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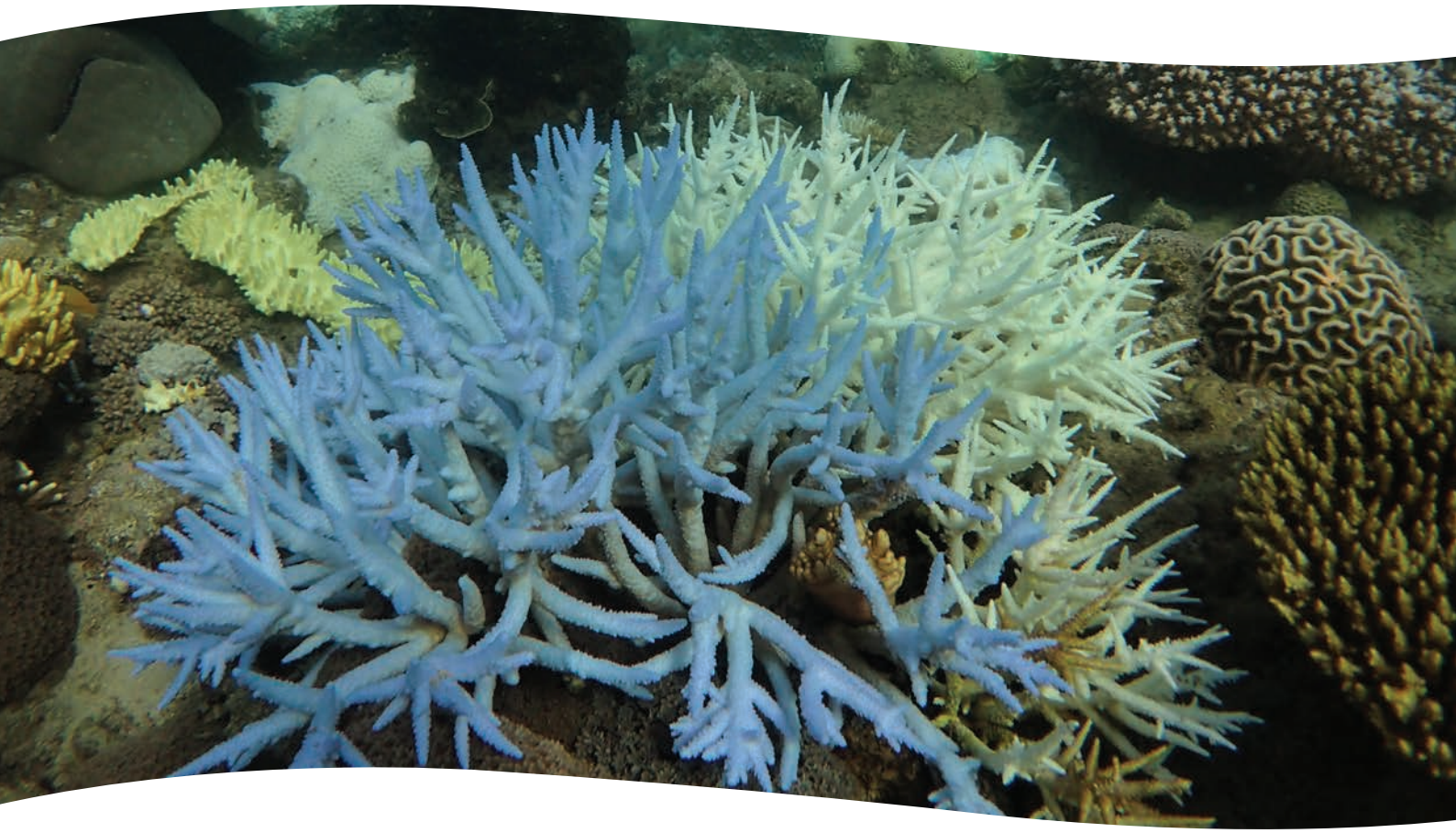
Neal E. Cantin, Mark E. Baird, Luke A. Morris, Daniela M. Ceccarelli, Veronique J.L. Mocellin, Renata Ferrari, Mathieu Mongin and Line K. Bay



Australian Government

Australian Institute of Marine Science

Assessing the linkages between water quality and coral bleaching on the Great Barrier Reef

Neal E. Cantin[1], Mark E. Baird[2], Luke A. Morris[1,4,5], Daniela M. Ceccarelli[3], Veronique J.L. Mocellin[1], Renata Ferrari[1], Mathieu Mongin[2] and Line K. Bay[1]

[1] Australian Institute of Marine Science, Townsville, QLD 4810, Australia
[2] CSIRO Oceans and Atmosphere, Hobart TAS 7001, Australia
[3] Marine Ecology Consultant, 36 Barton Street, Nelly Bay QLD 4819, Australia
[4] AIMS@JCU, Townsville, QLD 4811, Australia
[5] James Cook University, Townsville, QLD 4811, Australia

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Cover photographs: (front) A severely bleached inshore reef flat community at Russell Island in March 2017. Image: Neal E Cantin, AIMS; (back) Coral community bleaching observed at Pandora Reef, an inshore location near the Palm Islands in the Dry Tropics of Queensland. Image: Eric Matson, AIMS.

This report is available for download from the NESP Tropical Water Quality Hub website:
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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

CRW ..... Coral Reef Watch
CSIRO..... Commonwealth Scientific and Industrial Research Organisation
DHW ..... Degree Heating Weeks
DIN..... Dissolved Inorganic Nitrogen
DNRME..... Queensland Department of Natural Resources, Mines and Energy
DON..... Dissolved Organic Nitrogen
DIP..... Dissolved Inorganic Phosphorus
DOP ..... Dissolved Organic Phosphorus
EMS ..... Environmental Modelling Suite
ENSO..... El Niño Southern Oscillation
GBR..... Great Barrier Reef
JCU..... James Cook University
MMM..... Mean Monthly Maximum
MODIS ..... Moderate Resolution Imaging Spectroradiometer
NESP ..... National Environmental Science Program
NOAA ..... National Oceanographic and Atmospheric Administration
PAR ..... Photosynthetically Active Radiation
PIP..... Particulate Inorganic Phosphorus
PN..... Particulate Nitrogen
RECOM..... Relocatable Coastal Ocean Model
ROS ..... Reactive Oxygen Species
SST..... Sea Surface Temperature
WMIP ..... Water Monitoring Information Portal
WQ..... Water Quality

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EXECUTIVE SUMMARY

This research explores how the cumulative pressures, water quality inputs and thermal stress, interact to increase the risk of severe coral bleaching and subsequent mortality on inshore and mid-shelf coral reefs throughout the Great Barrier Reef (GBR) in a rapidly warming ocean.

Coral reefs around the world are threatened by climate change and cumulative local pressures such as overfishing and land-derived runoff which increases nutrients and sediment in the marine environment. At the time of writing this report, the GBR has just experienced a third severe and widespread coral bleaching event in five years.

Water quality is a known pressure for inshore reefs of the GBR, where elevated nutrient and sediment concentrations, can negatively impact the health and fitness of reef-building corals and promote shifts in reef community composition away from sensitive species and coral-dominated reef communities. Coastal and marine ecosystems of the GBR are closely connected to the adjacent river catchment areas and changes in land use since European settlement have led to increased pollutant loads delivered by river catchments into the GBR. This predominantly occurs during wet season rainfall events (Oct - May) and alters the marine water quality of the GBR, particularly on the inshore reefs.

We used a combination of approaches including: (i) coupled hydrodynamic-biogeochemical modelling of the nutrients, sediment and river plume transport into GBR lagoon to predict transport and coral bleaching risk, (ii) field collected water quality samples from the AIMS Marine Monitoring Program (MMP), (iii) field-based observations of coral reef communities during the 2016 and 2017 mass coral bleaching event and (iv) manipulative nutrient enrichment experiments in the National SeaSim aquarium facility at AIMS to evaluate the interactions and importance of temperature, nutrient enrichment, light and sediment concentration to influence the severity of coral bleaching for the GBR.

For the period leading up to the 2016 and 2017 bleaching years, the Queensland coastline was in a major drought phase of the climate cycle from 2013 – 2017. Exposure of the study reefs from Townsville to Port Douglas to fine sediment, nutrients and freshwater input was below average (Section 3), and wet season storms and cloud cover did not provide any respite from the extreme marine heatwave conditions that developed in 2016 and 2017. This report highlights that acute WQ inputs and extreme heat stress have not co-occurred with significant marine heatwaves. Past acute heat stress events leading to widespread mass bleaching on the GBR (1998, 2002, 2016, 2017) did not coincide with extreme rainfall and river flow events in the preceding year (Section 3 and 4).

Both nutrient enrichment and limitation can negatively affect the coral - algal symbiosis but the importance of these processes in relation to heat stress and bleaching susceptibility appears limited within the GBR.

Water quality gradients were assessed to explore if exposure frequency and history increases the risk of severe bleaching at elevated temperatures. In-water bleaching surveys and environmental data from the mass bleaching events in 2016 and 2017 were combined to model the relative importance of potential environmental water quality predictors. The models

developed for the 2016 and 2017 bleaching events, indicate that nutrient and sediment loads from human activities did not significantly alter the severity of modelled coral bleaching risk, as temperature stress was determined to explain bleaching severity and variability. Coral bleaching severity metrics: (i) % community bleached and (ii) % of corals at risk of mortality, significantly increased with accumulated heat stress, whilst water quality parameters generally had a much smaller influence on the severity of the bleaching response.

The percentage of bleached corals decreases significantly with depth (3.8% reduction / m) and increased significantly (by 9.7%) with each degree of longitude across the inshore-offshore gradient, with bleaching severity increasing in severity at reefs further offshore aligned with the distribution of temperature anomalies and accumulated heat stress. The total accumulation of temperature anomalies above the historical summer maximum was slightly higher on the inshore reef locations than the mid-shelf in 2017 (Figure 12). However, all reefs experienced severe levels of accumulated heat stress ranging from 5 - 9.5°C-weeks on the NOAA Degree Heating Weeks scale (DHWs), which resulted in major to severe coral bleaching at all study reef locations (50 - 90% of the community).

At the severe level of heat stress measured here, nutrient enrichment (total nitrogen and particulate nitrogen within the detritus) contributed only a minor influence on the severity of the bleaching response. After prolonged heat stress (>5°C-weeks), damage from excess heat overrides the WQ impacts, and thus widespread and severe community level bleaching becomes evident across the entire water quality gradient examined (Section 4).

Sampling of the common coral species *Acropora millepora in situ* at the time of peak heat stress during the 2017 bleaching event, revealed that inshore colonies exhibited greater relative bleaching (loss of symbionts within the host tissue) compared to the mid-shelf colonies, but conversely faster recovery of symbiont pigment content six months after bleaching (Section 5).

We also analyzed patterns of shelf position (inshore vs. mid-shelf) and catchment location (wet tropics vs dry tropics) to influence bleaching tolerance of GBR corals. The field-based sampling confirmed the expected negative relationship between visual signs of bleaching and decreasing photosynthetic pigment concentration (chlorophyll content). Shelf position had a significant effect on coral bleaching and recovery in 2017. Inshore corals contained the lowest pigment concentrations at peak heat stress (5.04 DHW at the time of sampling), and greater relative recovery of symbiont chlorophyll content, compared to the mid-shelf corals after four months at comparable levels of heat stress (4.22 DHW). There were no significant effects of catchment region on coral bleaching severity and recovery of photosynthetic pigments despite known variation in water quality parameters from inshore to offshore gradients and between river catchments in the Dry and Wet Tropics regions.

Experimentally, we explored two important factors of coral ecophysiology that determine healthy coral symbiosis and could influence thermal tolerance: (i) how corals respond to long-term nutrient enrichment, and (ii) if the availability of inorganic nutrients (especially nitrogen and phosphorus) affects the recovery of the coral host and its algal symbiont community within individual corals following bleaching at moderate temperature stress (Section 6). We exposed corals to 2 different scenarios of elevated dissolved inorganic nutrients, at reef relevant concentrations of dissolved nitrogen (28 µg/L), phosphate (7 µg/L) and combined nitrogen +

phosphate, which are above measured values from the MMP program, but within range at or below the water quality objectives for reefs in high ecological value locations in the Queensland Wet and Dry Tropics. Health of the coral symbiosis prior to and during a 'ramp and hold' temperature stress were monitored to assess bleaching tolerance following nutrient enrichment.

In the nutrient enrichment and bleaching experiment, the pre-experimental environmental history of the corals had the greatest influence on the severity of the coral bleaching response under heat stress. Inshore corals had a lower bleaching response, with less than a 50% decline in photosynthetic pigment content at high temperature, whereas the mid-shelf corals lost around 75% of pigments. Declines in photo-physiology were measured in the mid-shelf corals after the equivalent of 3.3°C-weeks heat stress accumulation.

Inorganic nutrient availability had comparatively little influence on the severity of the end-point bleaching response, however phosphate-enriched corals had the lowest levels of photoinhibition and the corals in the nitrate plus phosphate-enriched treatment had the highest levels of photoinhibition and would be at risk of increased levels of stress with further heat accumulation. The same inshore vs mid-shelf pattern of bleaching was observed in the 2019 bleaching and recovery experiment and could be detected after 2.0°C-weeks of heat stress was accumulated. Both inshore and mid-shelf corals experienced more severe levels of photoinhibition following nutrient enrichment, but recovery rates were largely consistent across all treatments indicating that nutrient effects are subtle following laboratory-based recovery from heat exposure at moderate levels of stress (3.1 and 4.5°C-weeks; inshore and mid-shelf heat stress treatments respectively).

We used these findings to guide a list of recommendations for consideration to inform future management decisions related to water quality and temperature impacts on reef-building corals and coral reef communities on the GBR. Mass coral bleaching is primarily driven by temperature and becomes severe both at colony and reef levels when accumulated heat stress exceeds 5°C-weeks. If ocean warming rates continue as projected by the IPCC, reef waters will exceed these upper temperature limits more frequently. Mass bleaching events will most likely increase in severity unless populations and communities adapt to the rapid rate of warming that is projected. Our experiments exposing corals to moderate heat stress of up to 4.5°C-weeks detected minor nutrient effects increasing photoinhibition with combined exposure to DIN and DIP. The best future for coral reefs requires a global stabilisation or a decrease in the current warming of the oceans, along with management actions of local inputs to the reef that will continue to improve water quality.

Widespread severe community coral bleaching at the peak of heat stress on an inshore reef at Russell Island in March 2017. Image: N. Cantin AIMS

1.0 INTRODUCTION

1.1 Water quality on the Great Barrier Reef

Coral reefs around the world are under pressure from cumulative global impacts related to climate change (Hughes et al., 2018a) and specific local and regional pressures such as overfishing and land-based runoff (Hughes et al., 2017). On the Great Barrier Reef (GBR), water quality is a major concern, especially for inshore reefs close to major river catchments and human population centers (Brodie et al., 2012). Nutrient and sediment concentrations are two water quality parameters with measurable detrimental effects on corals. Elevated inputs from terrestrial runoff can smother corals with increased sedimentation (Anthony 1999, Fabricius 2005), reduce light availability, which impacts phototrophic species with limited capacity for heterotrophic feeding (Anthony and Fabricius 2000), create negative effects on coral growth with weaker skeletal density (Rocker et al 2017), reproductive capacity, larval recruitment and juvenile coral survivorship and change the species composition of coral communities towards species with enhanced heterotrophic capacity (Fabricius, 2005).

On the GBR, nutrient and sediment concentrations are measured annually and expressed in a Water Quality Index (Gruber et al., 2019; Waterhouse et al., 2018). Across the length of the GBR, the observed declining water quality trend is driven by: 1) increasing concentrations of particulate phosphorus, dissolved organic carbon and particulate organic carbon; 2) concentrations of chlorophyll a (Chl*a*), total suspended solids and nitrogen oxides close to accepted guideline values; and 3) decreased water clarity (declining Secchi depth) across inshore reefs (Gruber et al., 2019). Concerns about the effects of terrestrial run-off in flood plumes entering the coastal GBR has facilitated the development and progressive review of the Reef Water Quality Protection Plan for catchments adjacent to the GBR (Queensland and Australian Government 2003, 2009, 2013). Water quality modelling indicated in 2014 that changes in land management practices were translating into progressive reductions of key pollutants, DIN and sediment compared to 2008 inputs (Queensland and Australian Governments 2018). Monitoring observations however indicate continued declines in water quality from 2006-2018 (Gruber et al. 2019), thus expanded water quality targets within the Reef 2050 Water Quality Improvement Plan 2017-2022 have been implemented to further reduce end of catchment loads entering the GBR marine environment. Updated targets include further reductions in anthropogenic end-of-catchment loads of DIN (60%), sediment (25%), particulate nutrients (20%) compared to 2008 levels, in addition to development of best practices in agriculture management and targeted catchment restoration efforts by industry and community stakeholders (Queensland and Australian Governments 2018).

Healthy coral reefs and inshore reef communities can exist over a range of natural wet season inputs, water quality gradients and nutrient concentrations, however high turbidity from fine sediments and nutrient levels are generally detrimental for corals, particularly fast growing phototrophic coral species (De'ath and Fabricius 2010, D'Angelo and Wiedenmann, 2014). On the GBR, inshore water quality is dynamic and changes seasonally, with pulse inputs correlated with extreme weather events, producing high rainfall and high river-flows (Lough et al. 2015). This interannual variation is superimposed upon spatial variability of regular annual river runoff events in the wet tropics compared to more pronounced cycling of drought-flood pulse events across the dry tropics (Schroeder et al. 2012). Regional variations in measured end-of-catchment sediment and nutrient loads reflect this spatial and temporal input variability,

with the Tully-Murray-Herbert basins dominating the DIN exports into the GBR and the Burdekin-Haughton basin contributing the highest inputs of total suspended solids and particulate nitrogen (Gruber et al. 2019).

Chronic and acute variation in water quality parameters can damage coral health by disrupting the balance of the coral-algal symbiosis, which can result in increased symbiont growth at the cost of the metabolic nutrient requirements of the coral host (D'Angelo and Wiedenmann, 2014). Water quality tolerance limits for coral reef communities are dependent upon the species and growth form composition of each individual reef, as clear differences exist among different coral species to withstand the effects of increased sedimentation. Tolerance limits for chronic suspended sediment concentrations range from <10mg L^{-1} at offshore reef locations with limited terrestrial input, up to >100mg L^{-1} at marginal inshore coral communities, which often have different coral species dominating the composition (See review Erftemeijer et al. 2012). Nutrient enrichment through particulate organic matter (POM) can benefit coral species with heterotrophic feeding capacity providing external energy sources to promote tissue and skeletal growth, however at high levels of sedimentation and POM, the associated reduction in light for photosynthesis and the energy required to clear tissues of settled sediment will outweigh the benefits gained from POM feeding (Fabricius 2005).Turbidity caused by suspended solids accounts for 74-79% of the annual variation in light intensity on coastal reefs of the GBR (Anthony 2004). Sedimentation stress also reduces photosynthetic yields in corals (Philipp and Fabricius 2003) and clearing of settled particulates increases the metabolic energy requirements to the coral host (Anthony and Fabricius 2000). Water quality gradients in nutrients (dissolved and particulates) and water clarity (sedimentation and light limitation) can shift the composition of coral reef communities from fast-growing, predominantly phototrophic coral species (such as branching *Acropora*) to others with increased heterotrophic capacity (for example *Turbinaria* and *Goniopora*; Fabricius et al. 2013), creating low-diversity inshore reef communities dominated by macro-algae.

1.2 Coral bleaching on the Great Barrier Reef

Coral bleaching is a general stress response where the endo-symbiotic dinoflagellates, of the family Symbiodiniaceae (LaJeunesse et al 2018), are actively expelled from the coral tissue through host mediated processes (Baird et al. 2009). While the coral-symbiont relationship can breakdown in response to cold temperatures (Howells et al 2013), periods of low salinity (Berkelmans et al. 2012) and other stressors, widespread mass bleaching events on regional and global scales are most often observed following acute periods of high temperature, combined with low winds and high benthic light levels (Hoegh-Guldberg, 1999). Direct temperature effects on the cellular health of the coral host can also initiate tissue necrosis and whole colony mortality rapidly during the peak of the heat wave (Hughes et al. 2018a). Under predicted future warming trajectories, the upper thermal limit will not only exceed the tolerance limits of the coral host-symbiont relationship causing coral bleaching, but will begin to exceed the upper thermal limit for survival of the coral host.

At the cellular level, coral bleaching occurs when levels of oxidative stress overwhelm the photoprotective repair mechanisms within the symbiont. Under this model, bleaching stress is initiated by heat damage to the carboxylation enzymes in the Calvin Cycle within the symbiont that disrupts the CO_2-fixation processes of photosynthesis. This heat damage is followed by reduced electron transport from the light harvesting Photosystem II into the Calvin Cycle, which

causes a chain of events leading to rapid production of reactive oxygen species (ROS) under calm, high light summer conditions (Jones et al. 1998, Smith et al., 2005, Hu et al. 2020). The algal symbionts of corals are known to regularly produce large amounts of ROS from the high rates of photosynthesis, in high light environments. Both symbiotic partners have antioxidant systems in place to eliminate excess photons through the Non-Photochemical Quenching (NPQ) xanthophyll cycle and reduce the concentrations of elevated ROS production with antioxidant proteins (e.g. heat shock proteins (HSPs) and mycosporine like amino acids (MAA's); Weis, 2008). However, during acute summer conditions of excess heat and light stress, elevated levels of daily ROS production overwhelm the antioxidant protective mechanisms of the symbiont and the coral host (Jones et al. 1998, Weis 2008). Excess ROS leak into the coral host tissues, signaling the expression of genes to increase the production of antioxidant stress response enzymes (Hu et al. 2020). Toxic ROS signaling processes initiates the bleaching response, which breaks the symbiotic relationship, expelling the damaging algal cells as a survival protection mechanism for the coral host (Weis, 2008). A bleached coral will eventually starve following moderate levels of heat stress if the coral host is not able to rebuild the beneficial symbiosis (Grottoli et al., 2006; Tremblay et al., 2016).

Long-term environmental exposure to temperature, light and nutrients influences corals' physiological state and can influence bleaching tolerance across environmental regimes (Oliver and Palumbi 2011, Kenkel 2013, Howells et al 2013; Safaie et al 2018). Variation in bleaching tolerance among species is likely determined by biological and environmental factors such as colony growth form and reef habitat influencing the exposure to light, flow and heat (Marshall and Baird 2000, McClanahan et al. 2005). Adaptation can result from changes in the genetic structure of populations of the coral host and their symbiont partners (Fuller et al 2020; Howells et al 2011; Palumbi et al 2014; Quigley et al. 2019). Key traits which have the ability to confer tolerance, including metabolic rates and antioxidant capabilities vary significantly between different coral host and symbiont species (Baird et al 2009; Suggett et al 2017). As bleaching events increase in severity, changes in the species structure of coral reef communities have become more dramatic, favouring only the most tolerant species following extreme levels of heat stress (DHW > 8°C-weeks) and thus altering the bleaching thresholds following bleaching and mortality events into the future since only the tolerant growth forms survive (Hughes et al 2018b).

The first widespread mass bleaching event was documented on the GBR in 1998. Before that, records show only isolated events in response to localised stressors and minor to moderate bleaching severity (Oliver et al 2009, Hughes et al., 2018a). Mass bleaching across latitudes occurred again in 2002, with localised bleaching on individual reefs occurring in other years (for example, 2006 in the southern GBR; Maynard et al. 2008). In 2016 and 2017, severe bleaching events occurred in consecutive years for the first time, resulting in the loss of approximately 35-40% of live coral cover GBR-wide (Hughes et al. 2017, 2018a, Sweatman 2018). At the time of writing this report, the GBR has suffered the third severe mass bleaching event in five years. Coral bleaching events are increasing in severity, with ocean heat waves that push coral reefs beyond historical summer maximum temperatures and that exceed bleaching tolerance limits are occurring more frequently (Hoegh-Guldberg 1999, Donner et al. 2005), reducing the time between disturbance events to enable sufficient recovery. Therefore, we need to identify if local management actions to improve water quality in the GBR will enhance thermal tolerance of coral communities and enhance recovery following disturbance events.

1.3 Water quality and coral bleaching dynamics

Water quality management on the GBR remains a priority for the Australian and Queensland Governments in order to maintain the resilience of coastal and inshore ecosystems from disturbance events and anthropogenic pressures (Gruber et al. 2019). The monitoring of flood plumes within the nearshore marine environment links concentrations of suspended sediment, nutrients, and particulate organic matter to end-of-catchment loads and is achieved through coordinated monitoring programs (Gruber et al. 2019). Maintaining water quality variables, dissolved and particulate nutrients, particulate organic matter and sediment concentrations, within guideline values (DERM 2009) are likely to deliver significant health benefits to benthic reef communities (Gruber et al 2019). These include a reduction in the risk of disease and limiting competition for corals with other heterotrophic filter feeders that could dominate the available reef substrate following a disturbance event (Fabricius, 2005; D'Angelo and Wiedenmann, 2014). Recent research shows that the recovery of corals from bleaching can be mediated by water quality, and reducing local chronic pressures may therefore benefit corals in a warming future (Carilli et al. 2010, D'Angelo and Wiedenmann, 2014; Bessell-Browne et al 2017, Morris et al., 2019). As a result, water quality management (especially nutrients, particulates and terrestrial sediment) has been proposed as an important mechanism to improve the thermal tolerance and recovery potential of coral reef communities within the GBR and the resilience of coastal ecosystems (Wooldridge and Done, 2009, Queensland and Australian Governments, 2018). The focus of resilience within this study assesses the thermal tolerance of coral reef communities and how this translates to survival potential during major marine heat waves.

It is likely that changes in environmental nitrogen to phosphorus (N:P) ratios, could be critical in affecting the coral bleaching process (Wiedenmann et al., 2013; D'Angelo & Wiedenmann, 2014; Morris et al 2019) but field studies seeking to link nutrient enrichment with coral bleaching patterns have produced mixed results. Wooldridge and Done (2009) found a positive correlation between ocean Chl *a* concentration gradients and coral bleaching in 1998 and 2002, however Hughes et al. (2017) determined that the effect of elevated Chl *a* concentrations was minimal compared to the effect of severe heat stress in during the recent 2016 bleaching event, suggesting that changes to water quality may not confer enhanced resistance to thermal bleaching. Current explanations for conflicting relationships between water quality variables and coral bleaching include subtle effects of WQ and the temporal incongruity between the timing of major flood events that lead to acute exposure to elevated WQ parameters along with heat stress (Donovan et al., 2020). We have yet to understand the main agents of water quality that affect bleaching susceptibility in the field and role of temperature and WQ regimes on the capacity of bleached corals to recover (e.g., nutrients/nutrient ratios, fine sediments, light, turbidity). Consequently, it is not yet clear if further water quality improvements on the inshore of the GBR would enhance the thermal tolerance of coral reefs and increase the potential for recovery following major heat stress events.

1.4 Project goals

This research project aims to improve knowledge around the links between water quality indicators and bleaching thresholds, to be used in the development of effective decisions and targets for the management of the GBR into the future. The outputs include this report, scientific publications, presentations and media.

Our activity aims to have the following outcomes:

- An increased understanding of how water quality gradients interact with coral bleaching severity and mortality during the 2016 and 2017 bleaching events throughout the GBR and directly test how nutrient enrichment affects the thermal heat tolerance and recovery potential following from thermal bleaching stress in the laboratory and under field conditions,

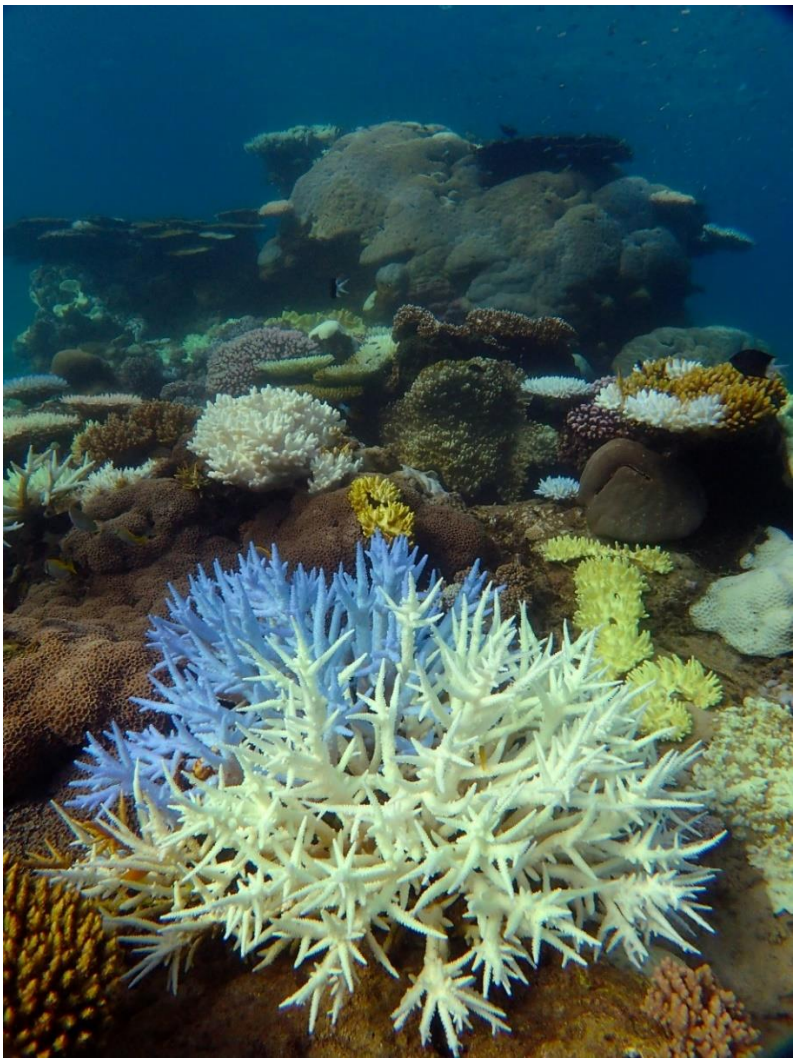
and,

- A modelling framework to identify management options that could mitigate the effects of warming on reefs through changes in water quality pressures.

Using SeaSim experiments and field data, we examine which water quality parameters (nutrients, particulates, sediment and water clarity) affect corals' thermal tolerance, and assess how temperature and water quality exposure histories affected coral bleaching and recovery during the 2016 and 2017 coral bleaching events. We refine the eReefs model to predict how anthropogenic nutrient and sediment loads influence coral bleaching severity on the GBR in a warming climate.

We will use these approaches to address the key questions:

1. Which water quality parameters (nutrients/nutrient ratios, light/turbidity, particulate organics) are most linked to reductions in coral bleaching, and what are the ecological and physiological mechanisms that underpin thermal tolerance?
2. Over which temperature and light exposure is nutrient enrichment a determinantal input which reduces coral colony and reef community heat tolerance?
3. Can improvements in water quality mitigate bleaching, and under which temperature regimes?



Severe bleaching and mortality of branching and sub-massive inshore hard and soft corals with tolerant Porites colonies at Russell Island in March 2017. Image: N. Cantin AIMS

2.0 A MECHANISTIC MODEL OF CORAL BLEACHING DUE TO NUTRIENT AND TEMPERATURE-MEDIATED LIGHT-DRIVEN REACTIVE OXYGEN BUILD-UP IN ALGAL SYMBIONTS

2.1 Introduction

As mass coral bleaching and other large-scale disturbance events have increasingly impacted coral reefs, emerging management strategies are seeking to identify and protect reefs that appear resilient to future threats (Hock et al., 2017). Modelling provides a tool for predicting the conditions that make individual corals or reefs more or less susceptible to thermal bleaching, but the impacts of cumulative stressors remain unclear. For example, we do not yet understand the relationship between coral bleaching and the nutrient and sediment loads delivered to the GBR, even if the physiological mechanisms are beginning to be understood (Bessell-Browne et al 2017; Morris et al 2019). To improve the understanding of drivers of mass bleaching events, and to support effective management actions to ameliorate these effects, we applied a process-based coral-symbiont model that considers temperature-mediated, light-driven oxidative stress within biogeochemical / ecosystem hydrodynamic models that are capable of predicting the time-varying light, nutrient and prey conditions of natural reef environments (Baird et al., 2018). We assess the ability of this coupled model system to capture a mass bleaching event against aerial surveys from 2016, indicating promise for predicting bleaching risk in real-time using the eReefs hydrodynamic model platform.

The objective of this component of the project was to further develop and apply a process-based coral-symbiont model to explore potential links of anthropogenic nutrient and sediment loads to influence coral bleaching severity (at the polyp scale) on reefs of the GBR. To quantitatively link dissolved nutrient concentration, sediment dynamics and water clarity to coral bleaching risk the coral model was required to link these factors to the photo-physiological processes within algal symbionts within the coral polyps. Therefore, the coupled, coral-hydrodynamic-biogeochemical models were also required to represent the complex processes (e.g. biological uptake, physical mixing) that modify river catchment loads of nutrients and sediments before they are delivered to reefs. This task of simultaneous hydrodynamic, biogeochemical and coral polyp modelling has never been undertaken before for any reef system in the world.

2.2 Methods

At the commencement of this project, the eReefs marine models were already modelling coral polyp growth and water quality on the GBR. Specifically, the coupled model contained:

1. A hydrodynamic-biogeochemical model of the Great Barrier Reef that tracked the transport and transformation of carbon, nitrogen and phosphorus from catchment delivery to biological fate throughout the Great Barrier Reef;
2. A coral model that represented the symbiotic relationship between coral hosts and the symbionts (Mongin and Baird, 2014). This 2016 version of the coupled model did not

represent the processes of photosystem adaptation and inhibition, or the build-up of reactive oxygen species.

As such, this NESP project funded the improved modelling of physiological response to thermal stress and the variation of this response with water quality parameters affected by pollutant loads from the catchment.

As a first step to enhancing the eReefs coral model for the purpose of this project, CSIRO modellers (Baird, Mongin, Soja-Wozniak and Rizwi) developed a new coral polyp model and presented this to the coral bleaching experts (Bay, Cantin and Morris) of the team. The first iteration of the improved coral model included:

1. Symbiont photoadaptation through changing rates of synthesis of chlorophyll a and a single xanthophyll accessory pigment.
2. Symbiont photoacclimation through the xanthophyll cycle adjusting between photoprotective and photosynthetic states of the accessory pigment.
3. A three-component reaction centre component that included active, reduced and inhibited reaction centre states and generation of reactive oxygen species (ROS).
4. Temperature mediation of the rate of ROS detoxification.

This initial coral model version was presented to the project team (Bay, Cantin and Morris) who suggested the following changes:

1. The addition of multiple symbiont accessory pigment types, in particular the pigment peridinin.
2. A shift to the primary means of temperature inhibition through the inactivation of the RuBisCO enzyme (rather than the rate of ROS detoxification).

After addressing these refinements, we (the whole project team) arrived at a model of coral polyp growth and reaction-centre dynamics summarised in Figs. 1, 3 and 4 of Baird et al. (2018).

In summary, we developed a mechanistic model of the coral-symbiont relationship that considers temperature-mediated build-up of reactive oxygen species due to excess light, leading to symbiont expulsion. The coral model explicitly represents the coral host biomass, as well as symbiont biomass, intracellular pigment concentration, nutrient status, and the state of reaction centres and the xanthophyll cycle. Photophysiological processes represented include photoadaptation, xanthophyll cycle dynamics, and reaction centre state transitions. Thus, in this representation, reactive oxygen stress builds up due to photons absorbed during periods above average summertime seabed maximum temperatures.

This coral bleaching model is explained in full detail in Baird et al. (2018), where we compare the bleaching rate in the coupled model to the JCU aerial surveys of the 2016 bleaching event. Rather than providing the published results from the 2016 event, below we provide a new summary of the behaviour of the eReefs model coral bleaching component during the current 2020 bleaching event.

2.3 Results: Simulation of the 2020 GBR-wide bleaching event

The eReefs 1 km coupled hydrodynamic – biogeochemical model has been run in near-real time since 2016 (eReefs.info). The latest version of the biogeochemical model, including the

coral bleaching submodule described above, has been run since 16 Oct 2019. In near-real time mode, the catchment nutrient and sediment loads are calculated from mean river concentrations and gauged flows. The simulation is referred to as GBR1_H2p0_B3p2_Cfur_Dhnd and is archived in near real time on the publicly accessible National Computing Infrastructure (see eReefs.info for details). The results have continued to be produced through the 2019/20 summer, being between 4 and 6 days behind present, a result of waiting for BoM forcing products and the ~1 real day duration to run 3 days of simulation time.

The simulation shows that at some reefs, some of the time, there was sufficiently high thermal stress, combined with elevated bottom light levels, for the reactive oxygen stress to become toxic and begin symbiont expulsion, or bleaching. The levels do not appear to be as high as in 2016 or 2017. The distribution of bleaching-level stress is restricted to the inshore and mid-shelf regions.

The greatest rate of symbiont expulsion (up to 0.3 cells d^{-1}, enough to significantly pale a coral skeleton in a few days) occurred on the inshore reefs between Cooktown and Princess Charlotte Bay on the 17 March during the neap tides of the 3rd quarter moon (Figures 1 and 2). Around this time seabed temperatures reached their maximum for the summer. Neap tides correspond to low current speeds, and less resuspension, resulting in greater light levels at the seabed. Furthermore, the few days preceding March 17 had low cloud cover (Figure 3). The comparison of the model predictions and observations for 2020 will be important future work.

The coral bleaching model developed in NESP Project 3.3.1 has proven to be sufficiently robust, and accurate, to predict the spatially resolved distribution and intensity of bleaching during the summers of 2016 (Baird et al., 2018), 2017 (see next chapter) and 2020 (Figure 1).

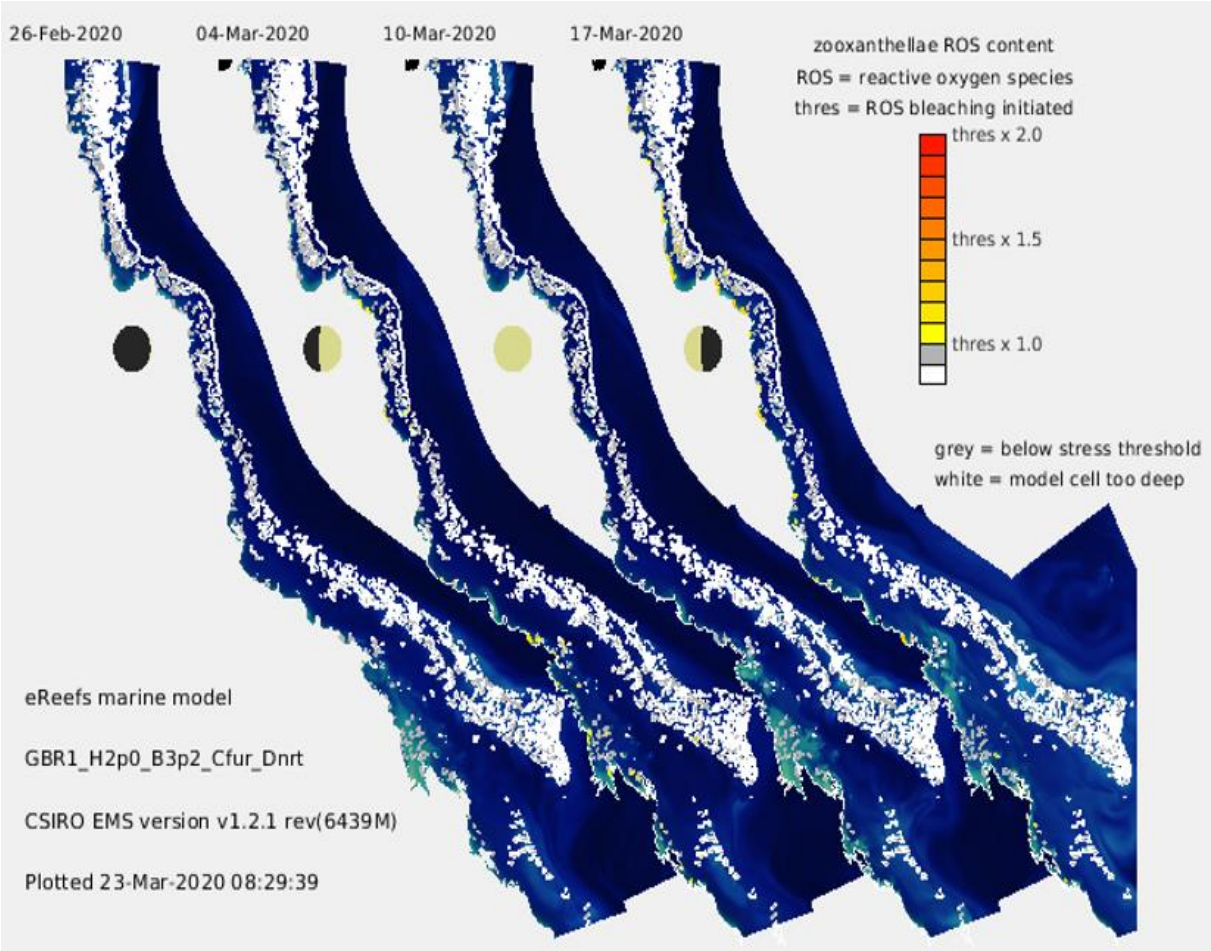


Figure 1: Water colour and reactive oxygen species [ROS] concentration in coral polyps on the 26 Feb (new moon), 4 Mar (1st quarter), 10 Mar (full moon) and 17 Mar (3rd quarter) 2020. The ROS concentration is normalised by the cellular content at which zooxanthellae expulsion is initiated. The water colour is the simulated true colour, a model-generated estimate of the colour of the ocean as seen from above, based on the normalised water leaving radiance of red, green and blue light, that has been calculated considering the 20+ optically active constituents in the marine model. The water looks greener due to suspended particles such as inorganic particulates and phytoplankton. Each model pixel with a reef community is assigned a colour, and rendered on top of the true colour image. White is used to show reefs that are too deep (z> 20 m) in the 1 km model to bleach. Grey shows pixels that are shallower than 20 m, but with ROS concentrations less than toxic levels. Yellow to Red shows increasing ROS levels resulting in bleaching.

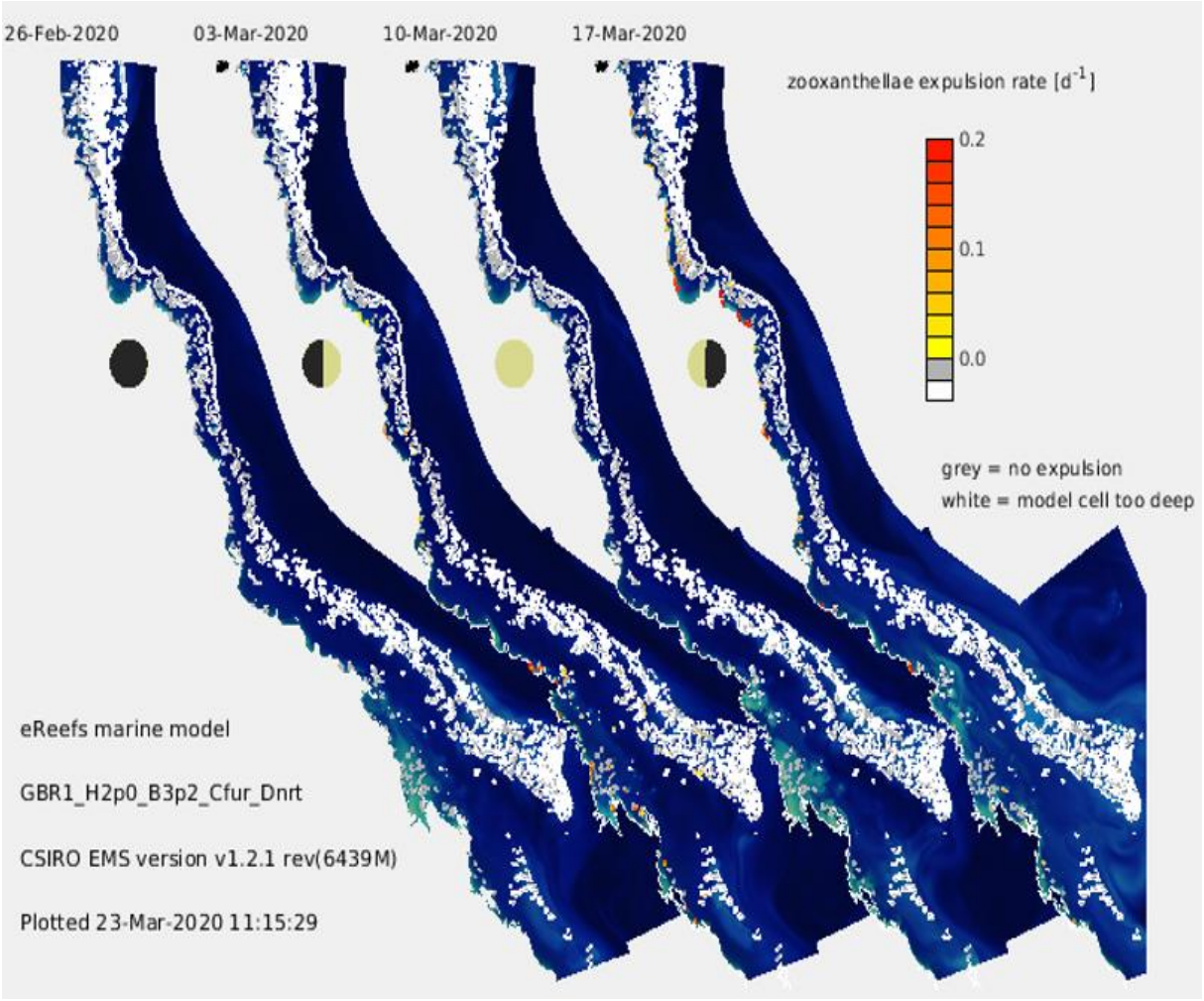


Figure 2: Water colour and symbiont expulsion rate in coral polyps on the 26 Feb (new moon), 4 Mar (1st quarter), 10 Mar (full moon) and 17 Mar (3rd quarter) 2020. White is used to show reefs that are too deep (z> 20 m) in the 1 km model to bleach. Grey shows pixels that are shallower than 20 m, but with no expulsion. Yellow to Red shows increasing expulsion. For more information see Figure 1.

Figure 3: Observed true colour on the 16 March 2020 from the MODIS sensor on the NASA satellite Terra. We acknowledge the use of imagery from the NASA Worldview application (https://worldview.earthdata.nasa.gov), part of the NASA Earth Observing System Data and Information System (EOSDIS).

2.4 Discussion and future applications

This represents the first application of a sophisticated coral bleaching model imbedded in a hydrodynamic – biogeochemical model applied across an entire shelf system. However, the coral model developed here does not consider all phenomena relevant to bleaching. For example, the coral model considers only one mechanism for thermal stress induced bleaching, but other mechanisms do exist, such as illustrated by bleaching under low light conditions (Tolleter et al., 2013). Further, only one generic coral-symbiont combination is considered at this stage, and therefore this simulation is not able to resolve the differences between temperature tolerances of different coral holobiont combinations (Bay et al., 2016). Finally, the eReefs biogeochemical model represents only a fraction of the processes affecting coral health. For example, the biogeochemical model represents only one coral type, and one macroalgae type, and considers only one interaction between the two: competition for nutrients and light. A more sophisticated, ecosystem or habitat style model would consider multiple coral and seagrass types inhabiting different habitats and interacting with each other and the fish assemblage (Bozec et al., 2018). However, despite all the simplifications and omissions, the coral model developed here does represent a comprehensive set of processes spanning scales from the polyp processes to the shelf-scale, and from nutrient and photochemical interactions to coral symbiosis.

The use of satellite-derived temperature exposure alone as a measure of coral bleaching severity has been broadly successful (Liu et al., 2014), and is used operationally at a global scale. Work is under way to include solar radiation in thermal bleaching algorithms (Skirving et al., 2018). Even with additional considerations such as solar radiation, satellite algorithms will always be limited to the estimation of near-surface properties, and their inability to consider factors affecting bleaching such as dissolved nutrients (D'Angelo and Wiedenmann, 2014) that cannot be remotely-sensed. Thus, the near real-time prediction and forecasting of coral bleaching by a biogeochemical model such as developed here that can consider the history of temperature, light and other environmental conditions across the entire water column, from the surface to the seafloor, provides a means to overcome some of these limitations.

Finally, a process-based model is capable of explicitly representing management strategies such as local shading (Coelho et al., 2017), marine cloud brightening, or increased stress tolerance of individuals and/or populations of coral or zooxanthellae (Anthony et al., 2017). The eReefs modelling framework has already been used to optimise catchment management for the purposes of improving water quality on the Great Barrier Reef (Brodie et al., 2017). The bleaching model derived here will next be used to quantify the impact of interventions designed to minimise the impacts of a warming ocean on the corals of the Great Barrier Reef.

3.0 EFFECTS OF NATURAL AND ANTHROPOGENIC SEDIMENT LOADS ENTERING THE GBR LAGOON ON CORAL BLEACHING AND BLEACHING RISK

3.1 Introduction

The most significant bleaching events on the GBR (1998, 2002, 2016 and 2017, excluding 2020) have occurred in low rainfall years. For catchment-derived sediments to affect coral bleaching on the GBR, they must be transported to the reef site in concentrations high enough to impact symbiont physiology and / or coral processes. The delivery of nutrients to a reef site involves physical factors such as large-scale circulation and local winds, tides, and river discharges (e.g., Bainbridge et al. 2018). River discharges on the GBR are driven by climatic cycles such as El Niño Southern Oscillation (ENSO). During the transport of sediment-laden water to a reef site, biogeochemical transformations change the nature of the pollutant loads that can impact the corals (Devlin et al., 2015), and these loads then mix with locally-generated processes.

A large, multi-agency collaboration has developed the eReefs coupled hydrodynamic, sediment and biogeochemical model that simulates the environmental conditions of the Great Barrier Reef at multiple scales (Schiller et al., 2014, Section 2). The model provides skillful predictions of the drivers of coral processes, such as temperature, spectrally-resolved bottom light, and water column concentrations of dissolved inorganic nutrients and particulate organic matter across the entire length of the GBR from 2011 to the present (Skerratt et al., 2019). Furthermore, the eReefs project includes bespoke model generation that allows high-resolution models to be nested within the 1 km regional hindcast (RECOM - Relocatable Coastal Ocean Model).

In order to capture the dynamics of bleaching on the GBR, a sophisticated coral sub-model was developed and implemented in the eReefs modelling system (Baird et al., 2018, Chapter 1). In this study we limit our analysis to the direct effect of anthropogenic sediment loads on ROS build-up. We emphasise model outputs of symbiont physiological status, such as cellular concentration of ROS build-up, as an indicator of bleaching processes.

This Chapter describes Methods and Results that are to be submitted for publication in June 2020. The draft manuscript is found in the Supplementary Material.

3.2 Methods

3.2.1 GBR-Wide Configuration

The eReefs model (Figure 4) simulates the circulation, optics, biogeochemistry and sediment dynamics using the CSIRO Environmental Modelling Suite (EMS, https://github.com/csiro-coasts/EMS, see Herzfeld and Gillibrand 2015 and Herzfeld 2015).The eReefs coupled hydrodynamic, optical, sediment and biogeochemical model was configured at 1 km resolution for the northeast Australian continental shelf and run from December 1, 2014 to present. The model's curvilinear grid has 2,389 cells in the alongshore direction, 510 in the offshore direction and 44 depth levels. The hydrodynamic model is run with a 1.2 s barotropic time step, and the

current fields used to calculate mass conserving fluxes of sediment and biogeochemical constituents (Gillibrand and Herzfeld, 2016). The sediment and biological processes are integrated using a 1-hour timestep.

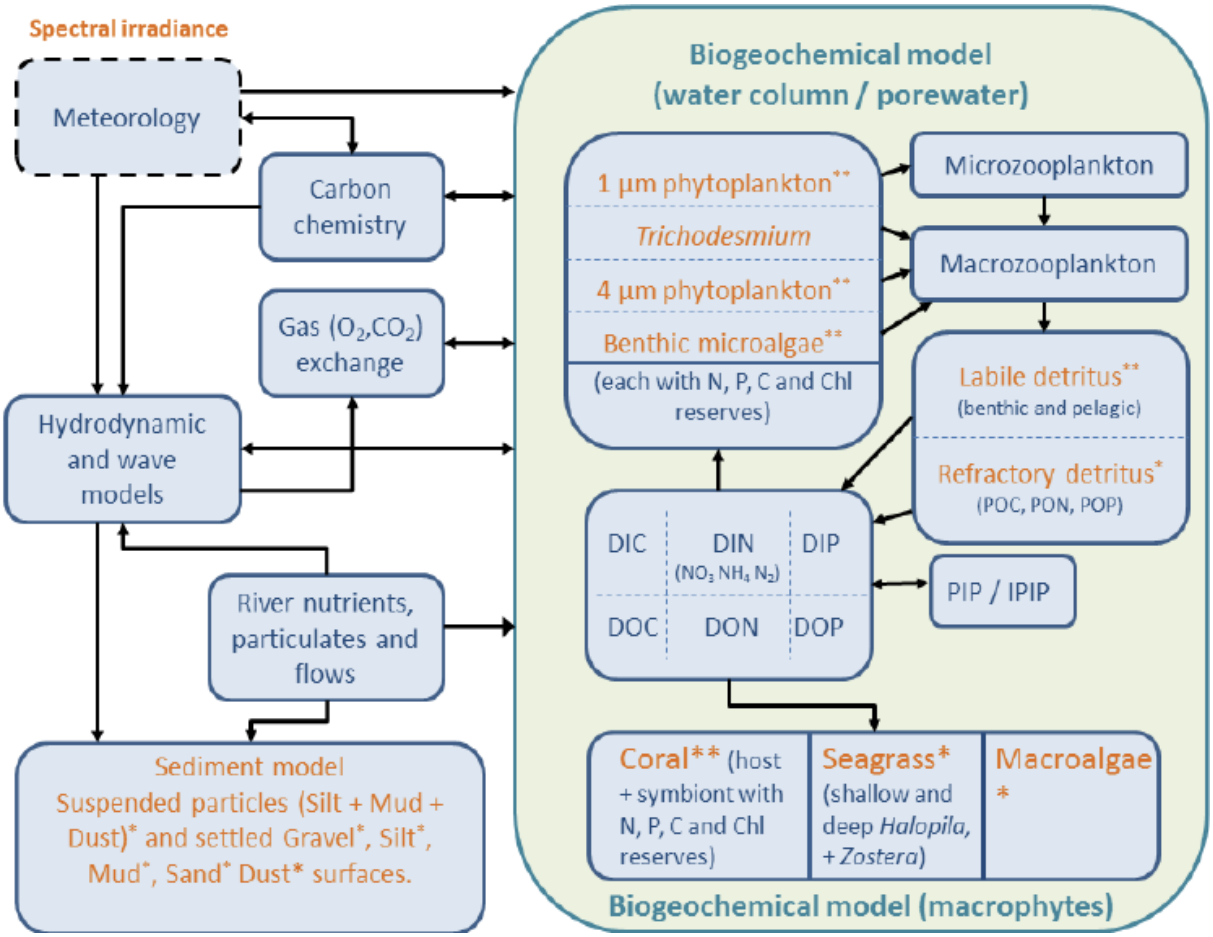


Figure 4: Schematic showing the eReefs coupled hydrodynamic, sediment, optical, biogeochemical model. Orange labels represent components that either scatter or absorb light, thus influencing seabed light levels.

The model is forced using atmospheric conditions from the BoM ACCESS-R and OceanMaps atmospheric and ocean products. Additionally, a 1.21 mg N m^{-2} d^{-1} flux of ammonium from the atmosphere into the ocean is applied uniformly in time and space across the entire grid, corresponding in regions with an annual rainfall of 1500 mm to a rainwater concentration of 0.3 - 149 mg L^{-1} (Packett, 2017). Both the Baseline and Pre-Industrial scenarios used identical atmospheric and ocean boundary conditions. The river flows for the two load scenarios were also the same, but the concentrations of [1]DIN, DON, PN, DIP, DOP, PIP and suspended sediments in the river water were adjusted to match the predicted loads of the two scenarios, described in the next two paragraphs.

The model considers inputs of dissolved and particulate constituents from 21 rivers along the Queensland coast (north to south: Normanby, Daintree, Barron, combined Mulgrave and

[1] Dissolved Inorganic Nitrogen, Dissolved Organic Nitrogen, Particulate Nitrogen, Dissolved Inorganic Phosphorus, Dissolved Organic Phosphorus, Particulate Inorganic Phosphorus.

Russell, Johnstone, Tully, Herbert, Haughton, Burdekin, Don, O'Connell, Pioneer, Fitzroy, Burnett, Mary, Calliope, Boyne, Caboolture, Pine, combined Brisbane and Bremer, and combined Logan and Albert rivers) and the Fly River in Papua New Guinea. River concentrations of sediment and nutrients for the 4 southern rivers and the Fly were based on mean values from observations over a 10 year period (Furnas, 2003) and multiplied by gauged flows to obtain river loads. Calculations of concentrations for the main rivers impacting our study region were based on loads calculated using the SOURCE Catchments model (Waters et al., 2014; Waterhouse et al., 2018). The full SOURCE Catchments model used for catchment load assessment uses paddock-scale models to determine flows into sub-catchments for the 1986-2014 time period. To extend the forcing through to 2017, an empirical approximation of the paddock-scales was used as inputs into the SOURCE Catchments model.

We use two sets of loads from the SOURCE Catchment model. The Present-day baseline loads are calculated using the SOURCE Catchments with 2017 catchment condition. Pre-Industrials loads were calculated using SOURCE Catchments with Pre-Industrial catchment condition that included restoration of vegetation to the 1850s but retaining modern water infrastructure.

The model uses a novel river boundary condition (Herzfeld, 2015) that discharges the river freshwater load in a brackish surface plume whose salinity and thickness is calculated to account for upstream flow in the salt wedge and in-estuary mixing between density layers. The coral distribution in the model is a combination of the eAtlas features map, or, where available, a satellite derived coral zonation (Roelfsema et al., 2018). A 5th-order Dormand-Prince ordinary differential equation integrator (Dormand and Prince, 1980) with adaptive step control is used to integrate the local rates of changes due to ecological processes. This requires 7 function evaluations for the first step and 6 for each step after. A tolerance of 10^{-5} mg N m^{-3} is required for the integration step to be accepted. The mass of carbon, nitrogen, phosphorus and oxygen are checked at each model timestep to ensure conservation.

3.2.2 Reef Configurations

Five individual reef sites were chosen to develop 200 m resolution configurations. We chose Jessie Island and High Island because in 2017 they were the sites along the central / northern GBR that were most impacted by river plumes. Further, Havannah Island, Otter Reef and Pandora Reef were chosen to represent a gradient in exposure to plumes, and because they were impacted by different rivers. The 200 m configurations were built using the eReefs Project RECOM automatic nesting capability[2].

The model bathymetry was interpolated from the GBR100 bathymetry (Beaman, 2010) version 4 with improved resolution of reef tops. Atmospheric forcing was the same as the 1 km model above. The initial conditions of each reef for the water column state variables was interpolated from a previous run of the 1 km model: GBR1_H2p0_B1p9_Cfur_Dhnd. Some benthic variables (seagrass and coral distributions) have distributions re-interpolated from the high-resolution benthic maps and assigned values from the nearest neighbour in the initialising model.

[2] https://research.csiro.au/ereefs/models/models-about/recom/

The 200 m nested model uses boundary conditions provided by a standard eReefs 1 km model simulations that did not include coral bleaching. Thus the model that generated the boundary conditions for the nested model is slightly different to the 1 km configuration described above, but the water column properties that are advected into the nested model, which depend primarily on nutrient / plankton processes in the water column, will be very similar. The boundary condition for all water column tracers was formulated using the advection scheme (Van Leer, 1977) used within the model domain itself. This consistency of boundary and advection schemes ensures diffusion and dispersion errors are minimised.

3.3 Results

To quantify the process of natural and anthropogenic loads in the runoff of multiple intermittently-flowing rivers affecting the physiology of distant symbionts on reefs of the Great Barrier Reef, we first characterised the catchment loads, then the extent of the river plumes, and finally the symbiont physiology at the 5 reef sites.

3.3.1 Catchment Loads in Early 2017

During the wet season (Dec-May) of 2017, northeast Australia was exposed to low to average rainfall (See Section 4 Figure 11). The Wet Tropics rivers (Herbert, Tully, Mulgrave, Johnstone) had relatively constant flows. The Burdekin, which discharges to the south of our region but flows past the designated reef sites, had low flow until the passage of Tropical Cyclone Debbie in the last week of March 2017. We calculate anthropogenic loads from the difference between the Baseline and Pre-Industrial loads (see Appendix 1). As noted elsewhere (Brodie et al., 2017), the Burdekin has small anthropogenic DIN and DIP loads, but large anthropogenic suspended sediment loads. The coupled catchment-hydrodynamic-biogeochemical model represents the greater plume transport of dissolved constituents relative to negatively buoyant particles. While the heavier suspended sediments loads tend to sink within 50 km of the Burdekin mouth (Margvelashvili et al., 2016), the finer component will be transported to the reef sites. In contrast, the anthropogenic nutrient and sediment loads from the wet tropics rivers in 2017 are about equal to the Pre-Industrial loads, due to the below average rainfall and decreased flows prior to peak heat stress. Thus, when exposed to flood water from these wet tropics river plumes, reefs will generally be exposed to both elevated nutrients and reduced light, whereas the reefs within the Burdekin region of influence may be exposed to reduced light and smothering risk from increased fine sediment inputs, but without significant additional nutrient input.

3.3.2 Extent of River Plumes in Early 2017

Although the plumes generally travel north from the river mouths, variation due to atmospheric forcing, tides and discharge strength ensured that each of the sites had exposure to multiple plumes (Figures 5 and 6). The High and Jessie Island reefs were chosen due to having the highest total river exposure on the central GBR in 2017, with Jessie Island having increased input from the Tully River, and High Island from the Johnstone River (Figure 5). The cumulative exposure reached a maximum of around 5%. In contrast, Otter, Havannah and Pandora reefs received smaller exposure until late in the simulation, after the start of the bleaching event, when the Burdekin plume reached the Pandora and Havannah sites in mid-April (Figure 6).

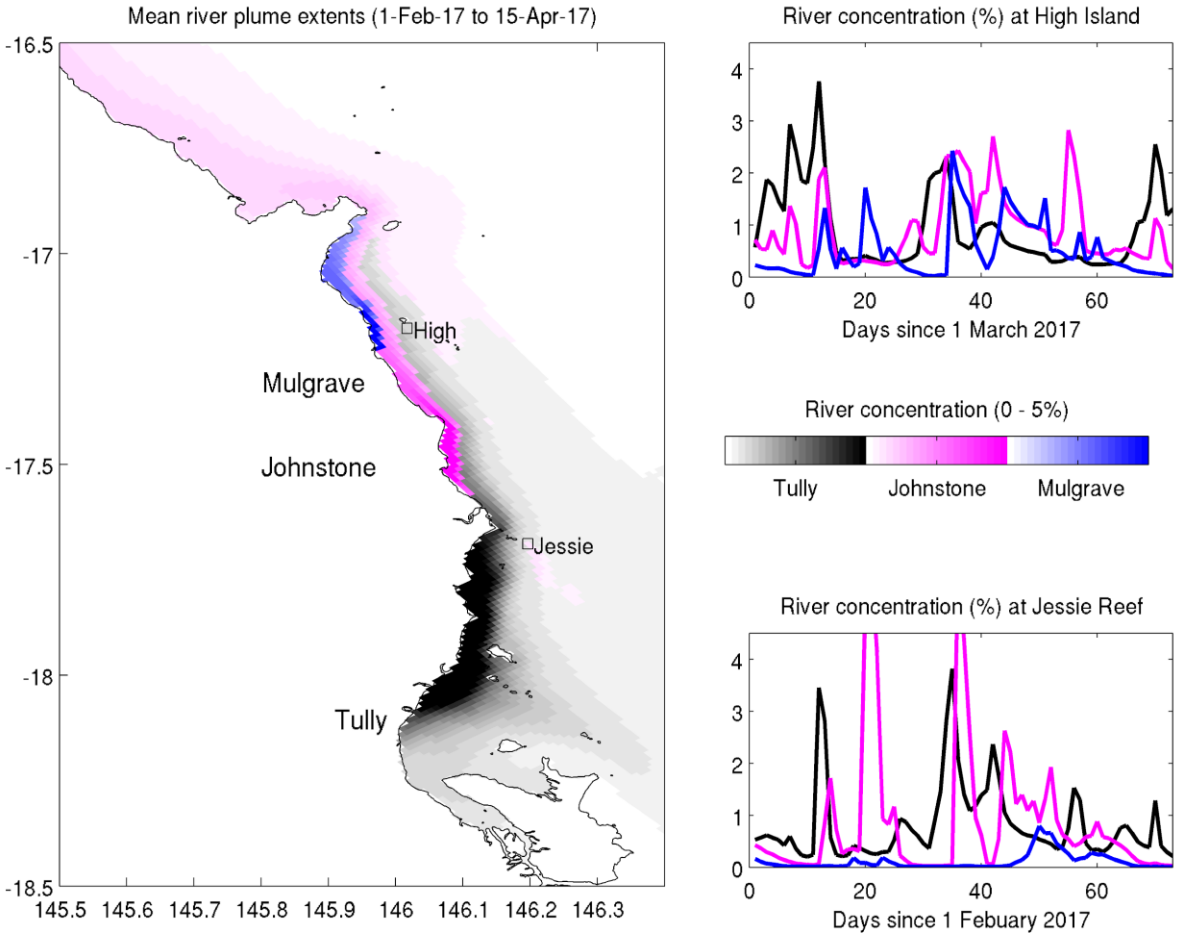


Figure 5: Temporal-mean spatial extent of the Tully, Mulgrave and Johnstone River plumes in the vicinity of High Island and Jessie Island reefs. Rivers are discharged with a concentration of 100%. At each location, the plume colouring only shows the dominant plume over the time period. For a view of river plumes across the whole GBR, and the techniques used to calculate the extent, see Baird et al. (2017).

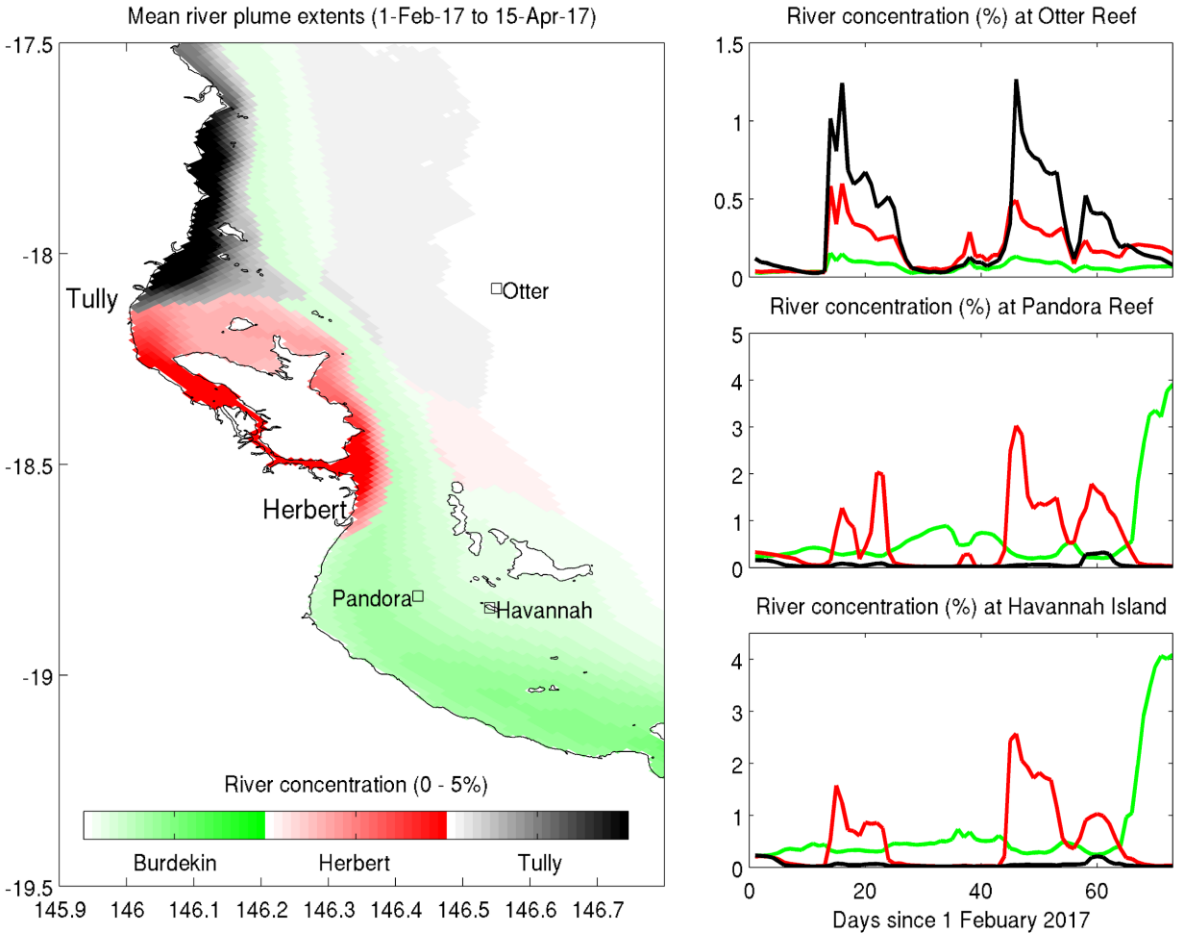


Figure 6: Temporal-mean spatial extent of the Burdekin (green), Herbert (red) and Tully (black) river plumes in the vicinity of Otter Reef, Havannah Island and Pandora Reef. Rivers are discharged with a concentration of 100%. At each location, the plume colouring only shows the dominant plume over the time period. For a view of river plumes across the whole GBR, and the techniques used to calculate the extent, see Baird et al. (2017).

3.3.3 Symbiont Physiological Response at Reef Sites

At Otter Reef, the concentrations of DIN were low relative to phosphorus for most of the summer of 2017. As a result, at the surface, symbionts were strongly N limited. Only the deepest corals show light (carbon) limitation R_C* < 0:5 at midnight. The shallowest reefs had the xanthophyll cycle switched to heat dissipating pigments ($X_h > X_p$), but otherwise the pigments were photoabsorbing. Reactive oxygen build-up was only occurring in the shallowest reef sites. In sites with a seabed depth greater than 20 m, the reaction centres were almost entirely oxidised, and for those less than 5 m they were inhibited. At intermediate depths reaction centres were spread across the oxidised, reduced and inhibited states.

The reef site with the greatest change in symbiont physiology as a result of anthropogenic loads was the 4 m deep site on Pandora. Anthropogenic nitrogen in the Herbert River, which impacted on Pandora during February, resulted in symbionts switching from being N limited to P limited. At Pandora, due to the anomalously high temperature, carbon fixation was zero, and carbon reserves became depleted in both load scenarios. However, when the temperature stress declined on day 98, with more nutrient reserves, the growth rate of symbionts increased more in the Baseline scenario than the Pre-Industrial scenario (Panel A, light blue). The very small change in light intensity resulted in more active xanthophyll switching on days 89-93.

With this acclimation, and only a small change in water clarity, the anthropogenic loads had a negligible effect on reactive oxygen stress.

The other reef sites did not show a switching of nutrient limitation, and had only small changes in photosynthetically active radiation (PAR) and reactive oxygen stress. Nonetheless there are significant differences between sites due to differing exposure to natural and anthropogenic loads.

3.5 Discussion

Anthropogenic loads of nutrients and sediments could potentially increase the reactive oxygen stress of corals, through increasing the symbiont density and therefore more reactive oxygen production within the coral host. Conversely, higher sediment concentrations could result in decreasedstress through shading, as the fraction of light absorbed by both the water column and through self shading mechanisms within the host tissue by the increased symbiont population could reduce oxidative stress production under high light conditions. However, the simulated impact of anthropogenic loads on symbiont physiology on the five runoff-exposed reefs studied in 2017 was small. The major reasons for this are:

(1) In 2017 catchment flows were low, resulting in relatively small natural and anthropogenic loads.

(2) The low flows led to small plume extents, which are constrained to propagate north in a thin inshore region by the rotation of the Earth; thus the reefs considered, some of the most exposed in the GBR, were generally exposed to waters of less than 4 % freshwater in 2017 prior to the peak of the heat stress event. Being less than 4% freshwater implies very limited terrestrial inputs to the marine environment of the reefs in this study.

(3) Reefs are generally not located within the 10-50 km downstream from the river mouth, due to the toxic effect of freshwater exposure on corals and the deposition of fine sediment near the river mouth. At this distance sediments have generally sunk out of the water column (Margvelashvili et al., 2016), although these deposits may be subsequently resuspended and transported further into the marine environment. Resuspended sediment is composed of a small fraction of anthropogenic loads (in these simulations considered anthropogenic only if exported after 1 December 2010), a much greater fraction of loads deposited over millennia. As a result of low flow inputs in the months prior to bleaching in 2017, baseline loads indicate suspended sediment concentrations were similar at the reef sites compared to the pre-industrial estimate and there was very little difference in bottom light intensity due to present day anthropogenic loads.

(4) Other than changes in the light environment, the main mechanism for runoff to affect symbiont physiology is through changing water column nutrient concentrations. Dissolved tracers propagate further in the plume than the sediments. However, during low flow years, when the coastal waters are depleted in nutrients, microalgae, seagrass and seaweeds all compete strongly for nutrients, resulting in very low concentrations even under anthropogenic loads (Skerratt et al., 2019).

(5) The excess nutrients that do make it to the reefs are then potentially absorbed by autotrophs in the coral communites, consumed through growth of the fixed carbon reserves, and allowing excess photons to be consumed by carbon fixation required to replenish the carbon reserves. Nitrogen and phosphorus reserves can only impact on the build-up of reactive oxygen by increasing the rate of carbon fixation, which only occurs if the RuBisCO enzyme is active ($a^*_{ox} > 0$). Thus, the inactivation of the RuBisCO enzyme during thermal stress conditions that could drive bleaching further prevents runoff-derived nutrients, either natural or anthropogenic, having an impact on the build-up of ROS.

(6) Coral symbionts can induce photoprotective mechanisms (xanthophyll cycling) to suppress the impact of light stress as temperatures increase, through the conversion of excess photons to heat, reducing oxidative stress. These processes, at least in the model, are independent
of nutrient status and thus runoff-derived loads.

(7) In the model, reactive oxygen stress is quantified by cell, and per host. The flux of photons to each cell, and carbon to the host, is reduced if the symbiont cell density, or host tissue biomass respectively, becomes large enough to self-shade. While the light and nutrient environment potentially varies cell and host density, this is constrained by the anatomy of the coral host that only allows 2 layers of symbiont cells.

Thus the model simulations suggest nutrient and sediment loads from human activities did not significantly alter the severity of coral bleaching on the GBR in 2017. This is most likely due to the significant drought phase of the climate cycle, low terrestrial input and transport of sediment and nutrients following well below median rainfall and river-ffow throughout the study region from 2013-2017. A similar conclusion was reached by Hughes et al. (2018b), based on field observations of the 2016 bleaching event and using a correlation of coral bleaching severity and seawater chlorophyll-a concentrations as a proxy for nutrient enrichment.

3.6 Comparison of physiological response of the model with laboratory and field observations.

The coral bleaching model developed in the previous section was undertaken in parallel with field and laboratory experiments (see following sections), and therefore reflects a consensus based on the physiological inputs driving the model variables. A number of insights have become apparent when comparing the model simulations with field and laboratory observations:

1. The observed Chl a to Chl c ratios are a constant 7.8 in the model, but vary between 2 and 6 in the field and laboratory observations. The model ratio was set on the assumption that the ratio of the Chl a molecule to accessory pigment in a particular species never changes. The value of 7.8 came from HPLC pigment analysis of one sample from the National Algal collection, but is probably accurate. The observed values of much less than 7.8 probably reflects that the laboratory UV Spectrometer technique used here does not distinguish well between accessory pigment types (i.e. it is measuring Chl a : peridinin rather than Chl a: Chl c). In this case, the model is therefore probably more representative. However, the variation in Chl a : accessory pigment in the observations has the intriguing possibility that it reflects a change in the

symbiont population after bleaching, a process that is not in the model. This, combined with the field component of this study emphasising the differing susceptibility of different coral species, reminds us that the model would be improved by considering multiple coral and symbiont types.

2. In theory, the model term Qox* (the fraction of oxidised reaction centres) corresponds to Fv/Fm (efficiency of photosystem II to convert photons of light into photochemistry). In the laboratory experiments, Fv/Fm varies by a maximum of approximately 0.1 between midday and early dark, while in the model, resolved every hour, in highly bleached corals Fv/Fm varies between 0.7 and 0.1. The maximum value of 0.7 seems to be an overestimate for highly bleached corals. There are two reasons the model corals have a greater drop in Qox*. Firstly, in resolving every hour, they pick up the time of highest stress (2-3 pm after light intensity peaks with daily warming). Secondly, the laboratory experiments were set up with light levels that were not as high as those experienced by the 2 m deep corals in clear waters. Given these caveats, the model may be performing well. However, it does appear that the model corals recover from inhibited reaction centres too quickly. If so, this may lead to underprediction of PSII damage and ROS build-up, and should therefore be investigated.

In summary, the comparison of field, laboratory and model outputs has not identified any major errors in the model, but rather emphasised subtle contributions of photophysiology that could improve the predictive capacity of the model. That is, representing multiple coral and symbiont species is necessary to predict the bleaching on the reef.

4.0 ASSESSING THE LINKS BETWEEN WATER QUALITY GRADIENTS AND CORAL BLEACHING SEVERITY AND MORTALITY ON THE GREAT BARRIER REEF

4.1 Introduction

Climate change, especially prolonged warming events that exceed historical summer maximum temperatures and cause coral bleaching and mortality, remain the biggest threat to the GBR (GBRMPA 2019). Guidelines based on past bleaching events suggest that accumulated heat stress using the NOAA DHW product from 0 - 4°C-weeks would cause little to no bleaching (ie. normal to just above average summer conditions), 4-8°C-weeks point to a risk of possible bleaching, and >8°C-weeks, will likely cause extreme bleaching and widespread mortality (Liu et al. 2003). Global emissions of greenhouse gases, particularly carbon dioxide (CO_2), have caused record breaking extreme summer temperatures and ocean warming at a rate faster than previously expected (Hoegh-Guldberg 1999; Donner et al. 2005). Current emissions trajectories and temperature stress metrics indicate that ocean warming will continue and that the GBR will reach heat stress levels capable of causing bleaching driven mortality events twice per decade by 2035 - 2041 and annually by 2044 - 2051 (Heron et al. 2017). Climate change is also predicted to increase the intensity of extreme weather events, which are significant drivers of terrestrial input into the coastal and marine ecosystems of the GBR. In combination with global efforts to mitigate and reduce the rate of ocean warming, reducing land-based pollution represents a consensus local action that could improve the resilience of marine ecosystems to cope with accelerating pressures associated with a changing climate (Waterhouse et al. 2017). A better understanding of the interactive role of extreme events (marine heat waves, storms and flooding events), climate change and end-of-catchment pollutant loads that reduce water quality is essential to determine if reaching the GBR end-of-catchment water quality targets (Queensland and Australian Governments, 2018) would contribute to reduce the risk of coral bleaching for the GBR into the future.

Nutrient cycling processes in coral reef ecosystems are complex and poorly quantified. Inputs of excess nutrients may elevate the availability of dissolved inorganic nutrients on coral reefs for brief periods of time (days to a week) but are quickly taken up by phytoplankton and converted to particulate and dissolved organic matter that further cycles through the system over weeks to months after the initial input (Furnas et al. 2005, 2011). Selective uptake of nutrients may also lead to changes in elemental ratios, such as the relative concentrations of nitrogen (N) to phosphorus (P), and potentially to nitrogen limitation (Furnas et al 2005, Schaffelke et al 2012). Both nutrient enrichment and nutrient limitation can undermine the stability of the coral-algal symbiosis. Elevated nutrients (particularly nitrate) may drive the algal symbionts into a state of rapid growth during which they become “selfish” and retain autotrophic carbon (Baker et al 2018; Ezzat et al 2015; Morris et al 2019; Wooldridge, 2009). Experimentally, it has been observed that nutrient limitation (particularly phosphorus limitation) can disrupt the manufacture and repair of important cellular components which leaves the symbiosis vulnerable to thermal stress (Ferrier-Pages et al 2016; Wiedenmann et al 2013). However, this is unlikely to occur in the coastal GBR, as the depletion times of P are much longer than DIN, indicating that P is rarely a limiting nutrient for phytoplankton growth in GBR waters (Furnas et al. 2005). Mechanistic links between nutrients and thermal bleaching are

largely based on laboratory experiments. Correlations of observational data for water quality and coral bleaching responses have yielded contrasti ng results at different intensities of heat stress (Wooldridge and Done, 2009; Hughes et al 2017), with the influence of water quality proxies becoming less important at extreme levels of accumulated thermal anomalies (>6°C-weeks; Hughes et al. 2017).

Inshore reef habitats exposed to terrestrial run-off are subjected to elevated levels of turbidity and reduced light intensity under ambient wind conditions, resulting in assemblages of corals associating with dark-adapted algal symbionts (Fabricius et al. 2016; Morgan et al. 2017). During warm, calm “doldrum” weather periods, still waters result in sediment settling out of suspension, causing abrupt increases in light intensity for corals usually adapted to turbid water (Gruber et al. 2019). This combination of acute light and heat stress could exacerbate bleaching in inshore corals compared with offshore corals, which are only exposed to a change in temperature. However, the contribution of this water quality-mediated mechanism to enhance the bleaching response of inshore corals is largely unknown.

On the other hand, terrestrial run-off may increase the capacity of inshore corals for heterotrophic feeding, by providing them with a greater availability of particulate organic matter compared to offshore oligotrophic locations (Fox et al. 2018). Increased heterotrophic feeding generally leads to increased tissue growth and greater tissue energetic reserves that can be metabolised during bleached conditions when photosynthate production has stopped (Anthony and Fabricius 2000; Grottoli et al 2006). Overall, there is plausible potential for particulate nutrients and organic matter to mitigate or exacerbate the thermal bleaching response of corals. However, the exact mechanisms and relative importance of the beneficial and negative effects are not known.

The aim of this chapter is to identify environmental drivers that influence the risk of coral bleaching at elevated temperatures, as an exploration of potential interactions between water quality and temperature. Using *in-situ* measurements of water quality (dissolved and particulate nutrients, turbidity, chl *a*) collected by the AIMS Marine Monitoring Program (MMP) on inshore reef locations and modelled estimates of water quality parameters from the eReefs BGC-hydrodynamic on both inshore and mid-shelf reef locations, we have applied a general hierarchical modelling approach to assess the relative contribution of temperature and water quality parameters to the overall observed patterns of bleaching severity during the 2016 and 2017 mass bleaching events. We seek to assess if future improvements in water quality have the potential to mitigate the severity of coral bleaching under current and future levels of heat stress.

4.2 Methods

4.2.1 In-water bleaching surveys

A total of 23 reefs were surveyed across a continental shelf gradient of water quality within the Dry and Wet Tropic regions of the central GBR during the 2016 and 2017 mass coral bleaching events. In-water surveys were conducted at 57 sites within 19 reefs in 2016 and 40 sites within 12 reefs in 2017 (Figure 10, Table 6 and Figure 24; Chapter 4). Surveys were conducted between March 13 and April 6 2016, and between March 14 and April 1 2017. To capture

bleaching at its full extent surveys coincided with the peak of maximum temperature stress accumulation, with an additional 0 – 2.2°C-weeks Degree Heating Weeks accumulated following the surveys. For each reef, we surveyed 3 habitats: (1) the shallow, sheltered reef flat at 2 m, (2) the exposed shallow reef slope at 3 m and (3) the deeper exposed reef slope at 7-9 m. The deeper slope sites coincided with permanent AIMS Long Term Monitoring (LTM) sites where available, or in equivalent habitats on the northern exposed flank of reefs. For image analysis, quadrats (1 m^2) at each site were photographed along five replicate 10 x 1m belt transects with 1m spacing between each replicate (e.g. Figure 7). Images were captured using an Olympus TG-4 camera within an Olympus PT-056 housing equipped with an INON UWL-H100 Type 2 wide conversion lens, in aperture priority mode. A manual white balance offset was captured at each survey depth against a white background colour card. Each image contained a meter stick for scale (1 m length, with 5 cm grid for size reference) and to set a consistent distance of the camera lens to the benthos. The Coral Watch (https://coralwatch.org/index.php/monitoring/using-the-chart/) colour coral health chart scales (D1-D5 and C1-C5) were included in each image as a consistent colour reference.

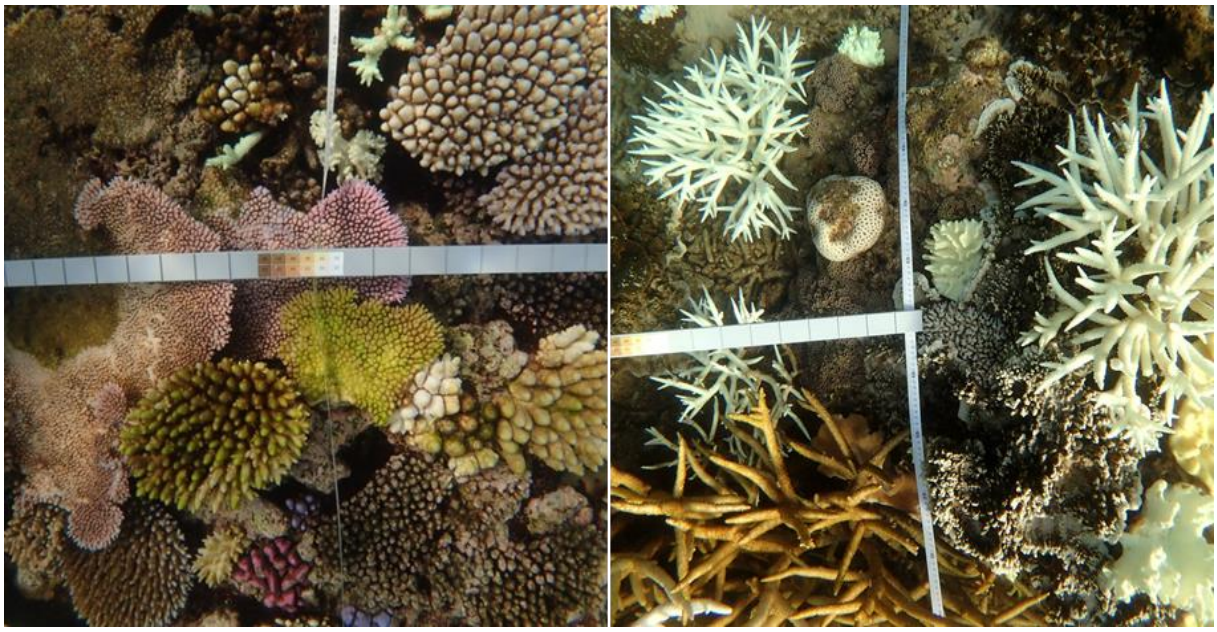


Figure 7: In-water bleaching survey photos capturing 1x1 m square quadrat images for analysis along a 10 x 1 m belt transect documenting coral bleaching severity and coral composition during the 2016 and 2017 bleaching events on the GBR.

Observers identified and counted each coral colony (hard and soft corals) >5 cm in diameter and recorded a categorical bleaching score for each individual colony based on the proportion of the visual pigment content (% individual colony area that was bleached white): (1) no bleaching (Figure 8A), (3) minor-moderate (1 - 50%; Figure 8B), (4) major (50 - 95%; Figure 8C) (5) severe (95 - 100% bleached white or fluorescent; Figure 8D) and (6) Bleached with complete or partial sections of the colony recently dead (Figure 8E), following the methods used by the National Bleaching Taskforce 2016 and detailed in Baird and Marshall (2002) based on the scheme of Gleason and Wellington (1993). During surveys in 2016, colonies appearing pale and recorded as (Bleached State 2) were considered as not bleached

(Bleached State 1), as differences in colour and / or shade can occur naturally between inshore and mid-shelf locations and may not be a result of thermal bleaching.

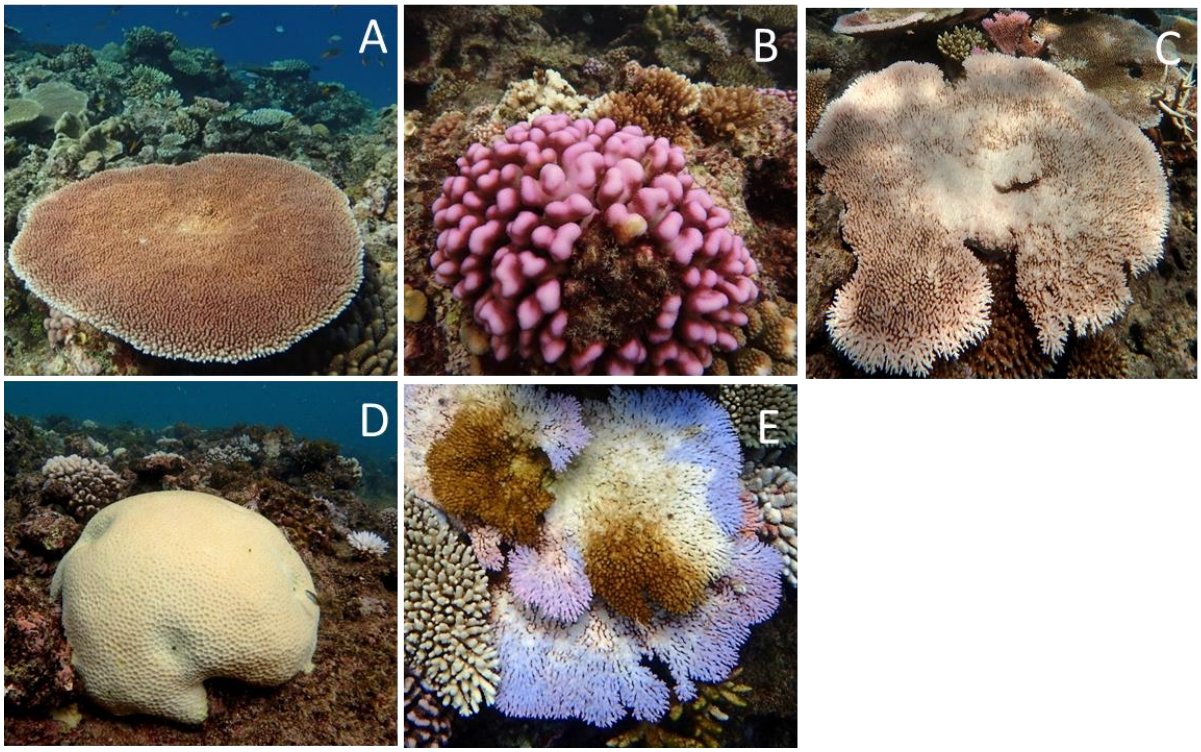


Figure 8: Individual colony bleaching severity categories used by in-water surveys. (A) Bleached State 1: No bleaching, (B) Bleached State 3: 1-50% colony area bleached, usually upper surfaces, (C) Bleached State 4: 50-95% of the colony area bleached, symbionts still visibly present in some tissue; (D) Bleached State 5: 100% white severely bleached (with or without fluorescent pigments) and (E) Bleached State 6 - Bleaching related recently dead colonies (full colony or partial sections of the colony).

For each transect, scores were recorded per bleached state and converted into: (1) proportion of the coral community not bleached (% Bleached State 1 and 2; Figure 8A); (2) proportion of the coral community bleached (% Bleached State 3-6; Figure 8B-E); (3) proportion of the coral community severely bleached (% Bleached State 5; Figure 8D); (4) proportion of coral mortality (% Bleached State 6) and (5) proportion of the coral community recently dead and severely bleached (% Bleached State 5 + 6). This combined metric of severely bleached corals and recently dead corals was used as an indicator of the direct impact of the heat stress event and coral bleaching on the reef community through the loss of living coral cover.

4.2.2 Temperature Data

Historical average maximum temperatures (climatologies) are a key feature in predicting summer extremes and are used to define the point when ocean temperature anomalies begin to exceed the upper thermal limit of each individual reef location. Historical summer maximum ocean temperatures generally occur in February for most of the GBR. Using daily 5km (0.05˚ resolution) satellite remote sensing data from the National Oceanographic and Atmospheric Administration (NOAA) Coral Reef Watch (CRW) 'CoralTemp' Version 3.1 (Liu et al. 2014), we assessed the annual Sea Surface Temperature (SST) anomalies at each reef where in-water bleaching surveys were conducted in 2016 and 2017. To assess the differences in thermal stress at each reef location, we extracted daily temperature records and computed the

following temperature metrics: (i) historical upper thermal limit using NOAA's Mean Monthly Maximum (MMM); (ii) maximum Sea Surface Temperature (max SST); (iii) maximum SST anomaly (max Anom) (iv) Degree Heating Weeks at the time of the survey (DHW.YTD); (v) Maximum Degree Heating Week accumulated at the end of the summer (maxDHW); and (vi) additional accumulation of DHW (DHW_Add) following the date of survey as an indicator of the timing of peak heat stress in relation to the survey date.

The NOAA Degree Heating Week (DHW) product is a coral reef heat stress index which combines the intensity of the temperature anomaly (Hotspot) at least 1˚C above the historical summer maximum (MMM, Version 3.1) temperature (upper thermal threshold) with the duration of time above this threshold over a rolling 12 week period. A heat stress accumulation of 4°C-weeks, essentially represents a reef experiencing 1 month at 1˚C above a normal summer maximum or 2 weeks at 2˚C above a normal summer maximum (Liu et al. 2003).

4.2.3 Water Quality Metrics

AIMS In Water sampling

The AIMS (MMP) quantifies temporal and spatial variation in inshore water quality conditions. Intensive sampling (5 - 10 times annually) occurs within the inshore GBR at increasing distance from river mouths in a northerly direction, to reflect the predominantly northward flow of surface water driven by the prevailing south-easterly winds. Detailed sampling methods are described in Gruber et al. (2019; Table 1). Samples collected in March, closest to the date of the in-water bleaching surveys in 2016 and 2017, were used as an indicator of the water quality state at the time of bleaching within the modelling analysis for this report.

eReefs Hydrodynamic model

Given, the dynamic nature of water quality inputs and the limited temporal resolution of in-situ water sampling intervals collected by the MMP program, daily estimates of water quality metrics were extracted from the nearest grid cell to each survey reef location from the eReefs Hydrodynamic model (GBR 4km domain[3]). These modelled daily water quality metrics during the wet-season (Oct - Apr) of each bleaching year, provide an integrated estimate over a longer time frame than the single in-situ water samples. Annual wet season mean, median, maximum and total range (max - min) and seasonal change (max - mean) were calculated for each reef for each year (2016 and 2017). Daily river flow data (megalitres) were obtained from the Queensland Department of Natural Resources, Mines and Energy (DNRME) Water Monitoring Information Portal (WMIP: Queensland Government). Scaling factors were used to adjust the daily river flow data to account for the proportion of the catchment area reflected by the location of the river gauge station. The discharge data for each catchment basin were upscaled using the difference between the gauged catchment area (% of total catchment area) and the total basin area to estimate flow for each basin following the approach developed by Gruber et al. (2019).

[3] https://research.csiro.au/ereefs/models/models-about/

Table 1: eReefs GBR 4km biogeochemical model water quality metrics used in analysis.

Name of metric	Symbol	Units	Description
Total Suspended Solids	TSS	kg m^{-3}	Ecological Fine Inorganics (EFI); inorganic fraction of total suspended solids used for TSS-dependent calculations such as phosphorus absorption
Total chlorophyll a	Chl a	mg m^{-3}	Sum of chlorophyll concentration of the four microalgae types
Vertical attenuation at 490nm	Kd490	m^{-1}	Vertical attenuation of light at 490nm (along z axis not along zenith angle)
Rubisco Enzyme activity of coral symbiont	CS_Tempfunc (CS_Q_{ox})	0-1	Normalized symbiont reaction centres in an oxidised state per m^2
Labile Detritus Benthic Nitrogen	DetBL_N	mg m^{-3}	Concentration of N in labile (quickly broken down) organic matter with C:N:P ratio of 550:30:1 from living seagrass and macroalgae
Labile Detritus Planktonic Nitrogen	DetPL_N	mg m^{-3}	Concentration of N in labile (quickly broken down) organic matter with C:N:P ratio of 106:16:1 derived from living microalgae, zooplankton, coral host tissue and symbionts.
Refractory Detritus Nitrogen	DetR_N	mg m^{-3}	Concentration of N as particulate refractory (slowly broken down) material. Sourced only from breakdown of labile detritus and from rivers.
Dissolved Organic Carbon	DOR_C	mg m^{-3}	Concentration of carbon in dissolved organic compounds
Dissolved Inorganic Carbon	DIC	mg m^{-3}	Concentration of dissolved inorganic carbon, composed chiefly at seawater pH of HCO_3^-
Dissolved Inorganic Nitrogen	DIN	mg N m^{-3}	Concentration of dissolved inorganic nitrogen
Total Nitrogen	TN	mg N m^{-3}	Sum of both dissolved and particulate nitrogen
Nitrate	NO_3	mg N	Concentration of nitrate. In the absence of nitrite [NO_2] in the model, nitrate represents [NO_3^-] + [NO_2^-]

pH	pH	Log_{10} mol m^{-3}	pH based on $[H^+]$ calculated from carbon chemistry equilibria at water column values of T,S, DIC and A_T
Particulate Inorganic Phosphorus	PIP	[mg P m^{-3}]	Phosphorus ions absorbed onto particles
Salinity	Salt [S]	PSU	Seawater salinity
Temperature	T	°C	Seawater temperature

4.2.4 Data Analysis

Initially, 152 spatial, temporal and environmental variables were selected according to their known ecological significance in driving bleaching sensitivity patterns. These explanatory variables were tested for collinearity using Pearson's correlation plots in R v3.4 (R Core team 2020), generated with package 'corrplot' (Taiyun and Simko 2017). If 2 or more variables were correlated by more than $|r| > 0.75$, only one was selected based on their expected influence on bleaching sensitivity from previous studies and their utility for being responsive to water quality management (Dormann et al. 2013); this resulted in 18 final continuous non-collinear variables (Figure 9).

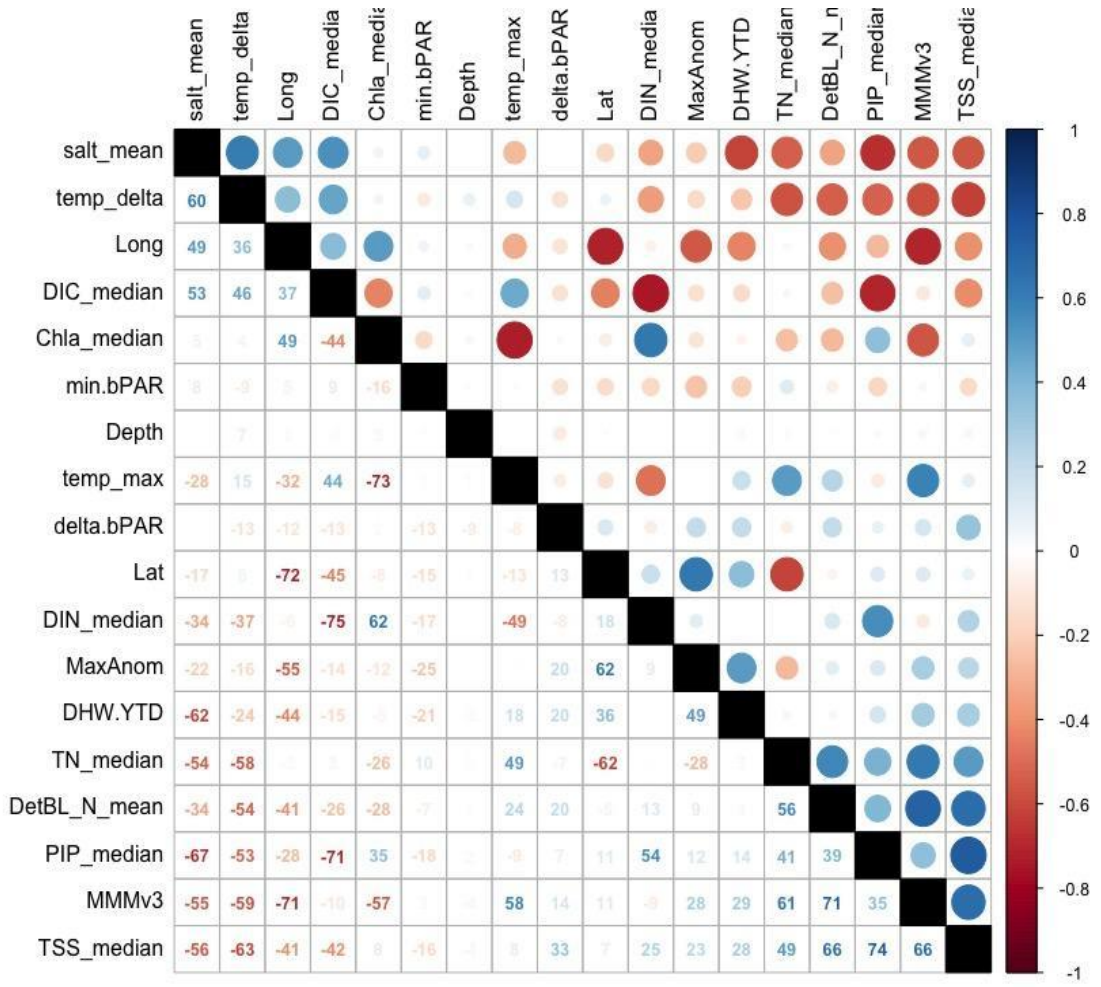


Figure 9: Correlation plot including data of both inshore and midshelf reefs, using the 18 explanatory variables (Appendix Table 1) tested in the hierarchical models for community bleaching (% hard and soft corals) and the proportion of severely bleached and recently dead corals (%). The lower left panel represents the correlation value as a percentage, while the upper right panel represents the correlation strength and relationship, with size and color of the circle related to magnitude of correlation. Blue denotes a positive correlation and red a negative correlation, correlations larger than 0.49 are solid, while correlations –below 0.49 magnitude fade in transparency with values below 0.1 being transparent.

General hierarchical models were used to explore the relationship between these 18 continuous explanatory variables and two response variables: (1) community bleached (% hard and soft corals >5cm), and (2) proportion of severely bleached and recently dead colonies (% hard and soft corals >5 cm; bleached state 5 + 6, Figure 8D & 8E). We also tested five different random effects: bleaching year (2016 and 2017), climate (Dry Tropics vs Wet Tropics), shelf (inshore vs mid-shelf), reef sector (Townsville, Tully, Innisfail and Cairns) and reef, where reef was nested within sector and shelf. Random effects were only retained if they improved the model fitness, which was tested via chi-square-test on likelihood ratios by comparing identical models with and without each random effect (Crawley 2012). No interactions were considered, and models were simplified using backward selection following the parsimony principle and tested using chi-square-test on likelihood ratios and AIC to determine the best model (Crawley 2012). Relative importance estimates and variance partitioned among predictor variables by averaging over orders, as described in Lindemann, Merenda and Gold (1980, p.119) for each predictor in the final hierarchical model. We used packages ‘mass’ (Venables and Ripley 2002) and ‘glmmADMB’ (Fournier et al. 2012) for modelling, and packages ‘corplot’ (Taiyun and Simko 2017), ‘ggplot2’ (Wickham 2009), ‘coefplot2’ (Bolker and

Su 2011) and ‘relaimpo’ (Gromping 2006) to visualize results. Assumptions of normality, heteroscedasticity and independence were tested on residuals of all final models using residual plots (package ‘graphics’, R-Core-Team 2013).

The modeling strategy described above was applied to the entire dataset, which used modelled water quality metrics from the eReefs BGC hydrodynamic model (https://research.csiro.au/ereefs/models/models-about/) for both the inshore and mid-shelf reef locations surveyed in this study (Figure 12) across the spatial cross-shelf gradient in temperature and water quality between Townsville and Port Douglas. We also ran models with a subset of the data from the inshore reefs only to investigate the role of water quality metrics from *in situ* water sampling, collected through the AIMS MMP, on bleaching and bleaching severity. Unfortunately, these in-situ seawater metrics were only available for a subset of inshore reefs (Table A1, Appendix 1), however they provide the opportunity to compare the contribution of in-situ seawater sampling and modelled estimates of water quality throughout the inshore reef locations. The same modeling strategy was applied, where correlations amongst explanatory variables were tested to shortlist uncorrelated explanatory variables. The subset models initially included 15 continuous variables (Figure 10).

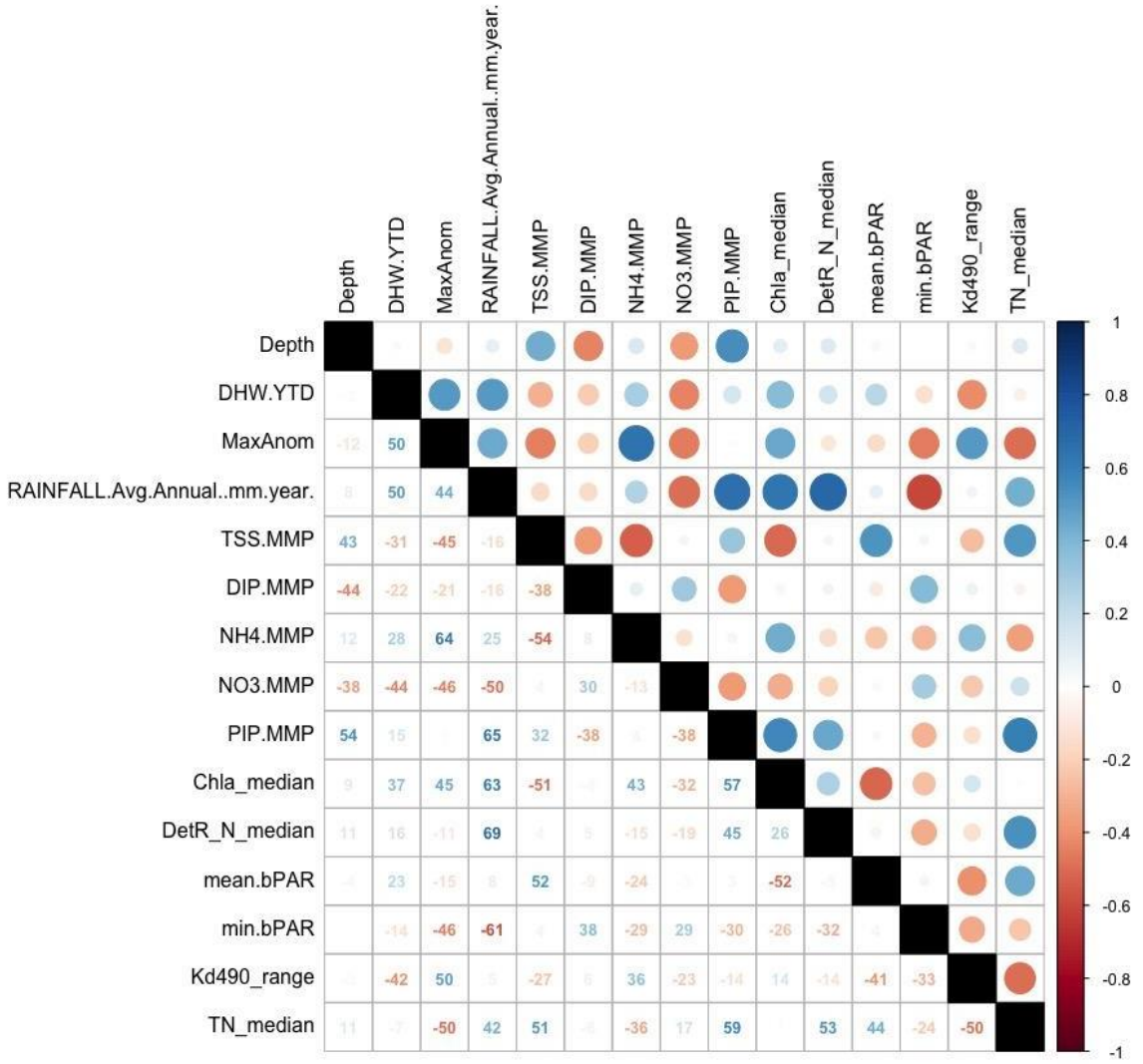


Figure 10: Correlation plot including data for inshore reefs only using the 15 explanatory variables tested in the hierarchical models for community bleaching (% hard and soft corals) and the proportion of severely bleached and recently dead coral (% hard and soft corals). *In situ* water sampling metrics have been labelled with .MMP, all other water quality metrics are modelled estimates from the eReefs BGC model. The lower left panel represents the correlation value as a percentage, while the upper right panel represents the correlations in circles. Blue denotes a positive correlation and red a negative one, correlations larger than 0.49 are solid, while correlations below –0.49 in magnitude fade in transparency with values below 0.1 being transparent.

4.3 Results

Temperature anomalies (SST exceeding the MMM by >1°C) during the summers of 2016 and 2017 exposed large areas of the GBR to prolonged thermal stress. Extreme levels of heat stress (DHW>5°C-weeks) occurred in the Far Northern GBR in 2016 (Figure 11A) and affected the Northern and Central sectors in 2017 (Figure 11B). Heat stress accumulation (measured as DHW) throughout the study region between Townsville and Port Douglas in 2016 ranged from mild to major (1.48-5.4°C-weeks) and increased during the second bleaching event in 2017, with all inshore and mid-shelf reefs exposed to severe DHW levels between 5.2-9.5°C-weeks (Figure 11). Moderate to severe community bleaching (% of both hard and soft corals) was observed during both bleaching events, with an increase in bleaching severity and mortality as the DHW exposure increased (Figure 12, 13 and 14). Severe bleaching (refer

Figure 8D) and mortality (refer Figure 8E) was low in 2016 <20% but increased with increased heat stress (DHW) throughout both inshore and mid-shelf locations in 2017 (15-70% of coral cover; Figure 14).

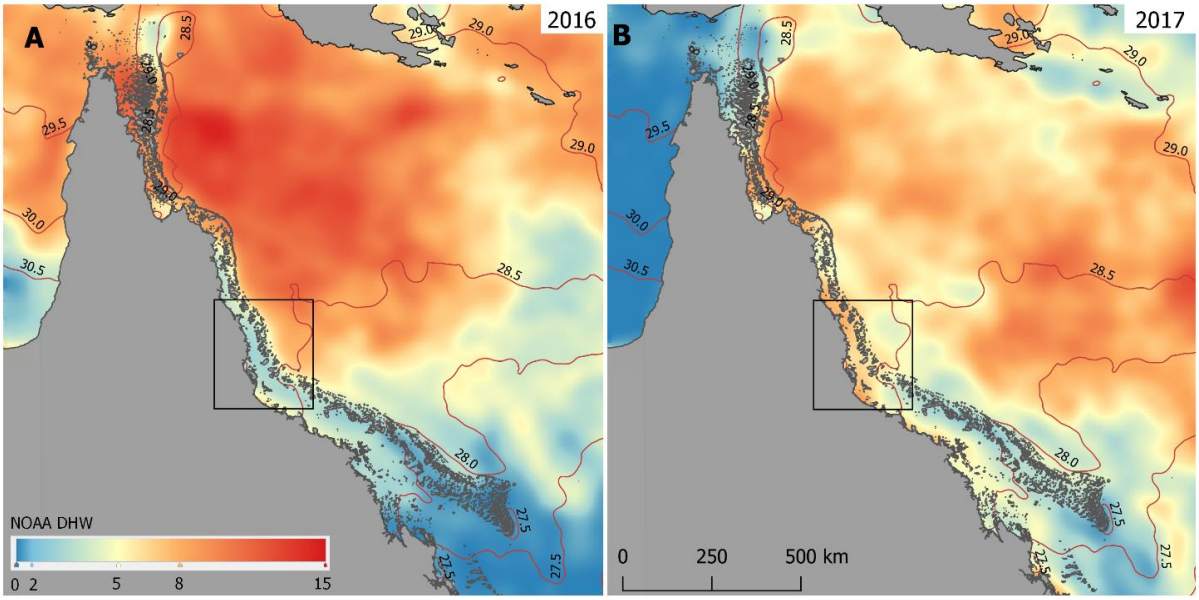


Figure 11: Spatial distribution of thermal heat stress measured by the NOAA Degree Heating Week (NOAA) product throughout the Great Barrier Reef in (A) **2016 and** (B) **2017. Iso-lines indicate spatial distribution of the historical upper thermal limit (NOAA MMM in °C) across the GBR. In water surveys were conducted within the central GBR (Bounding Box; Figure 12) between Townsville and Port Douglas for this study.**

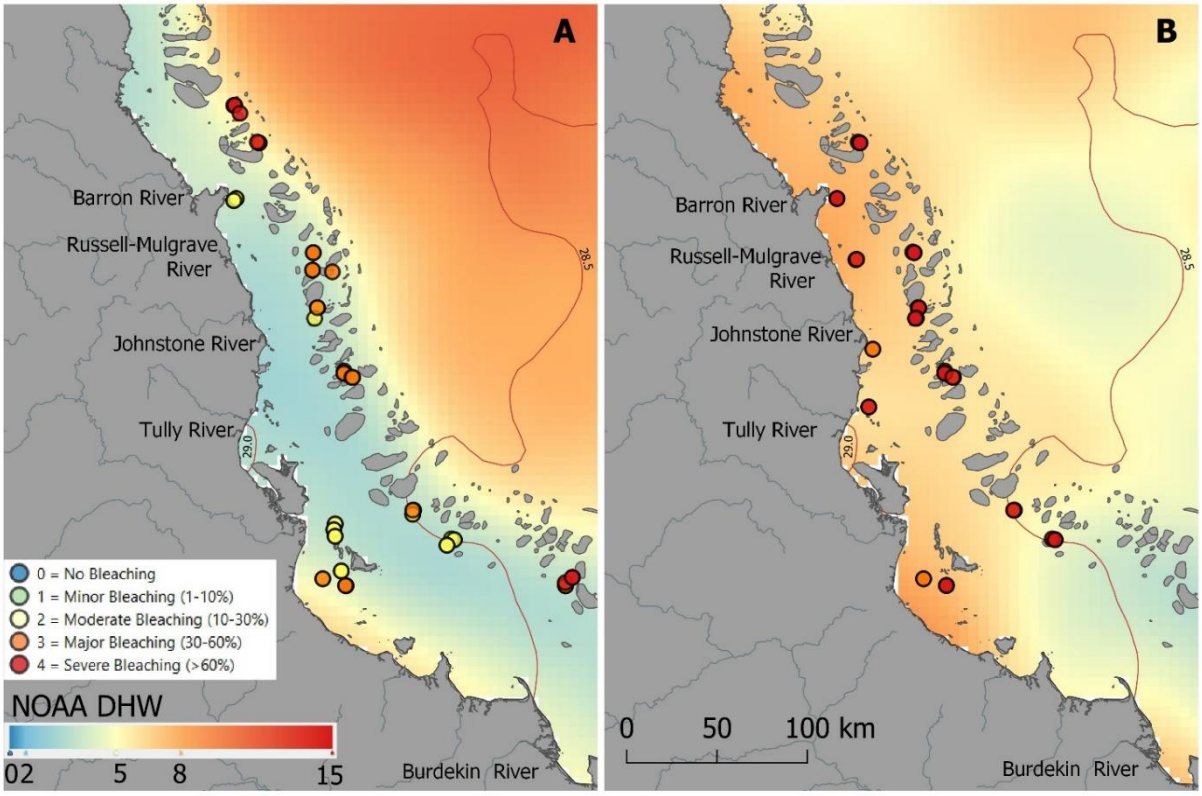


Figure 12: Coral community bleaching severity from in water surveys conducted in (A) **2016 and** (B) **2017 across the central GBR, using bleaching severity categories based on the proportion of hard and soft corals (>5cm diameter) bleached across a 5 category scale. Major river catchments across the survey locations include the Burdekin, Tully, Johnstone, Russell-Mulgrave and the Barron River. Thermal stress exposure and bleaching severity was greater at these reefs in 2017. Maximum accumulated annual heat stress indicated by the NOAA Degree Heating Week product.**

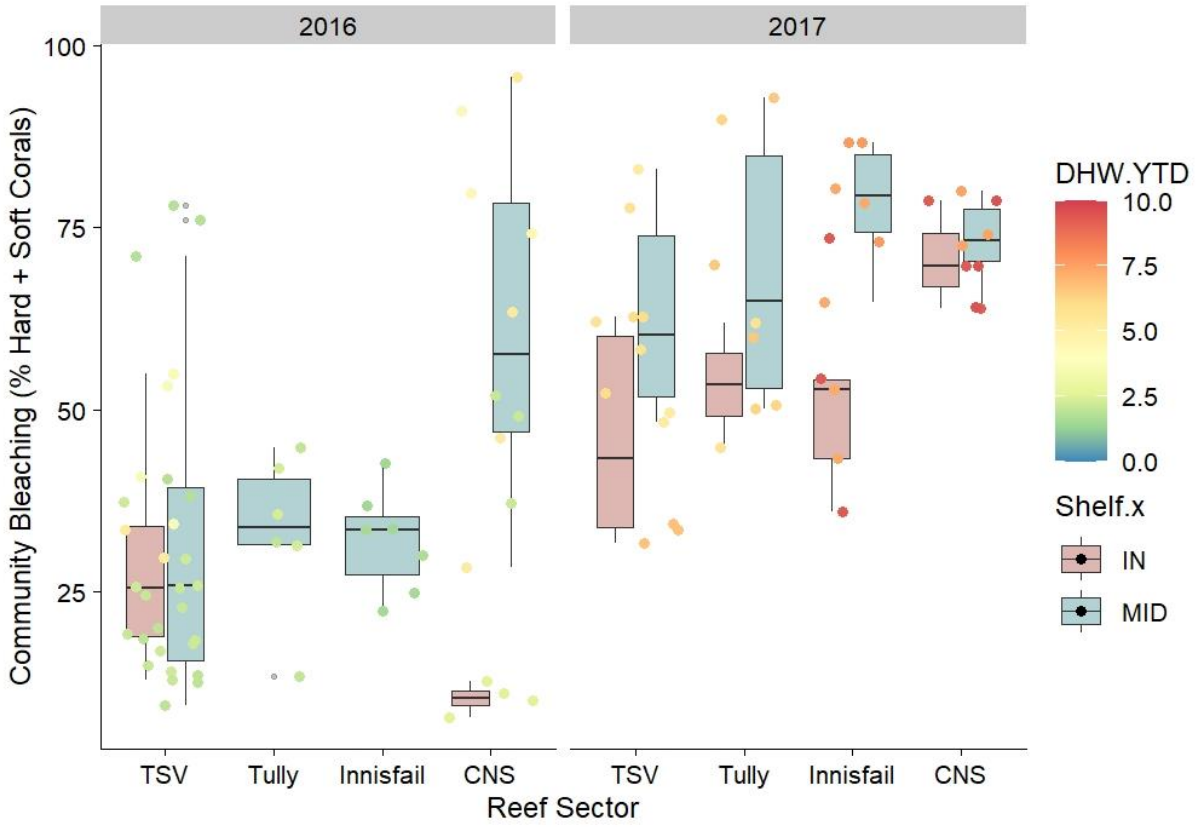


Figure 13: Community-level bleaching severity (% of both hard and soft corals > 5cm in diameter) by reef sector: Townsville (TSV), Tully, Innisfail and Cairns (CNS) from in-water transect based surveys in 2016 and 2017. Surveys grouped by inshore (green boxplots) and mid-shelf (grey boxplots) reef locations and individual bleaching severity plotted as a function of accumulated heat stress up to the date of survey (DHW.YTD).

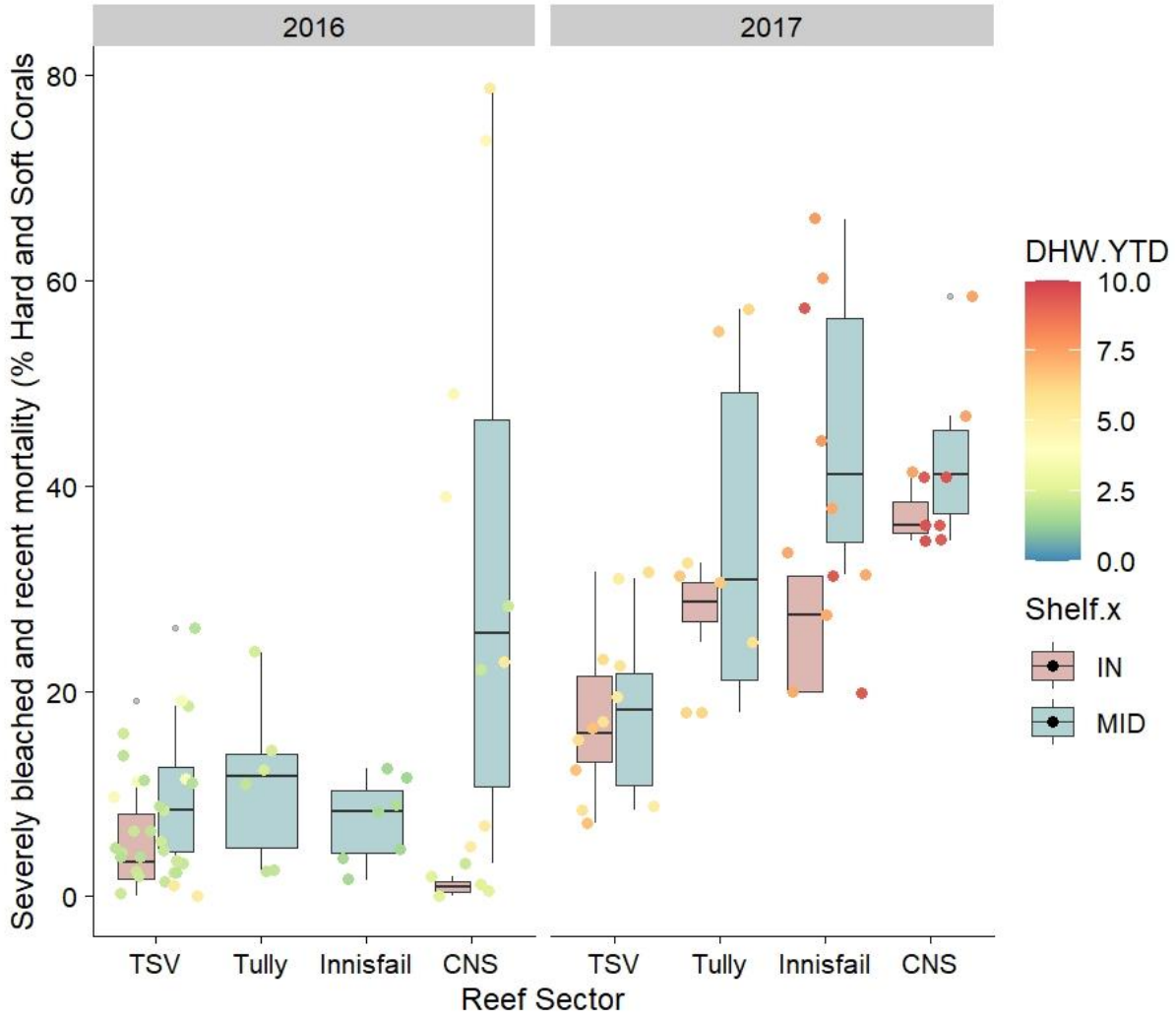


Figure 14: Proportion of the coral community severely bleached (Bleached State 5) and recently dead (Bleached State 6; % hard and soft corals >5cm in diameter) by reef sector: Townsville (TSV), Tully, Innisfail and Cairns (CNS) from in-water transect based surveys in 2016 and 2017. Surveys grouped by inshore (green boxplots) and mid-shelf (grey boxplots) reef locations and individual bleaching severity plotted as a function of accumulated heat stress up to the date of survey (DHW.YTD).

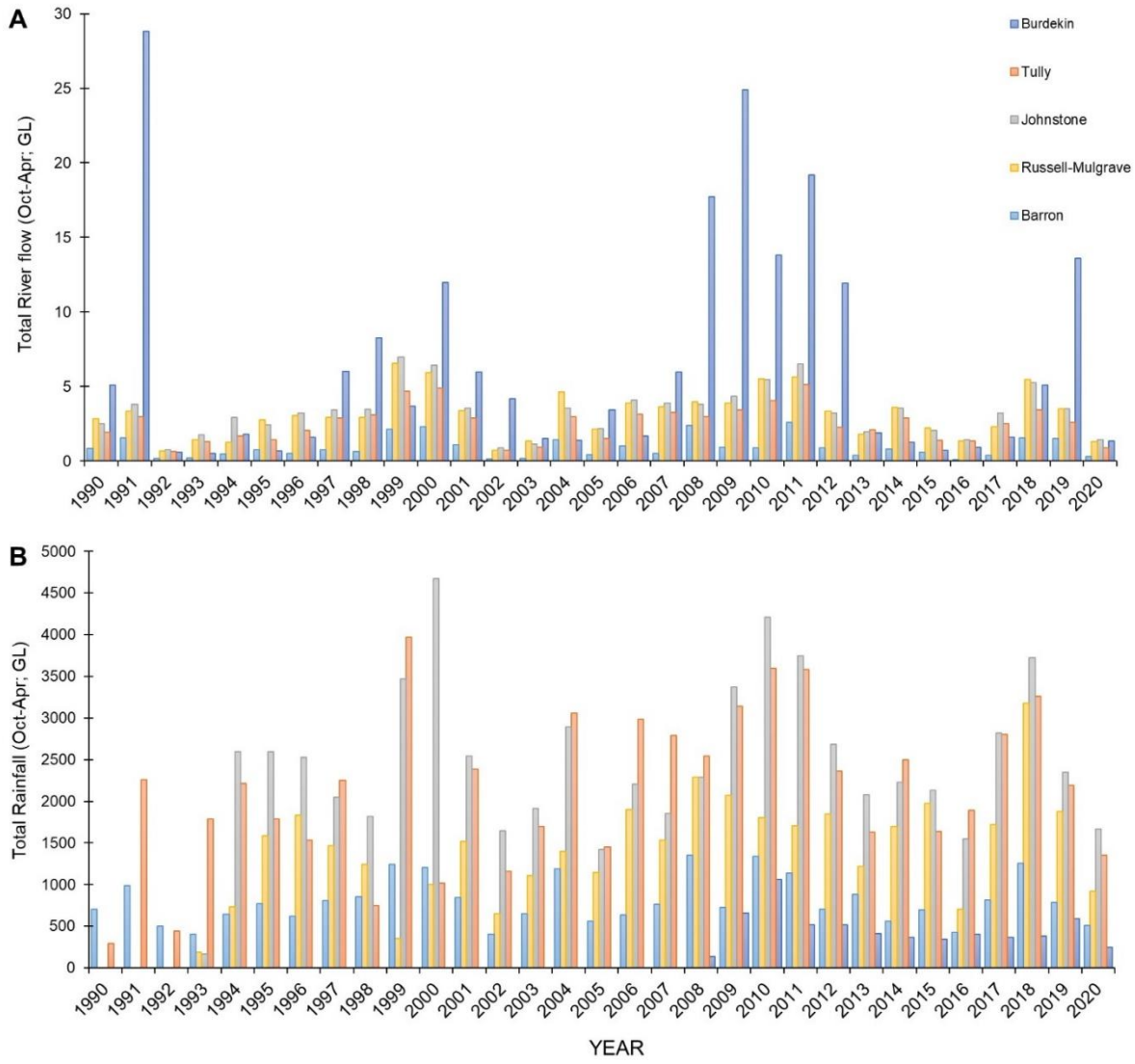


Figure 15: (A) **Annual wet season (Oct - Apr) river flow from the major rivers entering the GBR lagoon in the dry tropics (Burdekin River) and the wet tropics (Tully, Johnstone, Mulgrave and Barron Rivers) from 1990-2020.** (B) **Annual wet season (Oct - Apr) rainfall (mm) driving the river flow and terrestrial transport into the GBR lagoon throughout the study site regions, dry tropics (Burdekin) and wet tropics (Tully, Johnstone, Russell-Mulgrave and Barron).**

Variation in annual rainfall on both inter annual timescales and between the Dry Tropic and Wet Tropic regions of the GBR strongly influence riverflow into the GBR lagoon. Above median rainfall occurred in the Burdekin region from 2008-2012, which caused a 4-8 fold increase in riverflow and terrestrial transport into the marine lagoon of the GBR (Figure 15). In the years pre-ceding the 2016 and 2017 bleaching events, from 2013-2016, rainfall and river flow were below median. Interannual variability in river flow is greater in the dry tropics region compared to the wet tropics (Figure 15A), however river flow in 2015 and 2016 from each of the major rivers influencing the study region reached the lowest total discharge since 1995 (Figure 15A).

4.3.1 Coral community bleaching - Combined cross shelf gradient (mid-shelf and inshore)

Both the duration and severity of temperature stress are the major determining factors contributing to the severity of coral bleaching throughout the study region. The mixed effect

model explained 72.3% of the variance using the combined effect of temperature, eReefs modelled water quality metrics and spatial variables on proportion of reef community bleaching (% of bleached hard and soft corals), with seven of the 18 explanatory variables tested being retained in the final model. Of these seven explanatory variables two temperature metrics related to the accumulation of heat stress over time and the size of the temperature anomaly (DHW.YTD and Max.Anom) explained nearly three quarters of the variation in community bleaching response (47% of the total 72.3% variance explained; Figure 17). The remaining variance contributing to the increased bleaching response included minor contributions from the median concentration of Particulate Inorganic Phosphate (median_PIP; 2.5%) and the cross shelf gradient metric, longitude (5%; Figure 16). Nitrogen metrics were both inversely correlated to coral bleaching response, with Total Nitrogen explaining 9%, and DIN explaining 6% of the total variance in percent bleached corals. Community coral bleaching (%) decreased significantly with depth (3%). A significant amount of variation was explained by inter-reef variability (random effect 'reef'), the influence of some water quality variables, such as nitrogen metrics, also varied between inshore and midshelf reefs, however this variability was better captured by 'reef' (Figure 16 and Figure 17).

Table 2: Summary statistics for final hierarchical model of total community bleaching response (% hard and soft corals) across both inshore and mid-shelf reefs in this study. Significance indicated by ** >0.01 and *>0.001.**

% bleached	Estimate	Std. Error	Pr(>|z|)
(Intercept)	47.69	1.99	< 2e-16 ***
Long	9.71	2.31	2.5e-05 ***
Depth	-3.76	1.11	0.00069 ***
MaxAnom	5.93	1.93	0.00207 **
DHW.YTD	16.91	1.56	< 2e-16 ***
DIN_median	-10.16	2.27	7.9e-06 ***
PIP_median	9.72	2.38	4.3e-05 ***
TN_median	-11.2	2.34	1.8e-06 ***

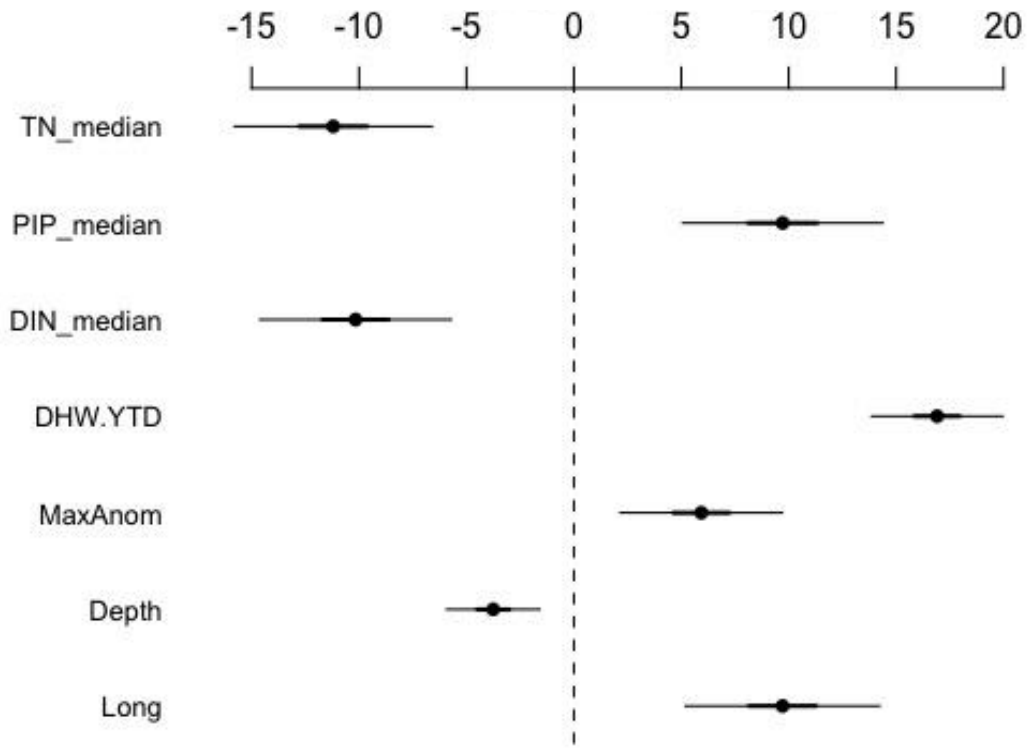


Figure 16: Coefficient plot for explanatory variables in the final hierarchical model for community coral bleaching (% hard and soft corals). The points are the coefficients with standard error (bold line) and 95% confidence intervals (thin line). Any value to the left of the zero (dashed) line represents a negative relation with bleached coral, and values on the right side of the dashed line are positive relations.

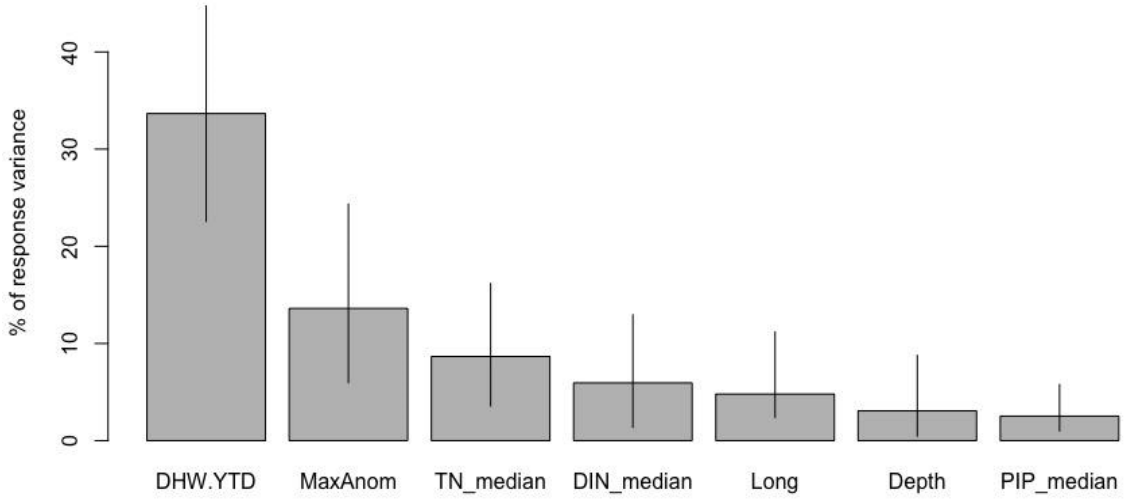


Figure 17: Relative importance estimates and 95% bootstrap confidence intervals (method LGM: R^2=72.3% partitioned for each predictor in the final hierarchical model for the proportion of the community bleached (% hard and soft corals) throughout the combined cross-shelf gradient.

4.3.2 Severely bleached and recently dead hard and soft corals - Combined cross shelf gradient (mid-shelf and inshore)

The mixed effect model investigating the effect of temperature, modelled water quality metrics and spatial variables on the proportion of the reef community that was severely bleached and recently dead (%) at the peak of the heat stress event (Bleached State 5+6) explained 63.9% of the variance in the response, with seven of the 18 explanatory variables tested being retained in the final model. Of these seven variables one was a temperature metric (DHW.YTD), four were modelled water quality metrics (median total nitrogen, DIN, mean concentration of Nitrogen in the labile benthic detritus, and median Chl *a*), and two were spatial metrics (longitude and latitude) (Table 3). Of the 5 random effects tested only 'reef' was retained as it was the only random effect that explained a significant amount of variance in the model; the influence of some water quality variables, such as nitrogen metrics, also varied between inshore and midshelf reefs, however this variability was better captured by 'reef' (Figure 18 and Figure 19).

The percentage of corals severely bleached and recently dead significantly increased with higher accumulation of heat stress (DHW.YTD), which explained most of the variance (36%) of severe bleaching and coral mortality. Median of total nitrogen was positively related to the proportion of the community severely bleached and recently dead and explained 5% of the variance, while median of dissolved inorganic nitrogen and the mean of labile detritus benthic nitrogen were both negatively related to percent of severely bleached and dead corals and explained 6% and 3% of the variance, respectively. Median chlorophyll *a* explained only 2% of the variance in severely bleached and dead corals but was positively and significantly linked. The percent of severely bleached and dead corals increased significantly with both latitude (8%) and longitude (4%) (Figure 18 and Figure 19).

Table 3: Summary statistics for final hierarchical model of percent of severely bleached and dead soft and hard corals in this study. This model used a negative binomial distribution to account for the low value inflation in the data, the corresponding parameter in the model was 5.7223 (se 1.45).

% Severely bleached and recently dead	Estimate	Std. Error	Pr(>|z|)
(Intercept)	2.602	0.103	< 2e-16 ***
Lat	1.366	0.4	0.00064 ***
Long	0.712	0.366	0.05186
DHW.YTD	0.567	0.084	1.4e-11 ***
DetBL_N_mean	-0.270	0.128	0.03562 *
DIN_median	-0.614	0.178	0.00057 ***
Chla_median	0.408	0.198	0.03922 *
TN_median	0.76	0.248	0.00216 **

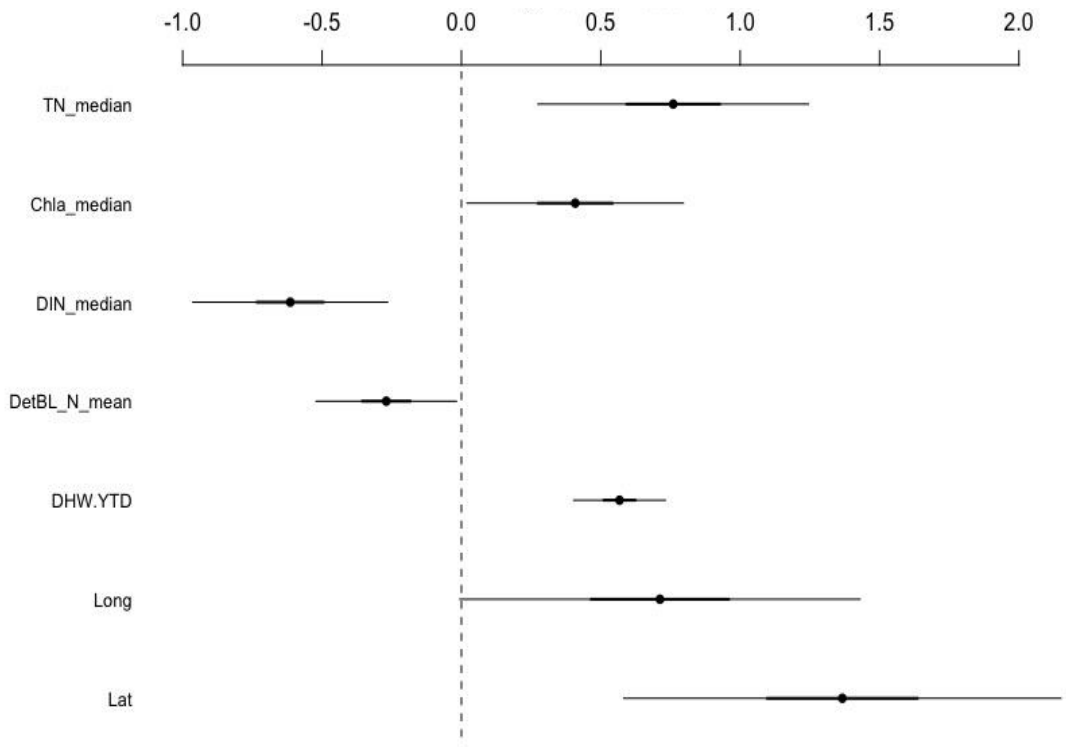


Figure 18: Coefficient plot for explanatory variables in the final hierarchical model for the severely bleached and recently dead (%) of hard and soft corals. The points are the regression coefficients with standard error (bold line) and 95% confidence intervals (thin line). Any value to the left of the zero (dashed) line represents a negative relationship with the response variable, and values on the right side of the dashed line indicate a positive relationship with the response variable. For instance, the percentage of severely bleached and dead corals decreases with DIN and increases with degree heating weeks (DHW.YTD).

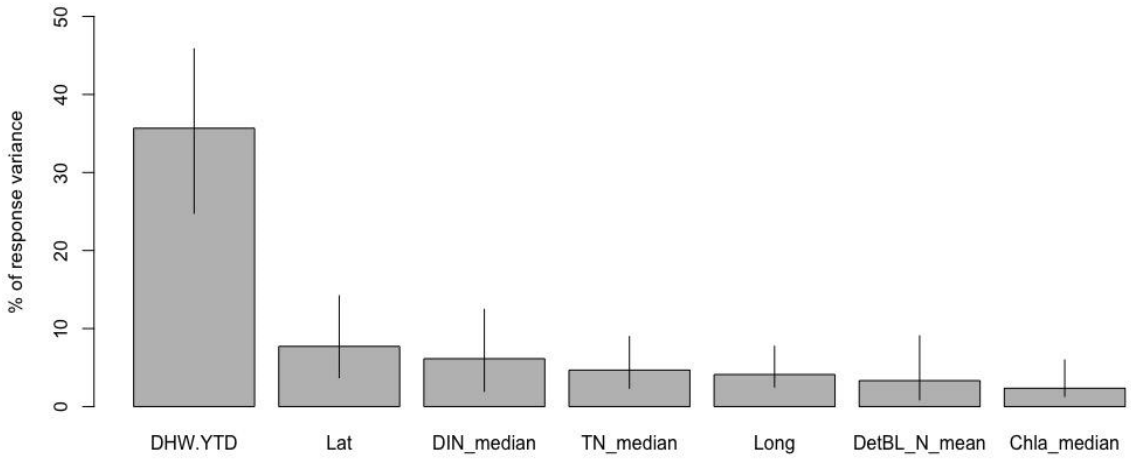


Figure 19: Relative importance estimates and 95% bootstrap confidence intervals (method LGM: R^2 = 63.99% partitioned by averaging over orders) for each predictor in the final hierarchical model for severely bleached and recently dead (% of both hard and soft corals). This model had reef as a random effect, the relative importance estimates are only for the fixed effects in the model and do not account for the variation in the importance of each predictor across reefs.

4.3.3 Coral community-level bleaching on inshore reefs

The final model that investigated the effect of temperature, eReefs modelled and *in situ* water quality metrics and spatial variables on the percent of bleached corals on inshore reefs explained 92.7% of the variance in the bleaching response, with nine of the 15 explanatory variables retained. Of these nine variables one was a temperature metric related to the accumulation of heat stress over time (DHW.YTD), three were modelled water quality metrics (median of total nitrogen, refractory detritus nitrogen and chlorophyll *a*), three were *in situ* water quality metrics (dissolved inorganic phosphorus, ammonium and nitrate) and two were spatial metrics (mean annual rainfall and depth; Table 4). Of the 5 random variables, none were retained (Figure 20 and Figure 21).

Coral bleaching significantly increased with temperature, with accumulated heat stress (DHW.YTD) explaining most (59%) of the variance. Coral bleaching significantly increased with more chlorophyll *a*, which explained 13% of the variance, but significantly decreased with rainfall, which explained 8% of the variance. These 3 predictors accounted for 80% of the variance in bleached corals of the inshore reefs, the remaining predictors in the final model only explained between 0-3% of the variance each (Figure 20 and Figure 21).

Table 4: Summary statistics for final model of percent of bleached corals (% of both hard and soft corals) on inshore reefs. DHW explains 59% in variance, Chla (13%), Rainfall (8%), NO3 (3%), NH4 (3%), TN (2%), DetR_N (2%), depth (2%) and DIP (1%). R^2 = 92.7%.

% Bleached	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	40.757	2.473	16.479	5.11e-11 ***
Depth	-8.132	2.610	-3.116	0.007082 **
DHW.YTD	18.138	1.764	10.283	3.46e-08 ***
RAINFALL.Avg.Annual	-28.655	6.143	-4.664	0.000305 ***
DIP.MMP	-3.555	2.090	-1.701	0.109645
NH4.MMP	7.742	2.822	2.743	0.015093 *
NO3.MMP	-8.741	3.202	-2.730	0.015509 *
Chla_median	11.813	2.227	5.303	8.85e-05 ***
DetR_N_median	9.207	2.739	3.361	0.004284 **
TN_median	11.495	2.781	4.133	0.000885 ***

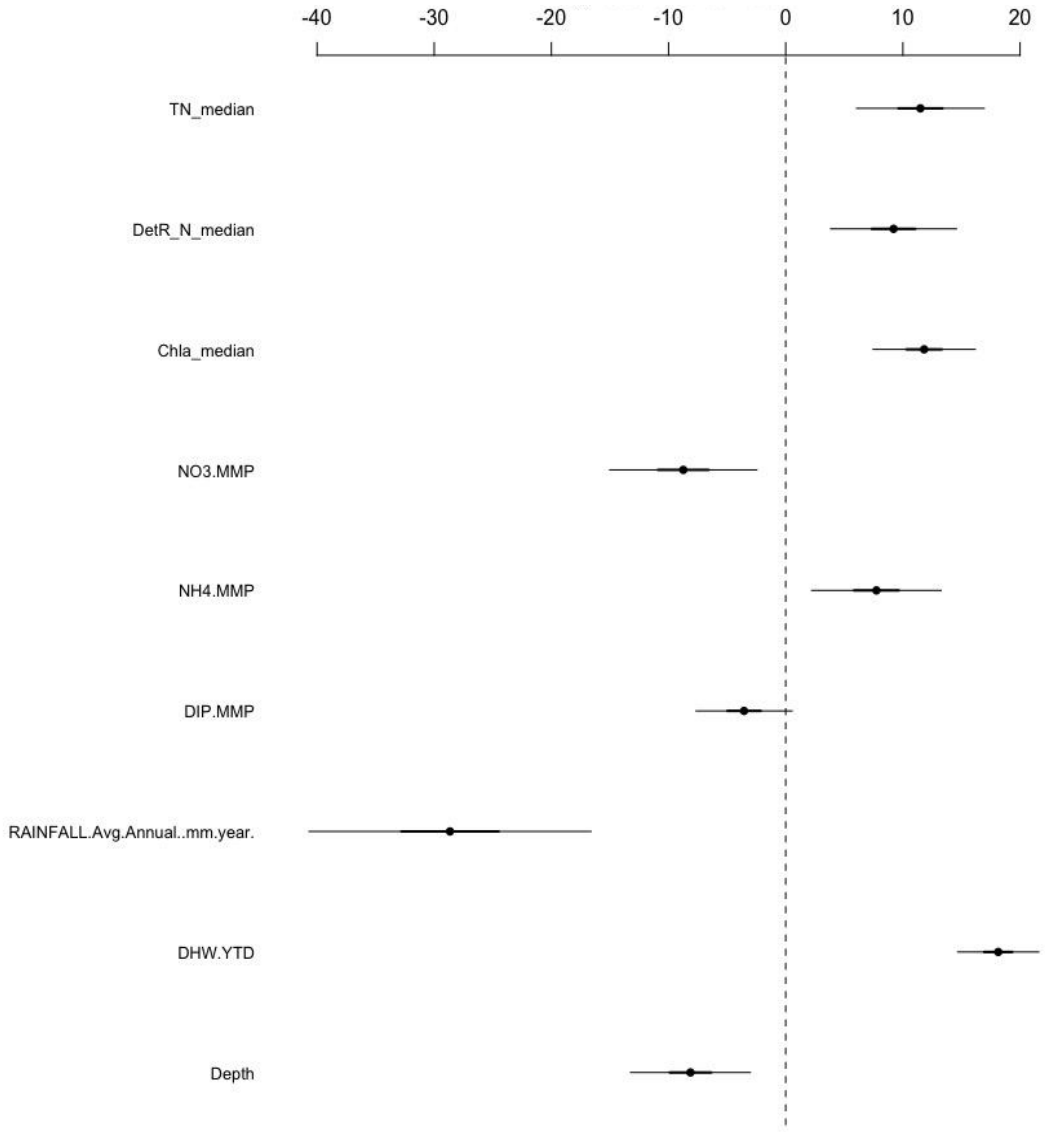


Figure 20: Coefficient plot for explanatory variables in the final linear model for percent of bleached corals (% of both hard and soft corals) on inshore reefs. The points are the regression coefficients with standard error (bold line) and 95% confidence intervals (thin line). Any value to the left of the zero (dashed) line represents a negative relation with bleached coral, and values on the right side of the dashed line are positive relations. For instance, the percent of bleached corals decreases with depth, and increases with accumulated temperature (DHW.YTD).

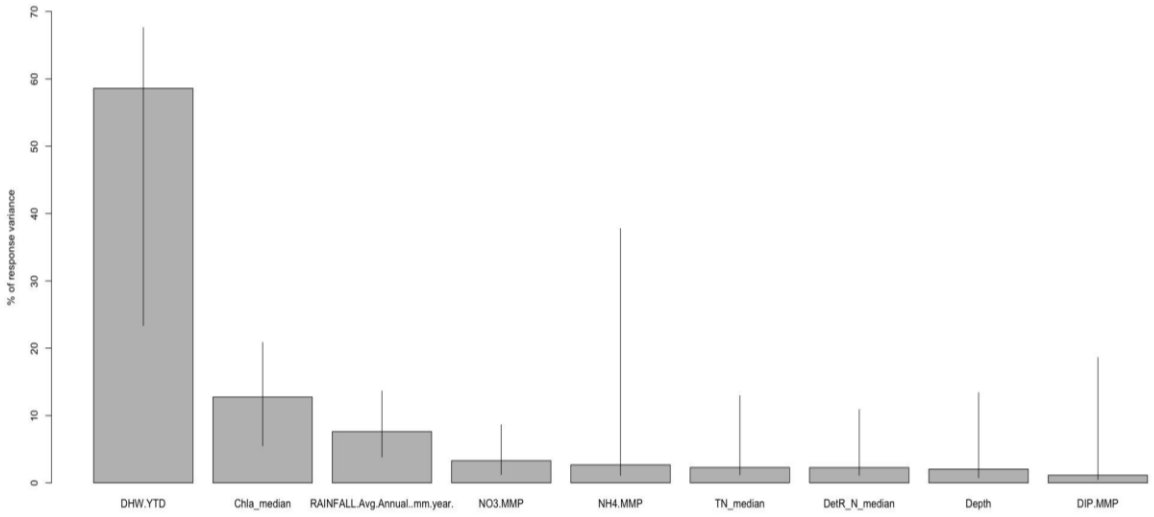


Figure 21: Relative importance estimates and 95% bootstrap confidence intervals (method LMG: R^2 = 92.7% partitioned by averaging over orders, like in Lindemann, Merenda and Gold (1980, p.119ff)) for each predictor in the final hierarchical model for percent of bleached corals (% of both hard and soft corals) on inshore reefs.

4.3.4 Severely bleached and recently dead hard and soft corals on inshore reefs

The mixed effect model that investigated the effect of temperature, modelled and *in situ* water quality variables and spatial variables on the percentage of bleached corals that were severely bleached and recently dead at the peak of the heat stress event (Bleached State 5+6) explained 90.38% of the variance in the response, with nine of the 15 explanatory variables tested being retained in the final model. Of these nine variables one was a temperature metric (DHW.YTD), three were modelled water quality metrics (median total nitrogen, labile detritus benthic nitrogen and median Chlorophyll *a*), three were *in situ* water quality metrics (mean total suspended solids, dissolved inorganic phosphorus and ammonium) and two were spatial metrics (mean annual rainfall and depth; Table 5). Of the 5 random effects tested only 'bleaching year' was retained. The influence of some water quality variables, such as nitrogen metrics, also varied between 'climate' and 'sector', however this variability was better captured by 'year' (Table 5).

The percentage of corals severely bleached and recently dead significantly increased with DHWs, which explained most of the variance (54%). The percent of severely bleached and dead corals also increased with the median chlorophyll *a* and with mean TSS, which explained 17% and 6% of the variance, respectively. Rainfall was negatively related to the percent of severely bleached and dead corals, explaining 7% of their variance. These 4 predictors accounted for 84% of the variance in severely bleached and dead corals of the inshore reefs, the remaining predictors in the final model only explained between 0-3% of the variance each (Figure 22 and Figure 23).

Table 5: Summary statistics for final model of percent of severely bleached and dead hard and soft corals in the inshore reefs only. DHW explains 54% in variance, Chla (17%), Rainfall (7%), TSS (6%), NH4 (3%), DIP (2%), DetR_N (1%), depth (1%) and TN (0.8%), amounting to R^2 = 90.38%.

% Severely bleached and recently dead	Estimate	Std. Error	Pr(>|z|)
(Intercept)	2.0369	0.1172	< 2e-16 ***
Depth	-0.1978	0.0979	0.0434 *
DHW.YTD	1.5534	0.1962	2.4e-15 ***
RAINFALL.Avg.Annual	-1.7323	0.3833	6.2e-06 ***
TSS.MMP	0.6261	0.1610	0.0001 ***
DIP.MMP	0.2434	0.0594	4.2e-05 ***
NH4.MMP	0.5424	0.1909	0.0045 **
Chla_median	0.9275	0.1482	3.8e-10 ***
DetR_N_median	0.6929	0.1530	5.9e-06 ***
TN_median	0.5312	0.1973	0.0071 **

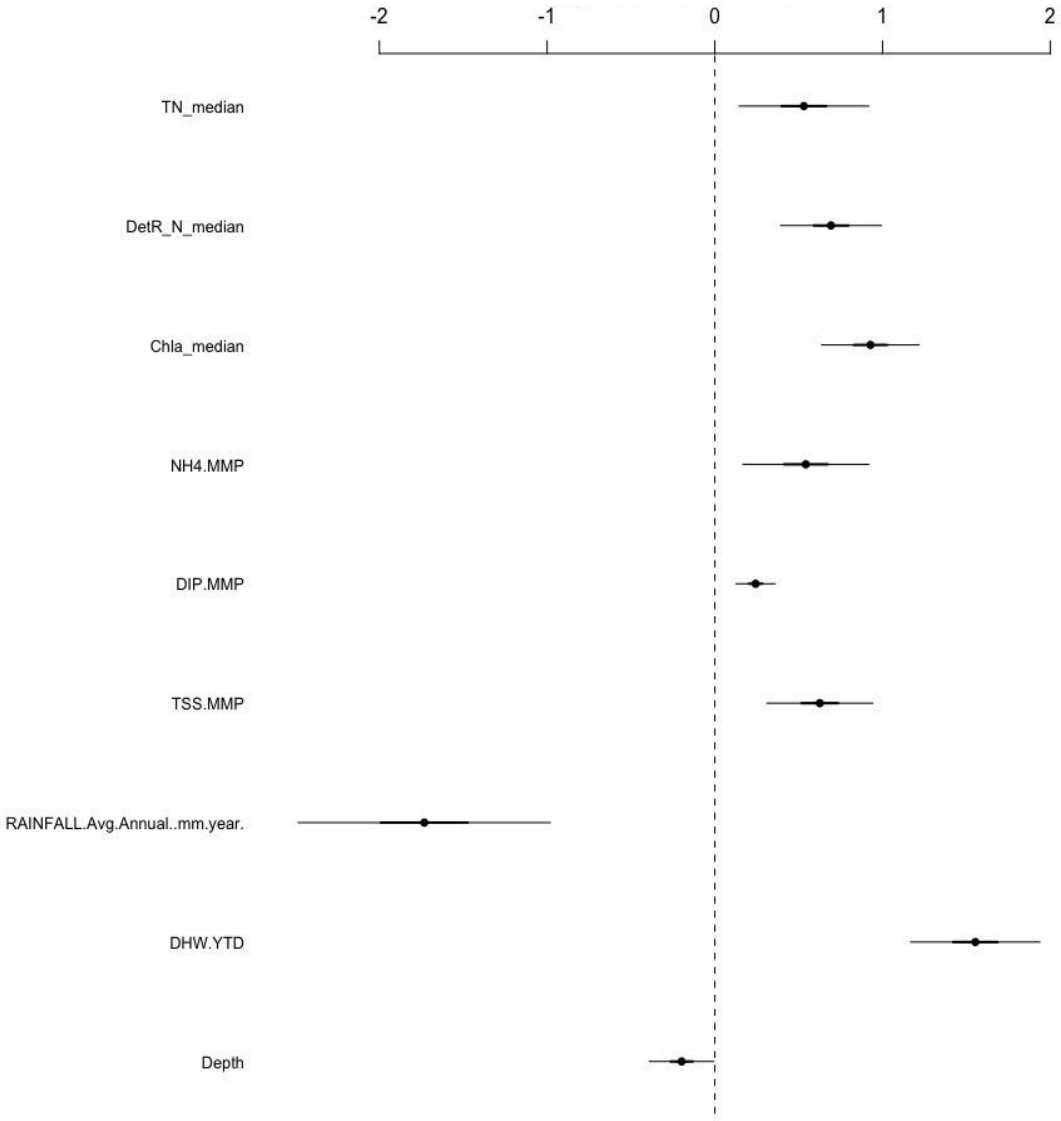


Figure 22: Coefficient plot for explanatory variables in the final hierarchical model for severely bleached and recently dead (% of both hard and soft corals) reef community on inshore reefs. The points are the coefficients with standard error (bold line) and 95% confidence intervals (thin line). Any value to the left of the zero (dashed) line represents a negative relation with bleached coral, and values on the right side of the dashed line are positive relations. For instance, the percent of severely bleached and dead corals decreased with depth and increased with accumulated temperature (DHW.YTD).

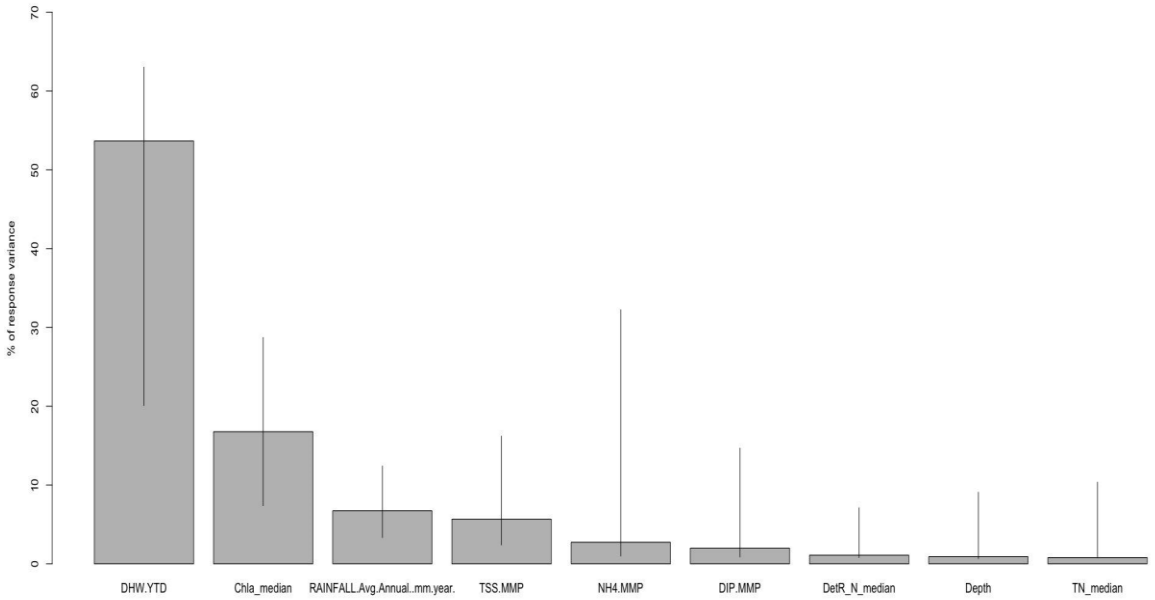


Figure 23: Relative importance estimates and 95% bootstrap confidence intervals (method LGM: R^2 = 90.38% partitioned by averaging over orders, like in Lindemann, Merenda and Gold (1980, p.119ff)) for each predictor in the final hierarchical model for severely bleached and recently dead (% hard and soft corals) reef community on inshore reefs. This model had bleaching year as a random effect, the relative importance estimates are only for the fixed effects in the model and do not account for the variation in the importance of each predictor across years.

4.4 Discussion

Severe heat stress was the dominant driver of coral bleaching in reef communities on both inshore and mid-shelf reef locations during the 2016 and 2017 mass bleaching events. The NOAA DHW at the time of the in-water surveys explained most of the variability in community level bleaching and the proportion of severely bleached and recently dead corals, with increasing mortality and risk of mortality at higher levels of heat stress, particularly when accumulated heat stress exceeds a DHW value of 5°C-weeks. Severe bleaching and mortality rates increased to 26-66% of the reef community in 2017, when heat stress exceeded 5°C-weeks.

Depending on rainfall patterns, nearshore reefs are regularly exposed to floodwaters with elevated concentrations of suspended material and dissolved nutrients from terrestrial sources, with the magnitude and influence of these pulse events varying spatially along the Queensland coast. The Wet Tropics regions (locations with >1,500mm rain / year) have less interannual variation in flow compared to the Dry Tropics regions, where interannual variation in annual rainfall and river flow into the GBR marine environment is high and depends on the intensity of the monsoon and the frequency of tropical cyclones (Burdekin River; Figure 15, Schroeder et al 2012). The 2016 and 2017 bleaching events followed a period of significant drought along the Dry Tropics region of Queensland, coinciding with low river flow from the Burdekin River from 2013-2018 and relatively lower flow from the rivers throughout the Wet Tropics (Figure 15).

In addition to heat stress, a suite of water quality parameters significantly contributed to exacerbate the prevalence and severity of coral bleaching and mortality. Total nitrogen (TN), Chl *a* concentration and particulate nitrogen sourced from the slowly broken-down labile

detritus (DETR_N) were positively correlated with bleaching severity in three of the 4 model analyses, particularly on inshore reefs. These water quality metrics are indicative of the transformation of terrestrial nutrient inputs by the pelagic plankton communities into organic matter, particulates, and detritus available to the benthic community.

Excess anthropogenic N was recently linked with lowering the temperature threshold for coral bleaching in Moorea, French Polynesia, but this additive effect of N enrichment diminishes at higher levels of accumulated heat stress (DHW>2.8; Donovan et al. 2020). Quantitative thresholds of nutrient enrichment within the GBR catchments have been proposed to link initial pulses of DIN to the subsequent chl a concentration as a useful indicator of degraded water quality on GBR reef from catchments inputs. Wooldridge (2009) proposed a chl *a* concentration >0.9 µg/L as an indicative degree of exposure to nutrient-enriched terrestrial inputs that correlates with localised reductions in hard coral species richness on the GBR and low macroalgal cover at reefs with chl a concentrations <0.45 µg/L (De'ath and Fabricius 2010). It was proposed that if end-of river DIN concentrations were reduced, cChl a concentrations would be lower and coral bleaching thresholds could be increased by 1.5-2.5°C (Wooldridge 2009). Maximum daily chl *a* concentrations in 2016 reached 0.75 µg/L, with median concentrations from Oct-Apr throughout the study region ranging from 0.05-0.2 µg/L in both 2016 and 2017, below guideline levels of 0.45 µg/L (De'ath and Fabricius 2010). Both eReefs modelled estimates and AIMS MMP in-water samples are indicative of drought conditions and low nutrient enrichment throughout the entire study region prior to the thermal stress events in 2016 and 2017 (Gruber et al. 2019), suggesting that nutrient enrichment prior to these mass bleaching events was low compared to guideline threshold targets.

There are strong spatial patterns in water clarity and cChl a concentration throughout the GBR. Water clarity decreases by more than threefold due to increased turbidity from suspended terrestrial solids from offshore to inshore waters, and cChl a concentrations decrease twofold from inshore to offshore and south to north gradients across the continental shelf of the GBR (De'ath and Fabricius 2010). The Wet Tropics region between Hinchinbrook and Cooktown contributes the highest annual DIN values and the Burdekin river region contributes the highest TSS surface load into the GBR (Gruber et al. 2018). Hard coral species richness and prevalence of phototrophic octocorals declines with increasing turbidity and chl a, whereas macroalgae and richness of heterotrophic species increase (De'ath and Fabricius 2010, Fabricius et al. 2012). Increased water clarity promotes the desired high richness of phototrophic hard coral and octocoral species in response to energy requirements from light driven photosynthetic metabolic demands, whereas heterotrophic species and macroalgae dominate light-limited reefs on the inshore reefs, with increased feeding capacity on available particulate organics (De'ath and Fabricius 2010). Differences among coral species in their bleaching susceptibility have been documented, with fast growing phototrophic dependent species (e.g. *Pocillopora, Seriatopora, Stylophora and Acropora spp.)* more severely affected by heat stress than slower growing species (e.g. *Galaxea*, *Goniopora* and *Porites*) that possess enhanced heterotrophic feeding capacity (Marshall and Baird 2000). The influence of water quality pressure on bleaching susceptibility of inshore reefs throughout the GBR has likely occurred over long-term chronic responses on decadal timescales, resulting in shifts in species composition, leading to inshore reefs with reduced species richness dominated by more thermally tolerant, slow growing heterotrophic species. In addition, as heat stress events increase in severity, the proportion of coral taxa within the reef community that can resist

bleaching decreases, with fewer coral species avoiding the impacts of warming oceans and coral bleaching (Hughes et al. 2017).

The overall conclusion from this analysis was that heat exposure was the main driver of coral bleaching in 2016 and 2017. Both the magnitude of temperature stress and the accumulation of prolonged heat stress above a normal historical summer maximum (DHW) are the major factors causing more severe coral bleaching and increased coral mortality throughout the GBR. Total Nitrogen enrichment, organic matter production and particulate inputs to inshore reefs provided minor contributions that increased the severity of the bleaching response. Continued efforts to meet the Reef 2050 Water Quality Improvement Plan targets for end of catchment loads of DIN, particulate organics and sediment should improve conditions that promote hard coral richness, reduce macroalgal cover following disturbance events and support healthy inshore reef communities.

5.0 BLEACHING PHYSIOLOGY OF *ACROPORA MILLEPORA* ACROSS THE GREAT BARRIER REEF

5.1 Introduction

The GBR coral bleaching events of 1998, 2002, 2016 and 2017 represent the biggest threat to the reef-building corals of the GBR (Hughes et al 2017). This bleaching has resulted from the rapid warming of coral reef waters which has occurred predominantly since 1980 (Lough et al 2018). However, the GBR has been subject to local anthropogenic stressors since the early 19th century, including 5.5 - to 8.9 fold increases in nutrient and sediment loading (Kroon et al., 2012) which has adversely affected inshore coral reef communities (Fabricius et al 2005; De'ath and Fabricius 2010). Laboratory experiments have implicated nutrient availability and metabolism as factors that modulate the bleaching and recovery responses of corals (Morris et al., 2019). Predictive models suggest that improvements to water quality may be able to help coral communities resist and recover from climate change on the GBR (Ortiz et al., 2018; Wolff et al., 2018), while others show that poor water quality initially protects inshore GBR corals from bleaching, before this effect is outweighed by reduced recovery (MacNeil et al., 2019). The evidence for interactions between water quality and bleaching may be limited to low-to-moderate heat stress events (Donovan et al., 2020) and remains controversial during severe bleaching events. However, direct physiological evidence of water quality and bleaching interactions in the field remain scarce, especially for the GBR.

We sampled a large number of Acropora *millepora* colonies during the 2017 bleaching event and again six months later to provide insights into physiological mechanisms of coral bleaching and recovery across water quality gradients. *A. millepora* is ubiquitous in the central GBR across wide latitudinal ranges and across the entire inshore and mid-shelf gradients of reef locations and therefore presents an ideal candidate for measuring spatial variability in coral bleaching physiology in relation to water quality. Our sampling region spanned almost 250 km of the central GBR and included inshore and mid-shelf reefs exposed to contrasting water quality regimes in the Dry Tropics and Wet Tropics catchments. We analysed the bleaching status of these corals by determining changes in the photosynthetic pigment concentration of the algal symbionts. We hypothesised that, if poor water quality mediates coral bleaching resilience, then inshore corals would experience greater bleaching and reduced recovery compared to those from mid-shelf environments. We found that inshore corals bleached more severely compared to mid-shelf corals. Interactions of nutrient enrichment across the inshore to mid-shelf water quality sampling gradientwere dampened by the severe levels of accumulated heat stress in 2017, making temperature the dominant factor causing the patterns in bleaching severity at both inshore and mid-shelf reef locations.

5.2 Methods

5.2.1 Coral collection

Acropora millepora samples from a total of 431 colonies were collected from 12 reefs in the central Great Barrier Reef during a coral bleaching event in March/April 2017 and six months later in September 2017 (Figure 24, Table 6). Six reefs were located inshore and impacted by riverine outflow. The other six were mid-shelf reefs and therefore exposed to clearer water and

only major flood events (Chapter 3). The latitudinal gradient between Townsville and Cairns was equally represented across both shelf positions, with paired inshore and mid-shelf reefs at each sampling latitude, including four reefs in the southern Dry Tropics region and eight reefs in the northern Wet Tropics region.

The *A. millepora* colonies were selected randomly for sampling under GBR Marine Park Permit G16/38488.1. Each sample was brought to the surface in individual zip-lock bags a maximum of 10 minutes after collection and immediately fixed in liquid nitrogen before long-term storage at -75 °C. Most reefs were sampled in March 2017 prior to the intensification of severe Tropical Cyclone (TC) Debbie. However, John Brewer Reef was sampled in April 2017 for safety reasons, after TC Debbie had crossed the GBR ~260 km to the south. The sampling was repeated in September 2017, but samples were lost from Feather Reef and thus excluded from the analysis.

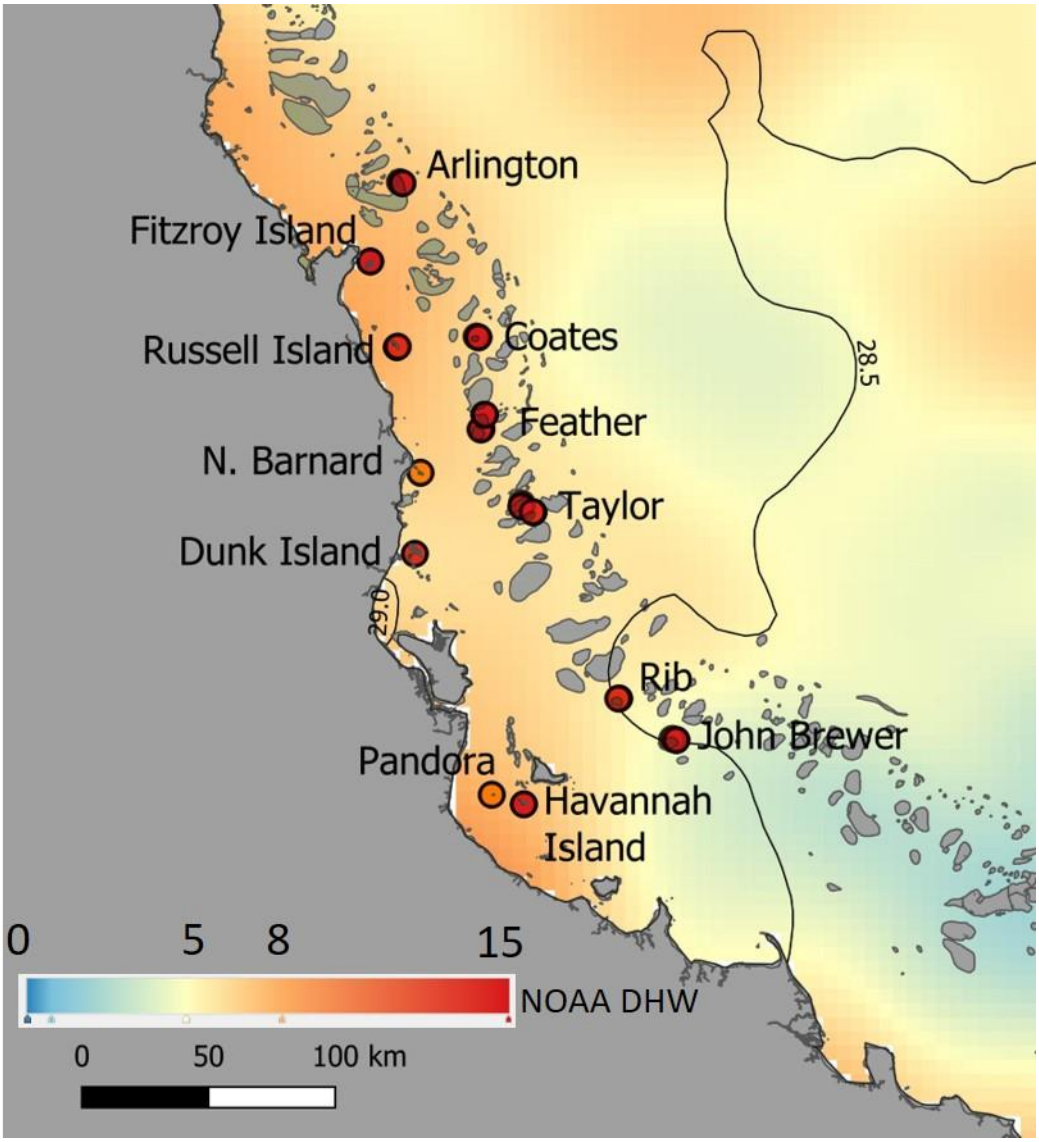


Figure 24: Map of reef sites visited for *A. millepora* collection and location within the Great Barrier Reef Marine Park during the 2017 summer bleaching event.

Table 6: *Acropora millepora* collection metadata.

Reef	Code	Latitude	Longitude	Acute Bleaching Dates	Recovery Dates
Arlington Reef	ARL	-16.7047	146.0473	24/03/2017	12/09/2017
Coates Reef	COA	-17.1888	146.3698	22 - 23/03/2017	11/09/2017
Dunk Island	DUN	-17.9558	146.1483	17/03/2017	09/09/2017
Feather Reef	FEA	-17.5286	146.3869	21/03/2017	N/A
Fitzroy Island	FIT	-16.9253	145.9891	24 - 25/03/2017	13/09/2017
Havannah Island	HAV	-18.8395	146.5345	14/03/2017	03/09/2017
John Brewer Reef	JBR	-18.6400	147.0415	01 - 02/04/2017	04/09/2017
North Barnard Islands	BAR	-17.7400	146.1562	20/03/2017	15/09/2017
Pandora Island	PAN	-18.8136	146.4324	15/03/2017	02/09/2017
Rib Reef	RIB	-18.4811	146.8713	16/03/2017	05/09/2017
Russell Island	RUS	-17.2236	146.0897	23/03/2017	14/09/2017
Taylor Reef	TAY	-17.8147	146.5678	18/03/2017	07/09/2017

5.2.2 Tissue blasting

Coral samples were removed from -75 °C storage and placed on ice under dim light. Coral holobiont tissues were removed from their skeletons with high-pressure air into approximately 10 ml of pre-chilled 0.04 µM ultra-filtered seawater (FSW). Tissues were then homogenised for 30 s using a post-mounted laboratory homogeniser (Bio-Gen PRO200, PRO Scientific, USA). A 1 ml aliquot of the coral homogenate was immediately taken, centrifuged (1,500 x g, 3 min, 4 °C), the supernatant discarded, and the resulting algal symbiont pellet stored at -75 °C for chlorophyll analysis. The remaining coral homogenate was centrifuged (1,500 x g, 3 min, 4 °C) and 500 µl of the supernatant aliquoted in triplicate into deep-well plates and stored at -75 °C for protein analysis.

5.2.3 Chlorophyll content

Algal symbiont samples were removed from -75 °C storage and placed on ice under dim light. The symbiont pellet was resuspended in 700 µl of pre-chilled 95% ethanol. The samples were then sonicated for 3 min in a custom-built ice bath attached to an ultrasonic generator (Sonic Power MU-600, Mirae Ultrasonic, South Korea), resuspended again and incubated on ice for 20 min. The samples were finally centrifuged (10,000 x g, 5 min, 4 °C) and 200 µl of the resulting supernatant was aliquoted in triplicate into clear 96-well plates. The absorbance was

read on a microplate reader (Synergy H4, BioTek Instruments, USA) at 665, 649 and 632 nm at 25 °C. The values were then corrected against 95% ethanol blanks and concentration of chlorophyll a (Chl *a*), chlorophyll c (Chl *c)* and total chlorophyll (Chl *t* = Chl *a* + *c*) were calculated using established equations (Ritchie, 2008).

5.2.4 Protein content

Deep-well plates containing coral host material were removed from -75 °C storage and defrosted overnight at 4 °C. NaOH was added to reach a final concentration of 0.5 M and the samples were resuspended. The plates were sonicated for 5 min in a custom-built water bath attached to an ultrasonic generator (Sonic Power MU-600, Mirae Ultrasonic, South Korea) and incubated in a laboratory oven (1 hr, 90 °C) prior to centrifugation (1,500 x g, 10 min, 25 °C). Total coral host protein was then measured using the DC Protein Assay (Bio-Rad Laboratories, USA) following the manufacturer's microplate protocol and measuring absorbance on a microplate reader (Synergy H4, BioTek Instruments, USA). Sample protein concentrations were calculated against a standard curve (50 – 750 $\mu g.ml^{-1}$) using Protein Standard II (Bio-Rad Laboratories, USA). Coral samples were repeated until < 10 % coefficient of variation was reached, and any samples that were more concentrated than the range of the standard curve were repeated after dilution. The measurements of coral host protein were used to normalise algal symbiont chlorophyll measurements as an approximate symbiont-to-host ratio or photosynthetic capacity of the symbiosis to diagnose coral bleaching (Cunning and Baker, 2014).

5.2.5 Additional metrics yet to be completed

Many analyses for this chapter are yet to be completed due to delays caused by COVID-19 and restricted access to AIMS laboratory facilities (Table 7). It is anticipated that collection of these data will resume in July 2020.

Prior to journal publication of this chapter we will include analyses of the symbiont densities and the surface area of all coral samples. The use of alternate metrics such as the absolute abundance of the algal symbionts, and potential for us to present coral host protein per surface area as a proxy for coral host biomass may provide us with additional insights regarding coral physiology across environmental gradients (Cunning and Baker, 2014). Furthermore, the final analysis will account for sample collection depth as a random factor, which may improve the ability of our models to explain any patterns due to shelf location.

We will also undertake a high-resolution analysis of variation in the algal symbionts hosted by our corals. We use amplicon sequencing of the ITS2 locus to provide us with detailed information on variation in identity and diversity of the algal symbiont communities and will underpin analyses of their roles in bleaching and recovery. A preliminary analysis using a lower resolution sequencing method has found a significant relationship between the bleaching status of the March coral samples and their algal symbiont complement, in which corals that hosted *Durusdinium* symbionts bleached less than those hosting *Cladocopium* (Fuller et al 2020). We will expand upon the scope of the preliminary analysis to investigate the impacts of shelf position (and thus water quality) on the algal community during acute bleaching and after a six-month period of recovery.

Table 7: Summary of additional metrics yet to be completed

Metric	Method	Importance
Algal symbiont density	Flow cytometry	Will disentangle symbiont population size from symbiont health, as Chl data is a function of both symbiont health and population size.
Surface area	Wax dipping	Will allow us to estimate coral host biomass (using protein as a proxy). We will also be able to disentangle symbiont density and Chl from host biomass.
Symbiont profiling	Illumina MiSeq	We will identify the algal symbiont diversity within corals and analyse their diversity in relation to cross-shelf bleaching and recovery patterns.

5.3.7 Statistical analysis

Data were analysed using linear mixed effects models in R (R Core Team, 2020) and the package "lme4" (Bates et al., 2015). Models were fit with total chlorophyll (Chl *t*), chlorophyll a (Chl *a*), chlorophyll c (Chl *c*) or chlorophyll a:c ratio (Chl *a*:*c* ratio) as the response variable, sampling period as the first fixed factor, either shelf position or catchment as the second fixed factor, and reef site as a random factor. Response data were first transformed based on the recommendations of the "bestNormalize" package (Peterson and Cavanaugh 2019): sqrt for Chl *t* and Chl *a*, arsinh for Chl *c* and orderNorm for Chl *a*:*c* ratio. For each combination of factors, both random intercepts and random slopes models were fitted, with the latter accounting for how the effect of sampling period may vary across reef sites. Model adequacy was tested by examining plots of residuals against fitted values for each of the fixed and random factors, and through a comprehensive model check in the "performance" package (Lüdecke et al. 2020). Model fit was also assessed in the performance package to select the best model for interpretation, which was the random slopes model in every case. Model effects were examined using the "effects" package (Fox 2019) and p values were generated using the "lmerTest" package (Kuznetsova et al. 2017). Post-hoc Tukey tests were conducted where interactive effects were detected using the "emmeans" package (Lenth 2020) and compact letter display generated using the "multcomp" package (Hothorn et al. 2008). Type III sum of squares and the Kenward-Roger method for calculating degrees of freedom were used throughout.

5.4 Results

The results of our field analysis of coral bleaching in *A. millepora* found that chlorophyll metrics were primarily affected by sampling time. Photosynthetic pigment concentrations were lowest during heat stress in March, indicative of coral bleaching, but had greatly increased in surviving corals by September. Shelf position had relatively minor but significant impact on Chl levels during coral bleaching and recovery, with inshore corals displaying the lowest pigment concentration during bleaching but greater pigmentation compared to the mid-shelf locations after recovery. In contrast, there were no significant impacts of different river catchments on coral bleaching and recovery.

5.4.1 Bleaching and recovery by shelf position

From the three chlorophyll forms measured (Chl *t*, Chl *a* and Chl *c*), a consistent trend was shown where bleaching was most severe on the inshore reefs, but after recovery this reversed and inshore corals had higher pigmentation. As such, coral pigmentation increased by 3.3 – 3.6 fold (depending on metric) on the inshore reefs between the bleaching and recovery periods compared to only 2.1 fold for the mid-shelf reefs (Figure 25), however, the differences between inshore and mid-shelf reefs were not statistically significant. For Chl *t*, a significant interaction was detected between shelf position and sampling period ($p = 0.027$), but Tukey tests revealed an insignificant difference between the inshore and mid-shelf reefs during bleaching ($p = 0.069$) and no significant difference during recovery ($p = 0.171$). For Chl *a*, there was also a significant interaction between shelf position and sampling period ($p = 0.014$). In this instance, inshore reefs had significantly lower Chl *a* levels compared to mid-shelf reefs during bleaching ($p = 0.039$), but not during recovery ($p = 0.200$). For Chl *c*, there was a significant effect of sampling period alone ($p < 0.001$) but a marginally insignificant interaction ($p = 0.055$) and no significant effect of shelf position ($p = 0.994$). Finally, Tukey's tests showed that Chl *t* and Chl *a* had increased after recovery, regardless of shelf position ($p < 0.001$).

Chl *a*:*c* ratio followed a similar pattern to the chlorophyll concentrations, increasing 1.4 fold on the inshore from bleaching to recovery but only 1.1 fold on mid-shelf reefs. There was no significant interaction between sampling period and shelf position ($p = 0.196$) or significant effect of shelf position ($p = 0.218$), but there was a significant effect of sampling period ($p = 0.003$).

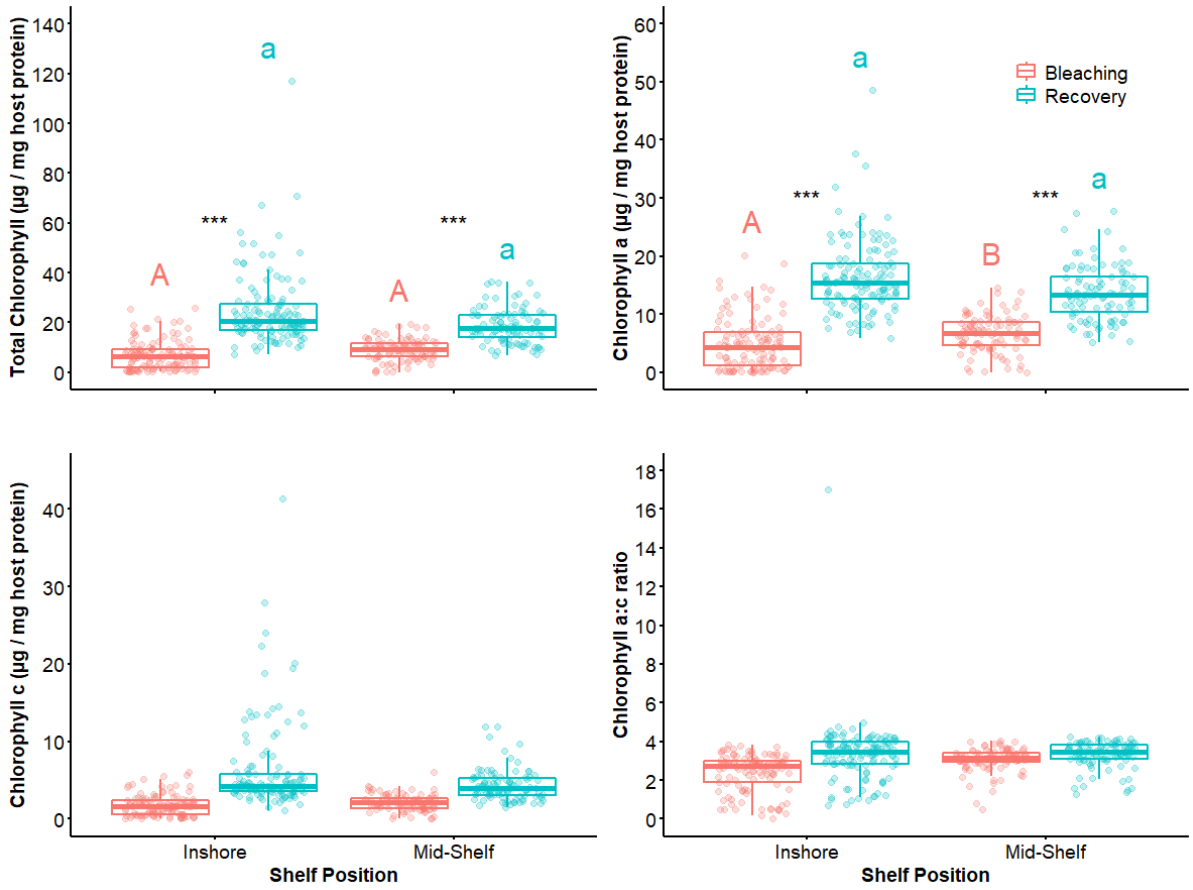


Figure 25: Bleaching and recovery of *A. millepora* according to shelf position as measured with Chl metrics. Box plots show the quartiles and range (excluding outliers) and dots show the raw the data points. Astersks denote Tukey test results showing statistically significant (p < 0.001) differences between bleaching and recovery, specific to each shelf location and letters denote statistically significant differences (p < 0.05) between inshore and mid-shelf reefs specific to the bleaching and recovery periods.

5.4.2 Bleaching and recovery by catchment

There was no evidence to suggest that the Dry Tropics and Wet Tropics catchments were differently impacted by coral bleaching in terms of the Chl metrics measured, or that they recovered differently (Figure 26). As a result, there were no significant effects of catchment nor any significant interactions between catchment and sampling period in any of the models. All Chl metrics increased between the bleaching and recovery sampling period ($p < 0.001$ for Chl *t*, *a* and *c*; $p = 0.007$ for Chl *a*:*c* ratio).

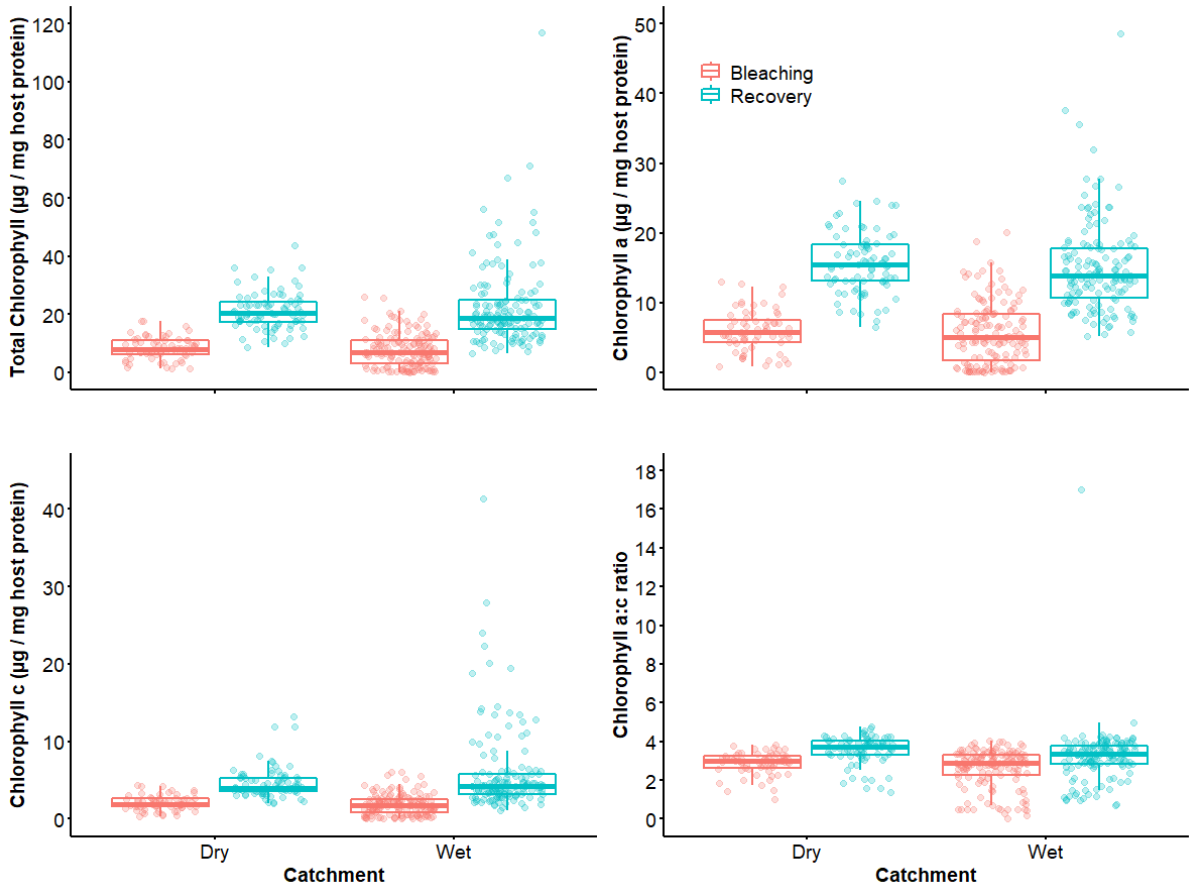


Figure 26: Bleaching and recovery of *A. millepora* according to water quality catchment as measured with Chl metrics. Box plots show the quartiles and range (excluding outliers) and dots show the raw data points.

5.5 Discussion

Our primary finding was that the sampling time was the strongest determinant of pigmentation in the algal symbionts of the coral *Acropora millepora* during and after the 2017 heat stress event at 12 reefs in the central GBR. That is, *A. millepora* colonies had much lower pigmentation during the peak of heat stress in March 2017 as opposed to six months later when surviving corals had increased photosynthetic pigment levels. This result was largely consistent regardless of shelf position or water catchment, implying that acute temperature stress was the dominant driver of bleaching in the corals examined. It has been proposed that degraded water quality conditions (nutrient enrichment) increases the risk of coral bleaching and mortality by lowering the heat tolerance of corals (Wooldridge, 2009), and later confirmed in aquarium experiments (Wiedenmann et al. 2013). Our field data agree with observations from the 2016 GBR bleaching event that seawater Chl *a* (as a proxy for water quality) did not significantly alter the relationship between seawater temperatures and the prevalence of coral bleaching (Hughes et al. 2017). A possible explanation for the disparity between laboratory results and field observations is that the high levels of heat stress across the central GBR in 2017 (Section 4) dampened any water quality influence on bleaching tolerance, as was the case for severely heat stressed corals in French Polynesia (Donovan et al 2020).

Although coral bleaching occurred across a gradient of water quality exposure, we found some evidence that inshore corals bleached more severely than mid-shelf corals. Although differences were largely insignificant and varied across Chl metrics, inshore corals tended to have the lowest pigment content in March, but had the highest pigment content in September. Inshore corals may be expected to have elevated symbiont populations under normal conditions due to an increased availability of nutrients and turbidity (Fabricius, 2005), which could increase their susceptibility to bleaching under heat stress (Cunning and Baker, 2014; but see Morris et al 2019). In contrast, the same inshore conditions could promote the recovery of algal symbiont populations following bleaching by shading the re-growing symbiont populations and providing corals with an alternative nutrient source (Grottoli et al 2006; Anthony 2007). These factors may explain the apparently elevated bleaching impact and subsequent elevated recovery of the inshore *A. millepora* colonies in March and September respectively. It is important to note that we have not included pre-bleaching samples to confirm baselines. It remains possible that the symbiont populations of corals sampled in September were yet to fully recover (Levas et al 2018) to reach new and stable densities (Cunning et al 2017). We did not detect an impact of catchment on coral bleaching and recovery despite differences in the primary water quality variables between the Wet and Dry Tropics catchments (Gruber et al. 2019).

6.0 EXPERIMENTAL ASSESSMENT OF THE ROLE OF NUTRIENT ENRICHMENT ON BLEACHING AND RECOVERY

6.1 Introduction

Inorganic nutrient availability can influence the physiological health of corals and their susceptibility to bleaching under heat stress (Wiedenmann et al 2013; Shantz and Burkepile 2014; Morris et al 2019). A diversity of (sometimes contradictory) nutrient effects on corals were observed in the late 20th century (Fabricius, 2005) and left some to conclude that the direct threat of nutrients to coral physiology could have been over estimated (Szmant, 2002). However, further developments in the 2010s clarified the equivocal responses shown in earlier studies, by linking specific nutrient forms and their ratios to coral health decline (D'Angelo and Wiedenmann, 2014; Shantz and Burkepile, 2014): An imbalanced diet of inorganic nutrients for the corals' algal symbionts was shown to be the Achilles heel of the coral holobiont (Ezzat et al 2016; Rosset et al 2017; Wiedenmann et al 2013), rather than their absolute levels. It is now clear that the response of corals to nutrient enrichment is more nuanced than originally observed and specifically dependent on the exact nature of available nutrients (Morris et al 2019).

The response of corals to inorganic nitrogen enrichment is a tale of two nutrient forms (Shantz and Burkepile, 2014). On one hand, ammonium (NH_4^+) as commonly supplied through the excretions of marine organisms (Allgeier et al 2017) can benefit the productivity of corals host and symbionts (Ezzat et al 2015; Shantz and Burkepile, 2014), and improve their heat tolerance (Ezzat et al 2019). On the other hand, nitrate (NO_3^-) often supplied from anthropogenic sources is less favoured by corals (Grover et al 2003), and can destabilise the coral-algal symbiosis (Ezzat et al 2015; Shantz and Burkepile 2014), especially under heat stress (Burkepile et al 2019; Wiedenmann et al 2013). The opposing impacts of these nitrogen sources likely stem from the requirement for nitrate to be reduced to ammonium prior to utilisation by the algal symbionts for growth, which incurs significant energetic costs (Dagenais-Bellefeuille and Morse, 2013). When nitrate triggers symbiont growth, the normal autotrophic release of organic carbon to the coral host may decline (Falkowski et al 1993), as the symbionts begin to retain carbon to meet their own metabolic needs (Baker et al 2018; Ezzat et al 2015). Nitrate therefore stimulates carbon limitation and destabilisation of the symbiosis rather than the vitality provided with ammonium (Ezzat et al 2015; Morris et al 2019; Wooldridge, 2009).

A compounding factor in the response of corals and inorganic nitrogen enrichment is the co-availability of inorganic phosphorus (Ezzat et al 2015; Morris et al 2019; Rosset et al 2017; Shantz and Burkepile 2014; Wiedenmann et al 2013). In addition to the energetic consequences detailed above, nitrate enrichment can drive the corals' algal symbionts into a state of phosphorus starvation (Wiedenmann et al 2013; Rosset et al 2017). Corals can bleach under conditions of high nitrate and low phosphate (Wiedenmann et al 2013) even without heat stress (Rosset et al 2017). A confirmed mechanistic explanation of this bleaching is through the substitution of unavailable phosphorus with sulphur in the symbiont's photosynthetic membranes (Wiedenmann et al 2013). Phosphorus is also vital for building fundamental biological molecules (Ferrier-Pagés et al 2016) including DNA and adenosine triphosphate (ATP) and promoting carbon translocation under heat stress (Ezzat et al 2016). Furthermore,

the addition of phosphorus to nitrate-enriched corals can stabilise the symbiosis (Rosset et al 2017; Shantz and Burkepile 2014), especially under stress (Wiedenmann et al 2013). Under this elevated but balanced nutrient condition it is likely that symbiosis will reach a new steady-state where symbiont density is high but carbon translocation is maintained (Krueger et al 2020; Morris et al 2019; Rosset et al 2017; Wiedenmann et al 2013), although some studies argue that growing and dense symbiont populations are more susceptible to bleaching (Cunning and Baker 2013, Cunning et al 2017; Wooldridge 2013, 2016). Regardless, phosphorus appears to be the vital nutrient for corals and their symbionts (Ezzat et al 2016; Ferrier-Pages et al 2016; Rosset et al 2017) and could be key to ensuring their survival under global warming.

Two relatively unexplored factors in coral ecophysiology are how corals adapt to water quality conditions and if inorganic nutrients impact the recovery of corals following bleaching. A study in the Florida Keys suggests that corals adjust through acclimatisation and adaptation to specific inshore temperature and water quality conditions and decline in health when moved (along- or off- shore) to sites with alternate environmental regimes (Kenkel et al 2015). In addition, nearshore Caribbean corals possess greater heat tolerance than their offshore counterparts (Kenkel et al 2013; Aichelman et al 2020). In other symbiotic reef organisms (foraminifera), some inshore populations are most tolerant of both nitrate and heat stress (Prazeres et al 2016, 2017). Some reef assemblages also appear to be pre-adapted to stress, with South Atlantic corals possessing greater tolerance to turbidity, nitrate and reduced bleaching-related mortality compared to those in the Indo-Pacific (Mies et al 2020). Altogether, it seems possible that inshore GBR corals may already possess greater resistance to poor water quality and high temperature scenarios and could perhaps even depend on an elevated availability of nutrients (Morris et al 2019). As for the influence of nutrients on bleaching recovery, the few studies conducted suggest that nitrate enrichment may trigger rapid growth of algal symbionts in bleached corals, and that this may prolong carbon limitation and increase the likelihood of coral mortality (Ezzat et al 2016; Cunning et al 2017, Burkepile et al 2019).

To examine the impacts of both chronic and acute water quality regimes on bleaching susceptibility and recovery we undertook laboratory-based experiments on corals collected from inshore and mid-shelf reefs with different environmental regimes (Chapter 3). We exposed the corals to nitrate and phosphate enrichment individually and in combination to test their impacts on corals before, during and following experimental heat stress. We used nutrient treatments that are environmentally relevant to flood-plume impacted areas of the GBR, but also manipulated nutrient ratios given prior evidence that these are fundamental to coral health. We tracked the response of corals to our experimental treatments through discrete measures of their photosynthetic pigments (Chl) concentrations, photosynthetic rates and continuous monitoring of photosynthetic efficiency (Fv/Fm).

6.2 Methods

6.2.1 Coral collection

Partial colonies of *Acropora millepora* were collected from 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) from 05 - 09 June 2018 and 23 - 24 June 2019 under permit (GBRMPA GB12/35236.1). These sites were located inshore

(FAL, HAV) and on the mid-shelf (HOP, JBR) in the Townsville sector of the central Great Barrier Reef. Corals collected in 2018 were used for a nutrient enrichment and bleaching severity experiment, and in 2019 the corals were used in a bleaching and recovery experiment (described below). At each reef individual colonies were collected (12 per reef in each experiment) from a depth of approximately 4 m. Colonies were assumed to be unique as they were collected > 5 m apart. Coral colonies were kept under shaded flow-through seawater conditions while in transit.

6.2.2 Coral acclimation

Coral colonies were transferred to outdoor aquarium facilities at the National Sea Simulator (SeaSim) of the Australian Institute of Marine Science on 10 June 2018 and 25 June 2019. At SeaSim they were kept under shaded conditions at the temperature of their collection sites (24.2 °C in 2018 and 23.25 °C in 2019). Each coral colony was cut into 16 (for the bleaching experiment) or 12 (for the recovery experiment) branch fragments (nubbins) of ~2 cm height using a diamond band saw and affixed to aragonite plugs using cyanoacrylate glue. In the bleaching and recovery experiment the temperature ramping was commenced whilst the corals were in outdoor tanks and gradually raised to 24.7 °C over one week. Later (three weeks after collection for the bleaching and recovery experiment and two weeks in nutrient enrichment and bleaching experiment), corals were transferred to an indoor experimental room containing 16 (for the bleaching experiment) or 12 (for the recovery experiment) 48 L tanks. In both experiments each tank was supplied with flow-through filtered seawater (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. Corals were initially supplied with 4.32 $mol.m^{-2}.d^{-1}$ of light (peak 150 $\mu mol.m^{-2}.s^{-1}$; Sol, Aqua Illumination, USA) which was doubled to 8.64 $mol.m^{-2}.d^{-1}$ (peak 300 $\mu mol.m^{-2}.s^{-1}$) over nine (for the bleaching experiment) or seven (for the recovery experiment) days. Temperature was initially maintained at 24.2 °C (bleaching experiment) or 24.7 °C (recovery experiment) and raised to 26 °C at a rate of +0.2 $°C.d^{-1}$. Corals were fed daily with freshly hatched *Artemia* at a concentration of ~0.5 $nauplii.ml^{-1}$. Corals were then acclimated to these temperature and light conditions for ~4 (bleaching experiment) or ~6 (recovery experiment) weeks prior to experimental treatments.

6.2.3 Nutrient treatments

Both experiments were initiated with exposure of corals to different nutrient conditions: nitrate (N) and phosphate (P) enrichment individually and in combination (NP), alongside a control condition without nutrient addition (C). Nutrient conditions were nominally increased by +2 µM nitrate (equivalent to 28 µg/L dissolved nitrogen) and +0.25 µM phosphate (7.7 µg/L dissolved phosphorus) using concentrated stock solutions of sodium nitrate (1 mM; Sigma-Aldrich, USA) and sodium phosphate monobasic (125 µM; Sigma-Aldrich, USA) dissolved in FSW and dosed continuously using a peristaltic pump (IPC 8, Ismatec, Germany). The levels of nutrient enrichment supplied were chosen based on those found in secondary water types of river plumes experienced across the whole GBR lagoon (Álvarez-Romero et al 2013; Gruber et al 2019) and are double the current water quality objectives targets for dissolved nitrogen and phosphorus at reef locations of high ecological value for both the Wet and Dry Tropics region of the GBR (State of Queensland 2020). Each nutrient condition was replicated across four (bleaching experiment) or three (recovery experiment) tanks. In the nutrient enrichment and bleaching experiment, each tank contained four randomly placed nubbins from 12 colonies, with each combination of genotypes replicated four times within the whole experiment but only

once per nutrient treatment. In the bleaching and recovery experiment, one nubbin from each of the 48 colonies was placed in each tank, meaning that each tank within the whole experiment contained identical genotypes. Nutrient conditions were monitored one to three times (bleaching experiment) or three times (recovery experiment) per week with triplicate water samples from each tank and filtering to 0.45 µM (Minisart NML, Sartorius, Germany). Ammonium, nitrite, nitrate and phosphate 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 of water and the contents were cleaned thoroughly two (bleaching experiment) or three (recovery experiment) times per week. Our nutrient dosing and cleaning regimes were effective at providing constant enrichment of the relevant inorganic nutrients in each treatment (excluding short periods of technical malfunction, most notably day 43 in the recovery experiment), despite natural variability in the nutrient content of the inshore FSW supplied to the experiment (Figures 27 and 28).

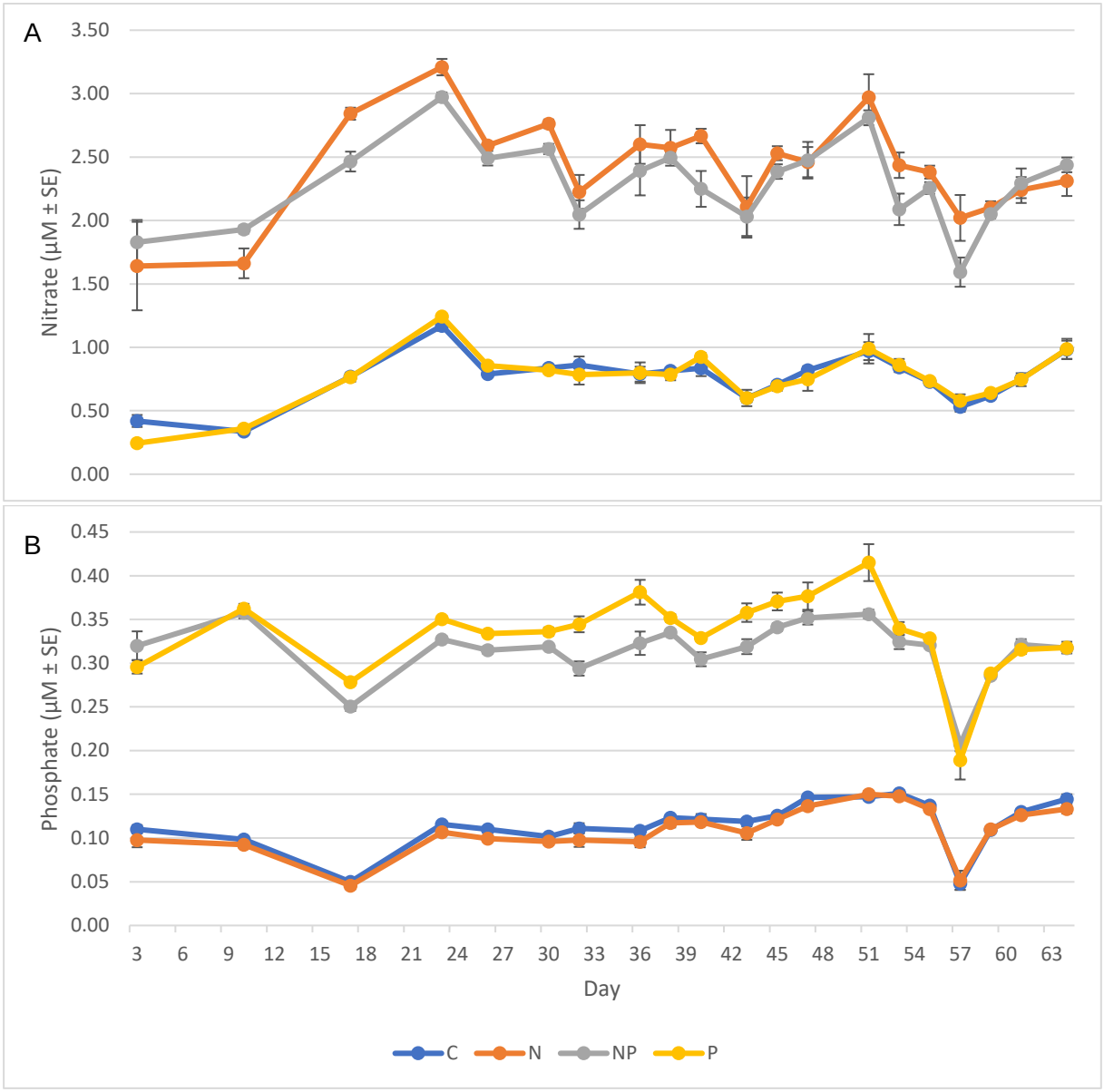


Figure 27: A) Mean nitrate and B) phosphate concentrations during the nutrient enrichment and bleaching severity experiment.

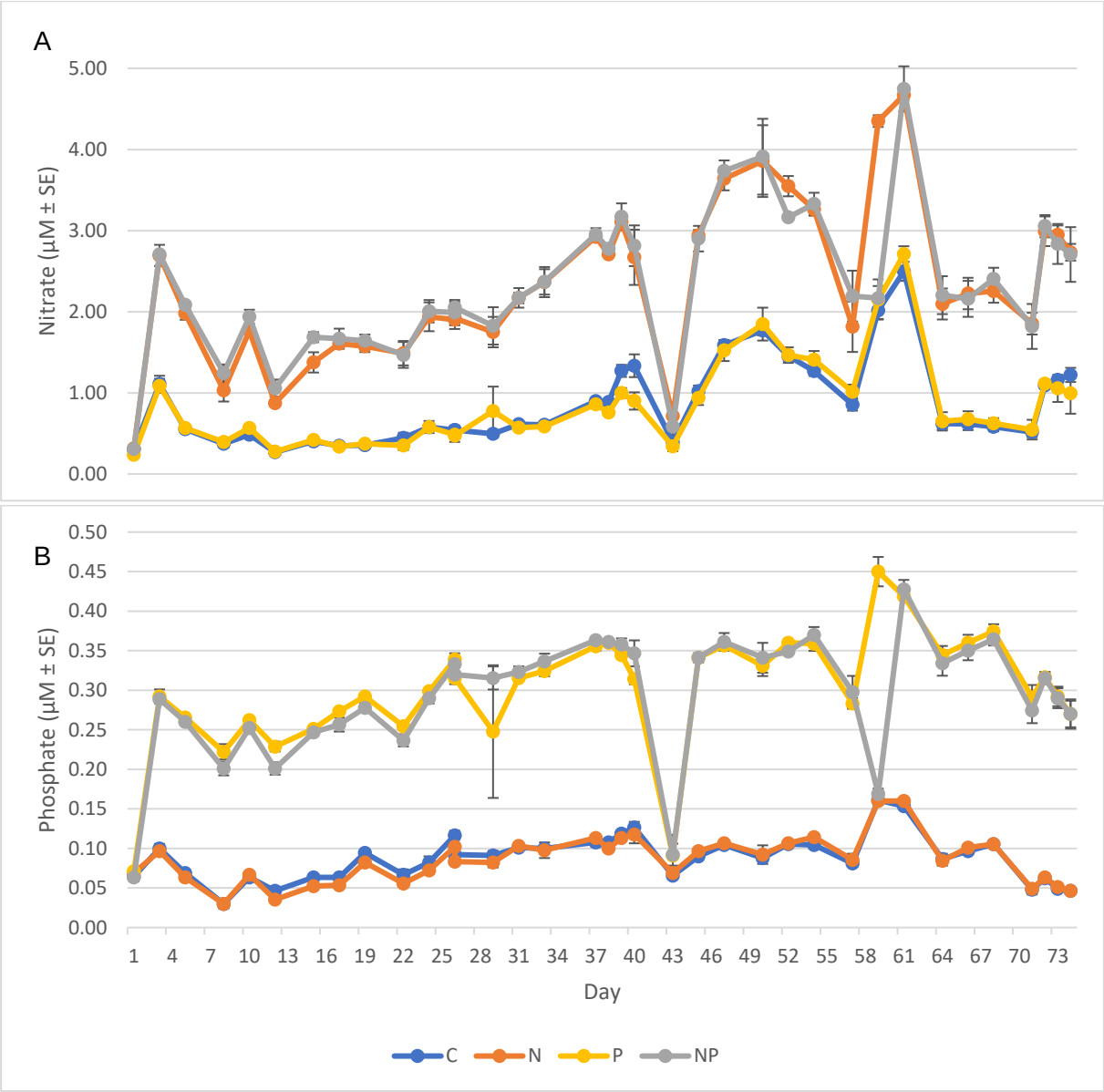


Figure 28: A) Mean nitrate and B) phosphate concentrations during the bleaching severity and recovery experiment.

6.2.4 Acute heat stress

On day 45 of nutrient treatment in the nutrient enrichment and bleaching experiment, the corals were additionally exposed to elevated temperature to elicit a bleaching response. The temperature in all tanks was raised by +1 °C.d^{-1} until 30 °C was reached (day 48), and thereafter the temperature was increased by +0.5 °C every week until a final temperature of 31.5 °C was reached (Figure 29; day 62). The temperature of each tank was measured weekly during the nutrient exposure and daily once temperatures were increased using a digital thermometer (376 Datalogger RTD Thermometer, CENTER Technology, Taiwan).

In the bleaching severity and recovery experiment heat stress was initiated simultaneously with the nutrient treatments. The temperature in all tanks was raised by +1 °C.d^{-1} until 30 °C was reached on day 4 and held for five days before being ramped down to 28 °C at the same rate starting day 9. On day 18 the temperature was then ramp was started back up again and

30 °C was reached again the following day. Thereafter, the temperature was increased by +0.5 °C every ~fifth day until a final temperature of 31.5 °C was reached on day 34. The maximum temperature was held for until day 39 when the temperature was reduced. A recovery temperature of 26.0 °C was reached on day 45 and kept constant until the end of the experiment on day 74 (Figure 30). The temperature in each tank was continuously monitored using in-water probes calibrated against a mercury thermometer.

By the end of the heat exposures the corals had experienced moderate accumulated heat stress, which was calculated from the historical summer maximum temperature (NOAA MMM upper thermal threshold) for each collection reef site, using the nearest pixel available from the 5km (0.05˚ resolution) satellite remote sensing data from the National Oceanographic and Atmospheric Administration (NOAA) Coral Reef Watch (CRW) 'CoralTemp' Version 3.1 (Liu et al. 2014). The experimental DHW accumulation was calculated following the methods described by NOAA Coral Reef Watch (Liu et al. 2006), using the experimental temperature profiles and the duration of heat stress that exceeds the source reef NOAA MMM value by at least 1˚C for the duration of time above this threshold over a rolling 12 week period during the experiment. In the bleaching experiment the inshore corals were exposed to the equivalent of 2.8°C-weeks, whereas the mid-shelf corals accumulated ~3.8 DHW. In the recovery experiment, the heat accumulation was ~3.1 DHW for inshore corals and ~4.5 DHW for the mid-shelf corals.. In comparison to the mass bleaching events on the GBR in 2016 and 2017, our inshore experimental corals experienced slightly less DHW than they had in 2016, and approximately half the heat accumulated in 2017 (Chapter 3). In contrast, the levels of heat stress in our mid-shelf corals were intermediate in magnitude between the 2016 and 2017 bleaching events (Chapter 3).

According to a meta-analysis of published heat stress experiments on corals (McLachlan et al 2020), the duration of heat stress applied here (six days of peak temperature) was classified as short-term corresponding to about half of previously published studies. However, corals accumulated heat stress (DHW) over 21 days in the bleaching experiment and 28 days in the recovery experiment, so our treatment conditions were similar to the 36% of experiments classified as moderate - term (8 to 30 days). In terms of the magnitude of heat stress, our peak experimental temperature increase of 5.5 °C was slightly higher than average, and within one standard deviation of the mean (McLachlan et al 2020). Our two experiments had different warming patterns in the temperature profiles applied, where DHW was accumulated consistently without interruption in the bleaching experiment (Figure 29) but allowed a brief respite from heat stress, prior to the main period of DHW accumulation in the recovery experiment (Figure 30). The shape of the temperature profile in the recovery experiment was most realistic in the context of central GBR (Ainsworth et al 2016).

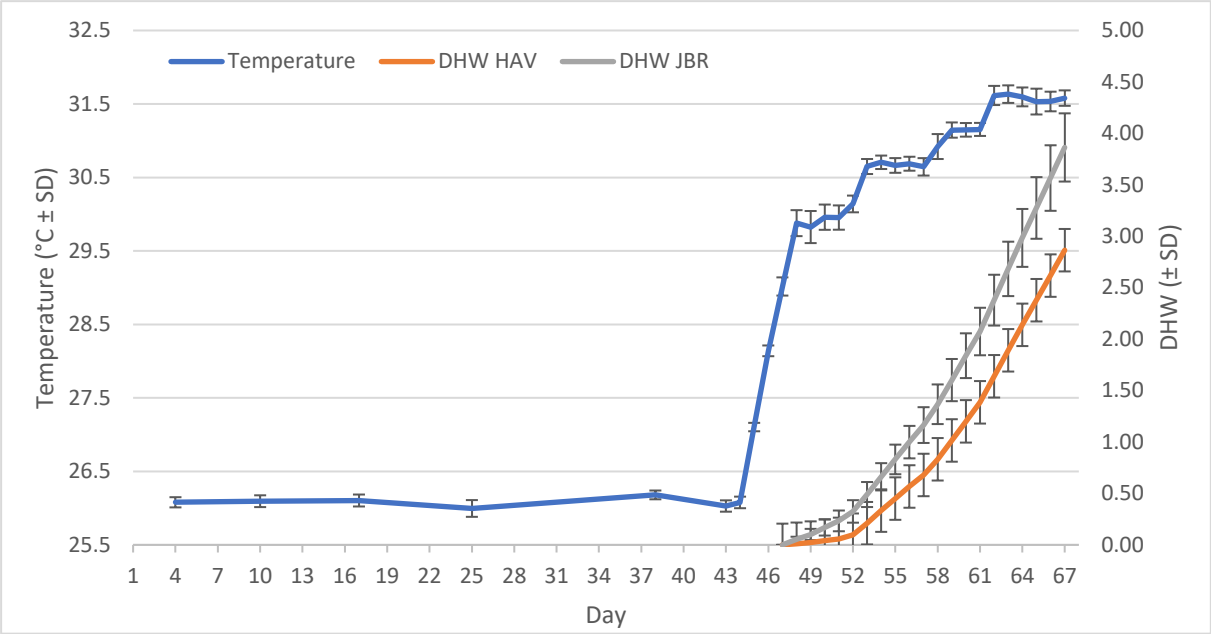


Figure 29: Mean measured temperature and accumulated heat stress (DHW) profiles during the nutrient enrichment and bleaching experiment. Temperature stress began after six-weeks of the nutrient enrichment which started on day 1. The inshore and mid-shelf corals were exposed to the same daily temperature conditions and the difference in DHW stress accumulation is a function of difference between long-term average temperature values at the different shelf locations.

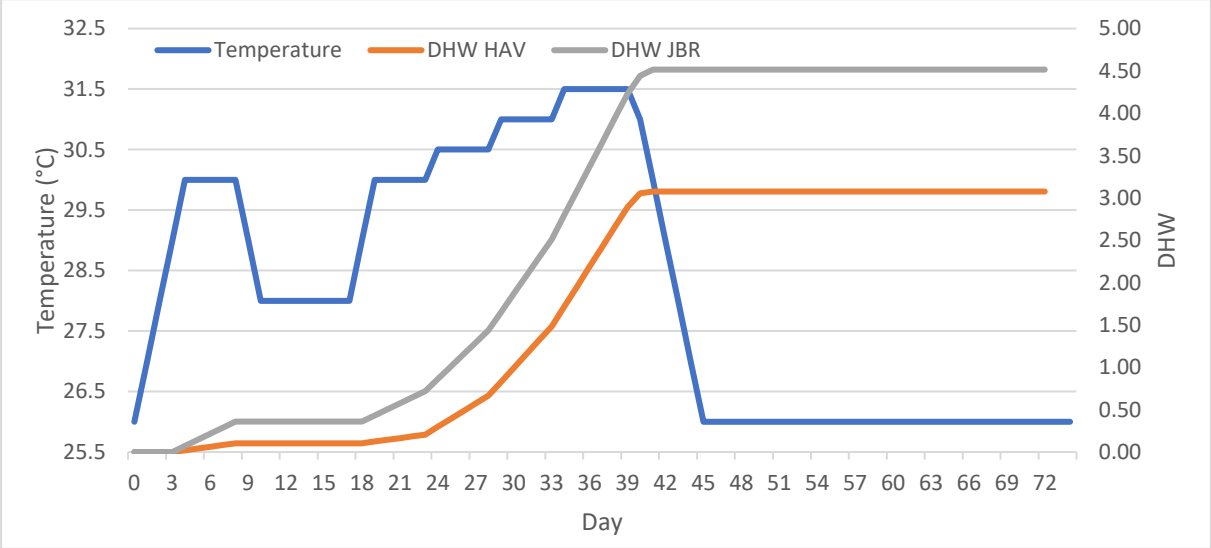


Figure 30: Set values for temperature and accumulated heat stress (DHW) profiles during the bleaching and recovery experiment. The inshore and mid-shelf corals were exposed to the same daily temperature conditions and the difference in DHW stress accumulation is a function of difference between long-term average temperature values at the different shelf locations.

6.2.5 Discrete sampling (nutrient enrichment and bleaching severity experiment)

In the nutrient enrichment and bleaching severity experiment, discrete sampling of corals for physiological analyses was undertaken twice: 1) after 1 month of nutrient treatment (day 31) and 2) after four days of exposure to 31.5 °C (day 66). For logistical reasons sampling occurred over 2 days. At each sampling point, sixteen unique colonies were sampled from each nutrient

treatment. The sixteen unique colonies chosen differed between sampling points but were kept the same across nutrient treatments at the same sampling point.

6.2.6 Coral respirometry

To measure light photosynthesis and dark respiration, corals were transferred to 600 ml transparent acrylic cylinders filled completely with treatment-specific seawater (TSW). Within each cylinder, a coral nubbin was mounted above a magnetic stir bar which was rotated when the cylinders were placed on tables containing a magnetic pulley system (Strahl et al., 2019). Each table was situated within an opaque flow-through water bath maintained at the corresponding experimental temperature. For photosynthesis, corals were exposed to the same peak lighting conditions of the experiment (300 µmol.m^{-2}.s^{-1}) for approximately 90 minutes. Following this, oxygen concentration was measured using a hand-held dissolved oxygen meter (HQ30D with Intellical LDO101 probe, Hach, USA). The cylinders were then resealed using new TSW and was repeated exactly in the dark (during the usual light period) to measure respiration. After respiration measurements were taken, corals were removed from the cylinders and immediately fixed in liquid nitrogen and stored at -75 °C. Net photosynthesis was calculated by subtracting the respiration rate from the gross photosynthesis rate for each coral.

6.2.7 Tissue blasting, chlorophyll and protein content

Tissue blasting, Chl and protein content were conducted as described in section 5.2.

6.2.8 Coral volume and surface area

The diameter of each coral branch was measured using digital calipers at multiple points along (e.g. top, middle and bottom) and across (e.g. on perpendicular faces) each branch. A single measurement of branch height was also taken. The volume of coral sample was calculated using the equation for the volume of a cylinder ($\pi r^2 h$), and the surface area was calculated using the equation for the lateral surface area of a cylinder ($2\pi rh$). Coral volume was used to correct for the volume of TSW in each acrylic cylinder and surface area was used to standardise coral health metrics.

6.2.9 Photosynthetic efficiency (both experiments)

Pulse-amplitude-modulation fluorometry (MINI-PAM, Walz, Germany) was repeatedly carried out on all corals, starting just prior to the temperature ramping, as a non-invasive measurement of coral bleaching (Warner et al., 1999). 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. During the nutrient enrichment and bleaching severity experiment, the effective quantum yield of PSII ($^{\Phi}$PSII) was measured ~daily in the middle of the light cycle whilst the maximum quantum yield of PSII (F_v/F_m) was measured ~every other night after at least one hour of darkness. During the bleaching severity and recovery experiment, measurements were taken ~every other day and the dark adaptation period reduced to 45 minutes.

6.2.10 Statistical analysis

Statistical analyses for the chlorophyll metrics (Chl *t*, Chl *a*, Chl c and Chl *a*:*c* ratio) were conducted in the same manner as Chapter 4 with minor modifications. Linear mixed effects were fit using each response variable, with nutrient treatment, shelf position and temperature as the fixed factors and tank as a random factor. For each combination of factors, both random intercepts and random slopes models were attempted, with the latter accounting for how the effect of temperature or shelf position may vary across tanks. For Chl c, Chl *a*:*c* ratio, gross photosynthesis per Chl *t* and respiration random intercepts models were selected, for Chl *t* and net photosynthesis shelf position random slopes models were selected and for Chl *a* and gross photosynthesis per protein temperature random slopes models were selected. Data were not transformed as they met model assumptions, except for gross photosynthesis per Chl *t* where log(x) transformation was performed. Type I sums of squares were used throughout due to the balanced design.

6.2.11 Additional metrics yet to be completed

Many analyses (Table 8) for this chapter are still to be completed due to delays caused by the continuing political unrest in Hong Kong (where our collaborator is based) followed by COVID-19, international travel restrictions and restricted access to AIMS laboratories.

Prior to journal publication of the nutrient enrichment and bleaching severity experiment we will additionally complete analyses of the algal symbiont density, symbiont composition, and calcification rates of our coral samples. The use of alternate metrics such as the absolute abundance of the algal symbionts, and potential for us to present coral host protein per surface area as a proxy for coral host biomass may provide us with additional insights (Cunning and Baker, 2014). The addition of coral calcification (as a proxy for growth) will provide us with additional information into how nutrients and temperature impact the coral host beyond bleaching. We will also conduct rigorous statistical analysis of our photosynthetic efficiency data to clarify our preliminary observations of possible nutrient impacts on coral bleaching.

We will also undertake a high-resolution genetic analysis of the community dynamics of algal symbionts hosted by our corals. The impacts of inorganic nutrient availability of the composition of a coral's algal symbiont have rarely been studied, despite their fundamental importance in mediating the stress responses of corals (Morris et al 2019; Suggett et al 2017). Amplicon sequencing of the ITS2 locus will provide us with detailed information on variation in identity and diversity of the algal symbiont communities in response to our experimental nutrient and temperature conditions.

For the recovery experiment, we will repeat most of the physiological analyses already shown for the nutrient enrichment and bleaching experiment. Additionally, we will measure inorganic carbon and nitrate assimilation by the algal symbionts of corals and their translocation as organic compounds to the coral host using stable isotope ratio mass spectrometry. We will also measure nitrate uptake from water samples taken from the stable isotope and respirometry incubations. The stable isotope analysis will be conducted in collaboration with A/Prof David Baker at the University of Hong Kong. David Baker is a leading expert in using stable isotope analysis to study the nutrient metabolism of corals and their algal symbionts (Baker et al 2013, 2018; McIlroy et al 2020). The combined physiological, molecular and biochemical approach to our recovery experiment will allow us to answer the outstanding

hypothesis laid out in our recent literature of the relationship between nutrient availability, nutrient metabolism and coral bleaching (Morris et al 2019).

Table 8: Summary of additional metrics yet to be completed

Metric	Experiment	Method	Importance
Algal symbiont density	Both	Flow cytometry	Will disentangle symbiont population size from symbiont health, as Chl data is a function of both symbiont health and population size
Symbiont profiling	Both	Illumina MiSeq	We will be able to identify the algal symbiont species associated with our corals and analyse their diversity in relation to cross-shelf bleaching and recovery patterns.
Surface area	Both	Wax dipping	Will allow us to estimate coral host biomass (using protein as a proxy). We will also be able to disentangle symbiont density and Chl from host biomass.
Calcification	Bleaching	Titration	We will be able to quantify how the skeletal growth rates of our corals responded to the experimental nutrient and heat stress conditions.
Chlorophyll	Recovery	Absorbance spectroscopy	Will enable us to quantify photosynthetic pigment responses of our corals to nutrient and heat stress as in our bleaching experiment.
Photosynthesis	Recovery	Respirometry	Will enable us to quantify the photosynthetic productivity response of our corals to nutrient and heat stress as in our bleaching experiment.
Respiration	Recovery	Respirometry	Will enable us (when combined with photosynthesis) to quantify the metabolic costs which our corals incur under nutrient and heat stress as in our bleaching experiment.
Protein	Recovery	Absorbance spectroscopy	Will allow us to standardise our coral traits to host biomass as in our bleaching experiment.

Stable isotope analysis	Recovery	Stable isotope ratio mass spectrometer	Will allow us to quantify the assimilation of inorganic carbon and nitrate by the algal symbionts and their translocation to the coral host.
Nitrate uptake	Recovery	Autoanalyser	An additional measure of nitrate assimilation by the algal symbionts.

6.3 Results

For each chlorophyll metric significant interactions between shelf position and temperature were found ($p < 0.001$) and investigated further using Tukey's tests (Figure 31). No significant effects of nutrient (Figure S1) or significant interactions involving nutrients were found for any Chl metric ($p > 0.05$). Tukey's tests revealed that Chl metrics significantly declined at 31.5 °C, regardless of shelf position ($p < 0.001$), and were also lower in mid-shelf corals when compared to inshore corals ($p < 0.001$). In contrast, at 26.0 °C, Chl *t*, Chl *a* and Chl *c* were slightly higher in the mid-shelf corals ($p = 0.003$, $p < 0.001$ and $p = 0.001$ respectively). Overall, the inshore corals lost less than 50% of their original pigmentation after heat stress, but the mid-shelf corals bleached more severely, losing around 75%.

Results from respirometry (Figure 32) agreed with the photosynthetic pigment content in showing that the impacts of heat stress were most profound in the mid-shelf corals. In all cases, nutrient availability (Figure S2) and its interactions with other factors produced non-significant results ($p > 0.05$). For gross photosynthesis normalised to Chl *t* (as a proxy for symbiont biomass), there were significant impacts of shelf position ($p = 0.003$) and temperature ($p < 0.001$) while the interaction between these two factors was marginally insignificant ($p = 0.051$). Photosynthesis per Chl *t* was highest in the mid-shelf corals, regardless of temperature, and increased at 31.5 °C, regardless of shelf position. However, this increase with temperature seemed higher in the mid-shelf than for the inshore corals (+272% as opposed to +121%). Similar trends were found regarding coral respiration normalised to protein (as a proxy for host biomass), with (slightly) higher respiration rates in mid-shelf corals ($p = 0.014$) and at 31.5 °C ($p > 0.001$) but no significant interaction ($p = 0.914$): respiration rates increased ~50% at 31.5 °C for both shelf locations. For both gross and net photosynthesis normalised to protein there were significant interactions between shelf position and temperature ($p > 0.001$). Tukey's test revealed that the mid-shelf corals had lower photosynthetic rates at 31.5 °C than the inshore corals ($p = 0.003$ and $p = 0.008$ for gross and net), whereas at 26.0 °C photosynthetic rates were highest in the mid-shelf corals ($p = 0.007$ and $p = 0.002$ for gross and net). The photosynthetic rates of inshore corals were maintained across temperatures ($p = 0.180$ and $p = 0.852$ respectively for gross and net photosynthesis), however for the mid-shelf corals photosynthesis significantly declined at 31.5 °C ($p < 0.001$), by 31% and 52% respectively for gross and net photosynthesis.

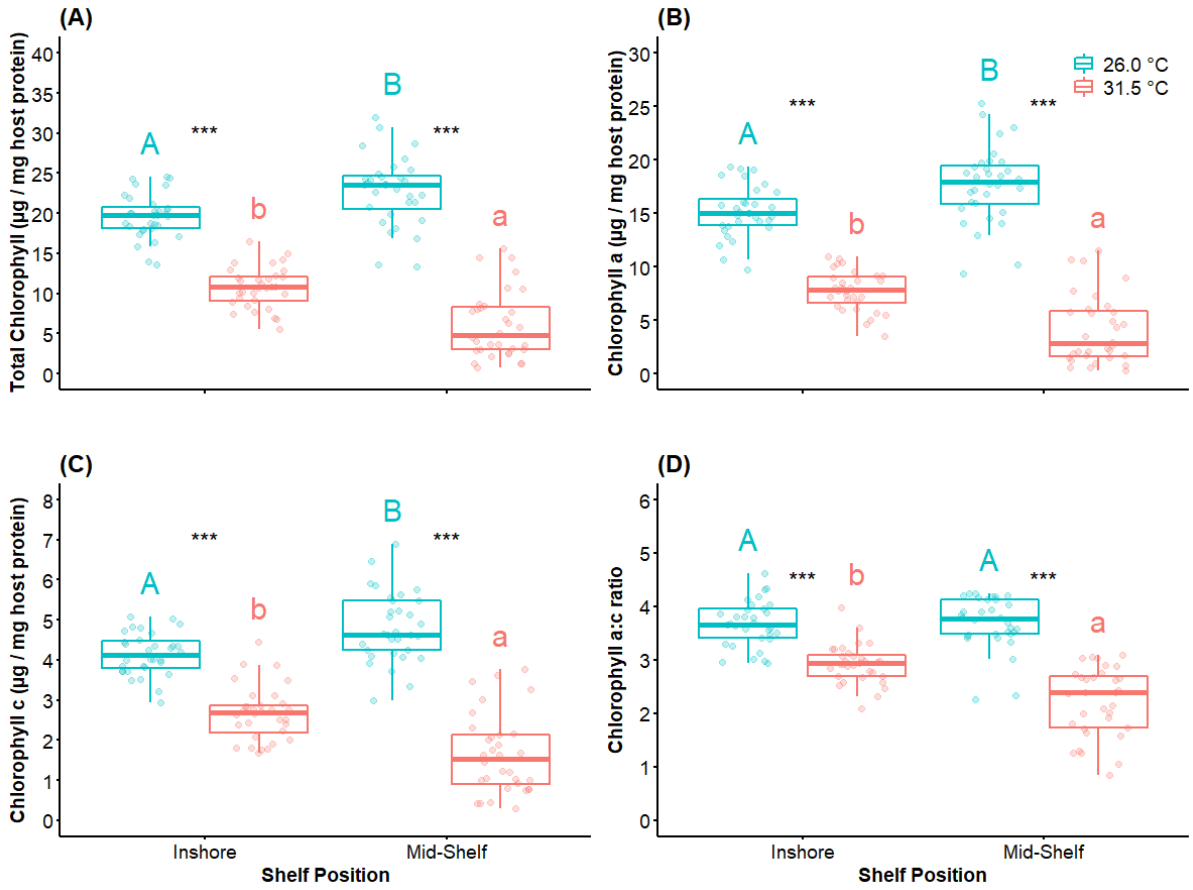


Figure 31: Chl metrics of A. *millepora* according to shelf position and temperature. Box plots show the quartiles and range (excluding outliers) and dots show raw the data points. Asterisks denote Tukey test results showing statistically significant ($p < 0.001$) differences between temperatures, specific to each shelf location and letters denote statistically significant differences ($p < 0.05$) between inshore and mid-shelf reefs specific to each temperature.

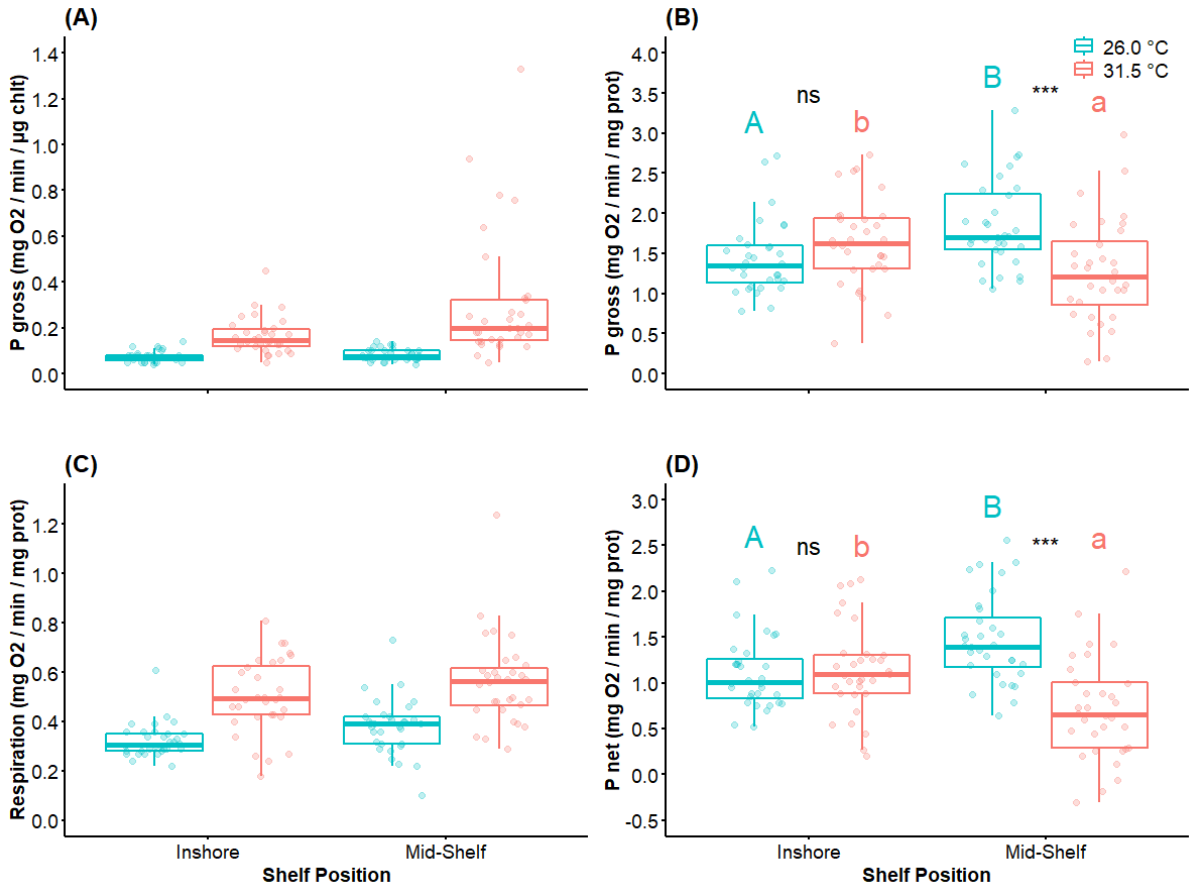


Figure 32: Photosynthesis and respiration of *A. millepora* according to shelf position and temperature. Box plots show the quartiles and range (excluding outliers) and dots show the raw data points. Asterisks denote Tukey test results showing statistically significant ($p < 0.001$) differences between temperatures, specific to each shelf location and letters denote statistically significant differences ($p < 0.05$) between inshore and mid-shelf reefs specific to each temperature.

Photosynthetic efficiency (Fv/Fm: $^{\Phi}$PSII and F_v/F_m) was measured throughout the heat stress exposure in both experiments and showed signs of photoinhibition in inshore and mid-shelf corals exposed to a maximum ~2.4 and ~3.3 DHW respectively in the nutrient enrichment and bleaching experiment (Figure 33), whereas in the bleaching severity and recovery experiment the maximum DHW was higher at ~3.1 and ~4.5 respectively and followed by a period of recovery at low temperature (Figure 34). In both experiments, the Fv/Fm data (Figures 33, 34) agreed with the findings that bleaching after exposure to 31.5 °C (in terms of chlorophyll loss) was much greater in mid-shelf corals and that their net photosynthesis declined. In both experiments, the light and dark Fv/Fm of inshore corals was slightly lower than mid-shelf corals during the initial stages of DHW accumulation, but once max temperature and DHW was reached Fv/Fm dropped sharply in mid-shelf corals whereas the response in inshore corals was comparatively gradual and smaller in magnitude. However, when Fv/Fm was considered in terms of relative heat exposure (accounting for differences in mean temperature across shelf positions), for equivalent DHW mid-shelf corals had higher Fv/Fm values than their inshore counterparts (Figures 33, 34). It is also notable that differences between $^{\Phi}$PSII and F_v/F_m values were most pronounced for the inshore corals. After heat stress abated in the recovery experiment, $^{\Phi}$PSII recovery appeared to be delayed in mid-shelf corals compared to inshore corals, but recovery of F_v/F_m was independent of shelf position (Figure 34).

Within each of the inshore and mid-shelf groups there was some variation between reefs: inshore coral from Falcon Island had consistently lower Fv/Fm values than those from Havannah (although the heat stress responses were identical), and in the recovery experiment declines to Fv/Fm due to heat stress were noticeably higher in Hopkinson Reef corals compared to John Brewer Reef (data not shown). Despite this, there were clear impacts of shelf position as described above.

There was also some variation in the magnitude of the Fv/Fm responses between the two experiments: overall Fv/Fm declines were greater in the bleaching severity and recovery experiment, when compared to the nutrient enrichment and bleaching experiment, due to greater maximum accumulated heat stress (Figures 33, 34). However, during the recovery experiment, declines in Fv/Fm values relative to DHW were lower than in the nutrient enrichment and bleaching experiment.

Despite the lack of significant nutrient impacts on coral pigmentation loss and photosynthetic rates, there was some evidence that nutrient availability affected the sensitivity of Fv/Fm to heat stress (Figures 33, 34). Prior to heat stress, Fv/Fm was identical under all nutrient conditions in both experiments. By the final Fv/Fm measurements for mid-shelf corals in the nutrient enrichment and bleaching severity experiment, it appeared that Fv/Fm was lowest in NP corals and highest in the P corals, whereas corals in the C and N treatments were intermediate (Figure 33). No such differentiation with nutrients was seen for inshore corals, which experienced a lower degree of heat stress. In the bleaching recovery experiment, the nutrient effects were different and more pronounced: Fv/Fm showed that corals in the C treatment were least impacted by heat stress, followed by N corals, and the P and NP groups were worst affected (Figure 34). Recovery rates were generally similar across all nutrient treatments, although there was an increased recovery of F_v/F_m in P corals in the latter stages of the experiment. Trends for inshore and mid-shelf corals were similar but the magnitude of differences was greater in the mid-shelf corals which were impacted by greater DHW.

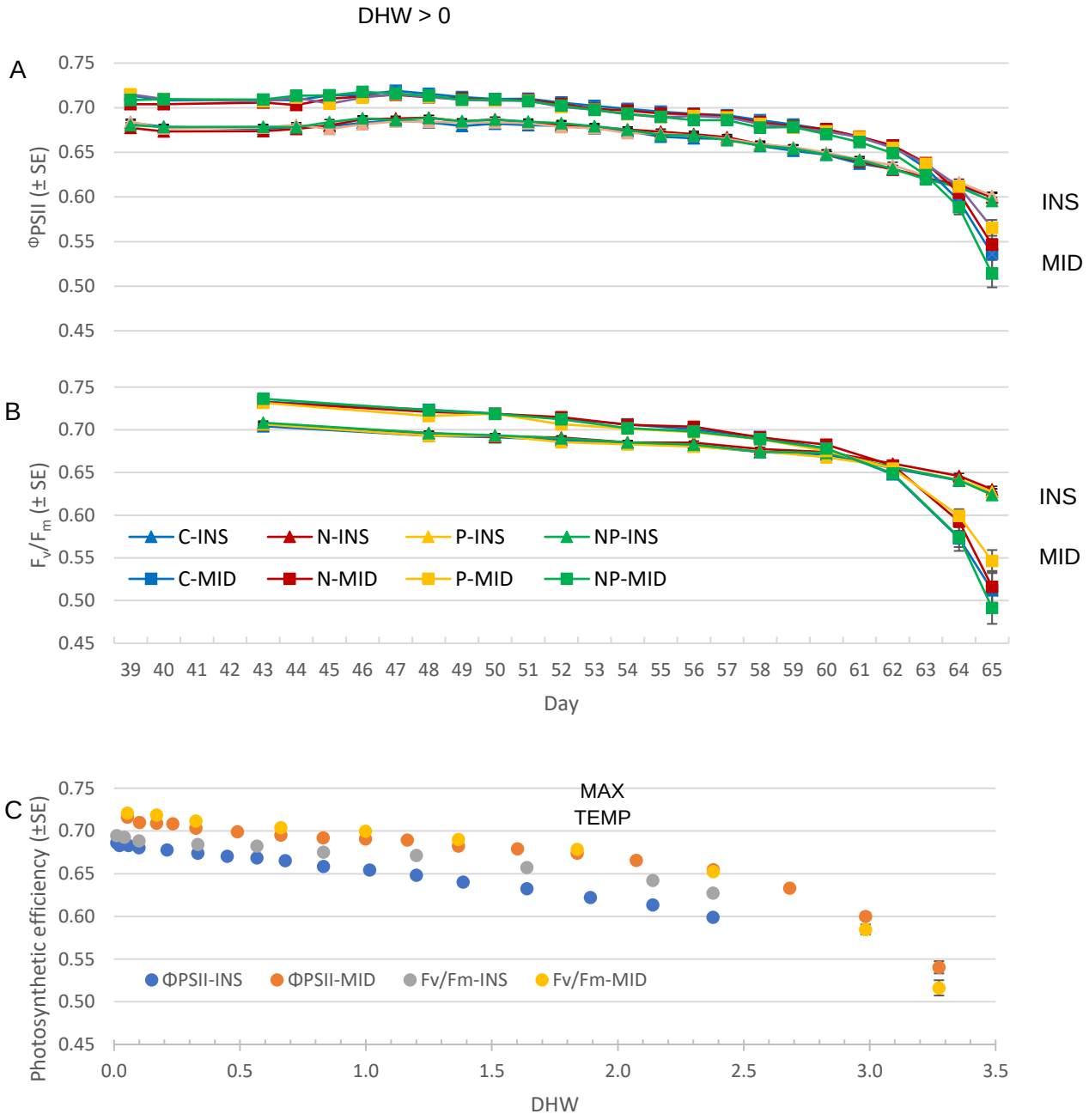


Figure 33: Light- (ΦPSII; panel (A)) and dark- (F_v/F_m; panel (B)) adapted photosynthetic efficiency of *A. millepora* symbionts during the nutrient enrichment and bleaching severity experiment grouped by nutrient treatment and source reef shelf position. Greater photoinhibition was observed for mid-shelf corals at the end of the experiment asDHW exposure reached a higher accumulated heat stress (panel C).

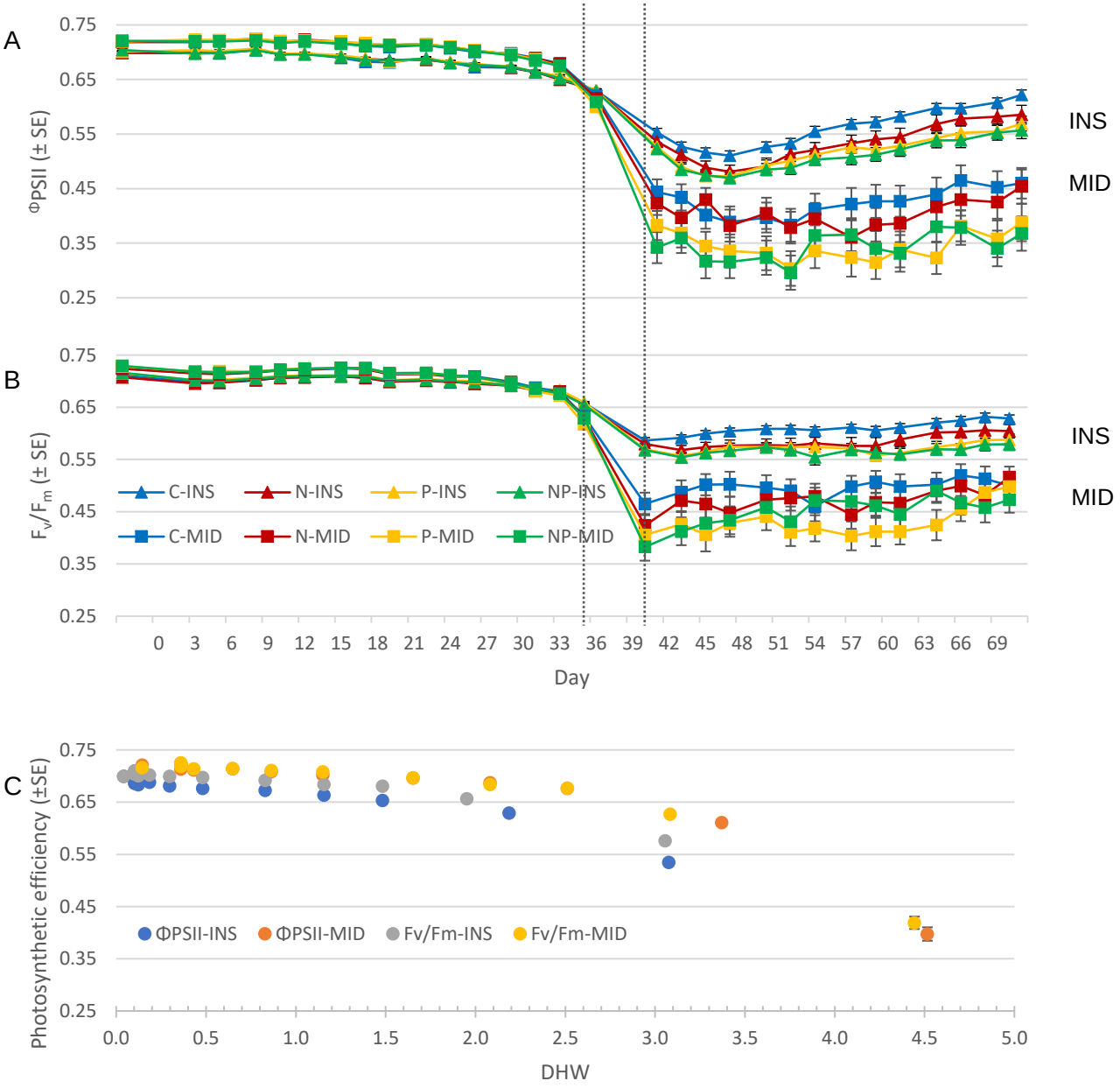


Figure 34: Light- ($^{\Phi}$PSII; panel (A)) and dark- (F_v/F_m; panel (B)) adapted photosynthetic efficiency of *A. millepora* symbionts during the bleaching severity and recovery experiment grouped by nutrient treatment and source reef shelf position. Greater photoinhibition was observed for mid-shelf corals after heat stress due to their greater DHW exposure and these differences were maintained during the recovery period.

6.4 Discussion

Our simulated heat stress events found that the bleaching and recovery of the coral *A. millepora* from the central GBR was primarily mediated by the environmental regimes of the reefs where corals were collected, rather than the exposure to inorganic nutrients (nitrate and phosphate) in our experiments. Corals from mid-shelf reefs exhibit higher pigmentation, photosynthetic rates and photosynthetic efficiency before heat stress than inshore corals but exhibited more severe bleaching under our experimental conditions due to their greater accumulated heat stress (DHW). In contrast, inshore corals suffered reduced losses of Chl and

Fv/Fm and were able to maintain their net photosynthetic rates despite due to their comparatively low DHW exposure. Despite this, inshore corals they had the lowest Fv/Fm values after normalising for differences in DHW. Contrary to expectations, we found that the immediate nutrient environment of the corals, where nitrate and phosphate concentrations and ratios were manipulated, had very little impact on the bleaching and recovery responses of *A. millepora*. Our results suggest that the short term inorganic nutrient environment does not materially affect bleaching in corals from the central GBR up to 4.5 DHWs. Instead, heat stress, influenced by the contrasting long-term environmental regimes of inshore and mid-shelf reefs is the main predictor of coral bleaching severity.

The impact of environmental history on the heat tolerance of corals has been demonstrated previously in Caribbean corals (Kenkel et al 2013; Aichelman et al 2020): In these instances, corals from inshore environments with greater levels and variation in heat and water quality stressors (Kenkel et al 2015; Kenkel and Matz, 2016; Baumann et al 2016) exhibited greater resistance to heat stress and bleaching than their offshore counterparts. Furthermore, it is now well established across a global scale that corals from high temperature environments with greater heat variability possess adaptations/acclimations that increase their bleaching thresholds (Kenkel and Matz, 2016; Safaie et al 2018; Howells et al 2011; Palumbi et al 2014); it is possible that similar mechanisms exist whereby long-term exposures to poor water quality conditions tune the metabolism of marine symbiotic organisms (including corals) to resist bleaching (Jin et al 2020; Kenkel et al 2015; Mies et al 2020; Morris et al 2019; Prazeres et al 2016, 2017). The inshore environments of the central GBR also expose corals to high levels of heat and water quality stresses (Chapter 3), which likely explains the increased heat tolerance of inshore *A. millepora* in our experiments. According to the commonly used DHW metric the mid-shelf corals experienced greater levels of accumulated heat stress in our experiments.

Although the inshore corals were clearly more tolerant of elevated temperatures, at equivalent DHW the mid-shelf corals had higher Fv/Fm values. It is possible that the lower values of Fv/Fm for inshore corals under equivalent DHW were a result of history of exposure to inshore environments. For example, the inshore corals may have contained a greater proportion of algal symbionts species with increased absolute heat tolerance compared to the mid-shelf corals (Cunning et al 2018). , Such symbiont species may potentially explain the lower Fv/Fm of inshore corals under ambient conditions (Cunning et al 2018) and equivalent DHW. Furthermore, there was a greater differential between $^{\Phi}$PSII and F_v/F_m measurements of inshore and mid-shelf corals taken at the same DHW. It is also possible that algal symbionts of corals from the inshore corals were relatively shade-adapted and therefore exhibited enhanced photoacclimation, whereas the mid-shelf corals were tolerant of light levels way above our experimental conditions (Hennige et al 2008; Tamir et al 2020). Additionally, we observed that both sets of corals exhibited lower declines in Fv/Fm under equivalent DHW in the bleaching recovery and experiment, compared to the nutrient enrichment and bleaching severity experiment. These differences increased with DHW exposure and were most pronounced in mid-shelf corals due to greater DHW exposure. Possible explanations for this include the nutrient pre-exposure of corals in the bleaching severity experiment, the relatively high rainfall conditions preceding coral collections for the recovery experiment (Bureau of Meteorology 2019) and the different heat stress trajectories used in each experiment (Ainsworth et al 2016).

Contrary to a number of aquarium and field experiments (reviewed by D'Angelo and Wiedenmann 2014, Morris et al 2019), inorganic nutrient availability had little effect on the health or heat tolerance of *A. millepora* in our experiments. Our experimental nutrient additions were designed to be ecologically relevant (Gruber et al 2019) and were at lower concentrations than many previous experiments (Shantz and Burkepile 2014). However, the range of our N:P manipulations were in line with previous studies demonstrating significant bleaching and other deleterious effects due to relative phosphate limitation (Rosset et al 2017). Regardless, there were some minor impacts of nutrients on Fv/Fm, particularly after the higher heat stress of our recovery experiment. Previous studies suggest that corals exposed to high and balanced levels of inorganic nitrogen and phosphorus have enlarged symbiont communities (Shantz and Burkepile, 2014) which could potentially increase their susceptibility to bleaching (Cunning and Baker, 2013). Furthermore, individual enrichments of nitrate and phosphate usually have opposing impacts with nitrate weakening the symbiosis (Ezzat et al 2015) and phosphate strengthening the symbiosis (Ezzat et al 2016), especially under heat stress (Wiedenmann et al 2013; Morris et al 2019). Based on values used in previous experiments (reviewed by Rosset et al 2017), we expected to see exacerbated bleaching under heat stress due to nutrient enrichment based on the magnitude of our experimental manipulations of nutrient concentrations and ratios.

Corals on the GBR have been chronically exposed to anthropogenically elevated nutrient loads since the 20th century (Lewis et al 2014) and may have developed a robustness to the direct impacts of inorganic nutrient enrichment on their physiology. Our findings are supported by a previous study on inshore central GBR *A. millepora* and *Montipora tuberculosa* showing no impact of nitrate enrichment on their heat tolerance (Fabricius et al 2013). Additionally, our Fv/Fm data show that the rainfall and flood plume events affecting our inshore sites prior to our recovery experiment (Figure 15, section X) did not modify the inshore and mid-shelf trends of coral bleaching that had been observed one year prior in the nutrient enrichment and bleaching experiment. In contrast, a recent study of *Stylophora pistillata* from the oligotrophic Red Sea found that their growth under heat stress was highly sensitive to nutrient enrichment (Hall et al 2018). Therefore, the heat tolerance of GBR corals appears to be unperturbed by changes to inorganic nutrient availability, but further study is warranted to confirm this and identify the potential mechanisms, particularly focused on the availability and assimilation of particulate nutrient sources. These may include minor adjustments to their photochemistry to suit nutrient conditions following bleaching, as we witnessed, but it remains possible that there are more subtle impacts like changes to the exchange of nutrients between corals and their symbionts (Baker et al 2018; Ezzat et al 2015; Morris et al 2019). Overall, reductions to anthropogenic nutrient enrichment of the GBR seems unlikely to improve coral heat tolerance at the levels of heat stress considered here.

Although low nutrient conditions did not improve the heat tolerance of our inshore corals, it did not decrease it either, in contrast to some experiments showing that the transfer of corals to a inorganic nitrogen and phosphorus depleted environment can exacerbate coral bleaching (Courtial et al 2018; Ezzat et al 2019). In some situations, the heat tolerance of corals is likely contingent on them remaining under the environmental conditions that they are adapted to, including salinity (D'Angelo et al 2015) and water quality (Kenkel et al 2015). In our control nutrient condition, inshore corals were not adversely impacted compared to our nutrient enriched treatments which may have better represented the conditions to which they are naturally adapted (Gruber et al. 2019). Therefore, our heat tolerant inshore corals may

represent good candidates for transplantation to improve the heat tolerance of mid-shelf coral reefs (Anthony et al 2017), although our control nutrient condition had nutrient levels similar to or higher than mid-shelf reefs (Furnas et al 2005) and other factors like turbidity need to be taken into account.

To conclude, we found that at low levels of heat stress inshore *A. millepora* from the central GBR possessed higher heat tolerance than their mid-shelf counterparts, potentially due to adaptation to higher summer maximum temperatures, and potentially due to exposure to poor water quality. Contrary to expectations, for the most part neither inshore nor mid-shelf corals were impacted by nitrate or phosphate availability. Therefore, reductions to inshore inorganic nutrient loading are unlikely to prevent coral bleaching on the GBR during ocean heatwaves, but at the same time inshore corals may represent prime candidates for transplantation to improve the heat tolerance of mid-shelf reefs.

7.0 CONCLUSIONS

This project supports the observation that temperature is the major driver of coral bleaching, however, our experiments identified variation in coral physiology in response to accumulated heat stress among shelf positions. The observed differences reflect long-term acclimation to a warmer upper thermal limit and different water quality regimes at the inshore reefs, but these differences were only evident at lower heat exposures, below DHW accumulation of 5°C-weeks. Bleaching prevalence, severity and mortality increased in the field as heat stress throughout the central GBR increased from moderate levels of DHWs in 2016 (1.5 – 5.3°C-weeks) to severe in 2017 (5.2 - 9.5°C-weeks). Bleaching severity was also more severe at the distant mid-shelf locations in both years compared to the inshore reefs, supporting the conclusion that during this event, heat stress was the dominant driver of bleaching severity, not water quality. If oceans continue to warm, corals will increasingly experience significant heat stress at an intensity that is currently masking acute water quality effects as examined here both experimentally and in the field.

The eReefs modelling framework was used to formulate targets for catchment loads of fine sediment and nutrients (Brodie et al., 2017). Here, the eReefs modelling framework was adapted to predict mass bleaching events on the GBR, taking into account a comprehensive set of processes spanning scales from the photochemistry and oxidative stress within individual corals to the GBR shelf, including spectrally resolved benthic light, the hydrodynamics of temperature, flood plume sediment transport and dissolved and particulate nutrient concentrations. It was used to determine the effects of anthropogenic nutrient and sediment loads entering the GBR lagoon on the likelihood of coral bleaching in 2017. The temporal dynamics of drought and flood drive the inputs of water quality parameters into the GBR, particularly in the Dry Tropics region. In general, low catchment flows and flood plume extents in the preceding years, from 2013-2017, led to low exposure of the measured reefs to anthropogenic sediment, nutrients and freshwater. In fact, over the last two decades, temperature stress leading to bleaching and high rainfall events have been mutually exclusive. Thus, the model simulations suggest that nutrient and sediment loads from human activities under chronic (non-pulse) conditions did not indicate transitions of nutrient limitation or significantly alter the predicted levels of ROS stress at the modelled reefs in 2017. A similar conclusion, based on field observations of the 2016 bleaching event, was reached by Hughes et al. (2017).

While accumulated heat was the main driver of bleaching severity across inshore and mid-shelf reefs in this study, a suite of water quality parameters also significantly contributed to exacerbate the prevalence and severity of coral bleaching and mortality. Nutrient inputs fluctuate on an annual, rather than decadal, basis, but sediment input varies over longer (probably ENSO-driven, with greater rainfall and transport during La Niña phases) time scales and is dependent on wind driven resuspension, the strength of the monsoon and frequency of tropical cyclones. Total nitrogen (TN), Chl *a* concentration and nitrogen sourced from the slowly broken-down labile detritus (DETR_N) were positively correlated with bleaching severity, particularly at the inshore reefs. These water quality metrics are indicative of the transformation of terrestrial nutrient inputs by the pelagic plankton communities into organic matter, particulates and detritus available to the benthic community. Quantitative thresholds of nutrient enrichment within the GBR catchments have been proposed to link initial pulses of

DIN to the subsequent Chl a concentration as a useful indicator of degraded water quality on GBR reef from catchments inputs. Observed Chl a concentrations during the 2016 and 2017 bleaching years throughout the Townsville to Cairns regions indicate low water quality impacts prior to these thermal stress events.

Analyses of bleaching physiology in the field and laboratory presented here corroborated that bleaching was mostly related to accumulated heat stress. There was some evidence that corals from inshore reefs bleached more severely with greater loss of photosynthetic pigments compared to mid-shelf corals, yet inshore survivors recovered to contain higher chlorophyll content than mid-shelf reef colonies in the months following peak heat stress. Inshore *A. millepora* from the central GBR showed higher thermal tolerance than their offshore counterparts within the aquarium experiments, indicative of long-term adaptation that hasresulted in a higher upper thermal limit (the inshore reefs used in this study historically have a 0.36°C higher annual summer maximum temperature). Contrary to expectations, the bleaching response from both inshore and mid-shelf corals was not enhanced by ecologically relevant dissolved nitrate or phosphate enrichment under experimental conditions. The results of this project showed that during severe heat stress events, like 2017, improvements to inshore water quality are unlikely to alleviate or mitigate the severity of coral bleaching events on the GBR as heat stress accumulation exceeds 5°C-weeks.

8.0 RECOMMENDATIONS

Coral bleaching is driven primarily by warming ocean temperature; however water quality issues are considered a key secondary factor influencing the composition, health and abundance of reef building coral species on the GBR. Direct relationships between water quality inputs and coral bleaching risk on the GBR become less apparent as heat stress increases in severity. However, inshore reefs impacted by water quality pressures have transitioned to a reef community with reduced ecological diversity. Continued focus to improve water quality by reducing terrestrial inputs to the marine environment will benefit the health of corals and coral reef communities on inshore reefs, since water clarity, chlorophyll and particulate nutrient concentrations below targeted guideline levels will promote hard coral diversity and lower macroalgal cover.

Key items resulting from this study for consideration to support focused efforts to deliver the Reef Plan 2050 Water Quality Management Plan are highlighted below. This study occurred during a prolonged drought period (2012-2018), as a result, water quality inputs to the GBR lagoon were low prior to the 2016 and 2017 bleaching events. Direct linkage between water quality pressure and coral bleaching risk was overwhelmed by severe heat stress.

eReefs model

1. The coupled hydrodynamic - biogeochemical eReefs model forced with realistic catchment load reductions showed that in 2017, when river flows were below average, the main driver of bleaching was thermal stress, and catchment inputs delivered during this low flow year had little impact on bleaching that year. Thus, improved catchment management to mitigate thermal bleaching will be most relevant during high flow years prior to an ocean heat wave.
2. In above average flow years, both modelling and previous observational studies have shown the effect of catchment loads persisting beyond the year of discharge. Furthermore, observed coral bleaching intensity in 2016 and 2017 was correlated to modelled water quality variables, especially particulate organic matter concentration overlying reefs, that are driven by river nutrient and sediment loads from preceding wet seasons. Thus, reduced catchment loads may lead to improve the health of corals leading up to a thermal stress event, which would likely mitigate the impacts of heat exposure at reefs exposed to moderate levels of heat stress (DHW < 5°C-weeks).

Water Quality effects:

3. Dissolved inorganic nutrients are rapidly converted to organic particulate sources in the water column, which are more likely to influence the nutrients assimilated by coral benthic communities. Field-based studies to determine how coral reef communities assimilate available particulate nutrients will assist in resolving the overall contribution of terrestrial nutrient inputs to the GBR lagoon and the response of inshore reef ecosystems.
4. There is limited evidence from the field-based observations of coral bleaching and mortality that the availability of nutrients to corals on the inshore reefs, following drought conditions from 2012-2017, remained at levels that could increase the adverse responses to thermal stress during the 2016 and 2017 bleaching events.

5. Different sediment and nutrient regimes within a nearshore reef or island group has, over time, established coral assemblages with less diversity, favouring coral species with enhanced heterotrophic capacity to cope with higher levels of sedimentation and low light conditions. Future research needs to take into account these compositional differences when predicting bleaching risk and include the influence of substrate composition on recruitment and recovery along water quality gradients and across different reef locations.

Management and adaptation

6. Our understanding of coral colony-level recovery will be enhanced if we track specific individual colonies through a bleaching event. Additionally, laboratory experiments need to test different species from across the bleaching and water quality sensitivity spectrum.
7. There is a need to determine genetic adaptation to water quality and possible implications for bleaching and recovery. For example, individual corals that are chronically exposed to poor water quality conditions on inshore reefs may possess genetic adaptations to stress including high temperatures.

Future outlook for coral reefs:

8. National and global greenhouse gas emission reduction strategies are required to rapidly reduce the global warming rate in the next 5-10 years in order to reduce the frequency and severity of ocean heat waves leading to widespread coral bleaching on the GBR.

Mid-shelf coral reef community at John Brewer Reef in April 2017 after the ocean heat wave was cooled by Cyclone Debbie, with high survivorship and some bleached corals remaining. Image: N. Cantin 2017

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APPENDIX 1

Table A1.1: Spatial, temporal and environmental water quality explanatory variables used in the hierarchical modelling analyses (Section 4.2.4). For all environmental variables from daily estimates we calculated an annual wet season (Oct-Apr) mean, median, maximum and total range (max - min) and seasonal change (max – mean; Delta) were calculated for each reef for each year (2016 and 2017).

Variable name	Symbol	Units	Description	Data Source
Spatial				
Latitude	Lat	degrees	Location longitude as a cross shelf position gradient	Garmin GPS
Longitude	Long	degrees	Location latitude as a climate sector position gradient (wet and dry tropics	Garmin GPS
Shelf Position	Shelf.x	Categorical	Inshore or Mid-shelf reef location	AIMS
Climate	Climate	Categorical	Reef within the Wet Tropics (>1500mm rain/year) or dry tropics (<1500mm rain/year) region of QLD	WMIP and BoM
Reef Sector	Sector.y	Categorical	Reefs within the Townsville, Tully, Innisfail and Cairns reef regions	AIMS
Habitat	Habitat	Categorical	Reef Flat (Sheltered 2-3m), Reef Crest (Exposed; 2-3m), Reef Slope (Exposed; 6-9m)	AIMS
Depth	Depth	(m)	Average depth of reef transect Lowest Astronomical Tide (LAT)	AIMS
Environmental				
Chlorophyll a	Chla.MMP	mg L^{-1}	In water sampled chlorphyll a concentration	AIMS MMP
Total Suspended Solids	TSS.MMP	mg L^{-1}	In water measured turbidity as total suspend solids concentration	AIMS MMP
Secchi Depth	Secchi.MMP	(m)	In water measure of water clarity	AIMS MMP
Dissolved Inorganic Phosphate	DIP.MMP	µmol	In water concentration DIP	AIMS MMP
Ammonium	NH4.MMP	µmol	In water concentration NH4	AIMS MMP
Nitrate	NO3.MMP	µmol	In water concentration NO3	AIMS MMP
Total Dissolved Nitrogen	TDN.MMP	µmol	In water concentration TDN	AIMS MMP

Total Dissolved Phophate	TDP.MMP	µmol	In water concentration TDP	AIMS MMP
Particulate Phosphate	PIP.MMP		In water concentration PP	AIMS MMP
Historical Maximum Summer Temperature	MMMv3	°C	Historical summer maximum average 1985-2012 NOAA CRW v3.1 climatology. Upper thermal limit threshold for each reef pixel.	NOAA CRW 5Km satellite v3.1
Accumulated heat stress at time of survey	DHW.YTD	°C-weeks	Accumulation of temperature hotspots and duration of anomalies at least +1.0°C above the MMM up to the date of in water survey	NOAA CRW 5Km satellite v3.1
Maximum annual accumulated heat stress	Max.DHW	°C-weeks	Total accumulation of DHW heat stress at the end of each summer	NOAA CRW 5Km satellite v3.1
Additional DHW accumulated after in water survey	DHW_Add_Accum	°C-weeks	Max.DHW – DHW.YTD as an indicator how the time of survey relates to peak heat stress	NOAA CRW 5Km satellite v3.1
Maximum annual Temperature	Max.Temp	°C	Maximum SST for each year	NOAA CRW 5Km satellite v3.1
Maximum Anomaly	Max.Anom	°C	Measure of the strength of temperature stress compared to historical summer max. Max.Temp-MMMv3	NOAA CRW 5Km satellite v3.1
Maximum seasonal change in temperature	Temp_delta	°C	Maximum change in seasonal Seawater temperature (Max – Min)	NOAA CRW 5Km satellite v3.1
Total Suspended Solids	TSS_mean, median, sd, range, delta	kg m^{-3}	Ecological Fine Inorganics (EFI); inorganic fraction of total suspended solids used for TSS-dependent calculations such as phosphorus absorption	eReefs BGC model
Total chlorophyll a	Chla_mean, median, sd, range, delta	mg m^{-3}	Sum of chlorophyll concentration of the four microalgae types	eReefs BGC model
Vertical attenuation at 490nm	Kd490_mean, median, sd, range, delta	m^{-1}	Vertical attenuation of light at 490nm (along z	eReefs BGC model

			axis not along zenith angle)	
Rubisco Enzyme activity of coral symbiont	CS_tempfunc mean, median, sd, range, delta	0-1	Concentration of symbiont reaction centres in an oxidised state per m^2	eReefs BGC model
Salinity	Salt_ mean, median, sd, range, delta	PSU	Wet season (Oct-Apr) mean salinity	eReefs BGC model
Temperature	Temp_ mean, median, sd, range, delta	°C	eReefs Temperature	eReefs BGC model
Dissolved Inorganic Carbon	DIC_mean, median, sd, range, delta	mg m^{-3}	Concentration of dissolved inorganic carbon, composed chiefly at seawater pH of HCO_3^-	eReefs BGC model
Particulate Nitrogen in the Labile Benthic Detritus	DetBL_N_ mean, median, sd, range, delta	mg m^{-3}	Concentration of N in labile (quickly broken down) organic matter with C:N:P ratio of 550:30:1 from living seagrass and macroalgae	eReefs BGC model
Labile Planktonic Detritus Nitrogen	DetR_N_ mean, median, sd, range, delta	mg m^{-3}	Concentration of N in labile (quickly broken down) organic matter with C:N:P ratio of 106:16:1 derived from living microalgae, zooplankton, coral host tissue and symbionts.	eReefs BGC model
Refractory Detritus Nitrogent	DetR_N_ mean, median, sd, range, delta	mg m^{-3}	Concentration of N as particulate refractory (slowly broken down) material. Sourced only from breakdown of labile detritus and from rivers.	eReefs BGC model
Dissolved Inorganic Nitrogen	DIN_ mean, median, sd, range, delta	mg N m^{-3}	Concentration of dissolved inorganic nitrogen	eReefs BGC model
Dissolved Organic Carbon	DOR_C_ mean, median, sd, range, delta	mg m^{-3}	Concentration of carbon in dissolved organic compounds	eReefs BGC model
Nitrate	$NO_{3\_}$ mean, median, sd, range, delta	mg N	Concentration of nitrate. In the absence of nitrite [NO_2] in the model, nitrate	eReefs BGC model

			represents [NO_3^-] + [NO_2^-]	
Total Nitrogen	TN_ mean, median, sd, range, delta	mg N m^{-3}	Sum of both dissolved and particulate nitrogen	eReefs BGC model
Particulate Inorganic Phosphorus	PIP_ mean, median, sd, range, delta	[mg P m^{-3}]	Phosphorus ions absorbed onto particles	eReefs BGC model
pH	pH_ mean, median, sd, range, delta	Log_{10} mol m^{-3}	pH based on [H^+] calculated from carbon chemistry equilibria at water column values of T,S, DIC and A_T	eReefs BGC model
Temporal				
Year	Bleaching.Year	yyyy	Year used for bleaching survey, in water WQ observations and eReefs modelled data	All data
Survey Date	IW.Date	dd/mm/yyyy	Date of in water survey or WQ sampling observations	AIMS

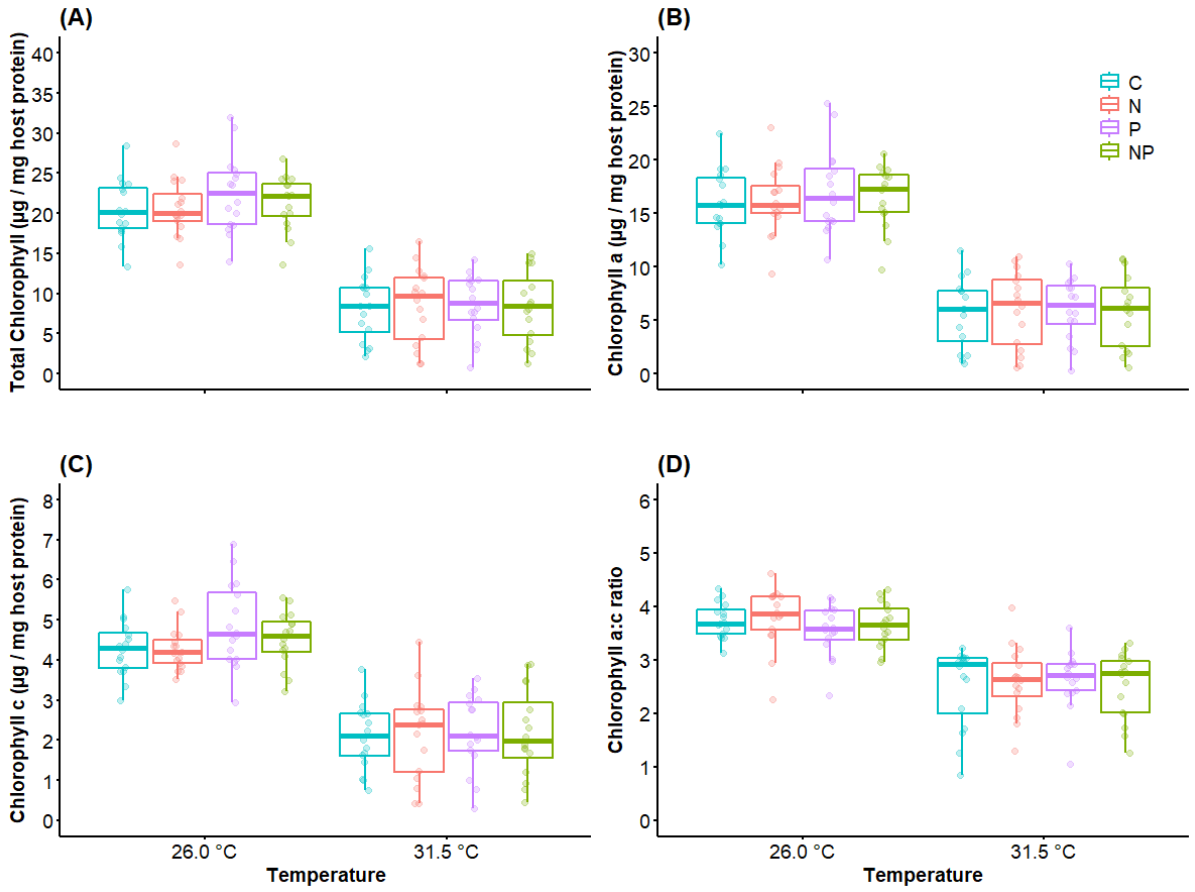


Figure A1.1: Lack of nutrient impacts on Chl metrics of *A. millepora* at each temperature. Box plots show the quartiles and range (excluding outliers) and dots show raw the data points.

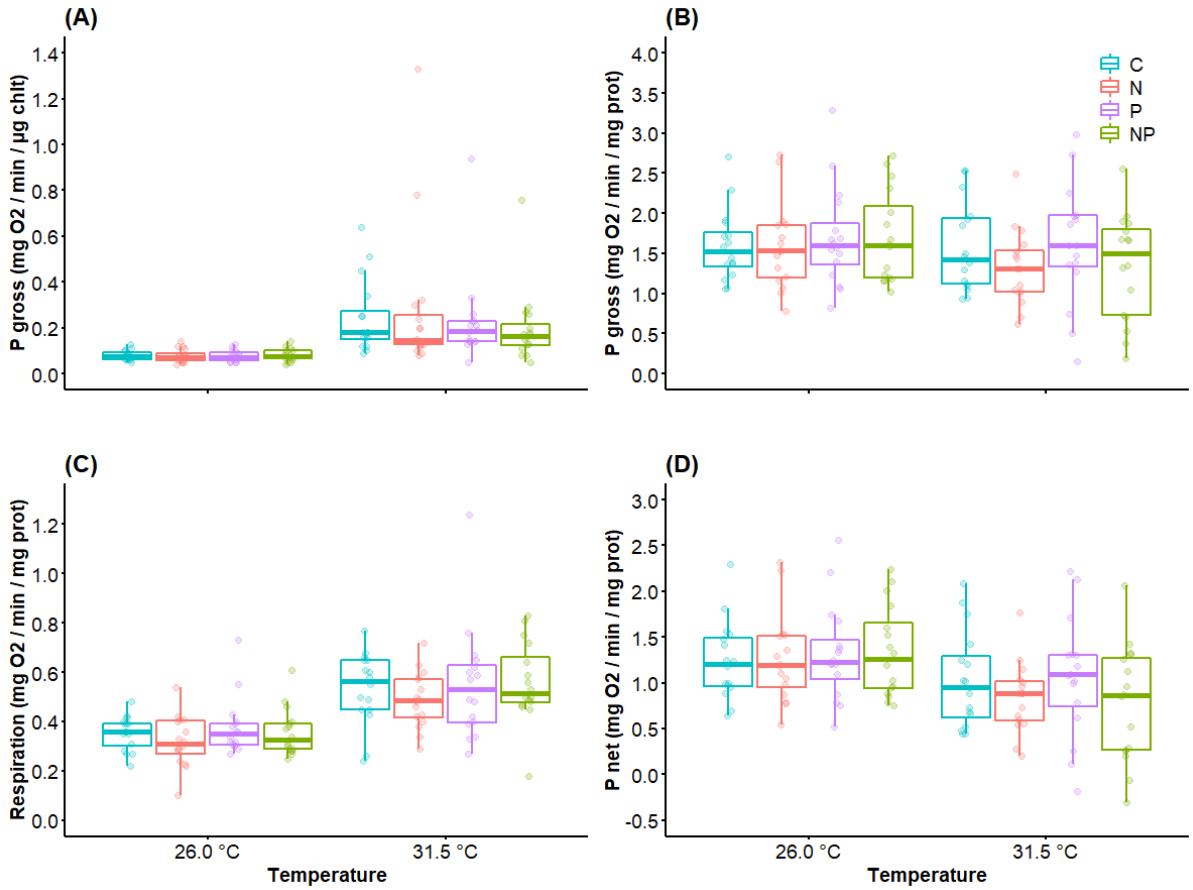
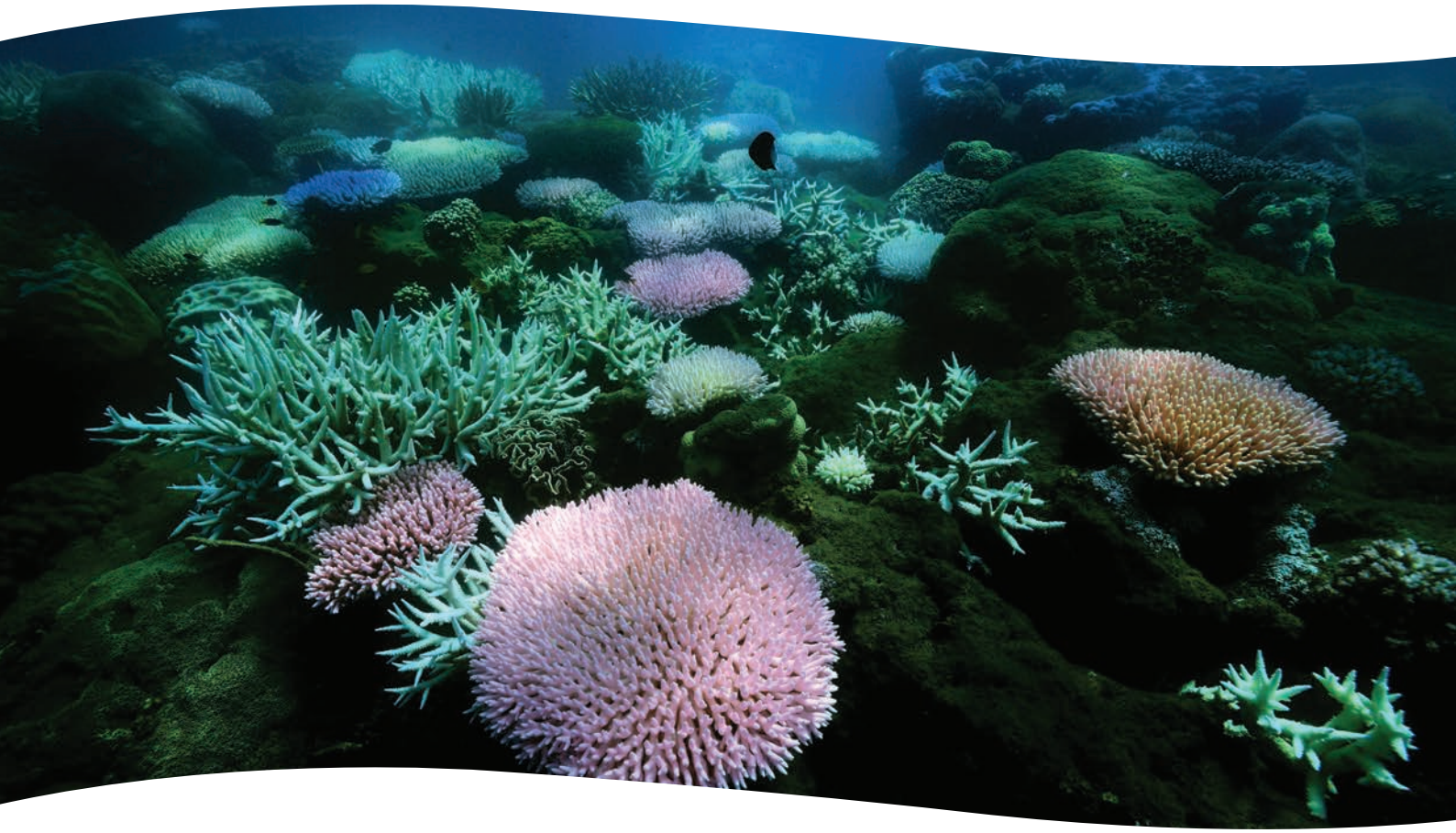


Figure A1.2: Lack of nutrient impacts on the photosynthesis and respiration of *A. millepora* at each temperature. Box plots show the quartiles and range (excluding outliers) and dots show raw the data points.



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