovery over cross-shelf and latitudinal gradients. Therefore, under the intensity of marine heatwave events seen recently on the GBR (Hughes et al. 2021), the effect of local factors like water quality are unlikely to influence the bleaching tolerance of corals as previously hypothesised (Wooldridge 2009b). However, increases in coral pigmentation (chlorophyll) following bleaching were stronger on inshore reefs, relative to the mid-shelf, likely related to the increased exposure of inshore corals to water quality inputs (high nutrients, turbidity and/or low light) which stimulate growth in the algal symbiont communities (Anthony 2000; DiPerna et al. 2018; Shantz and Burkepile 2014).

Contrary to the patterns of bleaching, the algal symbiont communities were spatially structured. Mid-shelf corals were dominated by *Cladocopium* C3/C50c symbionts on the warmer, northerly reefs and transitioned to *Cladocopium* C3/C3k further south. *Cladocopium* C3/C50c were present on many inshore reefs, but those in the middle of the study region hosted more diverse and dynamic communities of *Cladocopium* C1, C3, C21 and *Durusdinium*

D1 and D2, likely driven by local conditions including water quality (Cooper et al. 2011; Quigley et al. 2017a). Notably, corals at one inshore reef transitioned from *Durusdinium* D2 to *Cladocopium* C21 dominance, contrary to previous evidence that inshore *A. millepora* populations can transition from *Cladocopium* to *Durusdinium* during bleaching on the GBR (Bay et al. 2016; Jones et al. 2008). Although these previous studies on *A. millepora* helped to validate the paradigm that shifts in the algal symbiont communities can confer bleaching tolerance (Baker 2001), the potential for this to be an expansive mechanism of adaption to ocean warming (Logan et al. 2021) appeared to be limited on the central GBR in the current study.

Physiological response of GBR *Acropora millepora* to ecologically relevant nutrient and heat stress

Chapter 3 examined the heat tolerance of *A. millepora* under exposure to ecologically relevant levels of nutrient enrichment, and whether this was moderated by the long-term exposure of corals temperature and water quality on the GBR. The nutrient conditions applied (nitrate and/or phosphate) captured those which occur on the inshore GBR (Gruber et al. 2019) and matched previous experiments showing that elevated nitrate to phosphate ratios enhance coral bleaching. However, physiological impacts of nutrient exposure were subtle in the current study, supporting suggestions that GBR corals can adapt or acclimate to phosphorus limitation (Blanckaert et al. 2020) and/or nitrate enrichment (Jin et al. 2019). Instead, the environmental history of the corals dominated their response to heat stress, where mid-shelf corals experienced greater photosynthetic declines (chlorophyll loss, gross photosynthesis, photosynthetic efficiency) than their inshore counterparts. The underlying mechanisms were likely related to the local thermal acclimatisation and/or adaptation of inshore coral-algal symbioses to their historically warmer environment (Dixon et al. 2015), resulting in reduced levels of accumulated heat stress (2.9 °C-weeks) relative to corals sourced from the mid-shelf reefs (3.9 °C-weeks) under the single heat profile implemented. Additionally, the patterns of bleaching may have been explained through the observed cross-shelf differences in the identity of the *Cladocopium* symbionts hosted by *A. millepora* (Swain et al. 2017). Overall, the bleaching response of corals was dictated by accumulated heat stress

as previously identified (Hughes et al. 2017b) and the subtle nutrients effects observed here would likely be obscured (Donovan et al. 2020) at the severe levels of heat stress (≥ 4 °C-weeks) which increasingly threaten the GBR (Hughes et al. 2017b; Hughes et al. 2021).

Nutritional destabilisation during thermal bleaching and mortality of corals

Chapter 4 investigates whether the disruption of nutrient cycling is central to the processes of coral bleaching and mortality during and following heat stress, and how these are modulated by the environmental history and/or nutrient stress exposure of corals as outlined in Chapter 3. A relative lack of strong nutrient impacts, and the heat sensitivity of mid-shelf corals, were reconfirmed through measurements of chlorophyll and photosynthetic efficiency. Suppression of inorganic carbon and nitrate cycling by the algal symbionts was ubiquitous in corals under heat stress but most severe for mid-shelf corals. The divergent responses of inshore and mid-shelf corals were compounded after temperatures were reduced: mid-shelf corals experienced further chlorophyll loss, along with catabolism of host proteins, and severe losses to the overall carbon and nitrogen content of both the symbiont and host compartments. This nutritional starvation led to moderate levels of mortality in these corals, whilst inshore corals remained relatively stable in their nutrient stores following heat stress. The relative resilience of inshore corals to nutritional destabilisation may be underpinned by their hosting of specific communities of *Cladocopium* symbionts which remain relatively mutualistic under heat stress (Baker et al. 2013), or the thermal acclimatisation of the symbiosis against parasitic nutrient cycling (Gibbin et al. 2018) and protein catabolism (Dixon et al. 2020) after their exposure to warmer, inshore conditions. Overall, this study supports earlier findings of heat-stress-induced amino acid catabolism and destabilisation of nutrient cycling within corals (Rädecker et al. 2021). Evidence of a similar reversal of coral nitrogen metabolism from protein anabolism to catabolism during bleaching, which manifests in the starvation and mortality of corals after exposure to severe accumulated heat stress. These findings have broad implications for understanding mechanisms involved in the mass bleaching of reef corals by a providing a clear picture of how nutritional destabilisation extends from the onset of heat stress through to coral mortality. There is now strong evidence that starvation of the host drives coral mortality.

Further work should assess if the tolerance of reef corals to ocean warming can be predicted by the thermal performance of coral-algal nutrient cycling and metabolic potential of the hosts energy stores. Furthermore, a focus on the coral-algal nutrient cycling should be placed at the centre of novel efforts to enhance the resilience of corals to thermal bleaching.

New perspectives on coral bleaching and future research directions

Taken together, the thesis provides new and timely evidence for the involvement of nutritional mechanisms in coral bleaching. Existing hypotheses and experimental work suggested that the disruption to the internal nutrient metabolism of the coral-algal symbiosis by environmental nutrient enrichment can exacerbate the thermal bleaching response (Baker et al. 2018; D'Angelo and Wiedenmann 2014; Rädecker et al. 2021; Wiedenmann et al. 2013; Wooldridge 2009a; Wooldridge 2013). Within the environmental context of the GBR (Hughes et al. 2021; Waterhouse et al. 2021) the results of this thesis suggest that environmental nutrient pollution has little, if any, impact on coral bleaching on the GBR under mild to severe levels of heat stress, experimentally (2.9 to 4.5 °C-weeks) or in the field (5.2 to 11.0 °C-weeks). Conversely, the internal destabilisation of the coral-algal nutrient cycling was found to be a universal response of GBR *A. millepora* to heat stress and coincided with bleaching, as previously suggested (Rädecker et al. 2021). Mild to moderate heat stress (3.1 to 4.5 °C-weeks) clearly initiated the reversal of critical carbon and nitrogen cycling between corals and their algal symbionts, regardless of inshore or mid-shelf provenance. However, whether this dysbiotic nutrient cycling translates to coral starvation and mortality following bleaching appears to be determined by the initial severity of heat stress. It now seems likely that nutritional mechanisms are an integral part of the coral bleaching process, alongside known photo-oxidative stress pathways, and that impacts of environmental nutrient forcing can be overwhelmed under moderate heat stress.

Differential nutritional destabilisation and bleaching resilience in corals

A key factor underlying the experimental patterns of bleaching and nutritional destabilisation observed in this thesis was the environmental history of corals. Inshore corals, having

experienced greater water quality and temperature challenge, were more resistant to mildly elevated temperature than their mid-shelf counterparts. Local variation in temperature tolerance may be of little relevance during intense marine heat waves that have the potential to exceed the tolerance threshold of all coral populations on the GBR, leading to spatial homogeneity in the severity of bleaching (Hughes et al. 2021). Regardless, mild heat stress events are becoming increasingly common (Ainsworth et al. 2016), so it is important to understand the factors which regulate temperature tolerance in corals and how these relate to the nutritional mechanisms of bleaching. Differences in the *Cladocopium* species hosted by *A. millepora* were likely driven by long-term exposure to temperature and water quality and may contribute, in addition to host genetic effects, towards explaining the differences in coral heat tolerance (Cooke et al. 2020; Jin et al. 2019; Quigley et al. 2020). Future studies would benefit by surveying the baseline levels of metabolic compatibility within distinct coral-algal partnerships, along with identification of the specific genes (Fuller et al. 2020), transcripts (Rädecker et al. 2021) and proteins (Petrou et al. 2021) related to nutritional destabilisation in these diverse symbioses. These would enable characterisation of the pathways involved and their attribution to the host and/or symbiont compartments, and thereby strengthen the understanding of how dysbiotic nutrient cycling leads to heterogeneity in coral bleaching and mortality as observed here.

The current thesis focuses on one common GBR coral *A. millepora*, though at least 600 species that inhabit the GBR (Veron 2000). Each of these species likely vary in their degree of sensitivity to thermal bleaching and mortality (Hughes et al. 2017b; Hughes et al. 2018b; Marshall and Baird 2000), with *A. millepora* classified as moderately sensitive (Baird and Marshall 2002). Species-specific differences in tolerance to nutrient enrichment may also exist (Fox et al. 2021) and mediate potential interactions with heat stress (Burkepile et al. 2019; Donovan et al. 2020). To increase the generality of the findings presented here to diverse coral communities, further studies should include species with a broad range of stress tolerance (Hoey et al. 2016; Swain et al. 2016) as realised through variation in physiology, morphology and trophic plasticity (Conti-Jerpe et al. 2020; Loya et al. 2001; Putnam et al. 2017). It is likely that corals with large energy reserves and/or alternative nutrient acquisition strategies will be better equipped to maintain autotrophic nutrient cycling (Tremblay et al. 2016) and resist starvation and mortality (Grottoli et al. 2006) following nutritional

destabilisation. Future studies on the role of nutrients in the bleaching of GBR corals would therefore benefit from considering a range of taxa with increased and reduced susceptibility to environmental stress, to determine whether resistance to nutrient enrichment and the thermal impairment of nutrient cycling are universal for GBR corals.

Extending the ecological relevance of nutrient enrichment studies on corals

Although the conceptual links between nutrient availability and coral bleaching are clear (D'Angelo and Wiedenmann 2014; Morris et al. 2019; Wooldridge 2014), they are mainly restricted to observations of corals exposed to inorganic nitrogen and phosphorus along with carbon provided through heterotrophic feeding on zooplankton. Yet, corals can also derive nutrition from and be impacted by other nutrient sources including dissolved and particulate organic matter (Fabricius et al. 2013; Ferrier-Pagès et al. 2016; Pogoreutz et al. 2017a; Pogoreutz et al. 2018). These diverse nutrient sources are available on the GBR (Waterhouse et al. 2021) yet their impacts on coral physiology are poorly understood. In a similar vein, the experimental aspects of the thesis were highly controlled, yet for logistical reasons control corals were provided with a fluctuating inorganic nutrient supply through filtered inshore seawater, which may have obscured impacts of the nutrient-enriched treatments (Ezzat et al. 2019). Although experimental nutrient variation may be mitigated through additional filtration steps or alcohol enrichment (Ezzat et al. 2019; Wiedenmann et al. 2013) these may be difficult to implement at scale or have unintended impacts on coral physiology. Experiments were also conducted at a single light level, whereas on the GBR downwelling irradiance naturally covaries with nutrient availability and turbidity across water quality gradients on the GBR (Waterhouse et al. 2021) and has the potential to interact with nutrients and heat stress in determining the coral bleaching response (Rosic et al. 2020; Weis 2008; Wiedenmann et al. 2013). Future studies should consider a broad range of nutrient sources and light levels to further examine the relationship between anthropogenic nutrients and coral bleaching. These could take place outside of the laboratory using field plots (Burkepile et al. 2019) or portable aquarium systems (Voolstra et al. 2020) which would enable direct comparison of nutrient-enriched treatments to offshore oligotrophic reef waters.

Concluding remarks

The thesis finds that nutrient metabolism, but not nutrient availability, is an important factor in the bleaching and mortality of GBR corals exposed to heat stress. That is not to say that the water quality management is unimportant, since there is ample evidence that good water quality is crucial to coral health and reef ecology under non-bleaching conditions. Therefore, the best future for the GBR will require strong global actions to curb ocean warming, alongside local management actions to build reef resilience and enhance coral recovery. This comprehensive study has provided novel hypotheses and evidence for nutritional destabilisation of the coral-algal symbiosis as a fundamental mechanism of the coral heat stress response, extending the established photo-oxidative paradigm of bleaching and the role in which local adaptation plays in shaping coral physiology. Although cellular damage triggered by photo-oxidative stress is important to the bleaching process, it is now apparent that the reversal of coral-algal nutrient cycling is an initiating factor in coral bleaching and can later manifest in the starvation and mortality of the coral host. These nutritional insights can be applied to understand and predict mass bleaching and mortality events on coral reefs globally.

References

Abrego D, Ulstrup KE, Willis BL, van Oppen MJ (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress Proc Biol Sci 275:2273-2282 doi:10.1098/rspb.2008.0180

Aichelman HE et al. (2021) Exposure duration modulates the response of Caribbean corals to global change stressors Limnol Oceanogr 66:3100-3115 doi:10.1002/lno.11863

Ainsworth TD et al. (2016) Climate change disables coral bleaching protection on the Great Barrier Reef Science 352:338-342 doi:10.1126/science.aac7125

Alderdice R, Suggett DJ, Cardenas A, Hughes DJ, Kühl M, Pernice M, Voolstra CR (2021) Divergent expression of hypoxia response systems under deoxygenation in reef-forming corals aligns with bleaching susceptibility Glob Chang Biol 27:312-326 doi:10.1111/gcb.15436

Allgeier JE, Burkepile DE, Layman CA (2017) Animal pee in the sea: consumer-mediated nutrient dynamics in the world's changing oceans Glob Chang Biol 23:2166-2178 doi:10.1111/gcb.13625

Allgeier JE, Valdivia A, Cox C, Layman CA (2016) Fishing down nutrients on coral reefs Nat Commun 7:12461 doi:10.1038/ncomms12461

Álvarez-Romero JG et al. (2013) A novel approach to model exposure of coastal-marine ecosystems to riverine flood plumes based on remote sensing techniques J Environ Manage 119:194-207 doi:10.1016/j.jenvman.2013.01.036

Anderson KD, Cantin NE, Casey JM, Pratchett MS (2019) Independent effects of ocean warming versus acidification on the growth, survivorship and physiology of two *Acropora* corals Coral Reefs 38:1225-1240 doi:10.1007/s00338-019-01864-y

Anderson MJ, Walsh DCI (2013) PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecol Monogr 83:557-574 doi:10.1890/12-2010.1

Anthony KRN (2000) Enhanced particle-feeding capacity of corals on turbid reefs (Great Barrier Reef, Australia) Coral Reefs 19:59-67 doi:10.1007/s003380050227

Anthony KRN et al. (2017) New interventions are needed to save coral reefs Nat Ecol Evol 1:1420-1422 doi:10.1038/s41559-017-0313-5

Anthony KRN, Hoogenboom MO, Maynard JA, Grottoli AG, Middlebrook R (2009) Energetics approach to predicting mortality risk from environmental stress: a case study of coral bleaching Functional Ecology 23:539-550 doi:10.1111/j.1365-2435.2008.01531.x

Aranda M et al. (2016) Genomes of coral dinoflagellate symbionts highlight evolutionary adaptations conducive to a symbiotic lifestyle Sci Rep 6:39734 doi:10.1038/srep39734

Australian Institute of Marine Science (2021) Long-Term Monitoring Program - Annual summary report on coral reef condition for 2020/21

Avila-Magaña V et al. (2021) Elucidating gene expression adaptation of phylogenetically divergent coral holobionts under heat stress Nat Commun 12:5731 doi:10.1038/s41467-021-25950-4

Badgley BD, Lipschultz F, Sebens KP (2005) Nitrate uptake by the reef coral *Diploria strigosa*: effects of concentration, water flow, and irradiance Mar Biol 149:327-338 doi:10.1007/s00227-005-0180-5

Baghdasarian G, Muscatine L (2000) Preferential expulsion of dividing algal cells as a mechanism for regulating algal-cnidarian symbiosis Biol Bull 199:278-286 doi:10.2307/1543184

Baird AH, Bhagooli R, Ralph PJ, Takahashi S (2009) Coral bleaching: the role of the host Trends Ecol Evol 24:16-20 doi:10.1016/j.tree.2008.09.005

Baird AH, Marshall PA (2002) Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef Mar Ecol-Prog Ser 237:133-141

Baird ME et al. (2020) CSIRO Environmental Modelling Suite (EMS): Scientific description of the optical and biogeochemical models (vB3p0) Geosci Model Dev 13:4503-4553 doi:10.5194/gmd-13-4503-2020

Baker AC (2001) Reef corals bleach to survive change Nature 411:765-766 doi:10.1038/35081151

Baker AC (2003) Flexibility and specificity in coral-algal Symbiosis: Diversity, ecology, and biogeography of *Symbiodinium* Annual Review of Ecology, Evolution, and Systematics 34:661-689 doi:10.1146/annurev.ecolsys.34.011802.132417

Baker DM, Andras JP, Jordán-Garza AG, Fogel ML (2013) Nitrate competition in a coral symbiosis varies with temperature among *Symbiodinium* clades ISME J 7:1248-1251 doi:10.1038/ismej.2013.12

Baker DM, Freeman CJ, Wong JCY, Fogel ML, Knowlton N (2018) Climate change promotes parasitism in a coral symbiosis ISME J 12:921-930 doi:10.1038/s41396-018-0046-8

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR (2013) Genomic basis for coral resilience to climate change Proc Natl Acad Sci U S A 110:1387-1392 doi:10.1073/pnas.1210224110

Bartoń K (2013) MuMIn: Multi-model inference.

Bay LK, Doyle J, Logan M, Berkelmans R (2016) Recovery from bleaching is mediated by threshold densities of background thermo-tolerant symbiont types in a reef-building coral R Soc Open Sci 3:160322 doi:10.1098/rsos.160322

Bednarz VN, Grover R, Maguer JF, Fine M, Ferrier-Pagès C (2017) The assimilation of diazotroph-derived nitrogen by scleractinian corals depends on their metabolic status mBio 8 doi:10.1128/mBio.02058-16

Bednarz VN, Naumann MS, Cardini U, van Hoytema N, Rix L, Al-Rshaidat MMD, Wild C (2018) Contrasting seasonal responses in dinitrogen fixation between shallow and deep-water colonies of the model coral *Stylophora pistillata* in the northern Red Sea PLoS One 13:e0199022 doi:10.1371/journal.pone.0199022

Behrenfeld MJ et al. (2006) Climate-driven trends in contemporary ocean productivity Nature 444:752-755 doi:10.1038/nature05317

Bellantuono AJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012) Resistance to thermal stress in corals without changes in symbiont composition Proc Biol Sci 279:1100-1107 doi:10.1098/rspb.2011.1780

Beltrán VH et al. (2021) Physiological diversity among sympatric, conspecific endosymbionts of coral (*Cladocopium* C1acro) from the Great Barrier Reef Coral Reefs 40:985-997 doi:10.1007/s00338-021-02092-z

Benavides M, Bednarz VN, Ferrier-Pagès C (2017) Diazotrophs: Overlooked key players within the coral symbiosis and tropical reef ecosystems? Frontiers in Marine Science 4:10 doi:10.3389/fmars.2017.00010

Benjamini Y, Yekutieli D (2001) The control of the false discovery rate in multiple testing under dependency The Annals of Statistics 29:1165-1188, 1124

Béraud E, Gevaert F, Rottier C, Ferrier-Pagès C (2013) The response of the scleractinian coral *Turbinaria reniformis* to thermal stress depends on the nitrogen status of the coral holobiont J Exp Biol 216:2665-2674 doi:10.1242/jeb.085183

Berkelmans R, De'ath G, Kininmonth S, Skirving WJ (2004) A comparison of the 1998 and 2002 coral bleaching events on the Great Barrier Reef: Spatial correlation, patterns, and predictions Coral Reefs 23:74-83 doi:10.1007/s00338-003-0353-y

Berkelmans R, Oliver JK (1999) Large-scale bleaching of corals on the Great Barrier Reef Coral Reefs 18:55-60 doi:10.1007/s003380050154

Berkelmans R, van Oppen MJ (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change Proc Biol Sci 273:2305-2312 doi:10.1098/rspb.2006.3567

Berkelmans R, Weeks SJ, Steinberg CR (2010) Upwelling linked to warm summers and bleaching on the Great Barrier Reef Limnol Oceanogr 55:2634-2644 doi:10.4319/lo.2010.55.6.2634

Bhagooli R, Hidaka M (2004) Release of zooxanthellae with intact photosynthetic activity by the coral *Galaxea fascicularis* in response to high temperature stress Mar Biol 145:329-337 doi:10.1007/s00227-004-1309-7

Blackstone NW, Golladay JM (2018) Why do corals bleach? Conflict and conflict mediation in a host/symbiont community BioEssays 40:1800021

Blanckaert ACA, Fernandes de Barros Marangoni L, Rottier C, Grover R, Ferrier-Pagès C (2021) Low levels of ultra-violet radiation mitigate the deleterious effects of nitrate and thermal stress on coral photosynthesis Mar Pollut Bull 167:112257 doi:10.1016/j.marpolbul.2021.112257

Blanckaert ACA, Reef R, Pandolfi JM, Lovelock CE (2020) Variation in the elemental stoichiometry of the coral–zooxanthellae symbiosis Coral Reefs 39:1071-1079 doi:10.1007/s00338-020-01932-8

Bourne DG, Iida Y, Uthicke S, Smith-Keune C (2008) Changes in coral-associated microbial communities during a bleaching event ISME J 2:350-363 doi:10.1038/ismej.2007.112

Bourne DG, Morrow KM, Webster NS (2016) Insights into the coral microbiome: Underpinning the health and resilience of reef ecosystems Annu Rev Microbiol 70:317-340 doi:10.1146/annurev-micro-102215-095440

Brandl SJ et al. (2019) Demographic dynamics of the smallest marine vertebrates fuel coral reef ecosystem functioning Science 364:1189-1192 doi:10.1126/science.aav3384

Brodie J, Wolanski E, Lewis S, Bainbridge Z (2012) An assessment of residence times of land-sourced contaminants in the Great Barrier Reef lagoon and the implications for management and reef recovery Mar Pollut Bull 65:267-279 doi:10.1016/j.marpolbul.2011.12.011

Bronstein JL (2009) The evolution of facilitation and mutualism Journal of Ecology 97:1160-1170 doi:10.1111/j.1365-2745.2009.01566.x

Brooks ME et al. (2017) glmmTMB balances speed and flexibility among packages for zero-inflated Generalized Linear Mixed Modeling The R Journal 9:378-400

Bruno JF, Petes LE, Drew Harvell C, Hettinger A (2003) Nutrient enrichment can increase the severity of coral diseases Ecology Letters 6:1056-1061 doi:10.1046/j.1461-0248.2003.00544.x

Burkepile DE et al. (2019) Nitrogen identity drives differential impacts of nutrients on coral bleaching and mortality Ecosystems 23:798-811 doi:10.1007/s10021-019-00433-2

Bürkner P-C (2017) brms: An R Package for Bayesian Multilevel Models Using Stan Journal of Statistical Software 80:28 doi:10.18637/jss.v080.i01

Bürkner P-C (2018) Advanced Bayesian multilevel modeling with the R package brms The R Journal 10:395-411

Cai L et al. (2018) Season structures prokaryotic partners but not algal symbionts in subtropical hard corals Appl Microbiol Biotechnol 102:4963-4973 doi:10.1007/s00253-018-8909-5

Camp EF, Edmondson J, Doheny A, Rumney J, Grima AJ, Huete A, Suggett DJ (2019) Mangrove lagoons of the Great Barrier Reef support coral populations persisting under extreme environmental conditions Mar Ecol-Prog Ser 625:1-14

Camp EF, Schoepf V, Mumby PJ, Hardtke LA, Rodolfo-Metalpa R, Smith DJ, Suggett DJ (2018) The future of coral reefs subject to rapid climate change: Lessons from natural extreme environments Frontiers in Marine Science 5 doi:10.3389/fmars.2018.00004

Cantin NE et al. (2021) Assessing the linkages between water quality and coral bleaching on the Great Barrier Reef Report to the National Environmental Science Program Reef and Rainforest Research Centre Limited, Cairns:158

Cantin NE, van Oppen MJH, Willis BL, Mieog JC, Negri AP (2009) Juvenile corals can acquire more carbon from high-performance algal symbionts Coral Reefs 28:405-414 doi:10.1007/s00338-009-0478-8

Cardini U et al. (2015) Functional significance of dinitrogen fixation in sustaining coral productivity under oligotrophic conditions Proc Biol Sci 282:20152257 doi:10.1098/rspb.2015.2257

Cardini U, van Hoytema N, Bednarz VN, Rix L, Foster RA, Al-Rshaidat MM, Wild C (2016) Microbial dinitrogen fixation in coral holobionts exposed to thermal stress and bleaching Environ Microbiol 18:2620-2633 doi:10.1111/1462-2920.13385

Carpenter B et al. (2017) Stan: a probabilistic programming language Journal of Statistical Software 76:32 doi:10.18637/jss.v076.i01

Chan WY, Peplow LM, Menéndez P, Hoffmann AA, van Oppen MJH (2019) The roles of age, parentage and environment on bacterial and algal endosymbiont communities in *Acropora* corals Mol Ecol 28:3830-3843 doi:10.1111/mec.15187

Chumun PK, Casareto BE, Higuchi T, Irikawa A, Bhagooli R, Ishikawa Y, Suzuki Y (2013) High nitrate levels exacerbate thermal photo-physiological stress of zooxanthellae in the reef-building coral *Pocillopora damicornis* Eco-Engineering 25:75-83 doi:10.11450/seitaikogaku.25.75

Cinner JE et al. (2018) Gravity of human impacts mediates coral reef conservation gains Proc Natl Acad Sci U S A 115:E6116-E6125 doi:10.1073/pnas.1708001115

Claar DC et al. (2020) Dynamic symbioses reveal pathways to coral survival through prolonged heatwaves Nat Commun 11:6097 doi:10.1038/s41467-020-19169-y

Cleves PA, Shumaker A, Lee J, Putnam HM, Bhattacharya D (2020) Unknown to known: Advancing knowledge of coral gene function Trends Genet 36:93-104 doi:10.1016/j.tig.2019.11.001

Commonwealth of Australia and State of Queensland (2018) Reef 2050 Water Quality Improvement Plan 2017- 2022 Reef Water Quality Protection Plan Secretariat, Brisbane

Condie SA et al. (2021) Large-scale interventions may delay decline of the Great Barrier Reef R Soc Open Sci 8:201296 doi:10.1098/rsos.201296

Conlan JA, Bay LK, Severati A, Humphrey C, Francis DS (2018) Comparing the capacity of five different dietary treatments to optimise growth and nutritional composition in two scleractinian corals PLoS One 13:e0207956 doi:10.1371/journal.pone.0207956

Connolly SR, Lopez-Yglesias MA, Anthony KRN (2012) Food availability promotes rapid recovery from thermal stress in a scleractinian coral Coral Reefs 31:951-960 doi:10.1007/s00338-012-0925-9

Conti-Jerpe IE, Thompson PD, Wong CWM, Oliveira NL, Duprey NN, Moynihan MA, Baker DM (2020) Trophic strategy and bleaching resistance in reef-building corals Sci Adv 6:eaaz5443 doi:10.1126/sciadv.aaz5443

Cooke I et al. (2020) Genomic signatures in the coral holobiont reveal host adaptations driven by Holocene climate change and reef specific symbionts Sci Adv 6:eabc6318 doi:10.1126/sciadv.abc6318

Cooper TF et al. (2011) Environmental factors controlling the distribution of *Symbiodinium* harboured by the coral *Acropora millepora* on the Great Barrier Reef PLoS One 6:e25536 doi:10.1371/journal.pone.0025536

Courtial L, Planas Bielsa V, Houlbrèque F, Ferrier-Pagès C (2018) Effects of ultraviolet radiation and nutrient level on the physiological response and organic matter release of the scleractinian coral *Pocillopora damicornis* following thermal stress PLoS One 13:e0205261 doi:10.1371/journal.pone.0205261

Crossland CJ, Hatcher BG, Smith SV (1991) Role of coral reefs in global ocean production Coral Reefs 10:55-64 doi:10.1007/bf00571824

Cui G et al. (2019) Host-dependent nitrogen recycling as a mechanism of symbiont control in Aiptasia PLoS Genet 15:e1008189 doi:10.1371/journal.pgen.1008189

Cunning R, Baker AC (2013) Excess algal symbionts increase the susceptibility of reef corals to bleaching Nat Clim Change 3:259-262 doi:10.1038/nclimate1711

Cunning R, Baker AC (2014) Not just who, but how many: The importance of partner abundance in reef coral symbioses Front Microbiol 5:400 doi:10.3389/fmicb.2014.00400

Cunning R, Gillette P, Capo T, Galvez K, Baker AC (2015a) Growth tradeoffs associated with thermotolerant symbionts in the coral *Pocillopora damicornis* are lost in warmer oceans Coral Reefs 34:155-160 doi:10.1007/s00338-014-1216-4

Cunning R, Muller EB, Gates RD, Nisbet RM (2017a) A dynamic bioenergetic model for coral-*Symbiodinium* symbioses and coral bleaching as an alternate stable state J Theor Biol 431:49-62 doi:10.1016/j.jtbi.2017.08.003

Cunning R, Silverstein RN, Baker AC (2015b) Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change Proc Biol Sci 282:20141725 doi:10.1098/rspb.2014.1725

Cunning R, Silverstein RN, Baker AC (2017b) Symbiont shuffling linked to differential photochemical dynamics of *Symbiodinium* in three Caribbean reef corals Coral Reefs 37:145-152 doi:10.1007/s00338-017-1640-3

D'Angelo C, Hume BC, Burt J, Smith EG, Achterberg EP, Wiedenmann J (2015) Local adaptation constrains the distribution potential of heat-tolerant *Symbiodinium* from the Persian/Arabian Gulf ISME J 9:2551-2560 doi:10.1038/ismej.2015.80

D'Angelo C, Wiedenmann J (2014) Impacts of nutrient enrichment on coral reefs: new perspectives and implications for coastal management and reef survival Curr Opin Env Sust 7:82-93 doi:10.1016/j.cosust.2013.11.029

Damjanovic K, Menéndez P, Blackall LL, van Oppen MJH (2019) Bacterial and algal symbiont dynamics in early recruits exposed to two adult coral species Coral Reefs 39:189-202 doi:10.1007/s00338-019-01871-z

Damjanovic K, Menéndez P, Blackall LL, van Oppen MJH (2020) Mixed-mode bacterial transmission in the common brooding coral *Pocillopora acuta* Environ Microbiol 22:397-412 doi:10.1111/1462-2920.14856

Davies SW, Marchetti A, Ries JB, Castillo KD (2016) Thermal and pCO_2 stress elicit divergent transcriptomic responses in a resilient coral Frontiers in Marine Science 3 doi:10.3389/fmars.2016.00112

Davy SK, Allemand D, Weis VM (2012) Cell biology of cnidarian-dinoflagellate symbiosis Microbiol Mol Biol Rev 76:229-261 doi:10.1128/MMBR.05014-11

De'ath G, Fabricius K (2010) Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef Ecol Appl 20:840-850 doi:10.1890/08-2023.1

de Goeij JM, van Oevelen D, Vermeij MJ, Osinga R, Middelburg JJ, de Goeij AF, Admiraal W (2013) Surviving in a marine desert: the sponge loop retains resources within coral reefs Science 342:108-110 doi:10.1126/science.1241981

DeCarlo TM et al. (2020) Nutrient-supplying ocean currents modulate coral bleaching susceptibility Sci Adv 6:eabc5493 doi:10.1126/sciadv.abc5493

DeCarlo TM, Harrison HB (2019) An enigmatic decoupling between heat stress and coral bleaching on the Great Barrier Reef PeerJ 7:e7473 doi:10.7717/peerj.7473

Devlin MJ, McKinna LW, Alvarez-Romero JG, Petus C, Abott B, Harkness P, Brodie J (2012) Mapping the pollutants in surface riverine flood plume waters in the Great Barrier Reef, Australia Mar Pollut Bull 65:224-235 doi:10.1016/j.marpolbul.2012.03.001

Diaz JM et al. (2016) Species-specific control of external superoxide levels by the coral holobiont during a natural bleaching event Nat Commun 7:13801 doi:10.1038/ncomms13801

Dietzel A, Bode M, Connolly SR, Hughes TP (2020) Long-term shifts in the colony size structure of coral populations along the Great Barrier Reef Proc Biol Sci 287:20201432 doi:10.1098/rspb.2020.1432

DiPerna S, Hoogenboom M, Noonan S, Fabricius K (2018) Effects of variability in daily light integrals on the photophysiology of the corals *Pachyseris speciosa* and *Acropora millepora* PLoS One 13:e0203882 doi:10.1371/journal.pone.0203882

Dixon GB, Abbott E, Matz M (2020) Meta-analysis of the coral environmental stress response: *Acropora* corals show opposing responses depending on stress intensity Mol Ecol 29:2855-2870 doi:10.1111/mec.15535

Dixon GB, Davies SW, Aglyamova GA, Meyer E, Bay LK, Matz MV (2015) Genomic determinants of coral heat tolerance across latitudes Science 348:1460-1462 doi:10.1126/science.1261224

Dobson KL et al. (2021) Moderate nutrient concentrations are not detrimental to corals under future ocean conditions Mar Biol 168:98 doi:10.1007/s00227-021-03901-3

Donovan MK et al. (2020) Nitrogen pollution interacts with heat stress to increase coral bleaching across the seascape Proc Natl Acad Sci U S A 117:5351-5357 doi:10.1073/pnas.1915395117

Donovan MK et al. (2021) Local conditions magnify coral loss after marine heatwaves Science 372:977-980 doi:10.1126/science.abd9464

Dove S et al. (2006) Response of holosymbiont pigments from the scleractinian coral *Montipora monasteriata* to short-term heat stress Limnol Oceanogr 51:1149-1158 doi:10.4319/lo.2006.51.2.1149

Eddy TD et al. (2021) Global decline in capacity of coral reefs to provide ecosystem services One Earth 4:1278-1285 doi:10.1016/j.oneear.2021.08.016

Enríquez S, Méndez ER, Iglesias-Prieto R (2005) Multiple scattering on coral skeletons enhances light absorption by symbiotic algae Limnol Oceanogr 50:1025-1032 doi:10.4319/lo.2005.50.4.1025

Epstein HE, Smith HA, Cantin NE, Mocellin VJL, Torda G, van Oppen MJH (2019) Temporal variation in the microbiome of *Acropora* coral species does not reflect seasonality Front Microbiol 10:1775 doi:10.3389/fmicb.2019.01775

Ezzat L, Maguer JF, Grover R, Ferrier-Pagès C (2015) New insights into carbon acquisition and exchanges within the coral-dinoflagellate symbiosis under NH_4^+ and NO_3^- supply Proc Biol Sci 282:20150610 doi:10.1098/rspb.2015.0610

Ezzat L, Maguer JF, Grover R, Ferrier-Pagès C (2016a) Limited phosphorus availability is the Achilles heel of tropical reef corals in a warming ocean Sci Rep 6:31768 doi:10.1038/srep31768

Ezzat L, Maguer JF, Grover R, Rottier C, Tremblay P, Ferrier-Pagès C (2019) Nutrient starvation impairs the trophic plasticity of reef-building corals under ocean warming Functional Ecology 33:643-653 doi:10.1111/1365-2435.13285

Ezzat L, Towle E, Irisson J-O, Langdon C, Ferrier-Pagès C (2016b) The relationship between heterotrophic feeding and inorganic nutrient availability in the scleractinian coral *T. reniformis* under a short-term temperature increase Limnol Oceanogr 61:89-102 doi:10.1002/lno.10200

Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis Mar Pollut Bull 50:125-146 doi:10.1016/j.marpolbul.2004.11.028

Fabricius KE (2006) Effects of irradiance, flow, and colony pigmentation on the temperature microenvironment around corals: Implications for coral bleaching? Limnol Oceanogr 51:30-37 doi:10.4319/lo.2006.51.1.0030

Fabricius KE, Cseke S, Humphrey C, De'ath G (2013) Does trophic status enhance or reduce the thermal tolerance of scleractinian corals? A review, experiment and conceptual framework PLoS One 8:e54399 doi:10.1371/journal.pone.0054399

Fabricius KE, De'ath G, McCook L, Turak E, Williams DM (2005) Changes in algal, coral and fish assemblages along water quality gradients on the inshore Great Barrier Reef Mar Pollut Bull 51:384-398 doi:10.1016/j.marpolbul.2004.10.041

Falkowski PG, Dubinsky Z, Muscatine L, McCloskey LR (1993) Population control in symbiotic corals BioScience 43:606-611 doi:10.2307/1312147

Faxneld S, Jörgensen TL, Tedengren M (2010) Effects of elevated water temperature, reduced salinity and nutrient enrichment on the metabolism of the coral *Turbinaria mesenterina* Estuarine, Coastal and Shelf Science 88:482-487 doi:10.1016/j.ecss.2010.05.008

Fernandes de Barros Marangoni L, Ferrier-Pages C, Rottier C, Bianchini A, Grover R (2020) Unravelling the different causes of nitrate and ammonium effects on coral bleaching Sci Rep 10:11975 doi:10.1038/s41598-020-68916-0

Ferrario F, Beck MW, Storlazzi CD, Micheli F, Shepard CC, Airoldi L (2014) The effectiveness of coral reefs for coastal hazard risk reduction and adaptation Nat Commun 5:3794 doi:10.1038/ncomms4794

Ferrier-Pagès C, Godinot C, D'Angelo C, Wiedenmann J, Grover R (2016) Phosphorus metabolism of reef organisms with algal symbionts Ecol Monogr 86:262-277 doi:10.1002/ecm.1217

Ferrier-Pagès C, Sauzeat L, Balter V (2018) Coral bleaching is linked to the capacity of the animal host to supply essential metals to the symbionts Glob Chang Biol 24:3145-3157 doi:10.1111/gcb.14141

Ferrier-Pagès C, Schoelzke V, Jaubert J, Muscatine L, Hoegh-Guldberg O (2001) Response of a scleractinian coral, *Stylophora pistillata*, to iron and nitrate enrichment J Exp Mar Biol Ecol 259:249-261 doi:10.1016/s0022-0981(01)00241-6

Fine M et al. (2019) Coral reefs of the Red Sea — Challenges and potential solutions Regional Studies in Marine Science 25:100498 doi:10.1016/j.rsma.2018.100498

Fine M, Gildor H, Genin A (2013) A coral reef refuge in the Red Sea Glob Chang Biol 19:3640-3647 doi:10.1111/gcb.12356

Fisher R, O'Leary RA, Low-Choy S, Mengersen K, Knowlton N, Brainard RE, Caley MJ (2015) Species richness on coral reefs and the pursuit of convergent global estimates Curr Biol 25:500-505 doi:10.1016/j.cub.2014.12.022

Fordyce AJ, Ainsworth TD, Heron SF, Leggat W (2019) Marine heatwave hotspots in coral reef environments: physical drivers, ecophysiological outcomes, and impact upon structural complexity Frontiers in Marine Science 6 doi:10.3389/fmars.2019.00498

Fox MD et al. (2021) Differential resistance and acclimation of two coral species to chronic nutrient enrichment reflect life-history traits Functional Ecology 35:1081-1093 doi:10.1111/1365-2435.13780

Fox MD et al. (2018) Gradients in primary production predict trophic strategies of mixotrophic corals across spatial scales Curr Biol 28:3355-3363 e3354 doi:10.1016/j.cub.2018.08.057

Fuller ZL et al. (2020) Population genetics of the coral *Acropora millepora*: Toward genomic prediction of bleaching Science 369:eaba4674 doi:10.1126/science.aba4674

Gabay Y, Weis VM, Davy SK (2018) Symbiont identity influences patterns of symbiosis establishment, host growth, and asexual reproduction in a model cnidarian-dinoflagellate symbiosis Biol Bull 234:1-10 doi:10.1086/696365

Ganot P, Moya A, Magnone V, Allemand D, Furla P, Sabourault C (2011) Adaptations to endosymbiosis in a cnidarian-dinoflagellate association: Differential gene expression and specific gene duplications PLoS Genet 7:e1002187 doi:10.1371/journal.pgen.1002187

Gardner SG et al. (2019) Coral microbiome diversity reflects mass coral bleaching susceptibility during the 2016 El Nino heat wave Ecol Evol 9:938-956 doi:10.1002/ece3.4662

Gibbin EM, Krueger T, Putnam HM, Barott KL, Bodin J, Gates RD, Meibom A (2018) Short-term thermal acclimation modifies the metabolic condition of the coral holobiont Frontiers in Marine Science 5:10 doi:10.3389/fmars.2018.00010

Gintert BE, Manzello DP, Enochs IC, Kolodziej G, Carlton R, Gleason ACR, Gracias N (2018) Marked annual coral bleaching resilience of an inshore patch reef in the Florida Keys: A nugget of hope, aberrance, or last man standing? Coral Reefs 37:533-547 doi:10.1007/s00338-018-1678-x

Godinot C, Houlbrèque F, Grover R, Ferrier-Pagès C (2011) Coral uptake of inorganic phosphorus and nitrogen negatively affected by simultaneous changes in temperature and pH PLoS One 6:e25024 doi:10.1371/journal.pone.0025024

Goiran C, Al-Moghrabi S, Allemand D, Jaubert J (1996) Inorganic carbon uptake for photosynthesis by the symbiotic coral/dinoflagellate association I. Photosynthetic performances of symbionts and dependence on sea water bicarbonate J Exp Mar Biol Ecol 199:207-225 doi:10.1016/0022-0981(95)00201-4

Goulet TL, Cook CB, Goulet D (2005) Effect of short-term exposure to elevated temperatures and light levels on photosynthesis of different host-symbiont combinations in the *Aiptasia pallida*/*Symbiodinium* symbiosis Limnol Oceanogr 50:1490-1498

Gove JM et al. (2016) Near-island biological hotspots in barren ocean basins Nat Commun 7:10581 doi:10.1038/ncomms10581

Graham NA, Jennings S, MacNeil MA, Mouillot D, Wilson SK (2015) Predicting climate-driven regime shifts versus rebound potential in coral reefs Nature 518:94-97 doi:10.1038/nature14140

Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals Nature 440:1186-1189 doi:10.1038/nature04565

Grottoli AG et al. (2014) The cumulative impact of annual coral bleaching can turn some coral species winners into losers Glob Chang Biol 20:3823-3833 doi:10.1111/gcb.12658

Grover R, Maguer JF, Allemand D, Ferrier-Pagès C (2003) Nitrate uptake in the scleractinian coral *Stylophora pistillata* Limnol Oceanogr 48:2266-2274 doi:10.4319/lo.2003.48.6.2266

Gruber R et al. (2019) Marine Monitoring Program: Annual report for inshore water quality monitoring 2017-18

Guest JR et al. (2016) Coral community response to bleaching on a highly disturbed reef Sci Rep 6:20717 doi:10.1038/srep20717

Hall ER, Muller EM, Goulet T, Bellworthy J, Ritchie KB, Fine M (2018) Eutrophication may compromise the resilience of the Red Sea coral *Stylophora pistillata* to global change Mar Pollut Bull 131:701-711 doi:10.1016/j.marpolbul.2018.04.067

Hartig F (2021) DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models

Herfort L, Thake B, Taubner I (2008) Bicarbonate stimulation of calcification and photosynthesis in two hermatypic corals J Phycol 44:91-98 doi:10.1111/j.1529-8817.2007.00445.x

Higuchi T, Yuyama I, Nakamura T (2015) The combined effects of nitrate with high temperature and high light intensity on coral bleaching and antioxidant enzyme activities Regional Studies in Marine Science 2:27-31 doi:10.1016/j.rsma.2015.08.012

Hill R, Ralph PJ (2007) Post-bleaching viability of expelled zooxanthellae from the scleractinian coral *Pocillopora damicornis* Mar Ecol-Prog Ser 352:137-144 doi:10.3354/meps07159

Hillyer KE, Dias DA, Lutz A, Wilkinson SP, Roessner U, Davy SK (2016a) Metabolite profiling of symbiont and host during thermal stress and bleaching in the coral *Acropora aspera* Coral Reefs 36:105-118 doi:10.1007/s00338-016-1508-y

Hillyer KE, Tumanov S, Villas-Bôas S, Davy SK (2016b) Metabolite profiling of symbiont and host during thermal stress and bleaching in a model cnidarian–dinoflagellate symbiosis J Exp Biol 219:516-527 doi:10.1242/jeb.128660

Hoadley KD et al. (2016) High-temperature acclimation strategies within the thermally tolerant endosymbiont *Symbiodinium trenchii* and its coral host, *Turbinaria reniformis*, differ with changing pCO_2 and nutrients Mar Biol 163:134 doi:10.1007/s00227-016-2909-8

Hoadley KD et al. (2015) Physiological response to elevated temperature and pCO_2 varies across four Pacific coral species: Understanding the unique host+symbiont response Sci Rep 5:18371 doi:10.1038/srep18371

Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs Mar Freshwater Res 50:839-866 doi:10.1071/mf99078

Hoegh-Guldberg O, Smith GJ (1989) Influence of the population density of zooxanthellae and supply of ammonium on the biomass and metabolic characteristics of the reef corals *Seriatopora hystrix* and *Stylophora pistillata* Mar Ecol-Prog Ser 57:173-186 doi:10.3354/meps057173

Hoey AS et al. (2016) Recent advances in understanding the effects of climate change on coral reefs Diversity 8:12 doi:10.3390/d8020012

Hoogenboom M, Béraud E, Ferrier-Pagès C (2010) Relationship between symbiont density and photosynthetic carbon acquisition in the temperate coral *Cladocora caespitosa* Coral Reefs 29:21-29 doi:10.1007/s00338-009-0558-9

Houlbrèque F, Ferrier-Pagès C (2009) Heterotrophy in tropical scleractinian corals Biol Rev 84:1-17 doi:10.1111/j.1469-185X.2008.00058.x

Howe-Kerr LI, Bachelot B, Wright RM, Kenkel CD, Bay LK, Correa AMS (2020) Symbiont community diversity is more variable in corals that respond poorly to stress Glob Chang Biol 26:2220-2234 doi:10.1111/gcb.14999

Howells EJ, Bauman AG, Vaughan GO, Hume BCC, Voolstra CR, Burt JA (2020) Corals in the hottest reefs in the world exhibit symbiont fidelity not flexibility Mol Ecol 29:899-911 doi:10.1111/mec.15372

Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH (2011) Coral thermal tolerance shaped by local adaptation of photosymbionts Nat Clim Change 2:116-120 doi:10.1038/nclimate1330

Howells EJ, Willis BL, Bay LK, van Oppen MJ (2013) Spatial and temporal genetic structure of *Symbiodinium* populations within a common reef-building coral on the Great Barrier Reef Mol Ecol 22:3693-3708 doi:10.1111/mec.12342

Hughes AD, Grottoli AG, Pease TK, Matsui Y (2010) Acquisition and assimilation of carbon in non-bleached and bleached corals Mar Ecol-Prog Ser 420:91-101 doi:10.3354/meps08866

Hughes TP et al. (2018a) Spatial and temporal patterns of mass bleaching of corals in the Anthropocene Science 359:80-83 doi:10.1126/science.aan8048

Hughes TP et al. (2017a) Coral reefs in the Anthropocene Nature 546:82-90 doi:10.1038/nature22901

Hughes TP et al. (2017b) Global warming and recurrent mass bleaching of corals Nature 543:373-377 doi:10.1038/nature21707

Hughes TP et al. (2018b) Global warming transforms coral reef assemblages Nature 556:492-496 doi:10.1038/s41586-018-0041-2

Hughes TP et al. (2021) Emergent properties in the responses of tropical corals to recurrent climate extremes Current Biology doi:10.1016/j.cub.2021.10.046

Hughes TP et al. (2019) Ecological memory modifies the cumulative impact of recurrent climate extremes Nat Clim Change 9:40-43 doi:10.1038/s41558-018-0351-2

Hume BCC, D'Angelo C, Smith EG, Stevens JR, Burt J, Wiedenmann J (2015) *Symbiodinium thermophilum* sp. nov., a thermotolerant symbiotic alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf Sci Rep 5 doi:10.1038/srep08562

Hume BCC et al. (2019) SymPortal: A novel analytical framework and platform for coral algal symbiont next-generation sequencing ITS2 profiling Mol Ecol Resour 19:1063-1080 doi:10.1111/1755-0998.13004

International Coral Reef Initiative, Global Coral Reef Monitoring Network, Australian Institute of Marine Science, United Nations Environment Programme (2021) Status of Coral Reefs of the World 2020

Jin YK, Kininmonth S, Lundgren PB, van Oppen MJH, Willis BL (2019) Predicting the spatial distribution of allele frequencies for a gene associated with tolerance to eutrophication and high temperature in the reef-building coral, *Acropora millepora*, on the Great Barrier Reef Coral Reefs 39:147-158 doi:10.1007/s00338-019-01873-x

Jin YK et al. (2016) Genetic markers for antioxidant capacity in a reef-building coral Sci Adv 2:e1500842 doi:10.1126/sciadv.1500842

Jones AM, Berkelmans R (2010) Potential costs of acclimatization to a warmer climate: Growth of a reef coral with heat tolerant vs. sensitive symbiont types PLoS One 5:e10437 doi:10.1371/journal.pone.0010437

Jones AM, Berkelmans R (2011) Tradeoffs to thermal acclimation: Energetics and reproduction of a reef coral with heat tolerant *Symbiodinium* type-D Journal of Marine Biology 2011:185890 doi:10.1155/2011/185890

Jones AM, Berkelmans R (2012) The photokinetics of thermo-tolerance in *Symbiodinium* Marine Ecology 33:490-498 doi:10.1111/j.1439-0485.2012.00514.x

Jones AM, Berkelmans R, van Oppen MJ, Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: Field evidence of acclimatization Proc Biol Sci 275:1359-1365 doi:10.1098/rspb.2008.0069

Jones RJ, Giofre N, Luter HM, Neoh TL, Fisher R, Duckworth A (2020) Responses of corals to chronic turbidity Sci Rep 10:4762 doi:10.1038/s41598-020-61712-w

Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature-induced bleaching of corals begins with impairment of the CO_2 fixation mechanism in zooxanthellae Plant, Cell and Environment 21:1219-1230 doi:10.1046/j.1365-3040.1998.00345.x

Kemp DW, Hernandez-Pech X, Iglesias-Prieto R, Fitt WK, Schmidt GW (2014) Community dynamics and physiology of *Symbiodinium* spp. before, during, and after a coral bleaching event Limnol Oceanogr 59:788-797 doi:10.4319/lo.2014.59.3.0788

Kenkel CD, Almanza AT, Matz MV (2015) Fine-scale environmental specialization of reef-building corals might be limiting reef recovery in the Florida Keys Ecology 96:3197-3212 doi:10.1890/14-2297.1

Kenkel CD, Bay LK (2018) Exploring mechanisms that affect coral cooperation: symbiont transmission mode, cell density and community composition PeerJ 6:e6047 doi:10.7717/peerj.6047

Kenkel CD, Goodbody-Gringley G, Caillaud D, Davies SW, Bartels E, Matz MV (2013a) Evidence for a host role in thermotolerance divergence between populations of the mustard hill coral (*Porites astreoides*) from different reef environments Mol Ecol 22:4335-4348 doi:10.1111/mec.12391

Kenkel CD, Matz MV (2016) Gene expression plasticity as a mechanism of coral adaptation to a variable environment Nat Ecol Evol 1:14 doi:10.1038/s41559-016-0014

Kenkel CD, Meyer E, Matz MV (2013b) Gene expression under chronic heat stress in populations of the mustard hill coral (*Porites astreoides*) from different thermal environments Mol Ecol 22:4322-4334 doi:10.1111/mec.12390

Klueter A, Trapani J, Archer FI, McIlroy SE, Coffroth MA (2017) Comparative growth rates of cultured marine dinoflagellates in the genus *Symbiodinium* and the effects of temperature and light PLoS One 12:e0187707 doi:10.1371/journal.pone.0187707

Knowlton NC, E. et al. (2021) Rebuilding coral reefs: a decadal grand challenge International Coral Reef Society and Future Earth Coasts:56 doi:10.53642/NRKY9386

Knutson TR et al. (2010) Tropical cyclones and climate change Nature Geoscience 3:157-163 doi:10.1038/ngeo779

Kochman N-R, Grover R, Rottier C, Ferrier-Pagès C, Fine M (2021) The reef building coral *Stylophora pistillata* uses stored carbohydrates to maintain ATP levels under thermal stress Coral Reefs 40:1473-1485 doi:10.1007/s00338-021-02174-y

Koop K et al. (2001) ENCORE: the effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions Mar Pollut Bull 42:91-120 doi:10.1016/s0025-326x(00)00181-8

Kroon FJ et al. (2012) River loads of suspended solids, nitrogen, phosphorus and herbicides delivered to the Great Barrier Reef lagoon Mar Pollut Bull 65:167-181 doi:10.1016/j.marpolbul.2011.10.018

Krueger T et al. (2018) Temperature and feeding induce tissue level changes in autotrophic and heterotrophic nutrient allocation in the coral symbiosis - A NanoSIMS study Sci Rep 8:12710 doi:10.1038/s41598-018-31094-1

Krueger T, Horwitz N, Bodin J, Giovani ME, Escrig S, Fine M, Meibom A (2020) Intracellular competition for nitrogen controls dinoflagellate population density in corals Proc Biol Sci 287:20200049 doi:10.1098/rspb.2020.0049

Krueger T, Horwitz N, Bodin J, Giovani ME, Escrig S, Meibom A, Fine M (2017) Common reef-building coral in the Northern Red Sea resistant to elevated temperature and acidification R Soc Open Sci 4:170038 doi:10.1098/rsos.170038

LaJeunesse TC et al. (2004) Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients Mar Ecol-Prog Ser 284:147-161

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts Curr Biol 28:2570-2580.e2576 doi:10.1016/j.cub.2018.07.008

Lee STM, Keshavmurthy S, Fontana S, Takuma M, Chou WH, Chen CA (2018) Transcriptomic response in *Acropora muricata* under acute temperature stress follows preconditioned seasonal temperature fluctuations BMC Res Notes 11:119 doi:10.1186/s13104-018-3230-z

Lenth R (2021) emmeans: Estimated Marginal Means, aka Least-Squares Means

Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures Coral Reefs 16:187-192 doi:10.1007/s003380050073

Lesser MP (2021) Eutrophication on coral reefs: What is the evidence for phase shifts, nutrient limitation and coral bleaching BioScience doi:10.1093/biosci/biab101

Levas S, Schoepf V, Warner ME, Aschaffenburg M, Baumann JH, Grottoli AG (2018) Long-term recovery of Caribbean corals from bleaching J Exp Mar Biol Ecol 506:124-134 doi:10.1016/j.jembe.2018.06.003

Lewis SE et al. (2021) Land use change in the river basins of the Great Barrier Reef, 1860 to 2019: A foundation for understanding environmental history across the catchment to reef continuum Mar Pollut Bull 166:112193 doi:10.1016/j.marpolbul.2021.112193
Li J, Long L, Zou Y, Zhang S (2021) Microbial community and transcriptional responses to increased temperatures in coral *Pocillopora damicornis* holobiont Environ Microbiol 23:826-843 doi:10.1111/1462-2920.15168
Li M, Shi X, Guo C, Lin S (2016) Phosphorus deficiency inhibits cell division but not growth in the dinoflagellate *Amphidinium carterae* Front Microbiol 7:826
Lim C-S, Bachok Z, Hii Y-S (2017) Effects of supplementary polyunsaturated fatty acids on the health of the scleractinian coral *Galaxea fascicularis* (Linnaeus, 1767) J Exp Mar Biol Ecol 491:1-8 doi:10.1016/j.jembe.2017.02.009
Lin S et al. (2015) The *Symbiodinium kawagutii* genome illuminates dinoflagellate gene expression and coral symbiosis Science 350:691-694 doi:10.1126/science.aad0408
Little AF, van Oppen MJH, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals Science 304:1492-1494 doi:10.1126/science.1095733
Littman RA, Willis BL, Bourne DG (2011) Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef Environ Microbiol Rep 3:651-660 doi:10.1111/j.1758-2229.2010.00234.x
Liu G et al. (2014) Reef-scale thermal stress monitoring of coral ecosystems: New 5-km global products from NOAA Coral Reef Watch Remote Sensing 6:11579-11606
Liu G, Strong AE, Skirving W (2003) Remote sensing of sea surface temperatures during 2002 Barrier Reef coral bleaching Eos, Transactions American Geophysical Union 84:137-141 doi:10.1029/2003eo150001
Logan CA, Dunne JP, Ryan JS, Baskett ML, Donner SD (2021) Quantifying global potential for coral evolutionary response to climate change Nat Clim Change 11:537-542 doi:10.1038/s41558-021-01037-2
Lough JM, Anderson KD, Hughes TP (2018) Increasing thermal stress for tropical coral reefs: 1871-2017 Sci Rep 8:6079 doi:10.1038/s41598-018-24530-9
Lough JM, Lewis SE, Cantin NE (2015) Freshwater impacts in the central Great Barrier Reef: 1648–2011 Coral Reefs 34:739-751 doi:10.1007/s00338-015-1297-8
Loya Y, Sakai K, Yamazato K, Nakano Y, Sambali H, van Woesik R (2001) Coral bleaching: the winners and the losers Ecology Letters 4:122-131 doi:10.1046/j.1461-0248.2001.00203.x
Luo H, Lin X, Li L, Lin L, Zhang C, Lin S (2017) Transcriptomic and physiological analyses of the dinoflagellate *Karenia mikimotoi* reveal non-alkaline phosphatase-based molecular machinery of ATP utilisation Environ Microbiol 19:4506-4518 doi:10.1111/1462-2920.13899
Lyndby NH, Holm JB, Wangpraseurt D, Grover R, Rottier C, Kühl M, Ferrier-Pagès C (2020) Effect of temperature and feeding on carbon budgets and O_2 dynamics in *Pocillopora damicornis* Mar Ecol-Prog Ser 652:49-62
MacNeil MA et al. (2019) Water quality mediates resilience on the Great Barrier Reef Nat Ecol Evol 3:620-627 doi:10.1038/s41559-019-0832-3
Maor-Landaw K, Karako-Lampert S, Ben-Asher HW, Goffredo S, Falini G, Dubinsky Z, Levy O (2014) Gene expression profiles during short-term heat stress in the red sea coral *Stylophora pistillata* Glob Chang Biol 20:3026-3035 doi:10.1111/gcb.12592
Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef: Differential susceptibilities among taxa Coral Reefs 19:155-163 doi:10.1007/s003380000086

Marubini F, Ferrier-Pagès C, Furla P, Allemand D (2008) Coral calcification responds to seawater acidification: a working hypothesis towards a physiological mechanism Coral Reefs 27:491-499 doi:10.1007/s00338-008-0375-6

Matthews JL et al. (2017) Optimal nutrient exchange and immune responses operate in partner specificity in the cnidarian-dinoflagellate symbiosis Proc Natl Acad Sci U S A 114:13194-13199 doi:10.1073/pnas.1710733114

Matthews JL et al. (2018) Partner switching and metabolic flux in a model cnidarian-dinoflagellate symbiosis Proc Biol Sci 285:20182336 doi:10.1098/rspb.2018.2336

Matz MV, Treml EA, Aglyamova GV, Bay LK (2018) Potential and limits for rapid genetic adaptation to warming in a Great Barrier Reef coral PLoS Genet 14:e1007220 doi:10.1371/journal.pgen.1007220

Mellin C et al. (2019) Spatial resilience of the Great Barrier Reef under cumulative disturbance impacts Glob Chang Biol 25:2431-2445 doi:10.1111/gcb.14625

Meyer JL, Schultz ET, Helfman GS (1983) Fish schools: an asset to corals Science 220:1047-1049 doi:10.1126/science.220.4601.1047

Middlebrook R, Hoegh-Guldberg O, Leggat W (2008) The effect of thermal history on the susceptibility of reef-building corals to thermal stress J Exp Biol 211:1050-1056 doi:10.1242/jeb.013284

Mieog JC, Olsen JL, Berkelmans R, Bleuler-Martinez SA, Willis BL, van Oppen MJ (2009) The roles and interactions of symbiont, host and environment in defining coral fitness PLoS One 4:e6364 doi:10.1371/journal.pone.0006364

Moberg F, Folke C (1999) Ecological goods and services of coral reef ecosystems Ecological Economics 29:215-233 doi:10.1016/s0921-8009(99)00009-9

Morgan KM, Perry CT, Johnson JA, Smithers SG (2017) Nearshore turbid-zone corals exhibit high bleaching tolerance on the Great Barrier Reef following the 2016 ocean warming event Front Mar Sci 4:224 doi:10.3389/fmars.2017.00224

Morris LA, Voolstra CR, Quigley KM, Bourne DG, Bay LK (2019) Nutrient availability and metabolism affect the stability of coral-Symbiodiniaceae symbioses Trends Microbiol 27:678-689 doi:10.1016/j.tim.2019.03.004

Muller EM et al. (2021) Heritable variation and lack of tradeoffs suggest adaptive capacity in *Acropora cervicornis* despite negative synergism under climate change scenarios Proc Biol Sci 288:20210923 doi:10.1098/rspb.2021.0923

Muscatine L, Falkowski PG, Dubinsky Z, Cook PA, McCloskey LR (1989a) The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral Proc Biol Sci 236:311-324 doi:10.1098/rspb.1989.0025

Muscatine L, Falkowski PG, Porter JW, Dubinsky Z (1984) Fate of photosynthetic fixed carbon in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata* Proceedings of the Royal Society B: Biological Sciences 222:181-202 doi:10.1098/rspb.1984.0058

Muscatine L, Ferrier-Pagès C, Blackburn A, Gates RD, Baghdasarian G, Allemand D (1998) Cell-specific density of symbiotic dinoflagellates in tropical anthozoans Coral Reefs 17:329-337 doi:10.1007/s003380050133

Muscatine L, Porter JW (1977) Reef corals: Mutualistic symbioses adapted to nutrient-poor environments BioScience 27:454-460 doi:10.2307/1297526

Muscatine L, Porter JW, Kaplan IR (1989b) Resource partitioning by reef corals as determined from stable isotope composition I. $\delta^{13}C$ of zooxanthellae and animal tissue vs depth Mar Biol 100:185-193 doi:10.1007/Bf00391957

Newton K, Côté IM, Pilling GM, Jennings S, Dulvy NK (2007) Current and future sustainability of island coral reef fisheries Curr Biol 17:655-658 doi:10.1016/j.cub.2007.02.054

Nielsen DA, Petrou K, Gates RD (2018) Coral bleaching from a single cell perspective ISME J 12:1558-1567 doi:10.1038/s41396-018-0080-6

Nielsen JJV, Kenkel CD, Bourne DG, Despringhere L, Mocellin VJL, Bay LK (2020) Physiological effects of heat and cold exposure in the common reef coral *Acropora millepora* Coral Reefs 39:259-269 doi:10.1007/s00338-019-01881-x

Nordemar I, Nyström M, Dizon R (2003) Effects of elevated seawater temperature and nitrate enrichment on the branching coral *Porites cylindrica* in the absence of particulate food Mar Biol 142:669-677 doi:10.1007/s00227-002-0989-0

Oakley CA, Ameismeier MF, Peng L, Weis VM, Grossman AR, Davy SK (2016) Symbiosis induces widespread changes in the proteome of the model cnidarian Aiptasia Cell Microbiol 18:1009-1023 doi:10.1111/cmi.12564

Ochsenkühn MA, Röthig T, D'Angelo C, Wiedenmann J, Voolstra CR (2017) The role of floridoside in osmoadaptation of coral-associated algal endosymbionts to high-salinity conditions Sci Adv 3:e1602047 doi:10.1126/sciadv.1602047

Oksanen J et al. (2020) vegan: Community Ecology Package

Ortiz JC, Wolff NH, Anthony KRN, Devlin M, Lewis S, Mumby PJ (2018) Impaired recovery of the Great Barrier Reef under cumulative stress Sci Adv 4:eaar6127 doi:10.1126/sciadv.aar6127

Pernice M et al. (2015) A nanoscale secondary ion mass spectrometry study of dinoflagellate functional diversity in reef-building corals Environ Microbiol 17:3570-3580 doi:10.1111/1462-2920.12518

Petrou K, Nielsen DA, Heraud P (2018) Single-cell biomolecular analysis of coral algal symbionts reveals opposing metabolic responses to heat stress and expulsion Frontiers in Marine Science 5:110 doi:10.3389/fmars.2018.00110

Petrou K, Nunn BL, Padula MP, Miller DJ, Nielsen DA (2021) Broad scale proteomic analysis of heat-destabilised symbiosis in the hard coral *Acropora millepora* Sci Rep 11:19061 doi:10.1038/s41598-021-98548-x

Pogoreutz C, Rädecker N, Cárdenas A, Gärdes A, Voolstra CR, Wild C (2017a) Sugar enrichment provides evidence for a role of nitrogen fixation in coral bleaching Glob Chang Biol 23:3838-3848 doi:10.1111/gcb.13695

Pogoreutz C, Rädecker N, Cárdenas A, Gärdes A, Wild C, Voolstra CR (2017b) Nitrogen fixation aligns with *nifH* abundance and expression in two coral trophic functional groups Front Microbiol 8:1187 doi:10.3389/fmicb.2017.01187

Pogoreutz C, Rädecker N, Cárdenas A, Gärdes A, Wild C, Voolstra CR (2018) Dominance of *Endozoicomonas* bacteria throughout coral bleaching and mortality suggests structural inflexibility of the *Pocillopora verrucosa* microbiome Ecol Evol 8:2240-2252 doi:10.1002/ece3.3830

Pootakham W et al. (2018) Dynamics of coral-associated microbiomes during a thermal bleaching event MicrobiologyOpen 7:e00604 doi:10.1002/mbo3.604

Prazeres M, Roberts TE, Pandolfi JM (2017) Variation in sensitivity of large benthic foraminifera to the combined effects of ocean warming and local impacts Sci Rep 7:45227 doi:10.1038/srep45227

Prazeres M, Uthicke S, Pandolfi JM (2016) Influence of local habitat on the physiological responses of large benthic foraminifera to temperature and nutrient stress Sci Rep 6:21936 doi:10.1038/srep21936

Putnam HM, Barott KL, Ainsworth TD, Gates RD (2017) The vulnerability and resilience of reef-building corals Curr Biol 27:R528-R540 doi:10.1016/j.cub.2017.04.047

Quigley KM, Bay LK, van Oppen MJH (2020) Genome-wide SNP analysis reveals an increase in adaptive genetic variation through selective breeding of coral Mol Ecol 29:2176-2188 doi:10.1111/mec.15482

Quigley KM, Bay LK, Willis BL (2017a) Temperature and water quality-related patterns in sediment-associated *Symbiodinium* communities impact symbiont uptake and fitness of juveniles in the genus *Acropora* Frontiers in Marine Science 4 doi:10.3389/fmars.2017.00401

Quigley KM, Davies SW, Kenkel CD, Willis BL, Matz MV, Bay LK (2014) Deep-sequencing method for quantifying background abundances of *Symbiodinium* types: Exploring the rare *Symbiodinium* biosphere in reef-building corals PLoS One 9:e94297 doi:10.1371/journal.pone.0094297

Quigley KM, Willis BL, Bay LK (2017b) Heritability of the *Symbiodinium* community in vertically- and horizontally-transmitting broadcast spawning corals Sci Rep 7:8219 doi:10.1038/s41598-017-08179-4

Quigley KM, Willis BL, Kenkel CD (2019) Transgenerational inheritance of shuffled symbiont communities in the coral *Montipora digitata* Sci Rep 9:13328 doi:10.1038/s41598-019-50045-y

R Core Team (2021) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Rädecker N et al. (2021) Heat stress destabilizes symbiotic nutrient cycling in corals Proc Natl Acad Sci U S A 118:e2022653118 doi:10.1073/pnas.2022653118

Rädecker N, Pogoreutz C, Voolstra CR, Wiedenmann J, Wild C (2015) Nitrogen cycling in corals: The key to understanding holobiont functioning? Trends Microbiol 23:490-497 doi:10.1016/j.tim.2015.03.008

Rädecker N, Pogoreutz C, Wild C, Voolstra CR (2017) Stimulated respiration and net photosynthesis in *Cassiopeia* sp. during glucose enrichment suggests in hospite CO_2 limitation of algal endosymbionts Front Mar Sci 4:267 doi:10.3389/fmars.2017.00267

Rädecker N et al. (2018) Using *Aiptasia* as a model to study metabolic interactions in cnidarian-*Symbiodinium* symbioses Front Physiol 9:214 doi:10.3389/fphys.2018.00214

Ralph PJ, Gademann R, Larkum AWD (2001) Zooxanthellae expelled from bleached corals at 33 °C are photosynthetically competent Mar Ecol-Prog Ser 220:163-168

Ralph PJ, Larkum AWD, Kühl M (2005) Temporal patterns in effective quantum yield of individual zooxanthellae expelled during bleaching J Exp Mar Biol Ecol 316:17-28 doi:10.1016/j.jembe.2004.10.003

Rice MM, Maher RL, Vega Thurber R, Burkepile DE (2019) Different nitrogen sources speed recovery from corallivory and uniquely alter the microbiome of a reef-building coral PeerJ 7:e8056 doi:10.7717/peerj.8056

Ritchie RJ (2008) Universal chlorophyll equations for estimating chlorophylls *a*, *b*, *c*, and *d* and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents Photosynthetica 46:115-126 doi:10.1007/s11099-008-0019-7

Roberts CM et al. (2002) Marine biodiversity hotspots and conservation priorities for tropical reefs Science 295:1280-1284 doi:10.1126/science.1067728

Rodrigues LJ, Grottoli AG (2007) Energy reserves and metabolism as indicators of coral recovery from bleaching Limnol Oceanogr 52:1874-1882 doi:10.4319/lo.2007.52.5.1874

Rodriguez-Casariego JA et al. (2018) Coral epigenetic responses to nutrient stress: Histone H2A.X phosphorylation dynamics and DNA methylation in the staghorn coral *Acropora cervicornis* Ecol Evol 8:12193-12207 doi:10.1002/ece3.4678

Rosic N, Remond C, Mello-Athayde MA (2020) Differential impact of heat stress on reef-building corals under different light conditions Mar Environ Res 158:104947 doi:10.1016/j.marenvres.2020.104947

Rosset S, D'Angelo C, Wiedenmann J (2015) Ultrastructural biomarkers in symbiotic algae reflect the availability of dissolved inorganic nutrients and particulate food to the reef coral holobiont Front Mar Sci 2:103 doi:10.3389/fmars.2015.00103

Rosset S, Wiedenmann J, Reed AJ, D'Angelo C (2017) Phosphate deficiency promotes coral bleaching and is reflected by the ultrastructure of symbiotic dinoflagellates Mar Pollut Bull 118:180-187 doi:10.1016/j.marpolbul.2017.02.044

Rouan A et al. (2021) Telomere dysfunction is associated with dark-induced bleaching in the reef coral *Stylophora pistillata* Mol Ecol doi:10.1111/mec.16199

Ryle VD, Müller HR, Gentien P (1981) Automated analysis of nutrients in tropical seawater Australian Institute of Marine Science Townsville, Australia (AIMS Oceanogr Ser AIMS-OS-82–2)

Safaie A et al. (2018) High frequency temperature variability reduces the risk of coral bleaching Nat Commun 9:1671

Santos HF et al. (2014) Climate change affects key nitrogen-fixing bacterial populations on coral reefs ISME J 8:2272-2279 doi:10.1038/ismej.2014.70

Scheufen T, Iglesias-Prieto R, Enríquez S (2017a) Changes in the number of symbionts and *Symbiodinium* cell pigmentation modulate differentially coral light absorption and photosynthetic performance Frontiers in Marine Science 4 doi:10.3389/fmars.2017.00309

Scheufen T, Krämer WE, Iglesias-Prieto R, Enríquez S (2017b) Seasonal variation modulates coral sensibility to heat-stress and explains annual changes in coral productivity Sci Rep 7:4937 doi:10.1038/s41598-017-04927-8

Schlöder C, D'Croz L (2004) Responses of massive and branching coral species to the combined effects of water temperature and nitrate enrichment J Exp Mar Biol Ecol 313:255-268 doi:10.1016/j.jembe.2004.08.012

Schrameyer V, Wangpraseurt D, Hill R, Kühl M, Larkum AWD, Ralph PJ (2014) Light respiratory processes and gross photosynthesis in two scleractinian corals PLoS One 9:e110814 doi:10.1371/journal.pone.0110814

Seneca FO, Palumbi SR (2015) The role of transcriptome resilience in resistance of corals to bleaching Mol Ecol 24:1467-1484 doi:10.1111/mec.13125

Shantz AA, Burkepile DE (2014) Context-dependent effects of nutrient loading on the coral-algal mutualism Ecology 95:1995-2005 doi:10.1890/13-1407.1

Sinha E, Michalak AM, Balaji V (2017) Eutrophication will increase during the 21st century as a result of precipitation changes Science 357:405-408 doi:10.1126/science.aan2409

Slavov C et al. (2016) "Super-quenching" state protects *Symbiodinium* from thermal stress - Implications for coral bleaching Biochim Biophys Acta 1857:840-847 doi:10.1016/j.bbabio.2016.02.002

Smith EG, Gurskaya A, Hume BCC, Voolstra CR, Todd PA, Bauman AG, Burt JA (2020) Low Symbiodiniaceae diversity in a turbid marginal reef environment Coral Reefs 39:545-553 doi:10.1007/s00338-020-01956-0

Spalding M, Burke L, Wood SA, Ashpole J, Hutchison J, zu Ermgassen P (2017) Mapping the global value and distribution of coral reef tourism Marine Policy 82:104-113 doi:10.1016/j.marpol.2017.05.014

Sproles AE, Oakley CA, Krueger T, Grossman AR, Weis VM, Meibom A, Davy SK (2020) Sub-cellular imaging shows reduced photosynthetic carbon and increased nitrogen assimilation by the non-native endosymbiont *Durusdinium trenchii* in the model cnidarian Aiptasia Environ Microbiol 22:3741-3753 doi:10.1111/1462-2920.15142

Starzak DE, Quinnell RG, Cook CB, Davy SK (2020) Influence of symbiont species on the glycerol and glucose pools in a model cnidarian-dinoflagellate symbiosis Biol Bull 239:143-151 doi:10.1086/710349

Starzak DE, Quinnell RG, Nitschke MR, Davy SK (2014) The influence of symbiont type on photosynthetic carbon flux in a model cnidarian–dinoflagellate symbiosis Mar Biol 161:711-724 doi:10.1007/s00227-013-2372-8

Stat M, Loh WKW, LaJeunesse TC, Hoegh-Guldberg O, Carter DA (2009) Stability of coral–endosymbiont associations during and after a thermal stress event in the southern Great Barrier Reef Coral Reefs 28:709-713 doi:10.1007/s00338-009-0509-5

Strahl J, Rocker MM, Fabricius KE (2019) Contrasting responses of the coral *Acropora tenuis* to moderate and strong light limitation in coastal waters Mar Environ Res 147:80-89 doi:10.1016/j.marenvres.2019.04.003

Suggett DJ, Warner ME, Leggat W (2017) Symbiotic dinoflagellate functional diversity mediates coral survival under ecological crisis Trends Ecol Evol 32:735-745 doi:10.1016/j.tree.2017.07.013

Sully S, van Woesik R (2020) Turbid reefs moderate coral bleaching under climate-related temperature stress Glob Chang Biol 26:1367-1373 doi:10.1111/gcb.14948

Swain TD, Chandler J, Backman V, Marcelino L, Blanckenhorn W (2017) Consensus thermotolerance ranking for 110 *Symbiodinium* phylotypes: an exemplar utilization of a novel iterative partial-rank aggregation tool with broad application potential Functional Ecology 31:172-183 doi:10.1111/1365-2435.12694

Swain TD et al. (2016) Coral bleaching response index: a new tool to standardize and compare susceptibility to thermal bleaching Glob Chang Biol 22:2475-2488 doi:10.1111/gcb.13276

Szmant AM (2002) Nutrient enrichment on coral reefs: Is it a major cause of coral reef decline? Estuaries 25:743-766 doi:10.1007/bf02804903

Tagliafico A, Rangel S, Kelaher BP, Scheffers SR, Christidis L (2018) A new technique to increase polyp production in stony coral aquaculture using waste fragments without polyps Aquaculture 484:303-308 doi:10.1016/j.aquaculture.2017.09.021

Tagliafico A et al. (2017) Lipid-enriched diets reduce the impacts of thermal stress in corals Mar Ecol-Prog Ser 573:129-141 doi:10.3354/meps12177

Takahashi S, Whitney S, Itoh S, Maruyama T, Badger M (2008) Heat stress causes inhibition of the de novo synthesis of antenna proteins and photobleaching in cultured *Symbiodinium* Proc Natl Acad Sci U S A 105:4203-4208 doi:10.1073/pnas.0708554105

Tambutté S, Holcomb M, Ferrier-Pagès C, Reynaud S, Tambutté É, Zoccola D, Allemand D (2011) Coral biomineralization: From the gene to the environment J Exp Mar Biol Ecol 408:58-78 doi:10.1016/j.jembe.2011.07.026

Tanaka Y, Grottoli AG, Matsui Y, Suzuki A, Sakai K (2017) Effects of nitrate and phosphate availability on the tissues and carbonate skeleton of scleractinian corals Mar Ecol-Prog Ser 570:101-112 doi:10.3354/meps12079

Tanaka Y, Inoue M, Nakamura T, Suzuki A, Sakai K (2014) Loss of zooxanthellae in a coral under high seawater temperature and nutrient enrichment J Exp Mar Biol Ecol 457:220-225 doi:10.1016/j.jembe.2014.04.019

Tanaka Y, Suzuki A, Sakai K (2018) The stoichiometry of coral-dinoflagellate symbiosis: Carbon and nitrogen cycles are balanced in the recycling and double translocation system ISME J 12:860-868 doi:10.1038/s41396-017-0019-3

Tansik AL, Fitt WK, Hopkinson BM (2017) Inorganic carbon is scarce for symbionts in scleractinian corals Limnol Oceanogr 62:2045-2055 doi:10.1002/lno.10550

Thomas L, Palumbi SR (2017) The genomics of recovery from coral bleaching Proc Biol Sci 284:1865 doi:10.1098/rspb.2017.1790

Thummasan M, Casareto BE, Ramphul C, Suzuki T, Toyoda K, Suzuki Y (2021) Physiological responses (Hsps 60 and 32, caspase 3, H_2O_2 scavenging, and photosynthetic activity) of the coral *Pocillopora damicornis* under thermal and high nitrate stresses Mar Pollut Bull 171:112737 doi:10.1016/j.marpolbul.2021.112737

Tolleter D, Seneca FO, DeNofrio JC, Krediet CJ, Palumbi SR, Pringle JR, Grossman AR (2013) Coral bleaching independent of photosynthetic activity Curr Biol 23:1782-1786 doi:10.1016/j.cub.2013.07.041

Tonk L, Sampayo EM, Weeks S, Magno-Canto M, Hoegh-Guldberg O (2013) Host-specific interactions with environmental factors shape the distribution of *Symbiodinium* across the Great Barrier Reef PLoS One 8:e68533 doi:10.1371/journal.pone.0068533

Traylor-Knowles N, Rose NH, Sheets EA, Palumbi SR (2017) Early transcriptional responses during heat stress in the coral *Acropora hyacinthus* Biol Bull 232:91-100 doi:10.1086/692717

Tremblay P, Fine M, Maguer JF, Grover R, Ferrier-Pagès C (2013) Photosynthate translocation increases in response to low seawater pH in a coral–dinoflagellate symbiosis Biogeosciences 10:3997-4007 doi:10.5194/bg-10-3997-2013

Tremblay P, Gori A, Maguer JF, Hoogenboom M, Ferrier-Pagès C (2016) Heterotrophy promotes the re-establishment of photosynthate translocation in a symbiotic coral after heat stress Sci Rep 6:38112 doi:10.1038/srep38112

Ulstrup KE, van Oppen MJH (2003) Geographic and habitat partitioning of genetically distinct zooxanthellae (*Symbiodinium*) in *Acropora* corals on the Great Barrier Reef Mol Ecol 12:3477-3484 doi:10.1046/j.1365-294x.2003.01988.x

van der Zande RM, Achlatis M, Bender-Champ D, Kubicek A, Dove S, Hoegh-Guldberg O (2020) Paradise lost: End-of-century warming and acidification under business-as-usual emissions have severe consequences for symbiotic corals Glob Chang Biol 26:2203-2219 doi:10.1111/gcb.14998

van Hooidonk R et al. (2016) Local-scale projections of coral reef futures and implications of the Paris Agreement Sci Rep 6:39666 doi:10.1038/srep39666

van Oppen MJH, Mahiny AJ, Done TJ (2005) Geographic distribution of zooxanthella types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event Coral Reefs 24:482-487 doi:10.1007/s00338-005-0487-1

van Oppen MJH, Palstra FP, Piquet AM, Miller DJ (2001) Patterns of coral-dinoflagellate associations in *Acropora*: Significance of local availability and physiology of

Symbiodinium strains and host-symbiont selectivity Proc Biol Sci 268:1759-1767 doi:10.1098/rspb.2001.1733

van Rij J, Wieling M, Baayen R, H vR (2020) "itsadug: Interpreting Time Series and Autocorrelated Data Using GAMMs." R package version 2.4.

van Woesik R, Houk P, Isechal AL, Idechong JW, Victor S, Golbuu Y (2012) Climate-change refugia in the sheltered bays of Palau: Analogs of future reefs Ecol Evol 2:2474-2484 doi:10.1002/ece3.363

Vega Thurber RL et al. (2008) Metagenomic analysis indicates that stressors induce production of herpes-like viruses in the coral *Porites compressa* Proc Natl Acad Sci U S A 105:18413-18418 doi:10.1073/pnas.0808985105

Vega Thurber RL, Burkepile DE, Fuchs C, Shantz AA, McMinds R, Zaneveld JR (2014) Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching Glob Chang Biol 20:544-554 doi:10.1111/gcb.12450

Vega Thurber RL et al. (2009) Metagenomic analysis of stressed coral holobionts Environ Microbiol 11:2148-2163 doi:10.1111/j.1462-2920.2009.01935.x

Vehtari A, Gelman A, Gabry J (2017) Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC Statistics and Computing 27:1413-1432 doi:10.1007/s11222-016-9696-4

Veron JEN (2000) Corals of the World. Australian Institute of Marine Science, Townsville

Voolstra CR, Buitrago-López C, Perna G, Cárdenas A, Hume BCC, Rädecker N, Barshis DJ (2020) Standardized short-term acute heat stress assays resolve historical differences in coral thermotolerance across microhabitat reef sites Glob Chang Biol 26:4328-4343 doi:10.1111/gcb.15148

Voss JD, Richardson LL (2006) Nutrient enrichment enhances black band disease progression in corals Coral Reefs 25:569-576 doi:10.1007/s00338-006-0131-8

Wang JT, Douglas AE (1998) Nitrogen recycling or nitrogen conservation in an alga-invertebrate symbiosis? J Exp Biol 201 (Pt 16):2445-2453

Wang JT, Douglas AE (1999) Essential amino acid synthesis and nitrogen recycling in an alga–invertebrate symbiosis Mar Biol 135:219-222 doi:10.1007/s002270050619

Wang L, Shantz AA, Payet JP, Sharpton TJ, Foster A, Burkepile DE, Vega Thurber R (2018) Corals and their microbiomes are differentially affected by exposure to elevated nutrients and a natural thermal anomaly Front Mar Sci 5:101 doi:10.3389/fmars.2018.00101

Wang LH, Lee HH, Fang LS, Mayfield AB, Chen CS (2013) Fatty acid and phospholipid syntheses are prerequisites for the cell cycle of *Symbiodinium* and their endosymbiosis within sea anemones PLoS One 8:e72486 doi:10.1371/journal.pone.0072486

Wangpraseurt D, Holm JB, Larkum AW, Pernice M, Ralph PJ, Suggett DJ, Kuhl M (2017) In vivo microscale measurements of light and photosynthesis during coral bleaching: Evidence for the optical feedback loop? Front Microbiol 8:59 doi:10.3389/fmicb.2017.00059

Wangpraseurt D, Larkum A, Ralph P, Kühl M (2012) Light gradients and optical microniches in coral tissues Front Microbiol 3:316 doi:10.3389/fmicb.2012.00316

Wangpraseurt D, Pernice M, Guagliardo P, Kilburn MR, Clode PL, Polerecky L, Kühl M (2016) Light microenvironment and single-cell gradients of carbon fixation in tissues of symbiont-bearing corals ISME J 10:788-792 doi:10.1038/ismej.2015.133

Warner ME, Fitt WK, Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching Proc Natl Acad Sci U S A 96:8007-8012 doi:10.1073/pnas.96.14.8007

Waterhouse J et al. (2021) Marine Monitoring Program: Annual Report for Inshore Water Quality Monitoring 2019–20 Report for the Great Barrier Reef Marine Park Authority, Great Barrier Reef Marine Park Authority, Townsville

Weis VM (1993) Effect of dissolved inorganic carbon concentration on the photosynthesis of the symbiotic sea anemone *Aiptasia pulchella* Carlgren: Role of carbonic anhydrase J Exp Mar Biol Ecol 174:209-225 doi:10.1016/0022-0981(93)90018-j

Weis VM (2008) Cellular mechanisms of cnidarian bleaching: Stress causes the collapse of symbiosis J Exp Biol 211:3059-3066 doi:10.1242/jeb.009597

Wiedenmann J, D'Angelo C (2018) Symbiosis: High-carb diet of reef corals as seen from space Curr Biol 28:R1263-R1265 doi:10.1016/j.cub.2018.09.056

Wiedenmann J, D'Angelo C, Smith EG, Hunt AN, Legiret F-E, Postle AD, Achterberg EP (2013) Nutrient enrichment can increase the susceptibility of reef corals to bleaching Nat Clim Change 3:160-164 doi:10.1038/nclimate1661

Williams A et al. (2021) Metabolomic shifts associated with heat stress in coral holobionts Sci Adv 7:eabd4210 doi:10.1126/sciadv.abd4210

Wilson K et al. (2002) Genetic mapping of the black tiger shrimp *Penaeus monodon* with amplified fragment length polymorphism Aquaculture 204:297-309 doi:10.1016/s0044-8486(01)00842-0

Wolff NH, Mumby PJ, Devlin M, Anthony KRN (2018) Vulnerability of the Great Barrier Reef to climate change and local pressures Glob Chang Biol 24:1978-1991 doi:10.1111/gcb.14043

Wong JCY, Enríquez S, Baker DM (2021) Towards a trait-based understanding of Symbiodiniaceae nutrient acquisition strategies Coral Reefs 40:625-639 doi:10.1007/s00338-020-02034-1

Wood SN (2003) Thin plate regression splines Journal of the Royal Statistical Society: Series B (Statistical Methodology) 65:95-114 doi:10.1111/1467-9868.00374

Wood SN, Pya N, Säfken B (2016) Smoothing Parameter and Model Selection for General Smooth Models Journal of the American Statistical Association 111:1548-1563 doi:10.1080/01621459.2016.1180986

Woodhead AJ, Hicks CC, Norström AV, Williams GJ, Graham NAJ (2019) Coral reef ecosystem services in the Anthropocene Functional Ecology 33:1023-1034 doi:10.1111/1365-2435.13331

Wooldridge SA (2009a) A new conceptual model for the warm-water breakdown of the coral-algae endosymbiosis Mar Freshwater Res 60:483-496 doi:10.1071/mf08251

Wooldridge SA (2009b) Water quality and coral bleaching thresholds: Formalising the linkage for the inshore reefs of the Great Barrier Reef, Australia Mar Pollut Bull 58:745-751 doi:10.1016/j.marpolbul.2008.12.013

Wooldridge SA (2013) Breakdown of the coral-algae symbiosis: towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae Biogeosciences 10:1647-1658 doi:10.5194/bg-10-1647-2013

Wooldridge SA (2014) Formalising a mechanistic linkage between heterotrophic feeding and thermal bleaching resistance Coral Reefs 33:1131-1136 doi:10.1007/s00338-014-1193-7

Wooldridge SA (2020) Excess seawater nutrients, enlarged algal symbiont densities and bleaching sensitive reef locations: 1. Identifying thresholds of concern for the Great Barrier Reef, Australia Mar Pollut Bull 152:107667 doi:10.1016/j.marpolbul.2016.04.054

Wooldridge SA, Brodie JE, Kroon FJ, Turner RD (2015) Ecologically based targets for bioavailable (reactive) nitrogen discharge from the drainage basins of the Wet Tropics region, Great Barrier Reef Mar Pollut Bull 97:262-272 doi:10.1016/j.marpolbul.2015.06.007

Wooldridge SA, Done TJ (2009) Improved water quality can ameliorate effects of climate change on corals Ecol Appl 19:1492-1499 doi:10.1890/08-0963.1

Wooldridge SA, Done TJ, Thomas CR, Gordon II, Marshall PA, Jones RN (2011) Safeguarding coastal coral communities on the central Great Barrier Reef (Australia) against climate change: realizable local and global actions Climatic Change 112:945-961 doi:10.1007/s10584-011-0229-z

Xiang T et al. (2020) Symbiont population control by host-symbiont metabolic interaction in Symbiodiniaceae-cnidarian associations Nat Commun 11:108 doi:10.1038/s41467-019-13963-z

Xu H et al. (2020) Physiological characteristics and environment adaptability of reef-building corals at the Wuzhizhou Island of South China Sea Front Physiol 11:390 doi:10.3389/fphys.2020.00390

Yao Y, Vehtari A, Simpson D, Gelman A (2018) Using Stacking to Average Bayesian Predictive Distributions (with Discussion) Bayesian Analysis 13:917-1007, 1091

Yonge CM, Nicholls AG (1931) Studies on the physiology of corals: IV. The structure, distribution and physiology of zooxanthellae Scientific Reports / Great Barrier Reef Expedition 1928-29 1:135-176

Yu X et al. (2020) Thermal acclimation increases heat tolerance of the scleractinian coral *Acropora pruinosa* Sci Total Environ 733:139319 doi:10.1016/j.scitotenv.2020.139319

Zaneveld JR et al. (2016) Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales Nat Commun 7:11833 doi:10.1038/ncomms11833

Zaneveld JR, McMinds R, Vega Thurber R (2017) Stress and stability: applying the Anna Karenina principle to animal microbiomes Nat Microbiol 2:17121 doi:10.1038/nmicrobiol.2017.121

Zhang Y, Ip JC, Xie JY, Yeung YH, Sun Y, Qiu JW (2021) Host-symbiont transcriptomic changes during natural bleaching and recovery in the leaf coral *Pavona decussata* Sci Total Environ 806:150656 doi:10.1016/j.scitotenv.2021.150656

Zweifler A, O'Leary M, Morgan K, Browne NK (2021) Turbid coral reefs: Past, present and future—A review Diversity 13:251

Appendix S2: Supplementary material for Chapter 2

Table S2.1 Reefs from which *Acropora millepora* was sampled from during coral bleaching and recovery, including metadata on exact collection dates, sampling numbers used for each analysis, and the thermal characteristics of each reef.

Reef	Shelf-position	Latitude	Longitude	Bleaching	Recovery	March/April chlorophyll	September chlorophyll	March/April ITS2	September ITS2	MMM (°C)	DHW March/April	DHW max
Fitzroy	Inshore	-16.9253	145.9891	24/03/17	13/09/17	17	20	18	12			
				25/03/17						28.77	9.54	10.55
Russell	Inshore	-17.2236	146.0897	23/03/17	14/09/17	21	26	19	24	28.78	9.37	10.67
North	Inshore	-17.7400	146.1562	20/03/17	15/09/17	19	20	8	15			
Barnard										28.83	7.33	8.89
Dunk	Inshore	-17.9558	146.1483	17/03/17	09/09/17	28	20	24	18	28.93	5.81	7.38
Pandora	Inshore	-18.8136	146.4324	15/03/17	02/09/17	13	20	14	16	28.95	6.73	8.54
Havannah	Inshore	-18.8395	146.5345	14/03/17	03/09/17	19	19	17	17	28.86	6.03	8.24
Arlington	Mid-shelf	-16.7047	146.0473	24/03/17	12/09/17	4	21	3	18	28.65	9.31	11.00
Coates	Mid-shelf	-17.1888	146.3698	22/03/17	11/09/17	17	13	15	11			
				23/03/17						28.72	7.44	8.89
Feather	Mid-shelf	-17.5286	146.3869	21/03/17	N/A	20	N/A	20	N/A	28.72	7.35	N/A
Taylor	Mid-shelf	-17.8147	146.5678	18/03/17	07/09/17	19	21	17	21			
										28.68	6.25	7.74
Rib	Mid-shelf	-18.4811	146.8713	16/03/17	05/09/17	20	21	19	19	28.49	5.21	7.42
John	Mid-shelf	-18.6400	147.0415	01/04/17	04/09/17	12	20	16	15			
Brewer				02/04/17						28.50	5.72	5.72

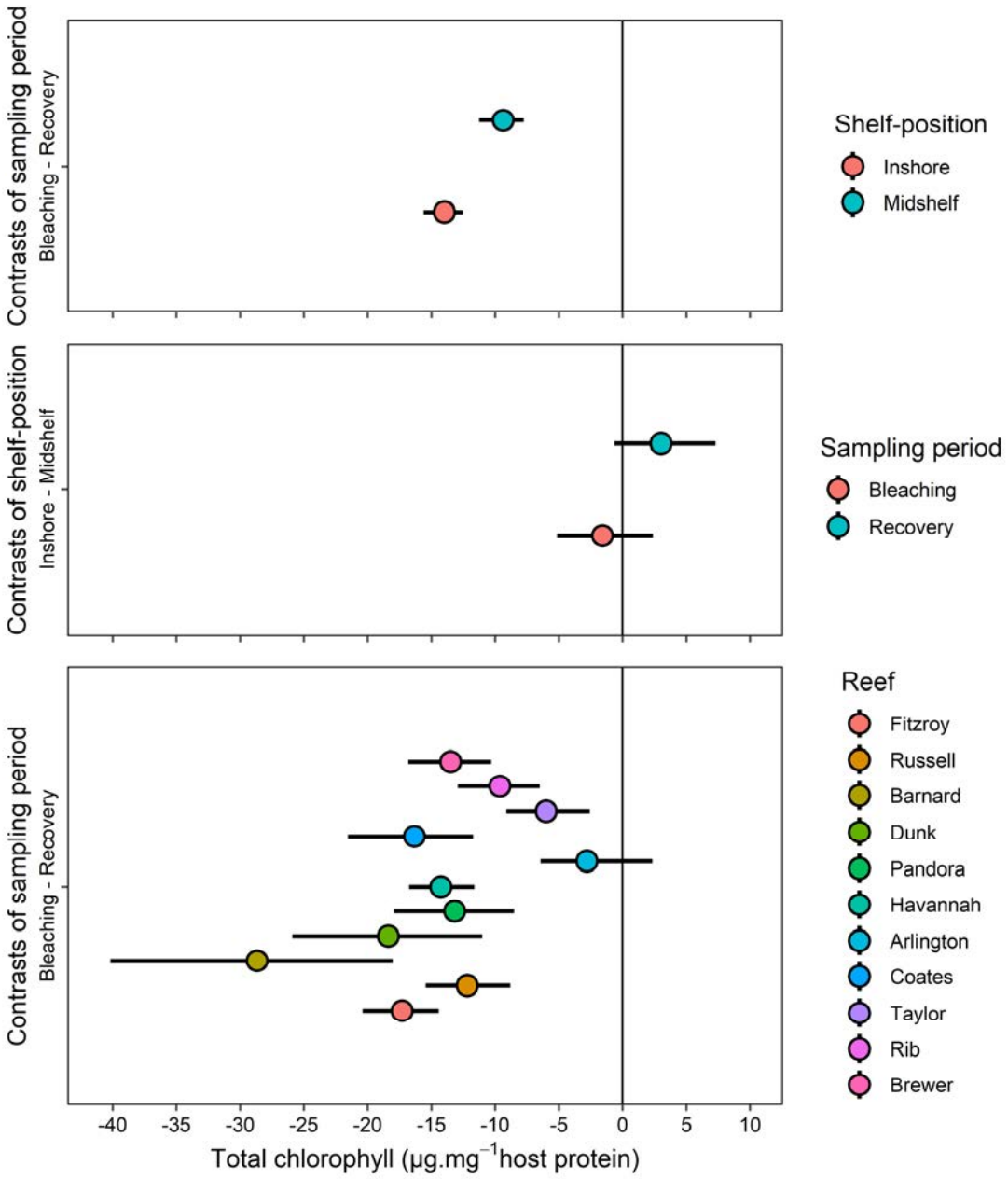

Figure S2.1 Total chlorophyll concentration (µg.mg^{-1} host protein) median effects size and 95% CIs for pairwise comparisons of interactions between shelf-position and sampling period., and sampling period within reef.

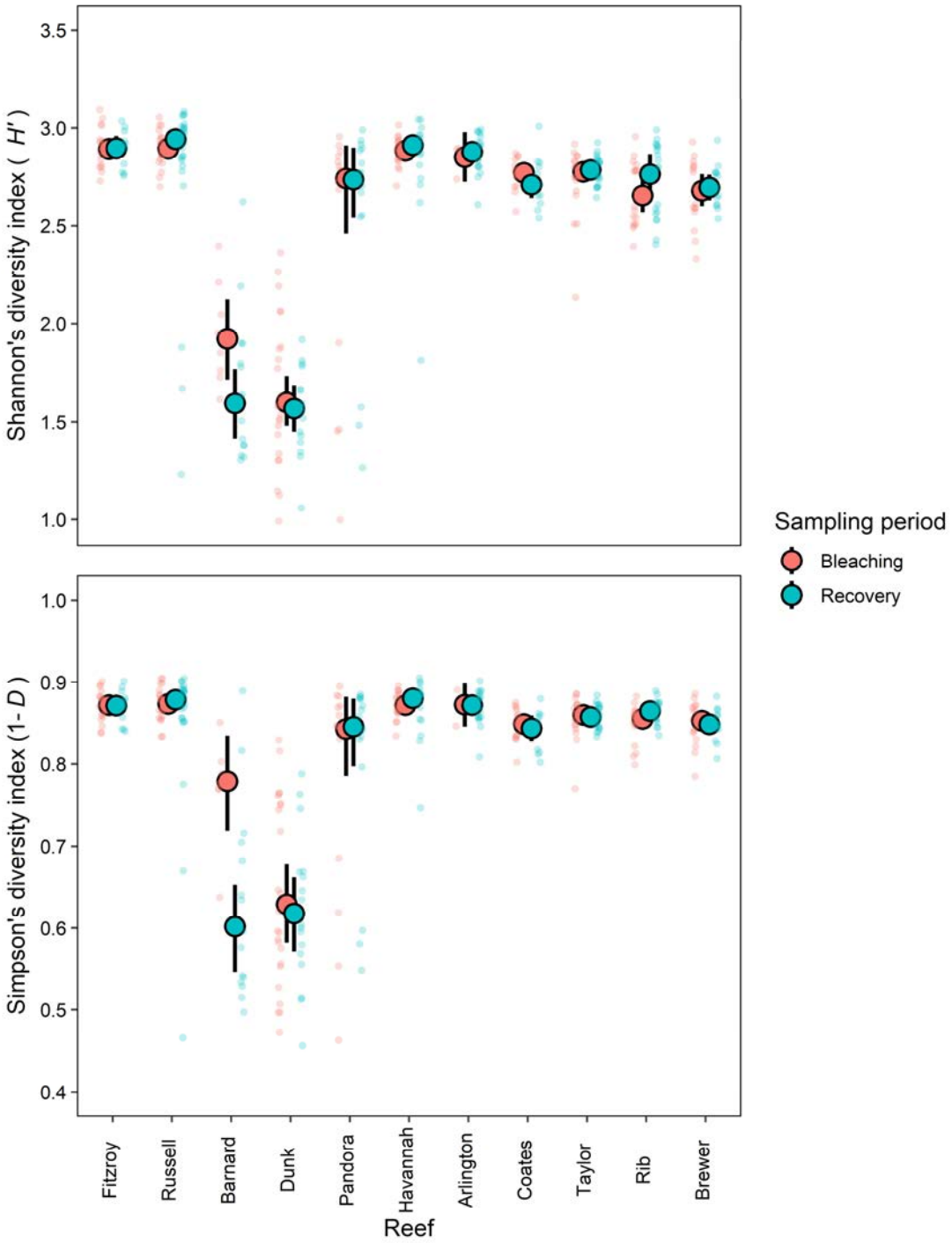

Figure S2.2 Shannon's (*H'*) and Simpson's (1-*D*) diversity indices of algal symbiont DIVs, by shelf-position and individual reef during the peak of bleaching in March/April 2017 and following six months of recovery in September 2017. Coloured points show median model simulations with 95% Cis and coloured dots show individual samples.

Appendix S3: Supplementary material for Chapter 3

Table S3.1 List of laboratory heat stress studies on the physiological responses of corals to nitrate and/or phosphate enrichment followed by heat stress. Details of coral species, source location and the application of nutrient treatments are included, with the current experiment and conditions on inshore and mid-shelf reefs of the central GBR *A. millepora* collection included for context. Physiological traits that increase or decrease due to nutrients during heat stress are noted in the last two columns. The Results from corals post-heat stress are not considered.

Study	Species	Source	Nutrients (days)	Nutrient + Heat (days)	Nutrient treatment	DIN / NO_3^- (µM)	PO_4^{3-} (µM)	N:P	Physiological increases	Physiological decreases
Chapter 3	*A. millepora*	GBR Burdekin region	32	67	Control Mean ± s.d.	0.76 ± 0.21	0.12 ± 0.03	7.19 ± 3.38	Cross-shelf difference in chlorophyll (all treatments)	Gross photosynthesis (all mid-shelf treatments)
					NO_3^-	2.40 ± 0.54	0.11 ± 0.03	21.80 ± 11.56		
					PO_4^{3-}	0.74 ± 0.25	0.33 ± 0.05	2.28 ± 0.81		
					$NO_3^- + PO_4^{3-}$	2.33 ± 0.36	0.32 ± 0.04	7.32 ± 1.08		
Gruber et al. (2019)	N/A	GBR Burdekin region	Inshore ~63	N/A	2017-2018 secondary waters	3.30	0.15	22.11	N/A	N/A
Nordemar et al. (2003)	*Porites cylindrica*	Philippines	12	14	Control	≤ 2.10	N/A	N/A	None	Gross photosynthesis
					NO_3^-	+ 15.00	N/A	N/A		
Schlöder and D'Croz (2004)	*Pocillopora damicornis* *Porites lobata*	Panama	30	30	Control	2.00	0.70	2.86	None	Algal density (*P. damicornis*)
					NO_3^-	≥ 20.81	0.70	≥ 29.73		

Faxneld et al. (2010)	*Turbinaria mesenterina*	Vietnam	1	1	Control	0.30	N/A	N/A	None	None
					NO_3^-	+ 5.00	N/A	N/A		
Chumun et al. (2013)	*P. damicornis*	Japan	2	2	Control	0.90	0.05	18.00	PSII excitation pressure	None
					NO_3^-	10.00	0.05	200.00		
Fabricius et al. (2013)	*A. millepora* *Montipora tuberculosa*	GBR	*A. millepora* 33 *M. tuberculosa* 59	*A. millepora* 33 *M. tuberculosa* 59	Control	0.44	0.08	5.79	None	None
					NO_3^-	0.48	0.06	8.28		
Wiedenmann et al. (2013)	*Acropora microphthalma* *Acropora polystoma* *Montipora foliosa*	Unknown	≥ 912	≥ 921	NO_3^- + PO_4^{3-} (control)	6.50	0.30	21.67	Mortality (*A. microphthalma, M. foliosa*)	Algal density and F_v/F_m (*A. polystoma*)
					NO_3^- (stress)	≥ 3.00	0.07	≥ 42.86		
Tanaka et al. (2014)	*Acropora tenuis*	Japan	25	34	Control	0.48	0.06	8.00	Algal density, chlorophyll	None
					NO_3^- + PO_4^{3-}	5.05	0.28	18.04		
Higuchi et al. (2015)	*Montipora digitata*	Japan	6	6	Control	≤ 1.00	N/A	N/A	Host oxidative stress	Algal density
					NO_3^-	10.00	N/A	N/A		
Ezzat et al. (2016a)	*P. damicornis*	Red Sea	28	45	Control	0.50	0.10	5.00	Carbon translocation, electron transport, non-photochemical quenching	None
					PO_4^{3-}	0.50	2.00	0.25		
Ezzat et al. (2016b)	*T. reniformis*	Red Sea	21	38	Control (fed and unfed)	≤ 0.50	≤ 0.10	5.00	N/A	N/A

Ezzat et al. (2016b) continued	*T. reniformis*	Red Sea	21	38	NO_3^- (fed and unfed)	2.00	0.10	20.00	None	Protein, host growth (fed) Gross photosynthesis, respiration (both)
					NO_3^- + PO_4^{3-} (fed and unfed)	2.00	0.50	4.00	Algal density, host growth (fed) Chlorophyll (both)	None
Dobson et al. (2021); Hoadley et al. (2016)	*A. millepora* *T. reniformis*	Fiji	33	33	Control	0.84	0.21	4.00	Chlorophyll (*A. millepora*)	None
					NO_3^- + PO_4^{3-}	3.87	0.32	13.82		
Hall et al. (2018)	*Stylophora pistillata*	Red Sea	10	10	Control	N/A	N/A	N/A	Algal density, respiration	Host growth
					NO_x^- + PO_4^{3-}	+1.00	+0.06	+16.67		
Rice et al. (2019)	*Pocillopora meandrina*	French Polynesia	21	21	Control	0.55	0.15	3.67	None	None
					NO_3^-	4.00	0.15	26.67		
Fernandes de Barros Marangoni et al. (2020)	*S. pistillata*	Red Sea	35	35	Control	0.50	0.20	2.50	Nitric oxide production, lipid peroxidation, protein nitration, lactate concentration	F_v/F_m
					NO_3^-	3.50	0.20	17.50		
Blanckaert et al. (2021)	*P. damicornis*	Unknown	56	56	Control	0.50	0.20	2.50	None	Non-enzymatic antioxidant capacity
					NO_3^-	3.50	0.20	17.50		
Thummasan et al. (2021)	*P. damicornis*	Japan	2.5	2.5	Control	1.05	0.08	12.56	None	Algal density
					NO_3^-	11.42	0.38	30.32		

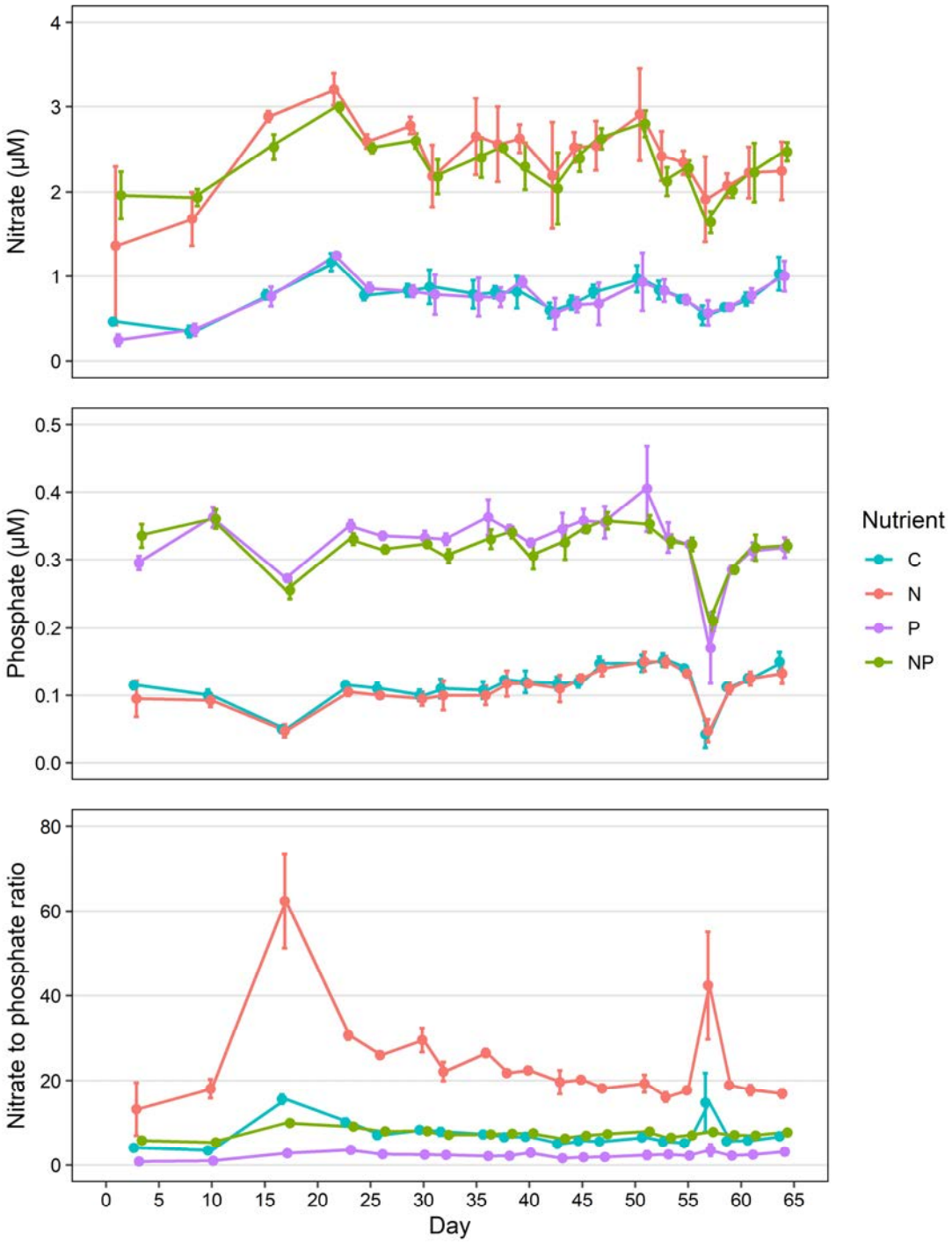

Figure S3.1 Nitrate and phosphate concentrations (µM) and ratios throughout the experiment, by nutrient treatment. Points and error bars represent the mean ± s.d. for replicate tanks within a treatment.

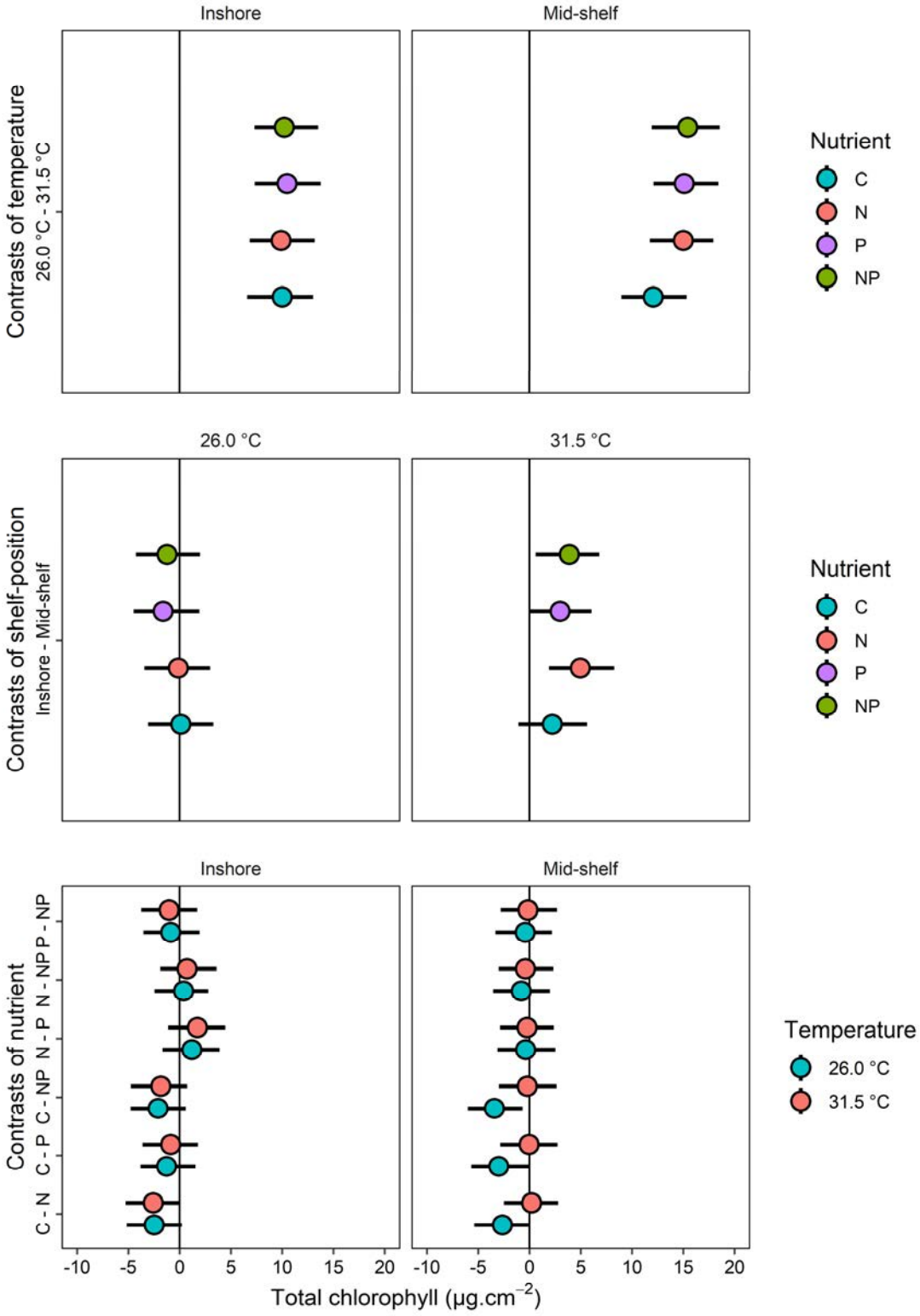

Figure S3.2 Total chlorophyll content (µg.cm^{-2}) median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

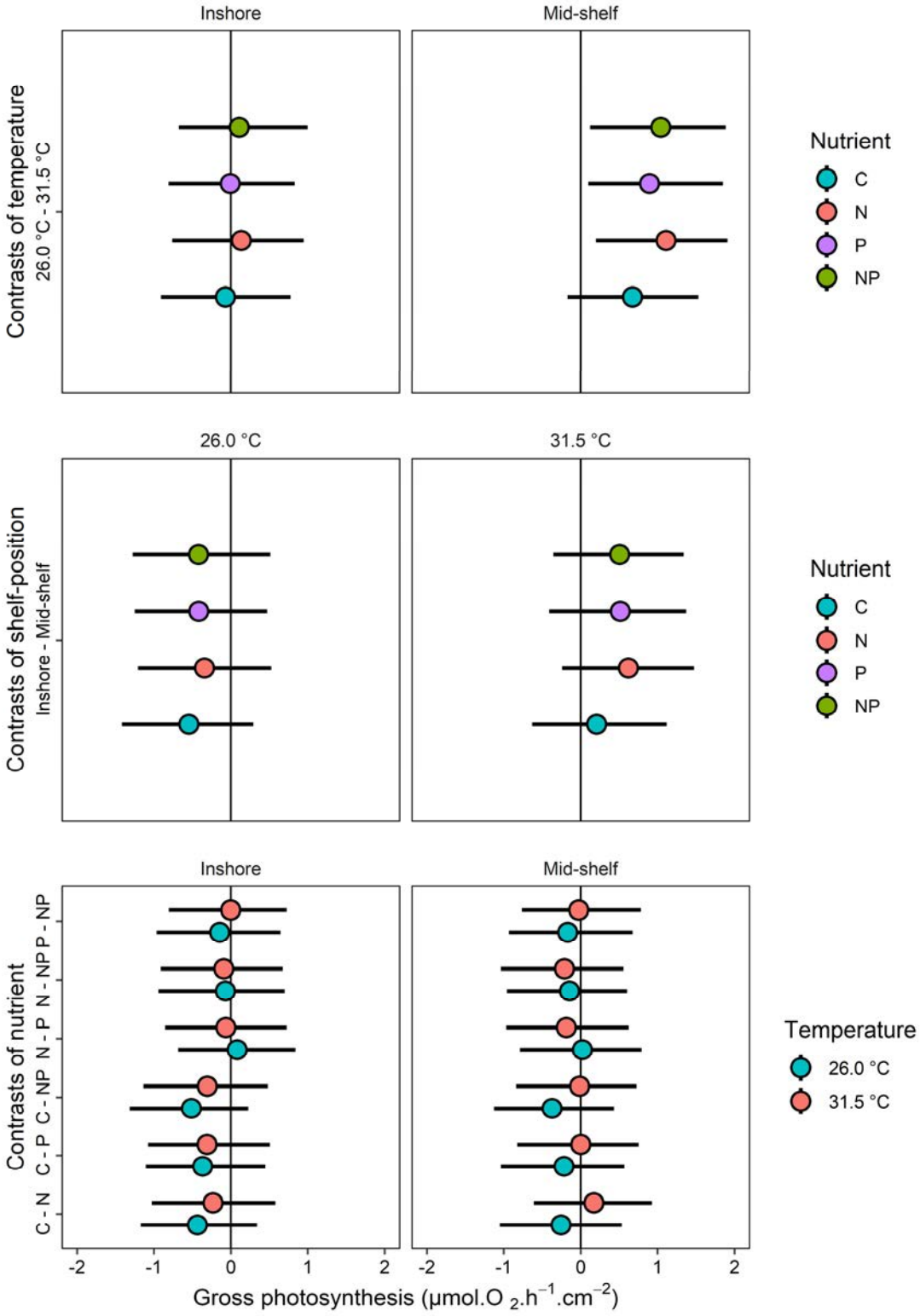

Figure S3.3 Gross photosynthesis (µmol $O_2.h^{-1}.cm^{-2}$) median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

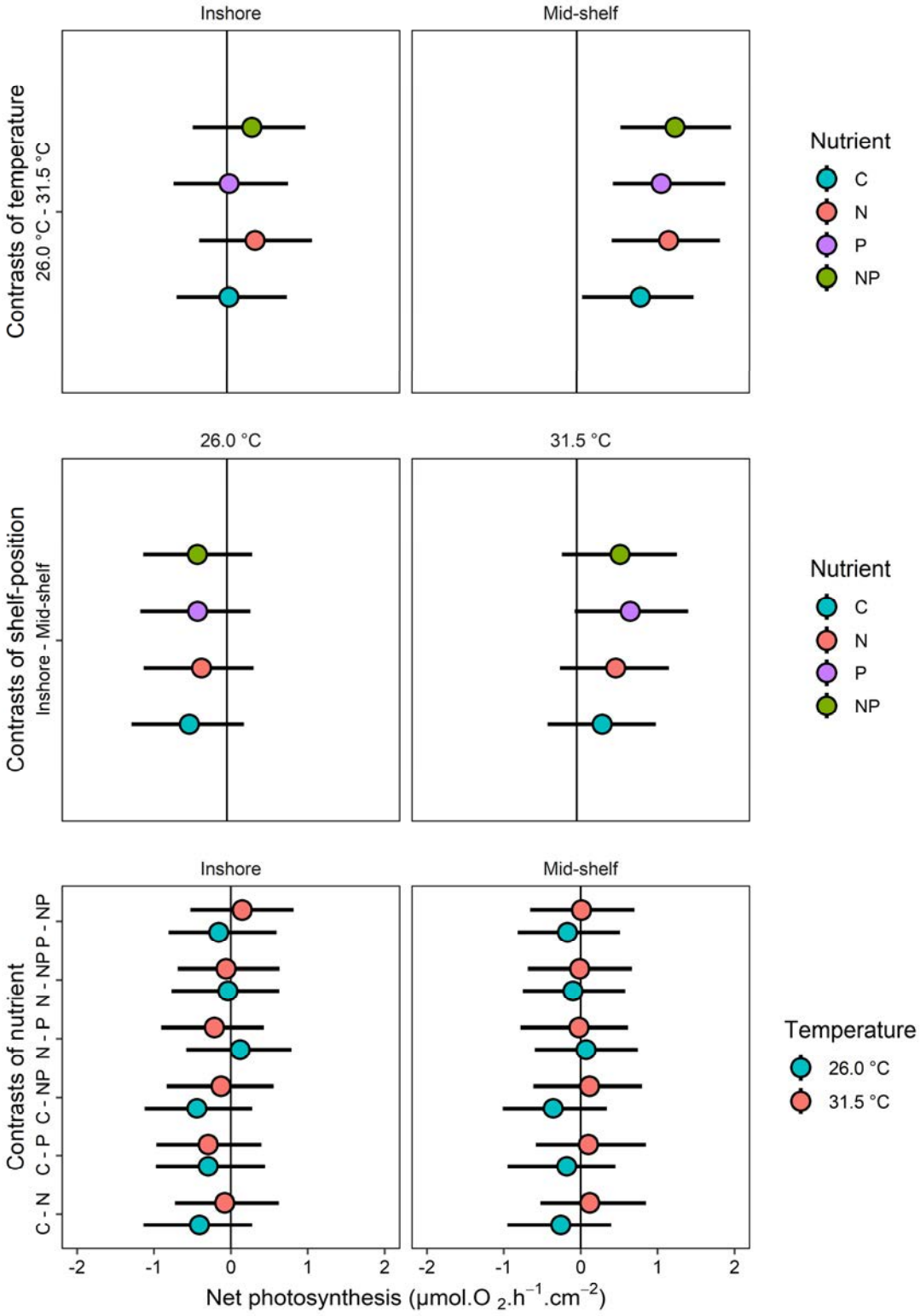

Figure S3.4 Net photosynthesis (µmol $O_2.h^{-1}.cm^{-2}$) median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

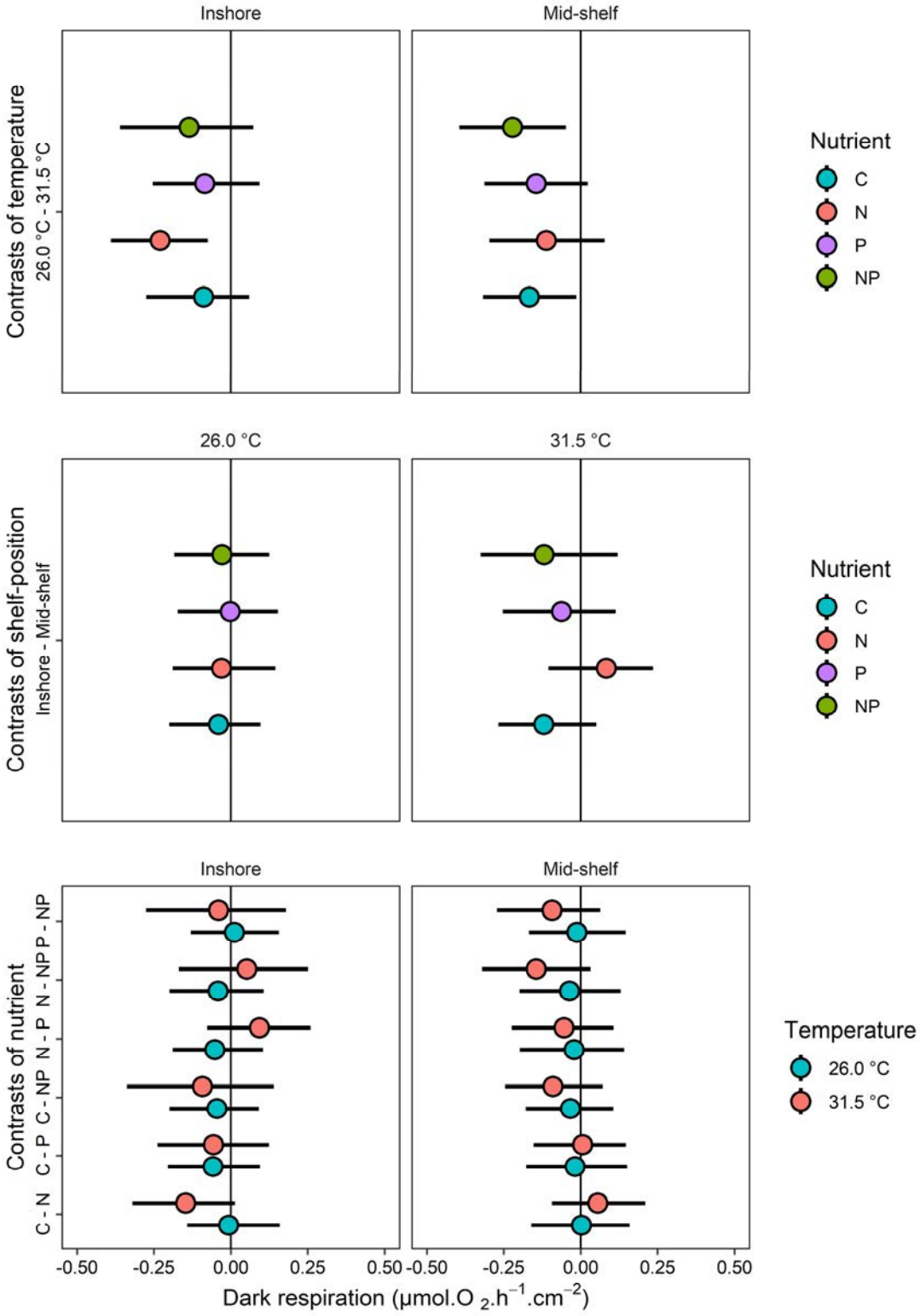

Figure S3.5 Dark respiration (µmol $O_2.h^{-1}.cm^{-2}$) median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

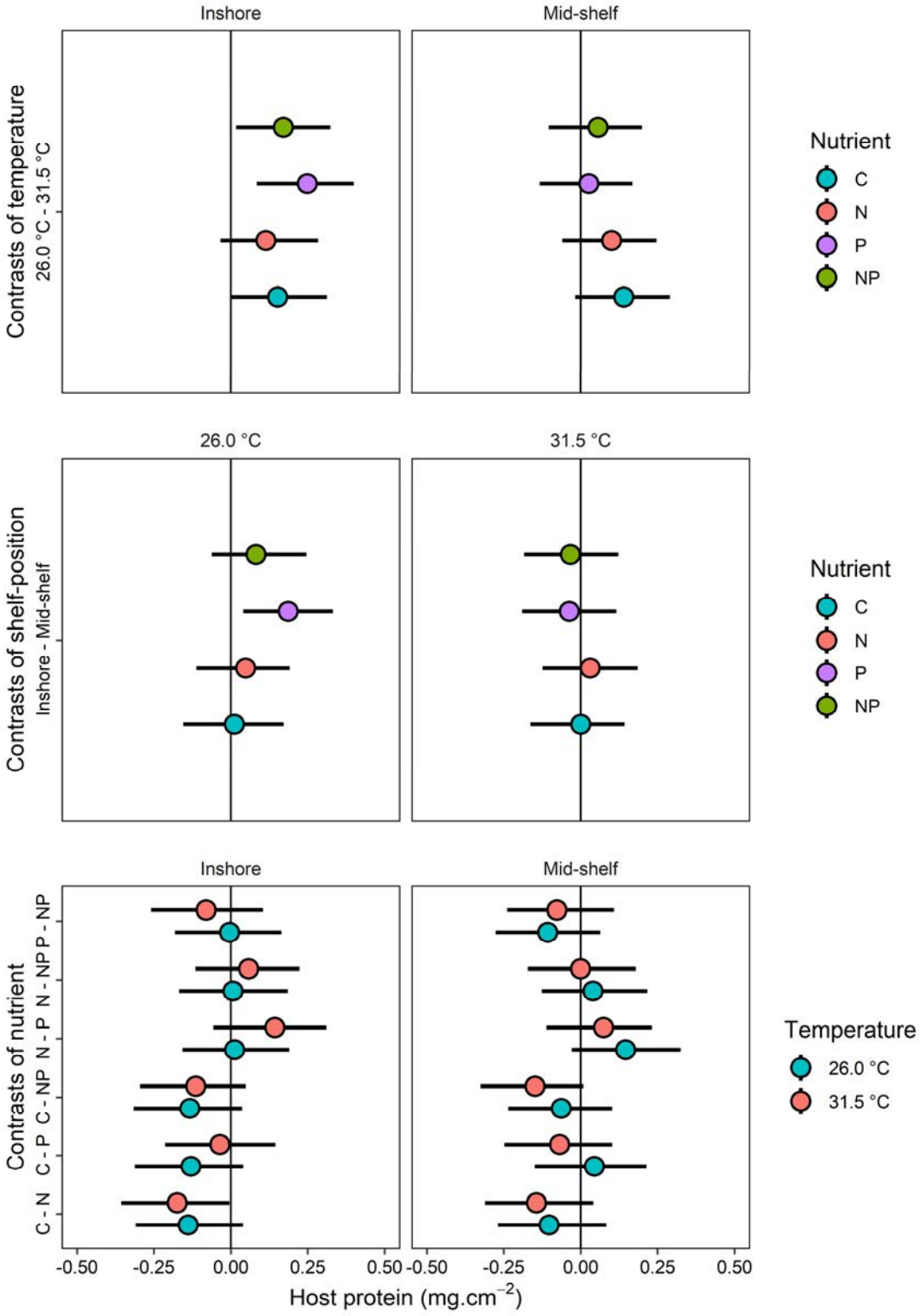

Figure S3.6 Coral host protein concentration (mg.cm^{-2}) median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

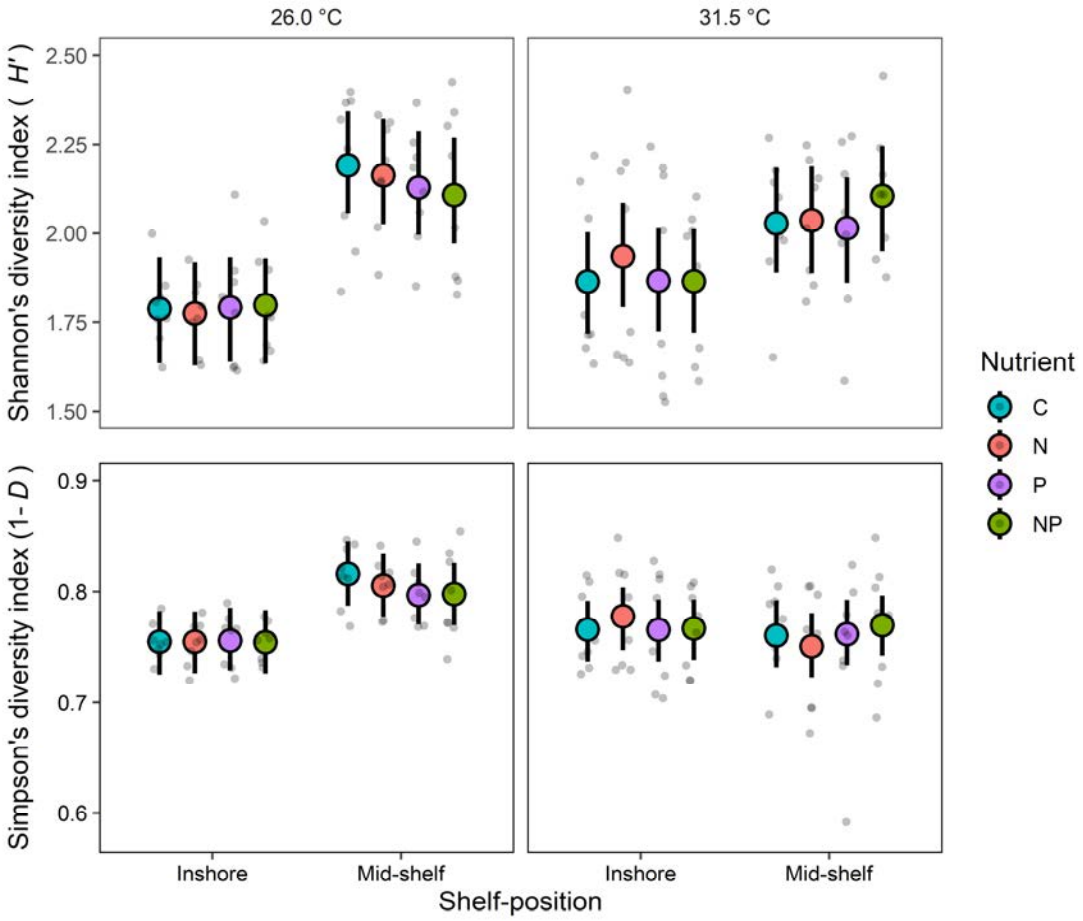

Figure S3.7 Shannon's (*H'*) and Simpson's (1-*D*) diversity indices of algal symbiont DIVs, according to temperature (left vs right) and nutrient treatment (colour). Coloured points and error bars show median model simulations with 95% CIs. Grey dots show the raw data points.

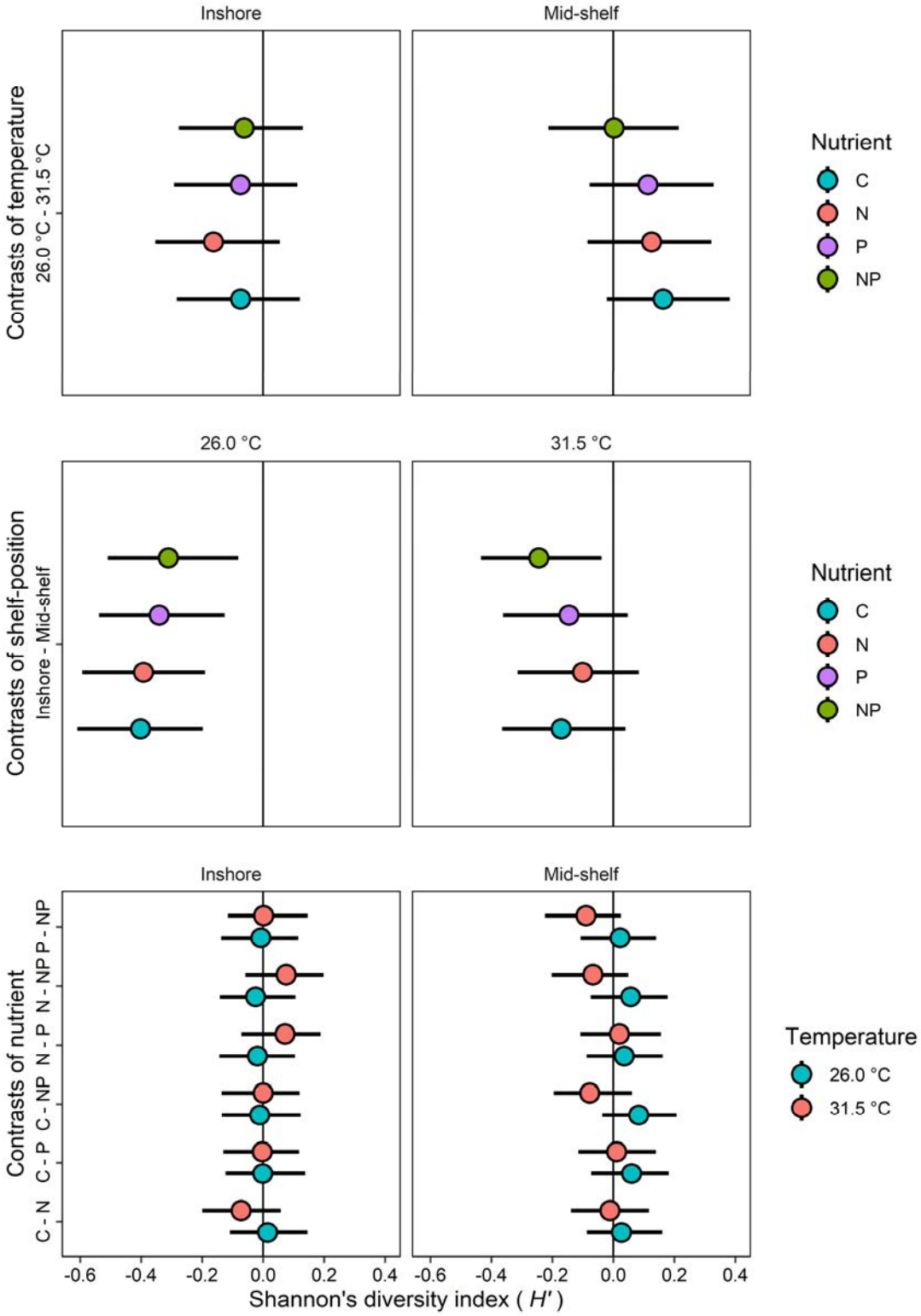

Figure S3.8 Shannon's diversity index (*H'*) of algal symbiont DIVs median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

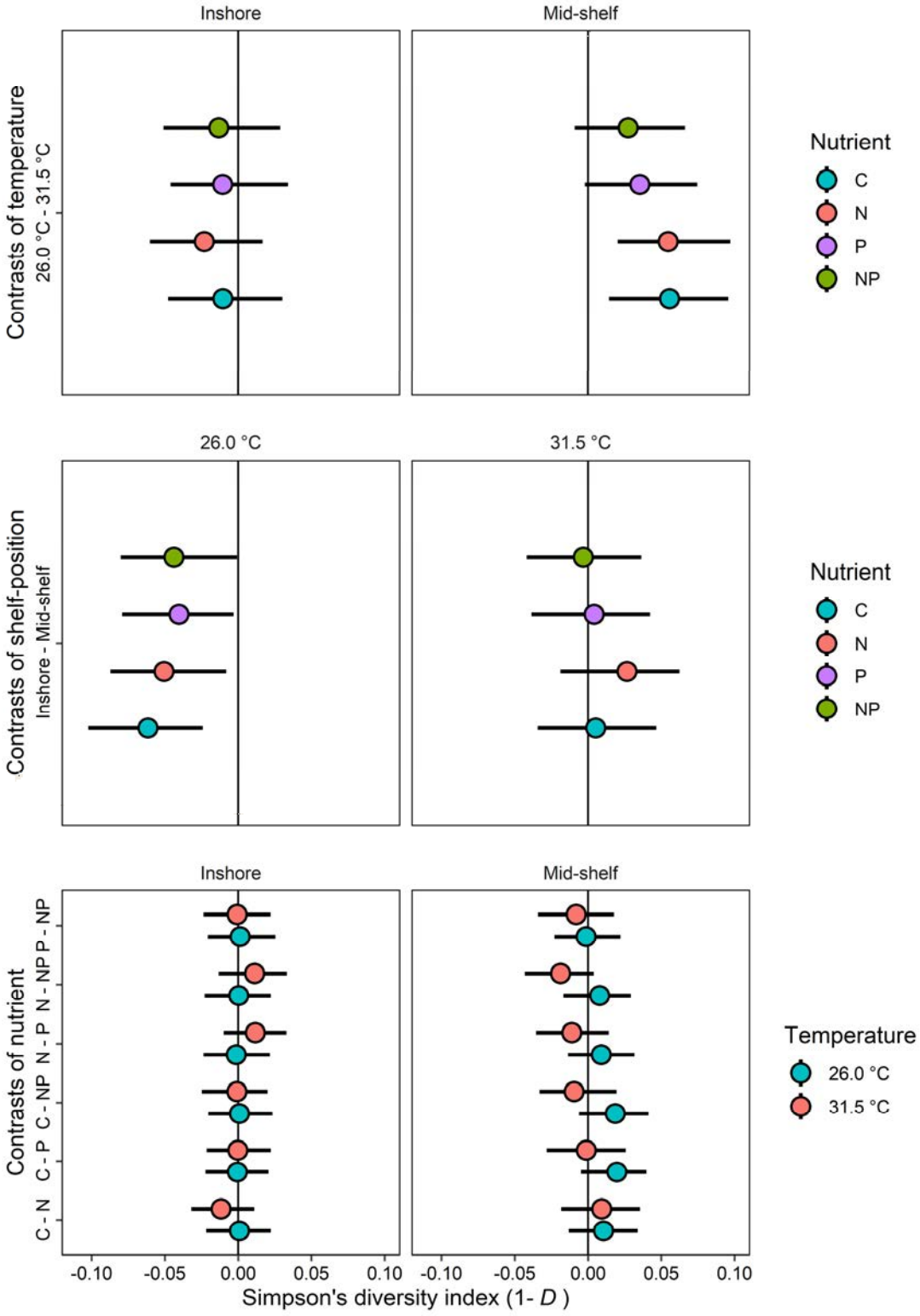

Figure S3.9 Simpson's diversity index (1-*D*) of algal symbiont DIVs median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

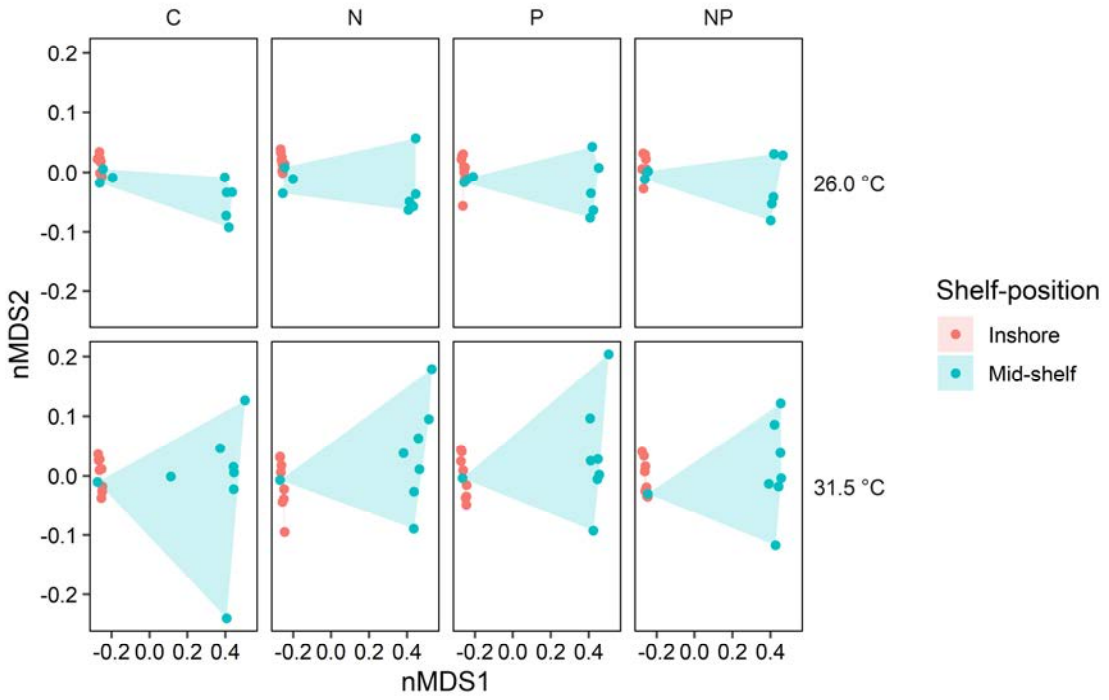

Figure S3.10 nMDS plots of inshore and mid-shelf algal symbiont DIVs from Bray-Curtis dissimilarities according to temperature (top vs bottom) and nutrient treatment (left to right).

Appendix S4: Supplementary material for Chapter 4

Table S4.1 Key details regarding the Bayesian (generalised) linear mixed models fit to each trait, including the model distributions, variance structures and R^2 values. Trait responses marked with an asterisk were made positive to conform with a Gamma distribution by adding a number just larger than the most negative value.

Trait	Compartment	Distribution	Variance structure	R^2 (95 % CIs)
Chlorophyll content (µg.cm^{-2})	Algal symbiont	Gamma	Sampling period x Shelf-position	0.73 (0.69-0.76)
Protein content (mg.cm^{-2})	Coral host	Gaussian	Sampling period x Shelf-position	0.65 (0.61-0.68)
Carbon content (%)	Algal symbiont	Student	Sampling period x Shelf-position	0.50 (0.44-0.55)
	Coral host	Student	Homogeneous	0.42 (0.35-0.48)
Nitrogen content (%)	Algal symbiont	Student	Sampling period x Shelf-position	0.33 (0.25-0.41)
	Coral host	Student	Homogenous	0.36 (0.29-0.43)
Ratio of carbon to nitrogen content (C:N)	Algal symbiont	Student	Sampling period x Shelf-position	0.13 (0.09-0.18)
	Coral host	Student	Homogenous	0.22 (0.16-0.27)
Inorganic carbon metabolism (δ^{13}C)	Algal symbiont	Student	Sampling period x Shelf-position	0.64 (0.60-0.67)
	Coral host*	Gamma	Sampling period x Shelf-position	0.44 (0.36-0.51)
Nitrate metabolism (δ^{15}N)	Algal symbiont*	Gamma	Nutrient condition x Sampling period	0.49 (0.37-0.59)
	Coral host	Gamma	Nutrient condition x Sampling period x Shelf-position	0.39 (0.31-0.47)
Shannon's diversity index (*H'*)	Algal symbiont	Student	Nutrient condition x Sampling period * Shelf-position	0.30 (0.24-0.36)
Simpson's diversity index (1-*D*)	Algal symbiont	Student	Sampling period x Shelf-position	0.27 (0.22-0.33)

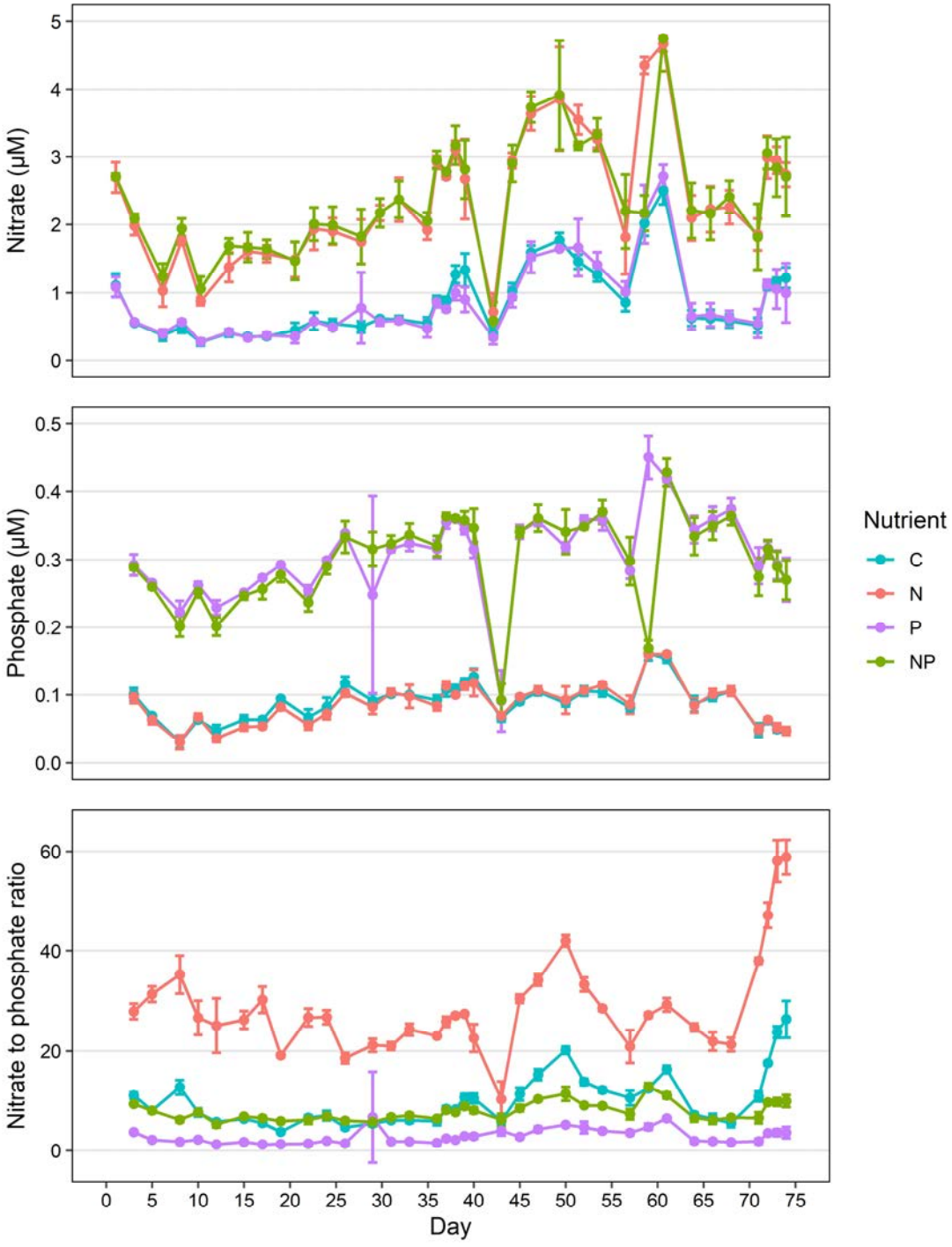

Figure S4.1 Nitrate and phosphate concentrations (µM) and ratios throughout the experiment, by nutrient treatment. Points and error bars represent the mean ± s.d. for replicate tanks within a treatment.

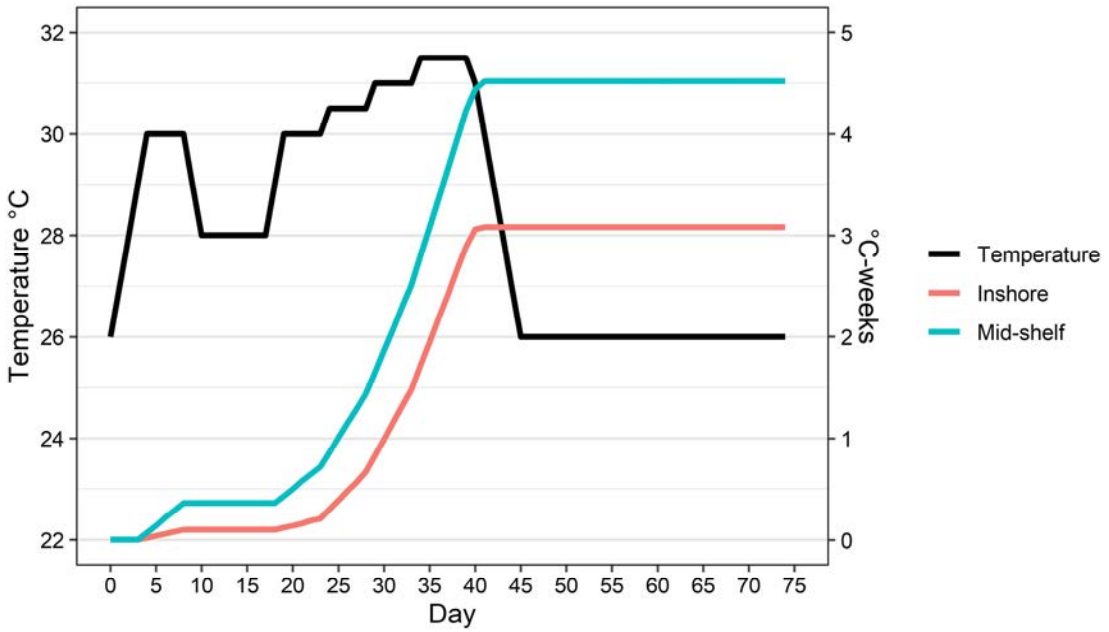

Figure S4.2 Experimental profiles for set values of temperature (°C) and corresponding accumulated heat stress (°C-weeks). Inshore heat stress is calculated relative to Havannah Island and mid-shelf heat stress relative to John Brewer Reef.

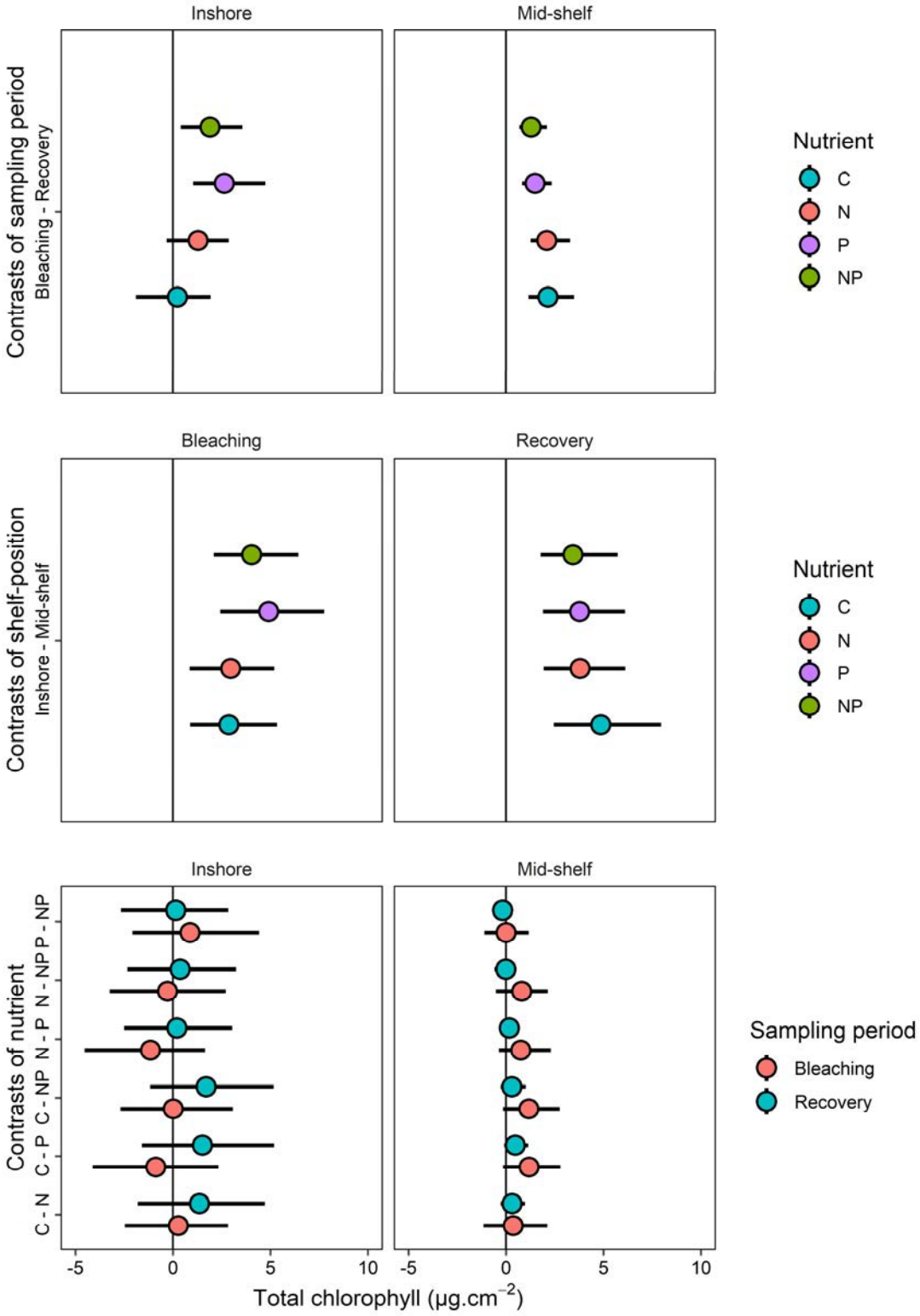

Figure S4.3 Total chlorophyll content (µg.cm^{-2}) median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

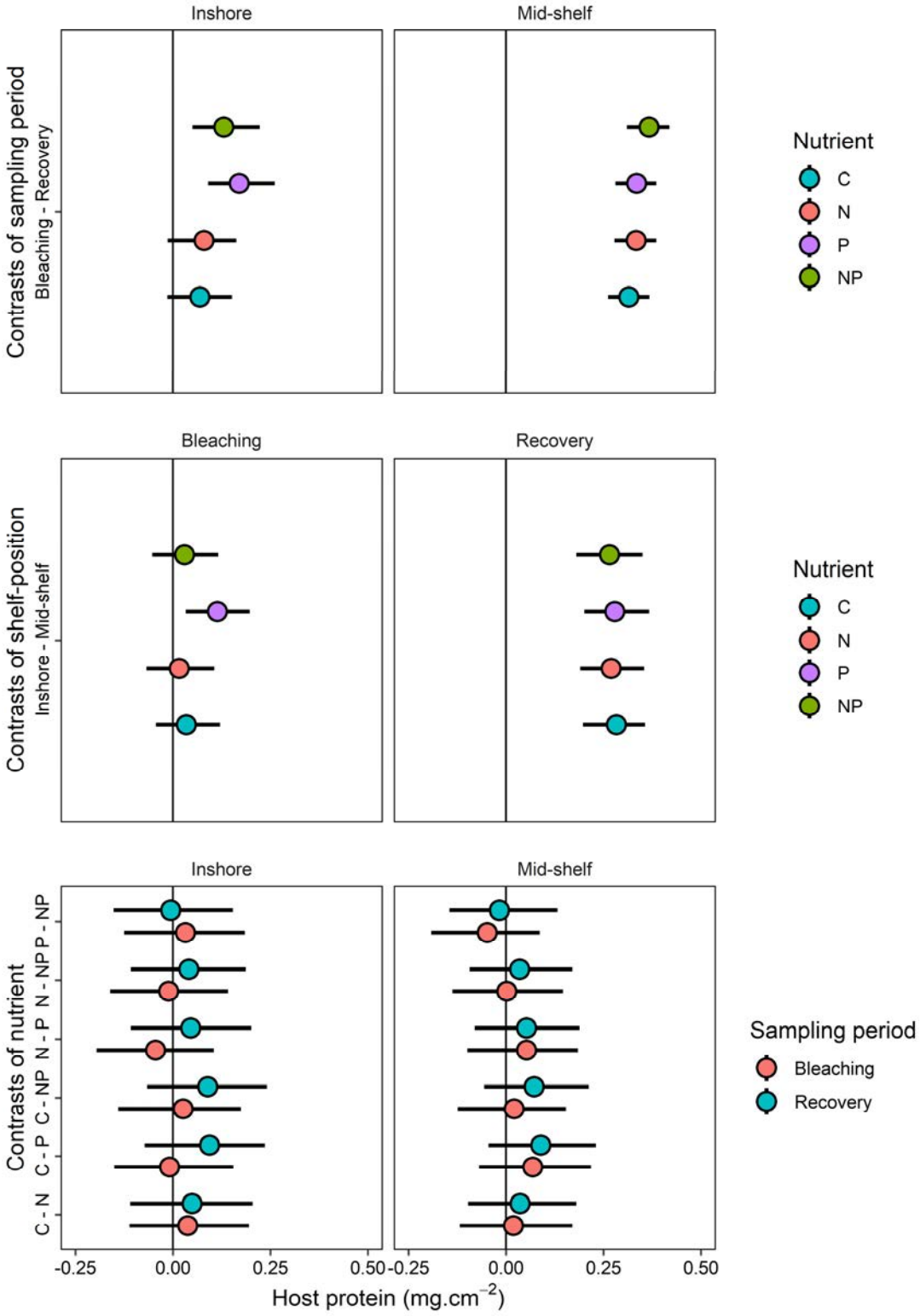

Figure S4.4 Coral host protein content (mg.cm^{-2}) median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

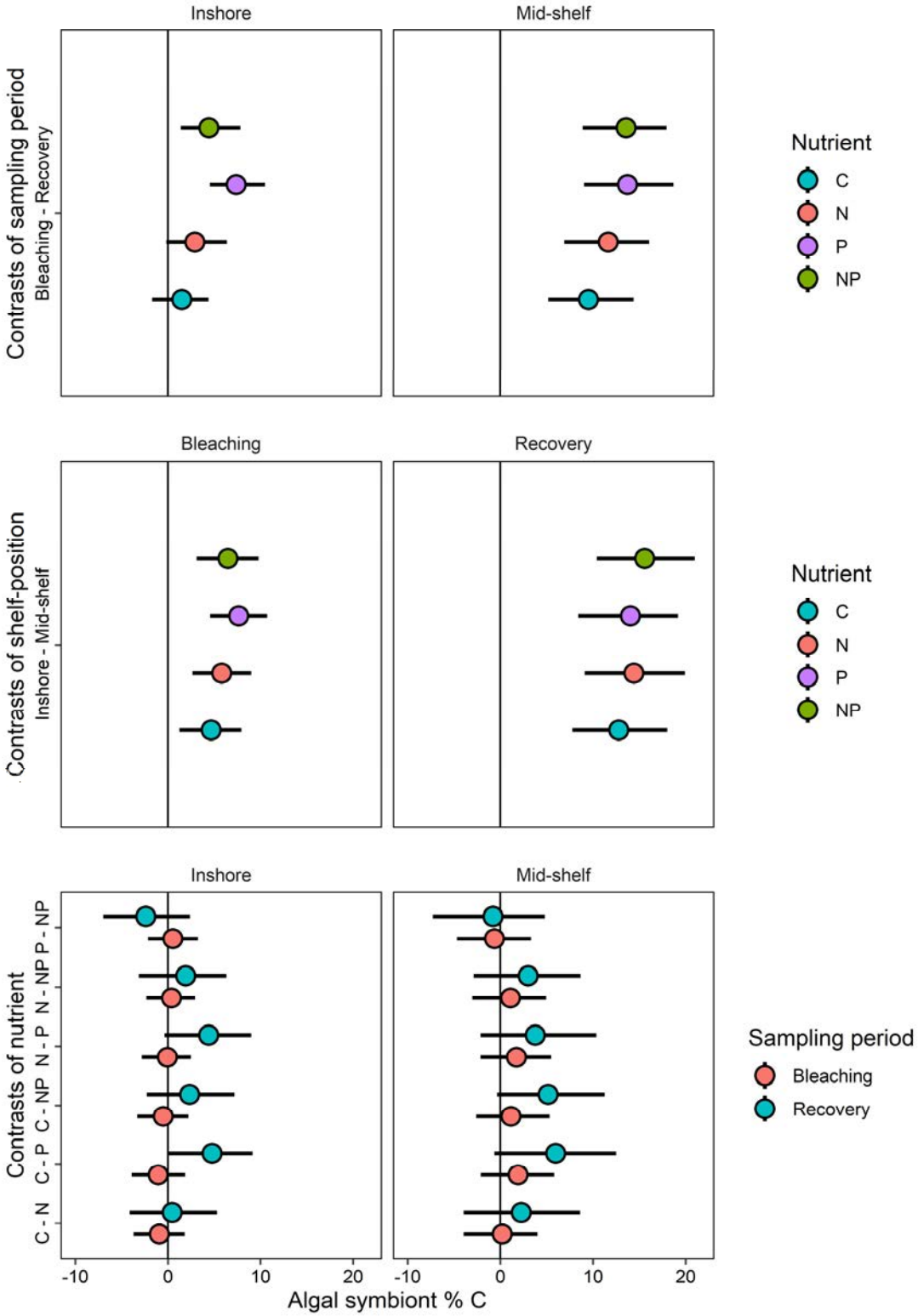

Figure S4.5 Algal symbiont percentage carbon median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

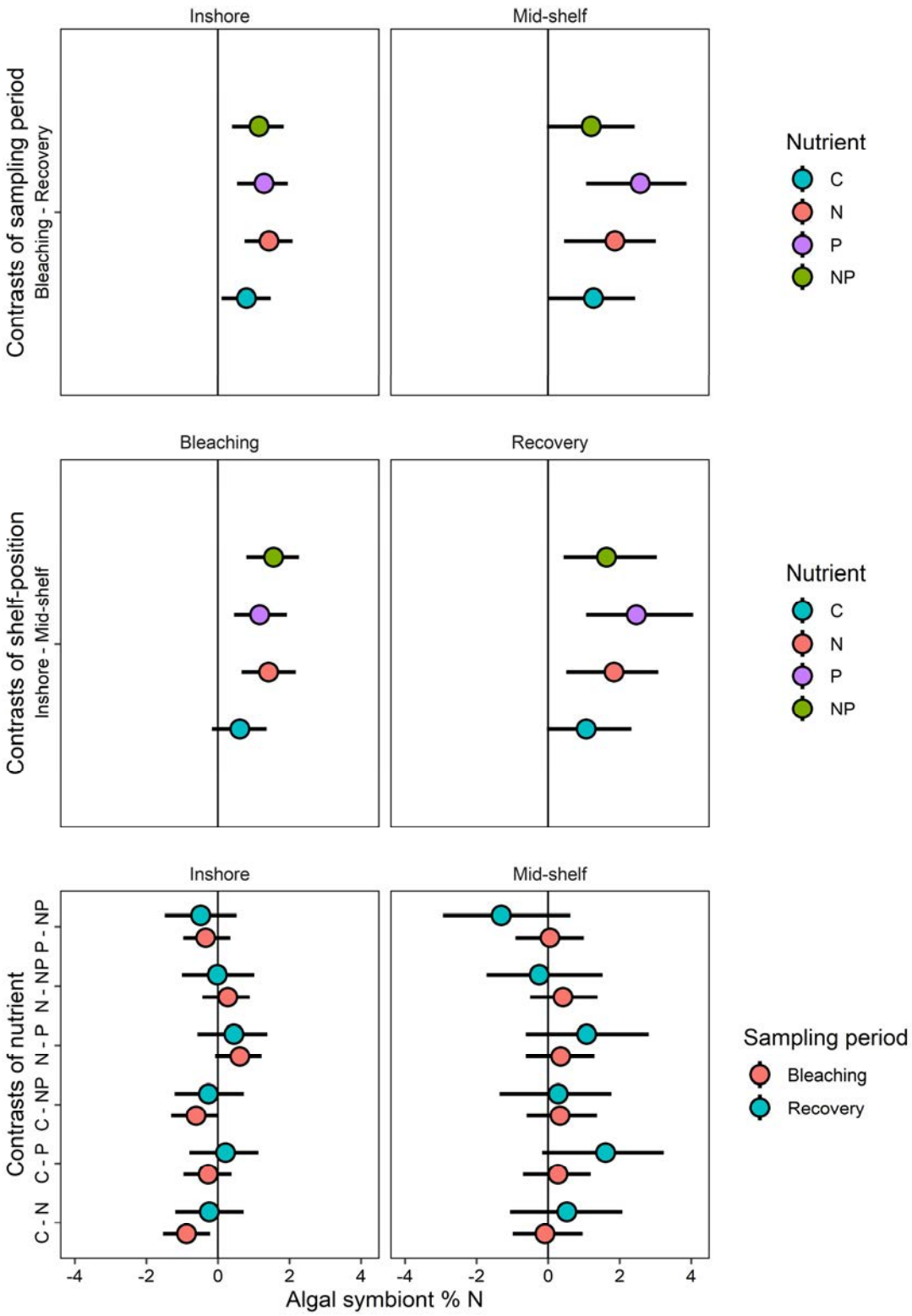

Figure S4.6 Algal symbiont percentage nitrogen median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

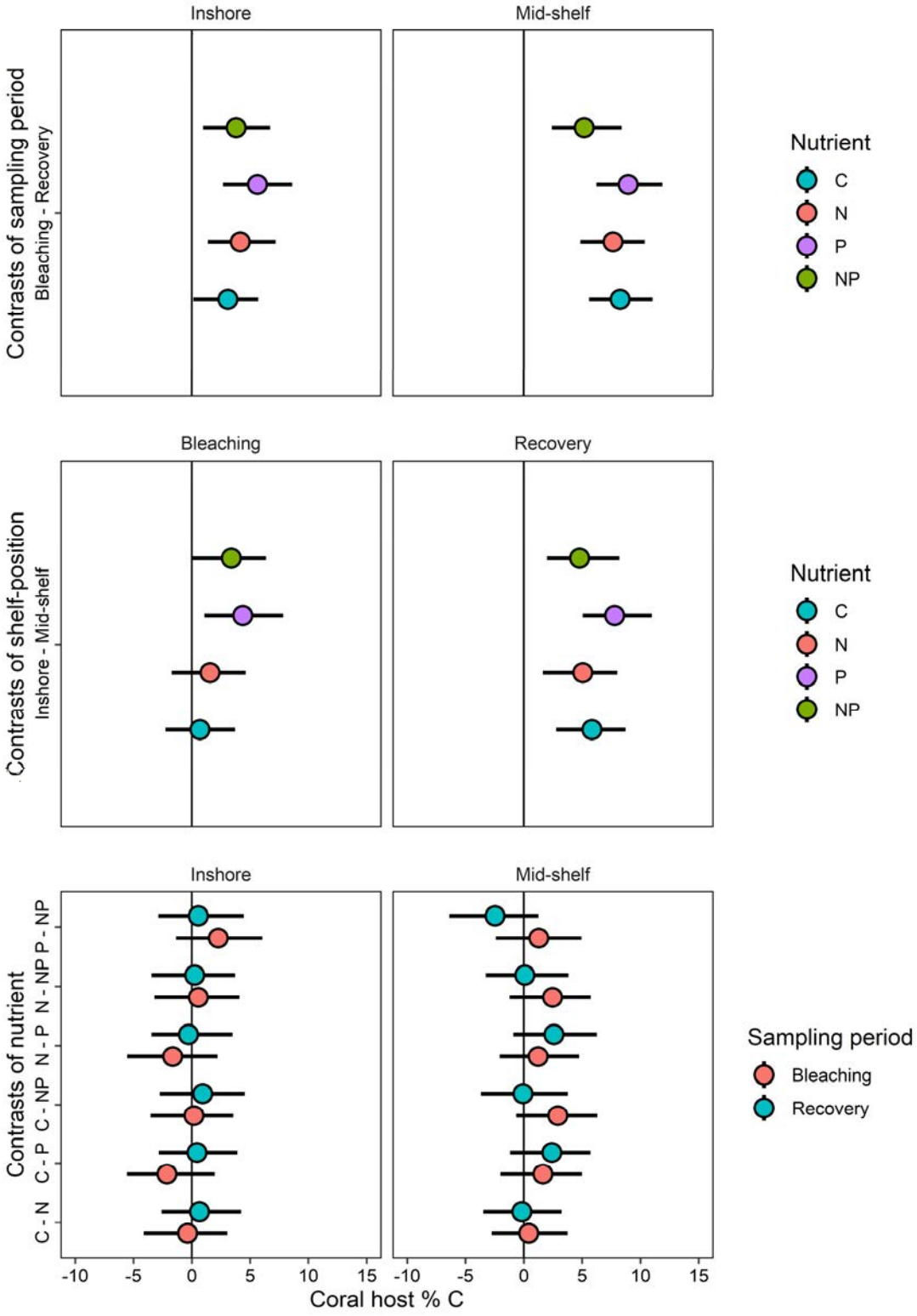

Figure S4.7 Coral host percentage carbon median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

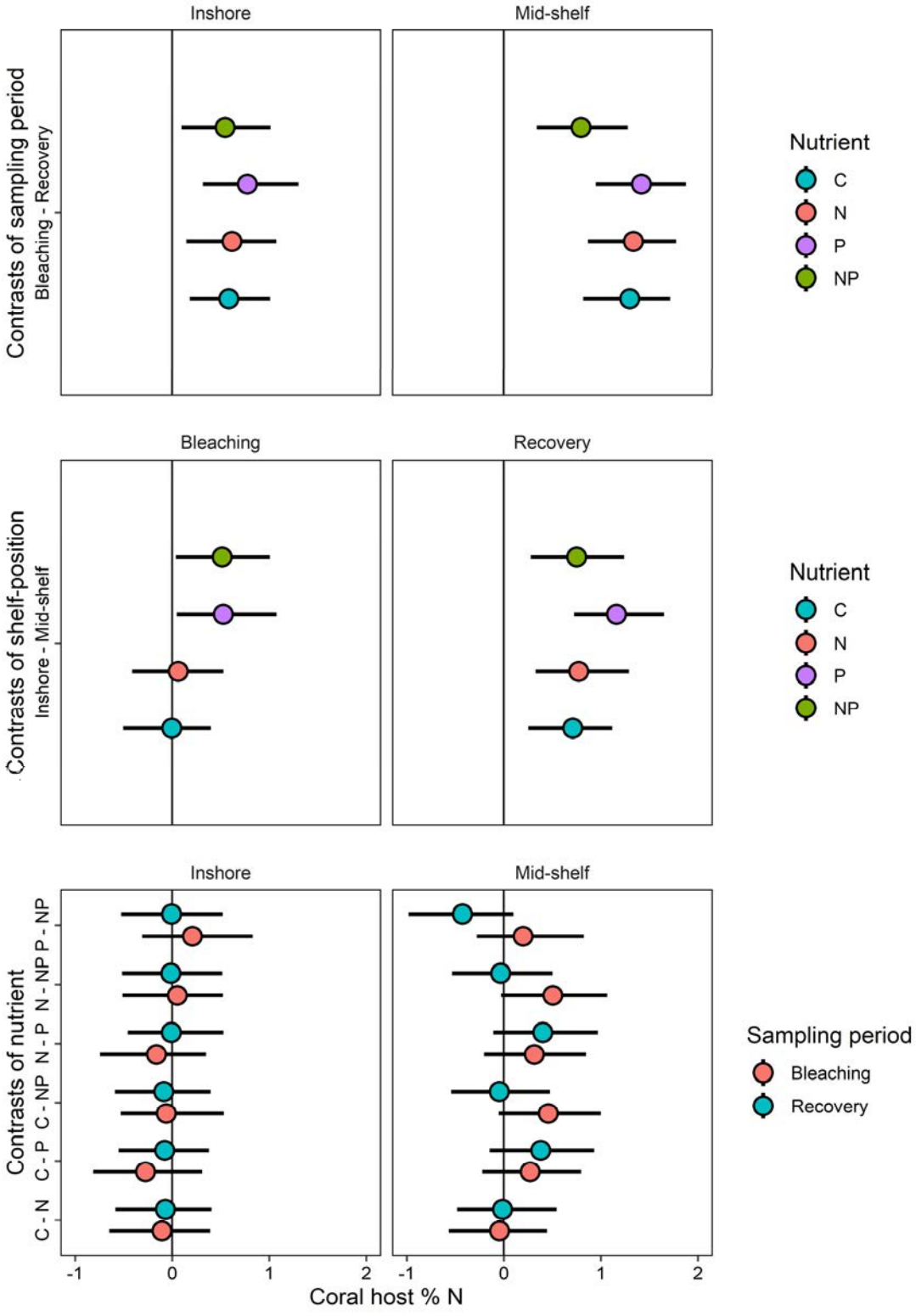

Figure S4.8 Coral host percentage nitrogen median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

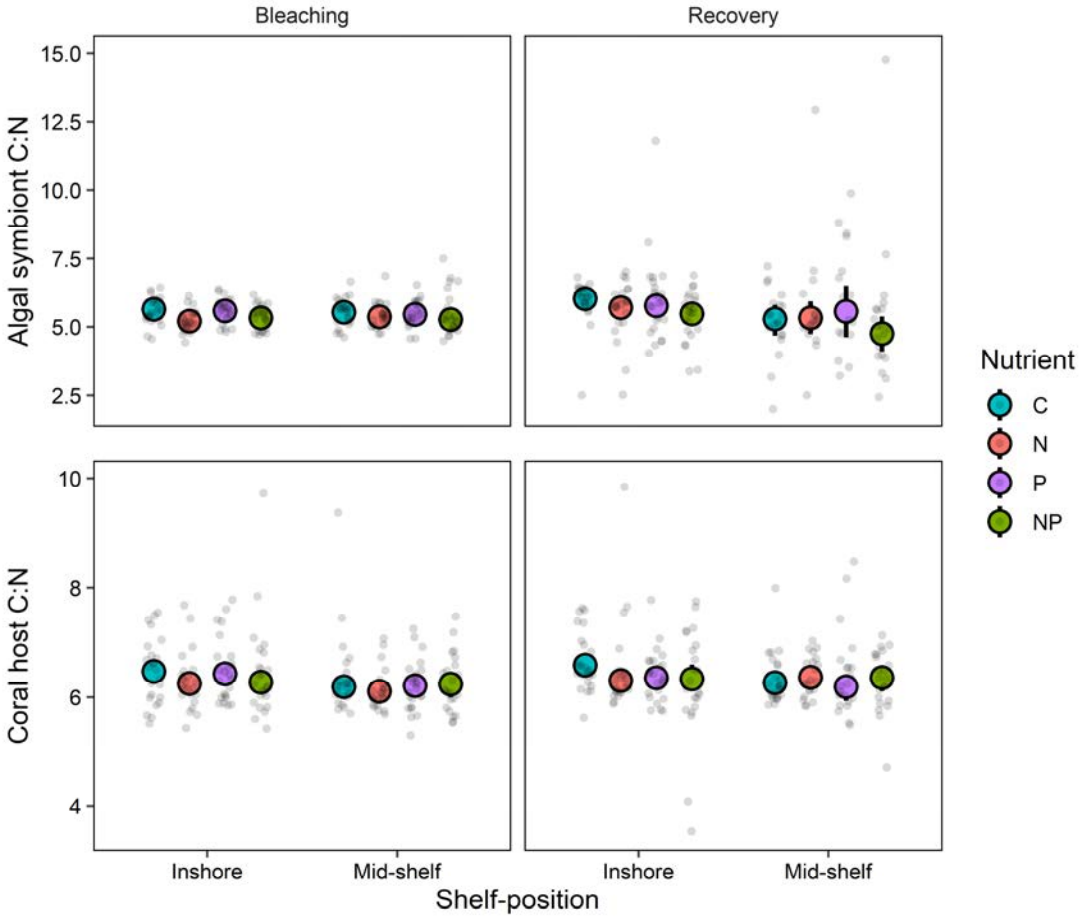

Figure S4.9 Algal symbiont and host carbon-to-nitrogen ratios within inshore and mid-shelf corals, according to sampling period (left vs right) and nutrient treatment (colour). Coloured points and error bars show median model simulations with 95% CIs. Grey dots show the raw data points.

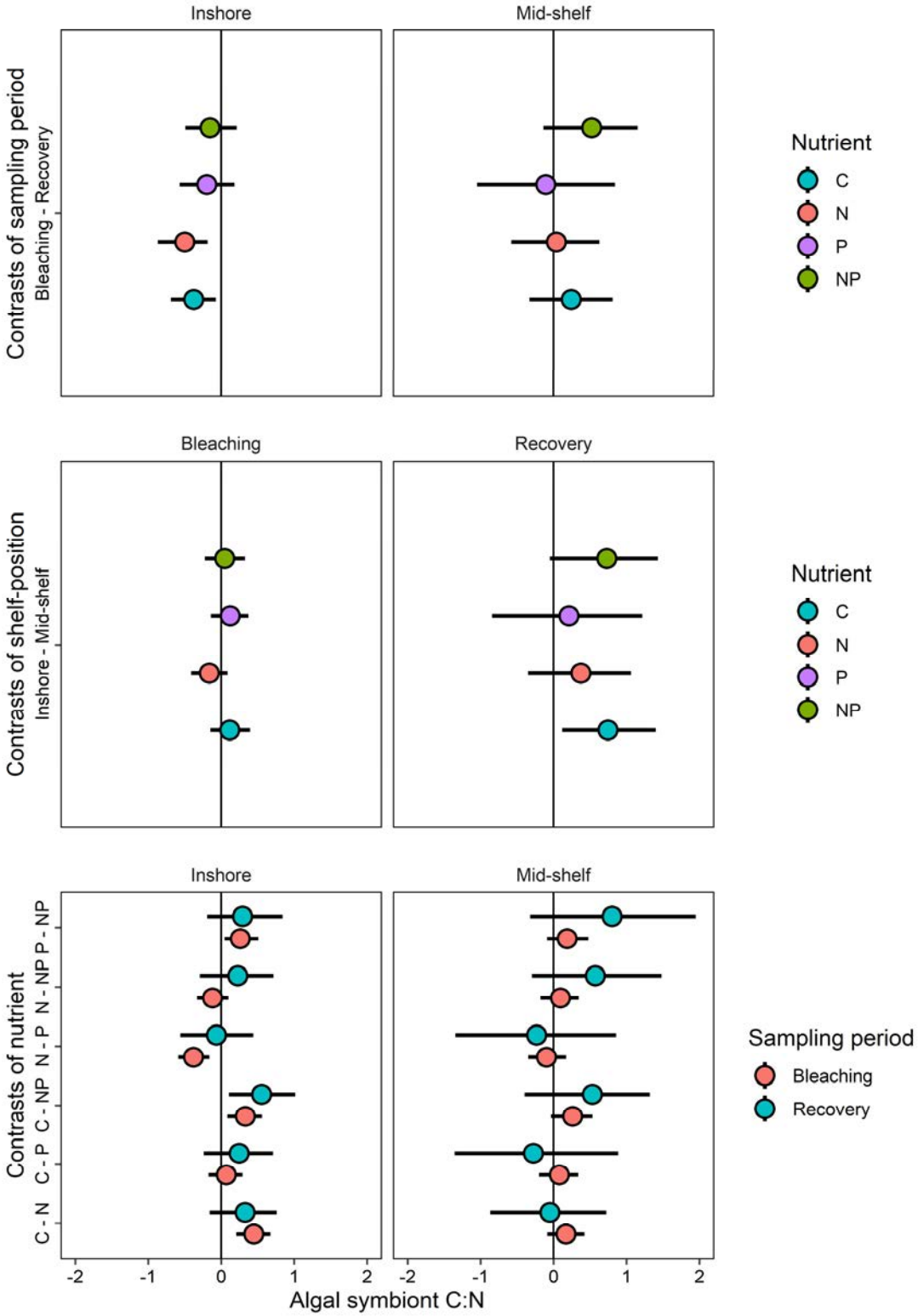

Figure S4.10 Algal symbiont carbon-to-nitrogen ratio median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

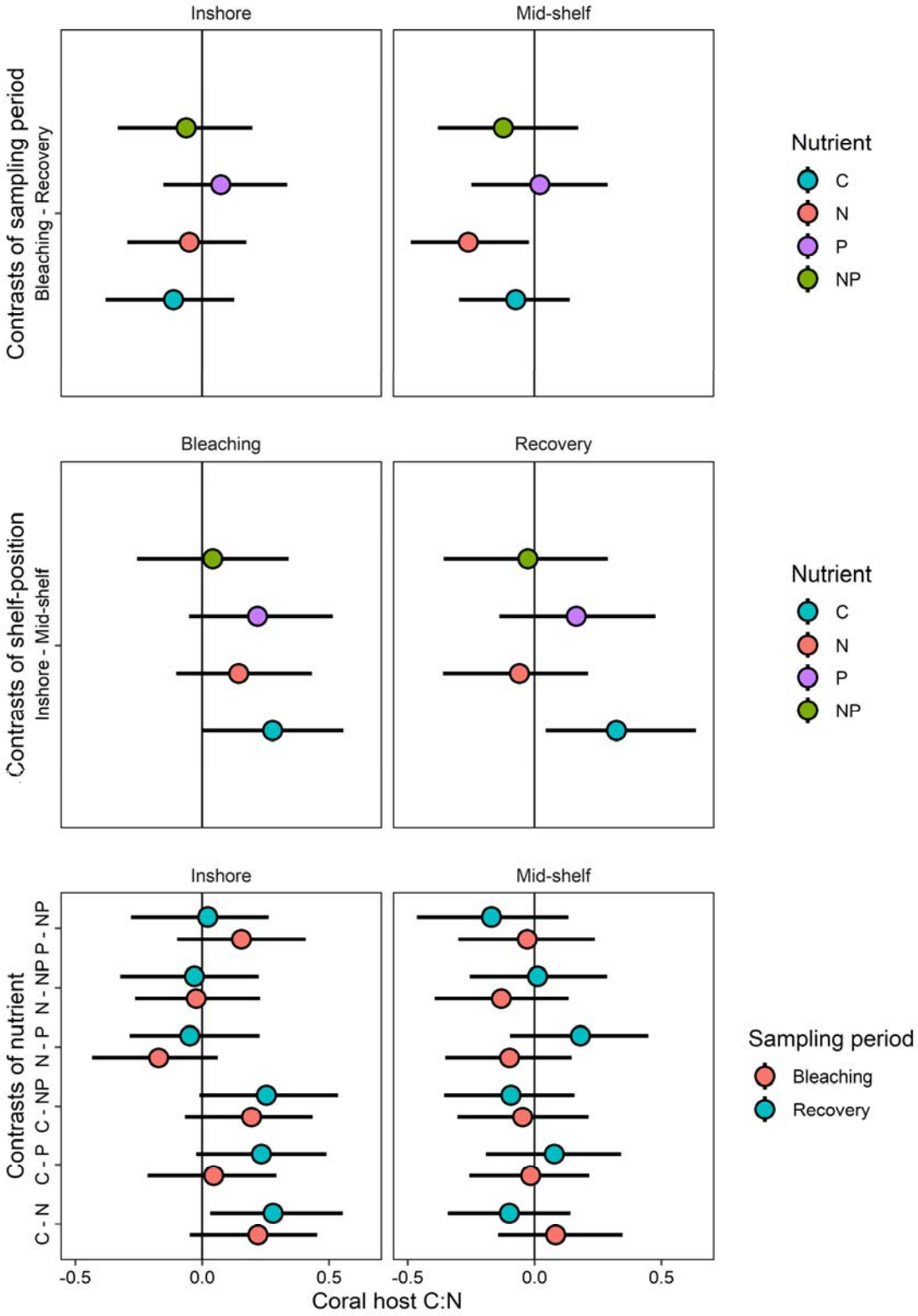

Figure S4.11 Coral host carbon-to-nitrogen ratio median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

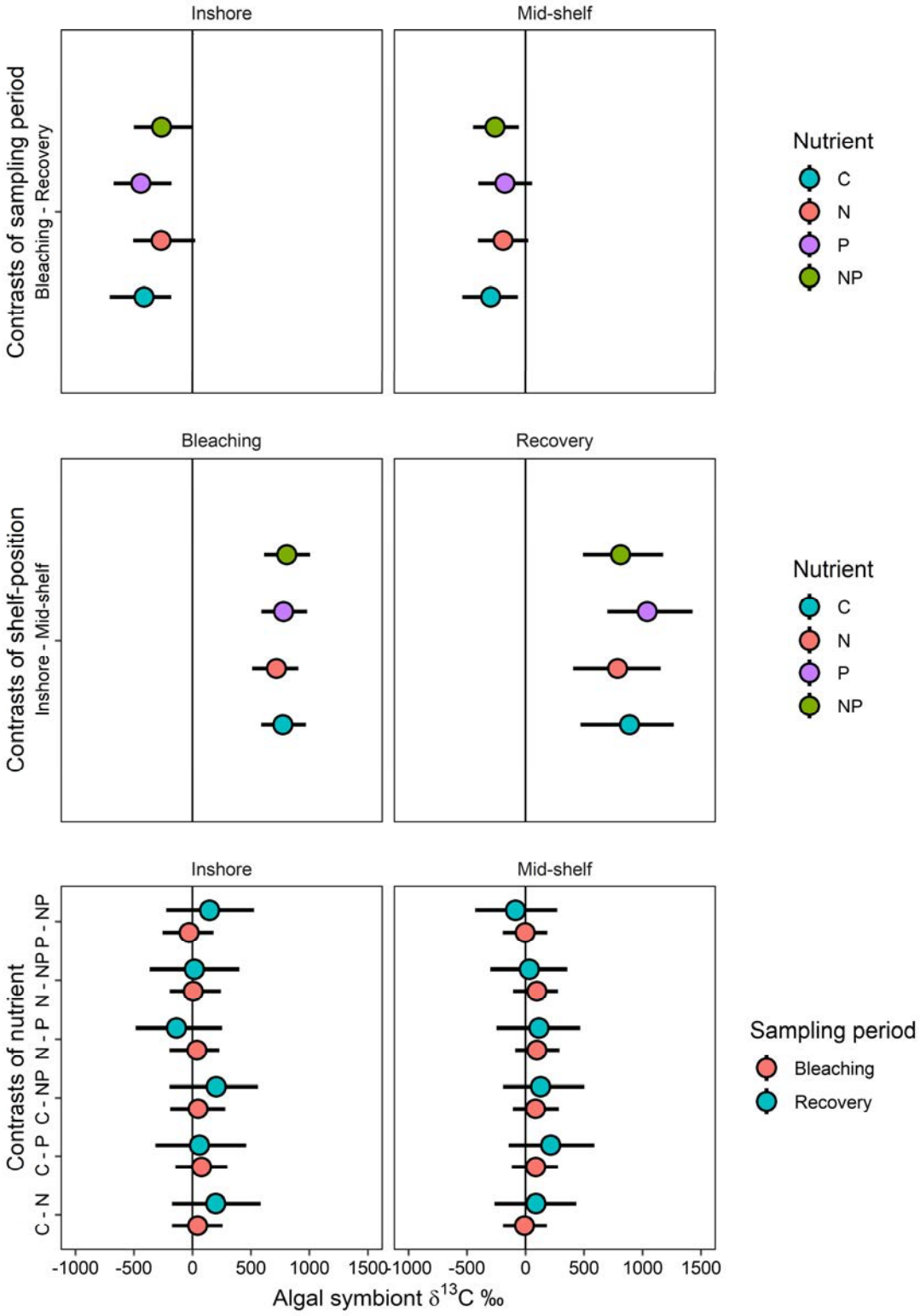

Figure S4.12 Algal symbiont $H^{13}CO_3^-$ enrichment median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

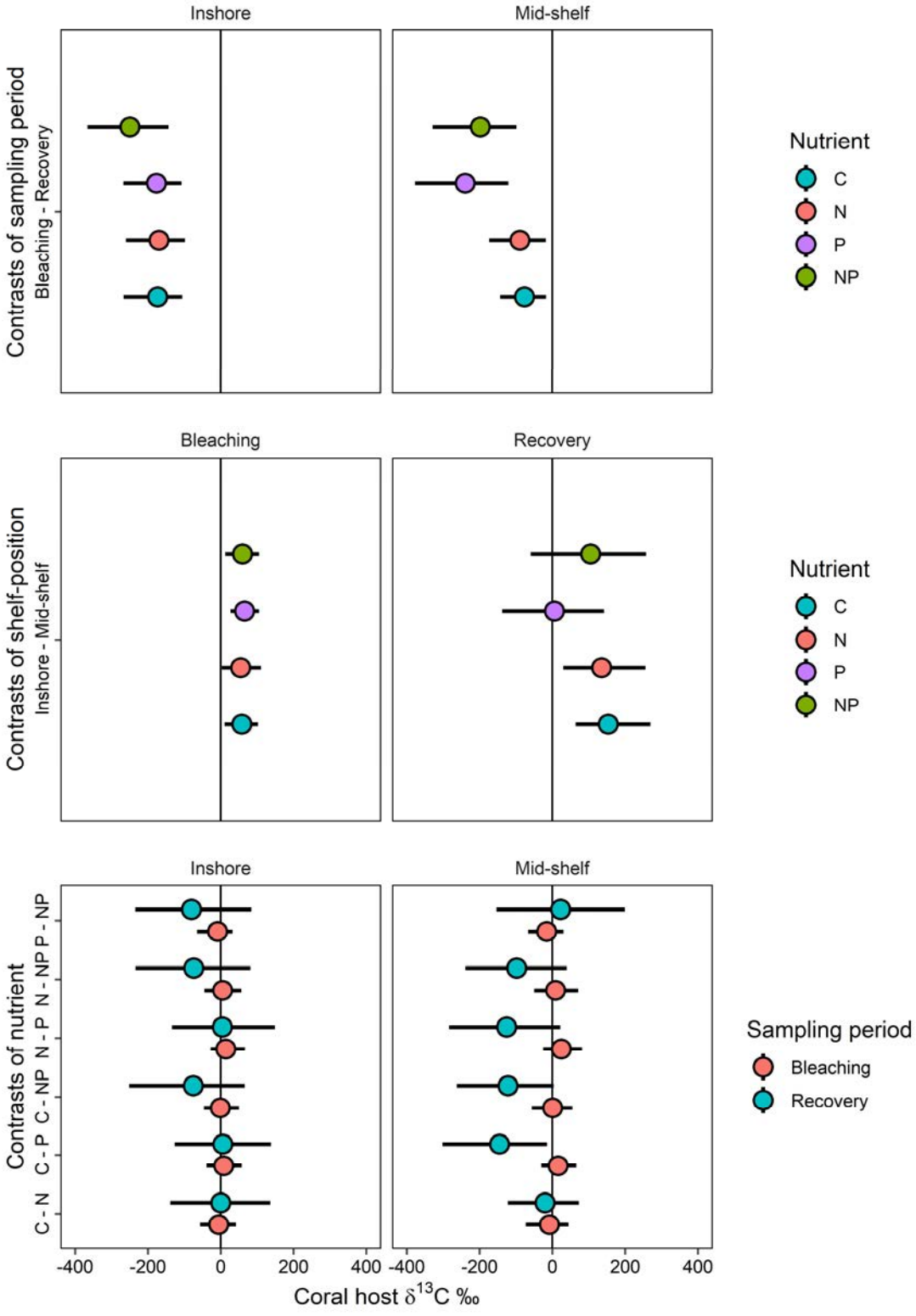

Figure S4.13 Coral host $H^{13}CO_3^-$ enrichment median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

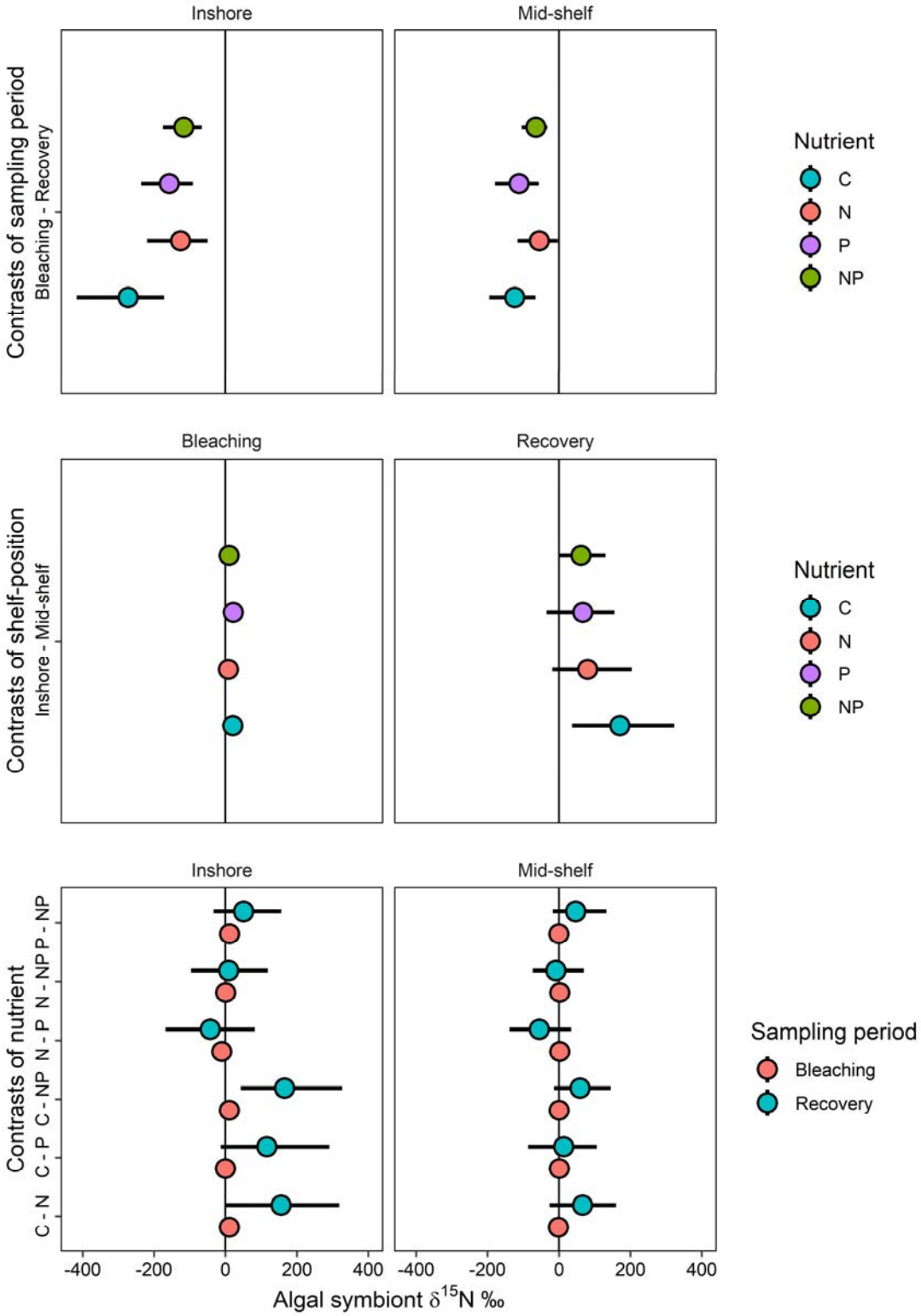

Figure S4.14 Algal symbiont $^{15}NO_3^-$ enrichment median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

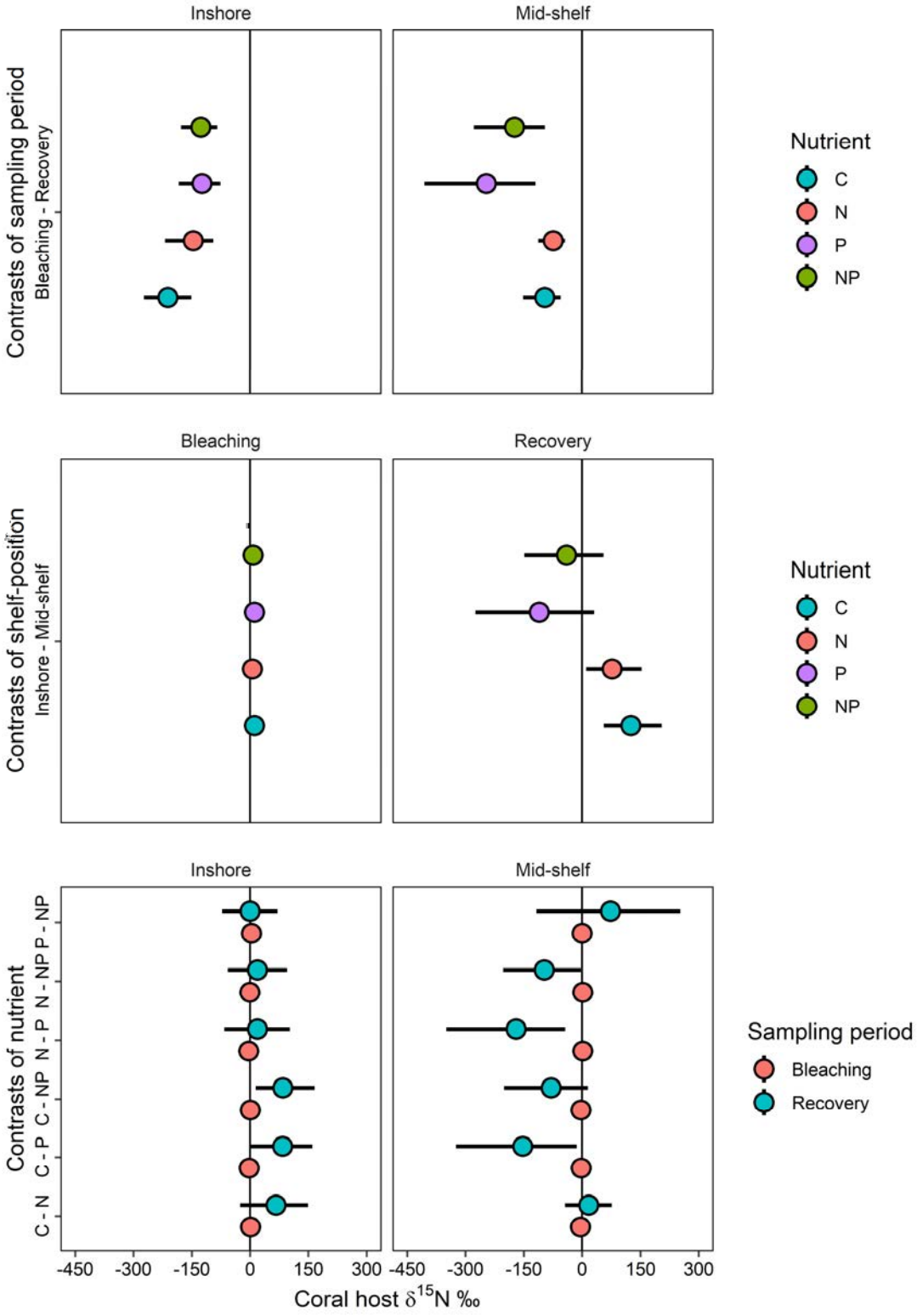

Figure S4.15 Coral host $^{15}NO_3^-$ enrichment median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

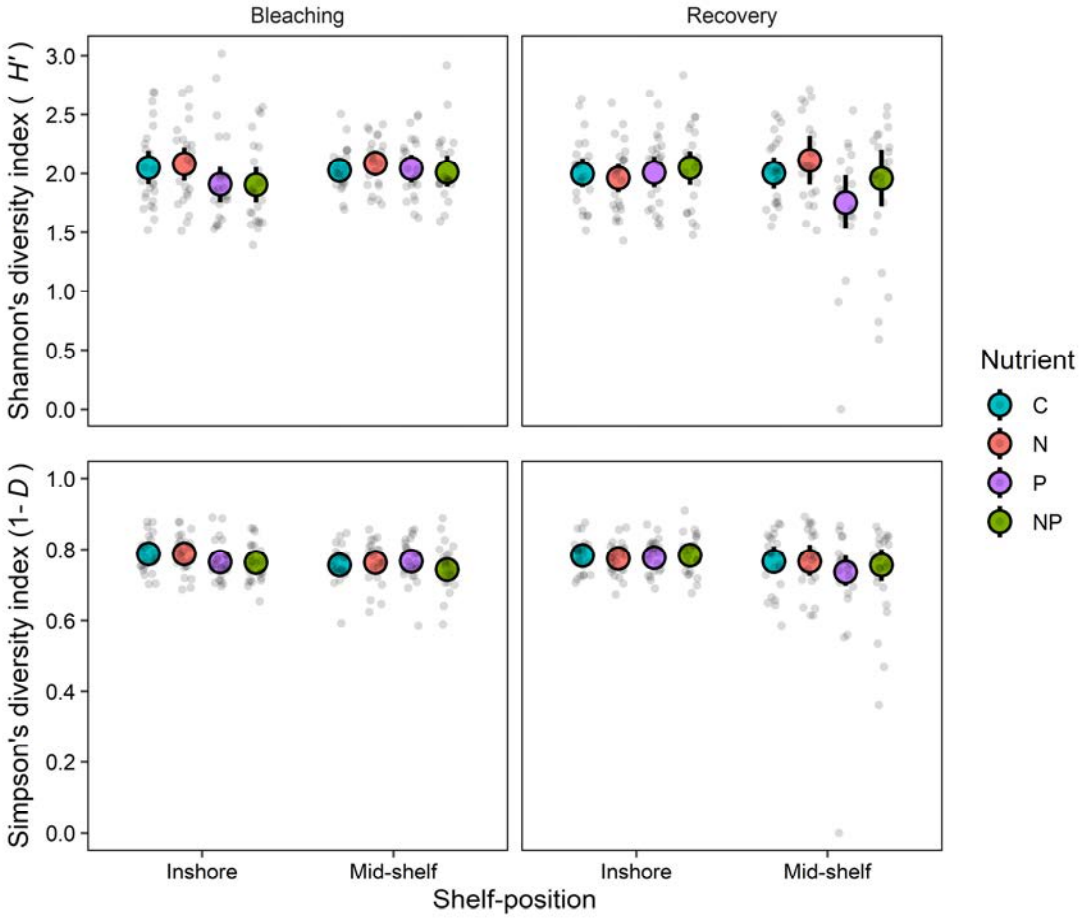

Figure S4.16 Shannon's (*H'*) and Simpson's (1-*D*) diversity indices of algal symbiont DIVs, according to sampling period (left vs right) and nutrient treatment (colour). Coloured points and error bars show median model simulations with 95% CIs. Grey dots show the raw data points.

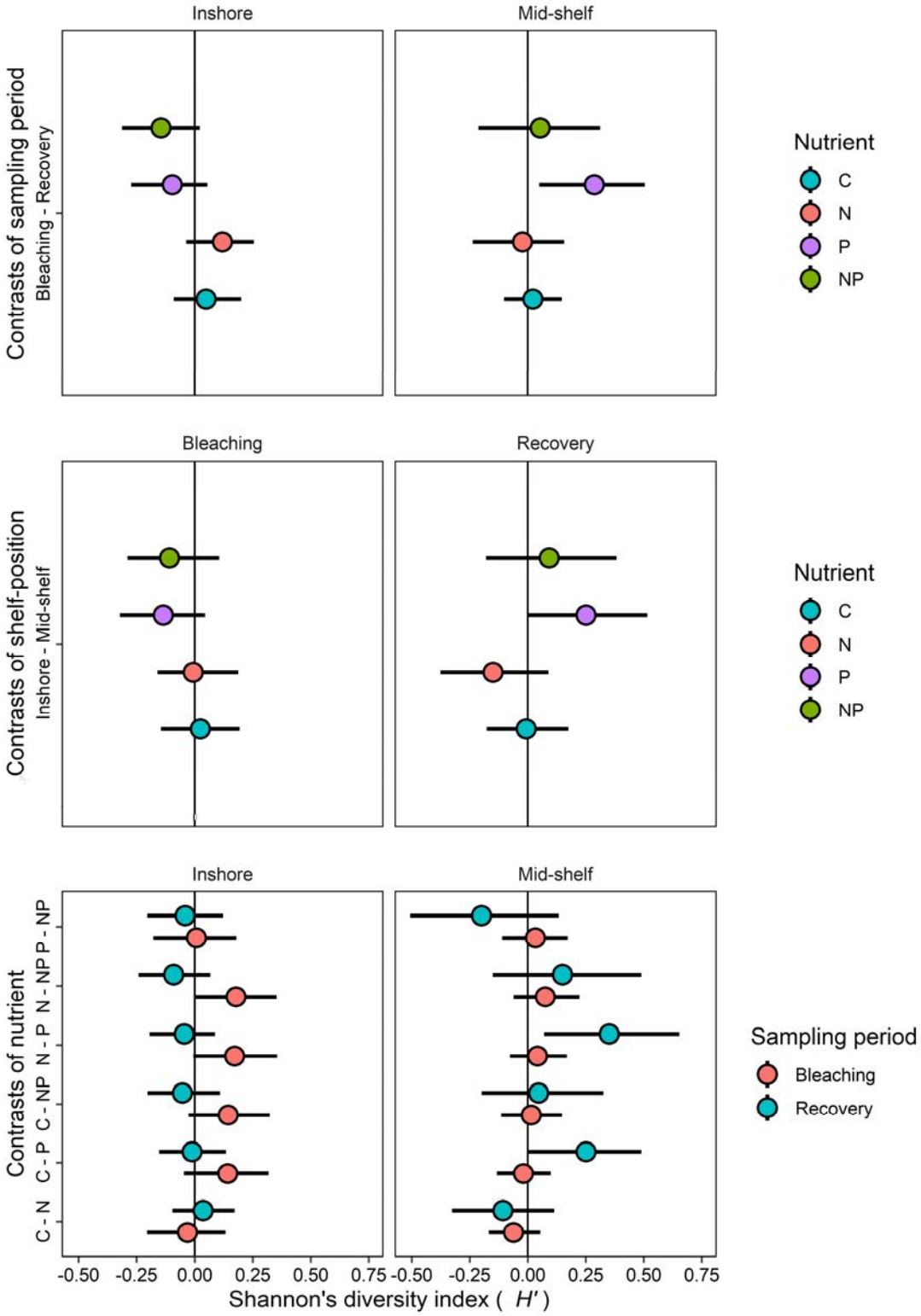

Figure S4.17 Shannon's diversity index (*H'*) of algal symbiont DIVs median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

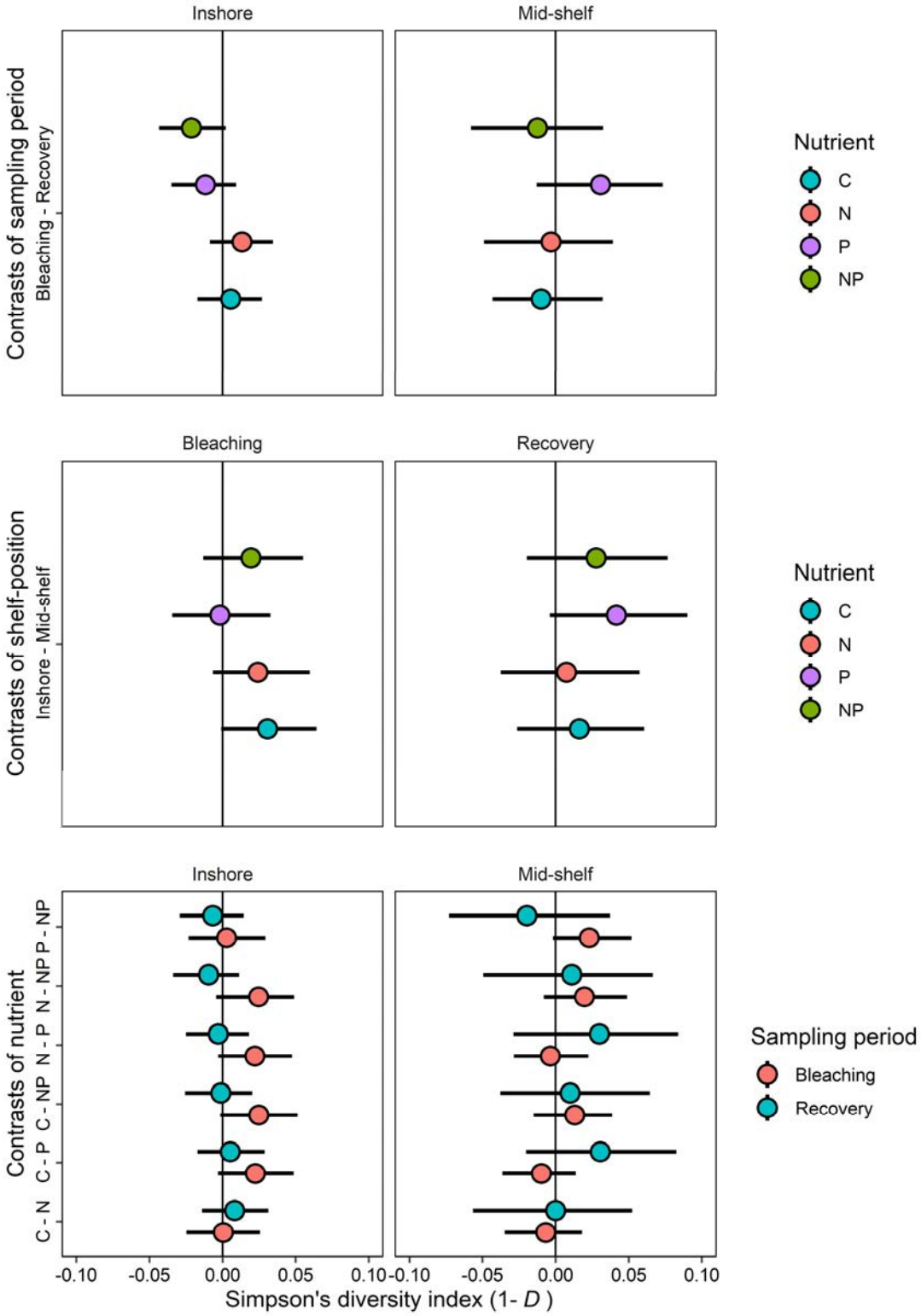

Figure S4.18 Simpson's diversity index (1-*D*) of algal symbiont DIVs median effects size and 95% CIs for pairwise comparisons of interactions between treatments.

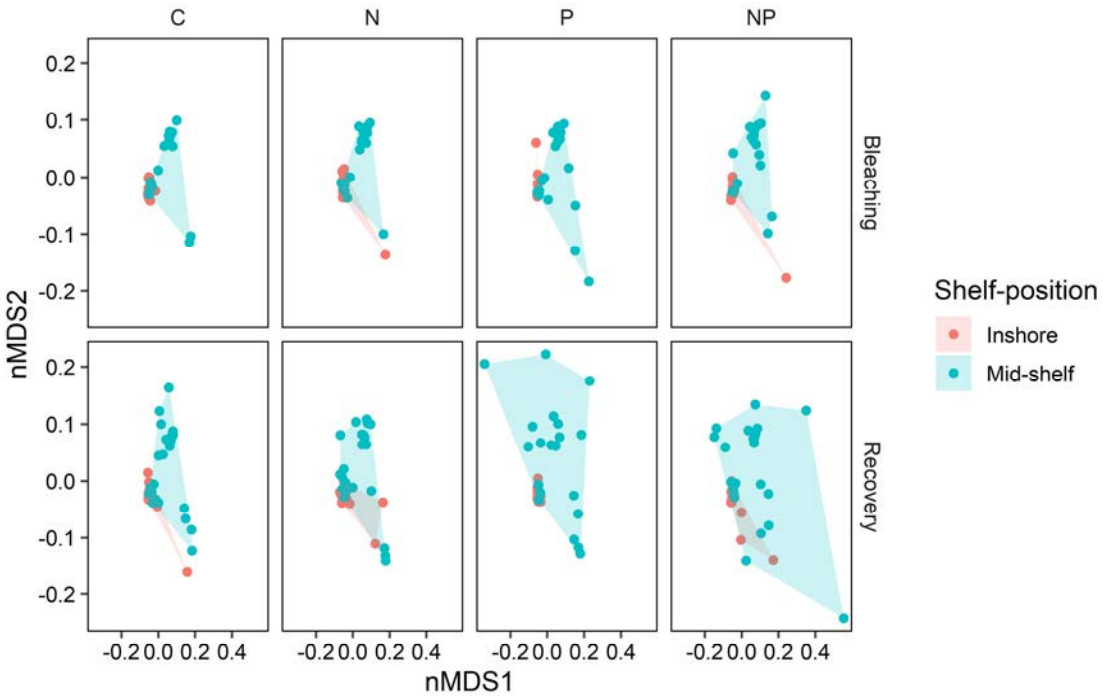

Figure S4.19 nMDS plots of inshore and mid-shelf algal symbiont DIVs from Bray-Curtis dissimilarities according to sampling period (top vs bottom) and nutrient treatment (left to right).