

From Exposure to Risk: Novel Experimental Approaches to Analyse Cumulative Impacts and Determine Thresholds in the Great Barrier Reef World Heritage Area (GBRWhA)

Sven Uthicke, Carolina Castro-Sanguino, Renata Ferrari, Katharina Fabricius, Eric Lawrey, Florita Flores, Frances Patel, Christopher Brunner and Andrew Negri



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determine thresholds in the GBRWH

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ACRONYMS

AIMS	Australian Institute of Marine Science
CA	Carbonic Anhydrase
CoTS	Crown-of-Thorns-Seastar
DHW	Degree Heating Weeks
DIN	Dissolved Inorganic Nutrients
DLI	Daily Light Interval
FSW	Filtered Seawater
GBR	Great Barrier Reef
GBRMPA	Great Barrier Reef Marine Park Authority
GLMM	Generalized Linear Mixed-Effects Model
GV	Guideline Values
IPCC	Intergovernmental Panel on Climate Change
JCU	James Cook University
LTMP	Long-Term Monitoring Program
MMP	Marine Monitoring Program
NESP	National Environmental Science Program
OA	Ocean Acidification
OW	Ocean Warming
PAR	Photosynthetic Active Radiation
PC	Protective Concentration
PT	Protective Temperature
QAEHS	Queensland Alliance for Environmental Health Sciences
RRRC	Reef and Rainforest Research Centre Limited
SSD	Species Sensitivity Distributions
TOC	Total Organic Content
TAC	Total antioxidant capacity
TWQ	Tropical Water Quality
WQ	Water Quality
WQGV	Water Quality Guideline Value

ABBREVIATIONS

CO₂	carbon dioxide
EC₅₀	50% effect concentration
km	kilometre
LC₅₀	50% lethal concentration
LD₅₀	50% of lethal dose
mg cm⁻²	milligram per square centimetre
mL⁻¹	per millilitre
mm	millimetre
mol m⁻² d⁻¹	mol per square metre per day
pCO₂	carbon dioxide partial pressure
ppm	parts per million
PCx	protective concentration values (where x = % effect)
PTx	protective temperature values (where x = % effect)
TTx	temperature threshold (where x = % effect)
µatm	micro atmosphere
µmol m⁻² s⁻¹ ..	micro mol per square metre per second

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EXECUTIVE SUMMARY

The overarching goal of these NESP TWQ Hub projects was “*To fill important experimental and conceptual gaps in understanding cumulative impacts on coral reef environments*”. This was achieved through a combination of three research approaches, namely

- a) intensive experimental work focusing on knowledge gaps identified in a preceding literature review;
- b) ecotoxicological modelling and exploration of novel approaches to combine species sensitivity distributions for multiple stressors; and
- c) collating information on environmental pressures available over the last > 20 years into pressure maps for the Great Barrier Reef (GBR).

The pressure maps were used in ecological modelling to explain changes in coral cover (*‘State’* of the reef) and *Performance* (potential for corals to recover from disturbance) of individual coral growth forms from a combined Long-Term Monitoring Program (LTMP) and Inshore Marine Monitoring Program (MMP) coral dataset. Lastly, we combined the results of the three approaches into an eAtlas-based interactive tool illustrating issues and concepts of cumulative impacts for managers and the interested public.

Experimental assessments of concentration-response relationships were undertaken for selected marine species within broad, controlled envelopes of local stressors under different climate scenarios. These experiments were targeted to fill knowledge gaps identified in the NESP TWQ Hub Project 1.6 review. These gaps were considered with stakeholders at three workshops where it was decided that the project should focus on cumulative effects between climate change and light/sedimentation or herbicides (Chapter 2). The experiments were conducted using a variety of key reef organisms with a focus on habitat builders (e.g. corals, seagrasses, macroalgae and foraminifera), simultaneously exposing these organisms to one or multiple stressors; climate change, sediments or herbicides. Although, as expected, the response depended on the organisms and response variable, the underlying tenor of experimental responses was that the combined stress of climate and local stress worsens the outcome for the organism. From a climate perspective, additional local runoff-induced stress worsens the outcome of climate change stress for the reef. From the runoff perspective, lower concentrations of sediments or pesticides led to negative impacts on the organisms under future climate scenarios. We propose that Guideline Values (GV) for pollutants will need to be reduced in the future, to derive ‘climate adjusted thresholds’. The need to reduce GV represents a currently hidden cost of climate change for society and management.

The concept of ‘climate adjusted thresholds’ was further explored using ecotoxicological modelling of Species Sensitivity Distributions (SSDs). An important management strategy to improve the resilience and recovery of coral reefs is to ensure contaminant concentrations remain below water quality GV. However, current GV are derived from toxicity experiments performed under optimal conditions and do not account for co-occurring pressures such as heatwave conditions that can affect the toxicity of contaminants (which will become more prevalent as the climate changes). This project adapted a method, usually applied to estimate the total toxicity of multiple contaminants, to be used here to adjust water quality GV for contaminants to account for elevated temperatures. This was achieved by combining a new

tropical SSD for temperature stress with existing SSDs for contaminants. Combining these predicts that more species are affected by contaminants as temperatures increase. As an example, we used this method to predict how the national water quality GVs for common contaminants, such as the herbicide diuron and the metal copper, could be adjusted for heatwave conditions. For example, the concentration at which 95% of the species are protected ('protective concentration', PC95) GV for diuron would need to be adjusted from 0.67 $\mu\text{g L}^{-1}$ to 0.59 $\mu\text{g L}^{-1}$ if temperature rose by 1°C above present day temperatures, while the PC90 of copper would require adjustment from 3.0 to 1.0 $\mu\text{g L}^{-1}$ under a of 2°C temperature increase.

A set of 25 important environmental stress/pressure variables were collated and combined into exposure maps available through the eAtlas. These data range from acute stressors (e.g., degree heating weeks, Crown of Thorns Seastars (CoTS) numbers, cyclones, and several combinations of these) to land runoff-based indices (e.g., River DIN) or eReefs-derived concentrations of chlorophyll-a, sediments, salinity and nutrients in the GBR lagoon. Not all variables exist for the period investigated (1992-2017), and some were thus only used in the ecological modelling as average over the available period. All data are available were made available via the eAtlas. Coral community data were obtained from the Australian Institute of Marine Science (AIMS) LTMP and the MMP, uniquely combining offshore and inshore data. We identified several disturbances as acute modulators of coral cover ('*State*') of individual coral growth forms. Not surprisingly the best predictors were a) a cumulative index for CoTS pressure on the reef, b) cyclones (frequency and magnitude), and c) temperature (degree heating week accumulated over summer). Several water quality parameters consistently exhibited high importance scores in initial model runs, but few made it into the final model selection. We presume this is mainly due to a) the overwhelming effect of acute pressures and b) insufficient temporal and spatial resolution of water quality parameters. Filling the latter gap should clearly be considered in future monitoring designs. For example, the most informative parameter ('River DIN') is available over the entire time span modelled, and had a significant influence on *State* and *Performance* of several coral growth forms.

The results of the three combined research approaches broadens our understanding of cumulative impacts on coral reef communities and provide guidance for management and further research. However, the nature of cumulative impacts on GBR organisms, and our results, are complex and often difficult to communicate to a wider audience. The purpose of the 'interactive tool' developed as part of this project is thus two-fold. Firstly, it is used to explain the outcomes of this report in a graphic and accessible fashion on several linked web pages accessible through eAtlas. Secondly, drawing upon our results it explains general concepts of cumulative impact management using interactive maps such as 'clickable' exposure layers and it also illustrates the current reef condition.

1. INTRODUCTION

1.1 Cumulative Impacts

Cumulative impacts result from one or more stressors and their interactions can accumulate in time and space, posing a threat to ecosystem resilience. Cumulative impacts represent added ecological risks and potential surprises not accounted for in conventional risk assessments. Understanding cumulative impacts from multiple stressors will be critical for successful management of the Great Barrier Reef (GBR) under the Reef 2050 Plan (Commonwealth of Australia, 2015; GBRMPA, 2017), and has become a priority for environmental policy, management and conservation globally (GBRMPA, 2014a, 2014b; Halpern, Walbridge et al., 2008). The Reef 2050 Long-Term Sustainability Plan highlights that in Australia a deeper understanding of cumulative impacts from global and local stressors, and the ability to attribute those impacts to specific drivers and activities, are now a priority at both federal and state (Queensland) government levels. Several actions and targets in the Reef 2050 Long-Term Sustainability Plan explicitly address cumulative impacts. For example:

- *Develop guidelines for assessing cumulative impacts (including climate change pressures) on matters of national environmental significance including ecosystem and heritage values in the World Heritage Area (EH 19).*
- *Key direct human-related activities are managed to reduce cumulative impacts and achieve a net benefit for the Reef (EHT7).*
- *Reduce cumulative impacts on coastal dolphin populations and their supporting habitats especially Australian humpback and snubfin dolphins (BA15).*

While reef managers recognize that cumulative impacts are an added risk dimension, large knowledge gaps exist in our understanding of the effect of individual stressors on key reef species, the interplay of local and global stressors and the way individual stressors combine into cumulative impacts. However, at present managers largely rely on qualitative tools to assess risks from cumulative impacts associated with development proposals (GBRMPA, 2009). Addressing the need for cumulative impact management with net-benefit under the Reef 2050 plan, the GBRMPA recently released a policy document (GBRMPA, 2018a).

In general, coastal marine ecosystems are subjected to a multitude of pressures (Duarte, 2014; Jackson, Kirby et al., 2001). Overfishing (including intertidal harvesting) was the early anthropogenic pressure to coastal areas, dating back hundreds if not thousands of years (Jackson et al., 2001). Other pressures such as eutrophication and pollution, physical habitat destruction and marine invasive species added to the pressures, many only in the last 200 years. Today, most areas of the coastal seas and open ocean are exposed to cumulative pressures caused by human activities or natural pressures. Cumulative pressures have increased demonstrably over as little as 5 years from 2008 to 2013 in large areas of the world's oceans (Halpern, Frazier et al., 2015). This is mainly caused by climate change and ocean acidification (Duarte, 2014; Jackson et al., 2001).

Usually the historic pressures persist as new pressures are being added, Thus, the overall outcome was conceptualised simply as a “stacking up” or a growing set of cumulative pressures (Figure 1.1). While a simple way to illustrate cumulative pressures, Figure 1.1 suggests an additivity of pressures and neglects acute impacts, two assumptions that may not apply given factors can interact in a non-additive fashion.

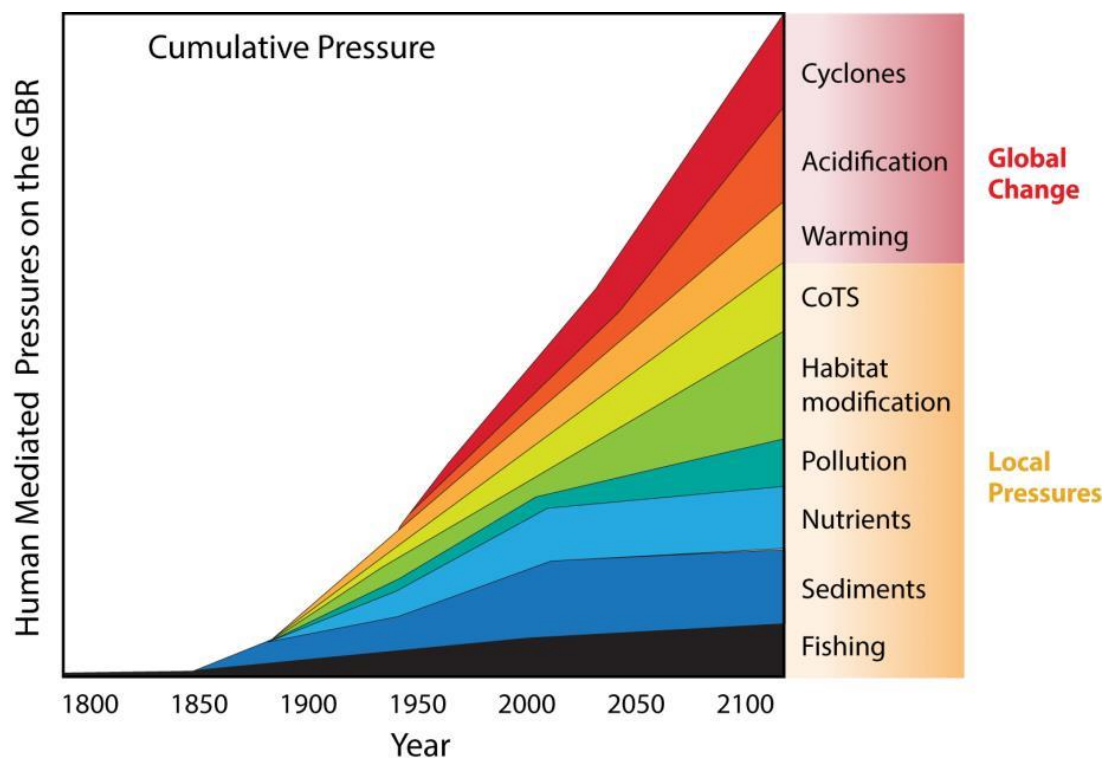


Figure 1.1: Pressures on coastal ecosystems, modified from Duarte (2014) in The Conversation, <http://theconversation.com/auditing-the-seven-plagues-of-coastal-ecosystems-13637>.

A suite of pressures or threats affect the GBR are listed in GBRMPA's Outlook reports and Strategic Assessments (e.g., GBRMPA, 2014a, 2019). Those pressures and threats ranked as “Very High” and ‘High’ are listed in Table 1.1. The drivers or causes of these pressures can be global or local. Many global pressures are due to anthropogenically increased greenhouse gasses in the atmosphere, which leads to climate change and ocean acidification (OA). The most recent GBR Outlook Report update see table below highlights that climate change is the most significant threat for the future of the GBR (GBRMPA, 2019). Addressing climate change

requires international collaborations and commitments to a shift to alternative and renewable energy sources (IPCC, 2014a). However, the Outlook report also acknowledges that addressing water quality at Regional scale is crucial. Many threats to the GBR are local or regional, caused by changing land-use practices, fishing, coastal developments and human population growth in the coastal zone.

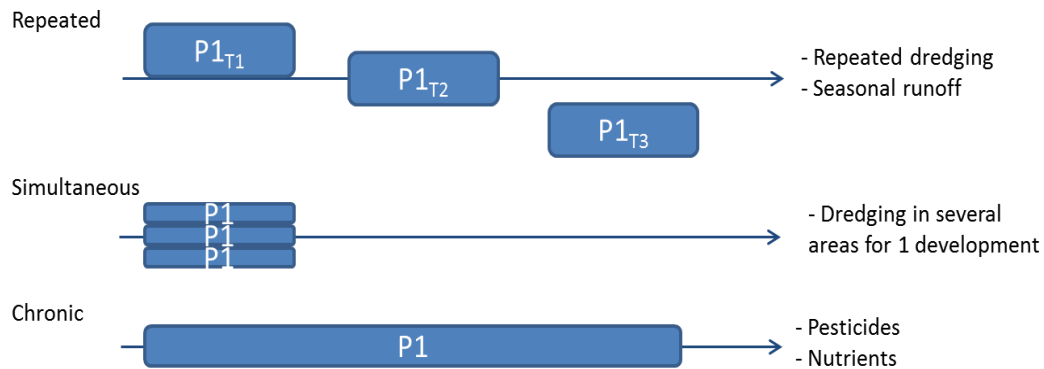
Table 1.1: List of pressures relevant for the Great Barrier Reef World Heritage Area. Only those threats identified in the GBR Outlook Report (GBRMPA, 2019) as “Very High” or “High” risks to the GBR Region’s ecosystem are listed. L = local, G = global, A = acute and C = chronic.

Pressure/Threat	Local vs. Global cause	Acute/ Chronic	Risk to ecosystem
Ocean warming	G	C/A	Very High
Ocean acidification	G	C	Very High
Cyclones/alterd weather patterns	L (G)	A/C	Very High
Illegal fishing and poaching	L	C	Very High
Incidental catch of species of conservation concern	L	A	Very High
Nutrient runoff	L	C/A	Very High
Outbreak of Crown-of-Thorns-Seastars	L	A	Very High
Sediment runoff	L	C/A	Very High
Coastal habitat modification	L	C	Very High
Sea level rise	G	C	Very High
Pesticide pollution	L (G)	C/A	High
Barriers to flow	L	C	High
Discarded catch	L	A	High
Extraction of predators	L	A/C	High
Extraction of particle feeders	L	A/C	High
Marine debris	L (G)	A/C	High
Extraction from spawning aggregations	L	A/C	High
Outbreak of disease	L (G)	A	High
Artificial light	L	A/C	High
Damage to reef structure	L	A	High

A variety of different types of cumulative pressures are listed in the literature and environmental impact statements (Anthony, Dambacher et al., 2013; Brown, Saunders et al., 2013; Crain, Kroeker et al., 2008; Halpern et al., 2015; Halpern, McLeod et al., 2008). We synthesised the types of cumulative pressures in Figure 1.2. Cumulative effects can be caused by the same pressure, or by multiple pressures with complex interactions (Figure 1.2). In the former case, the same pressure accumulates through time or space. For example, sequential acute events

of the same pressure, such as those caused by repeated maintenance dredging campaigns in the same area or seasonally occurring runoff events affecting the same coral reefs repeatedly. Other examples may be simultaneous pulses of the same pressure, or chronic (long term) exposure to the same pressure.

Same pressure accumulating over time/space



Multiple pressures, complex interactions

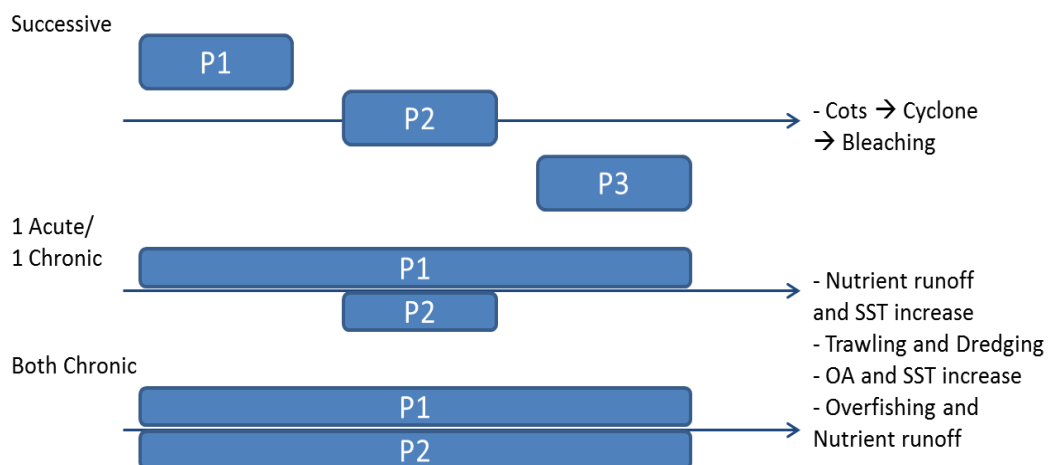


Figure 1.2: Different types of cumulative pressures (P1, P2) on marine ecosystems. Modified from Uthicke, Fabricius et al. (2016).

An example of multiple pressures may be simultaneous or successive effects by several acute events. Combined effects from land runoff and climate change have been much debated (e.g., Fabricius, Cseke et al., 2013; Wooldridge & Done, 2009a) and are the main subject of the experimental and modelling work of the current project. In its recent policy document (GBRMPA, 2018a), The GBRMPA uses the following working definition of cumulative impacts:

“Cumulative impacts are defined as the interaction of effects between one or more impacts and past, present and reasonably foreseeable future pressures.

Cumulative impact assessment takes into account direct, indirect and consequential impacts and the incremental and compounding effects of these impacts over time, including past, present and reasonably foreseeable future pressures?”

Project 2.1.6, and its extension Project 5.2, produced experimental analyses of concentration-response relationships for selected species under broad, controlled envelopes of local stressors at different climate scenarios. These experiments were targeted to fill knowledge gaps identified in project 1.6. Considering these gaps, and in discussion with stakeholders (3 workshops were conducted throughout the time of this project), it was decided to focus on cumulative effects between climate change and light/sedimentation or herbicides (Chapters 2.2, 2.3 and 2.4). Results are now available to inform assessments of ecological risks, and management opportunities for a range of activities in the coastal zone and on the GBR. Exploring tools used in ecotoxicology, we tested a method to derive climate adjusted thresholds for herbicides. This method was published in a high impact journal and can be applied or adapted to other pressure combinations (Chapter 3). We used exposure maps and ecological models, based on our developed and existing databases and experimental results, to advance our understanding of the contribution of individual disturbances in a cumulative framework (including both acute and chronic disturbances) to the current status of the reef (Chapter 4). In turn, this new knowledge can now inform management on setting future water quality thresholds, identifying catchments and regions as priorities for management action aimed at ameliorating impacts to reef health and building resilience for large-scale cumulative disturbances. A summary of all publications, published as well as in preparation, stemming from NESP TWQ Hub Projects 2.1.6 and 5.2 can be found in Table A1 in the Appendix.

1.2 Objectives

The objectives of these NESP TWQ Hub projects are to:

- 1) fill important experimental and conceptual gaps in understanding cumulative impacts on coral reef environments
- 2) provide climate-adjusted thresholds for key stressors and key organisms
- 3) support cumulative risk analyses for selected key areas of the GBR
- 4) provide guidance for how exposure and risk should be analysed and mapped to inform:
 - a) spatial risk assessments of cumulative impacts on key species underpinning reef ecosystem health, resilience and outstanding universal values
 - b) assessments of targeted risk mitigation associated with development proposals
 - c) indicator development to monitor early warnings of cumulative impacts.

2. EXPERIMENTAL GAPS

2.1 Summary of experimental work in Projects 2.1.6 and 5.2

Experimental work conducted under this project was guided by a gap analysis conducted in NESP TWQ Hub Project 2.1.6 (Figure 2.1). In consultation with the end-users we focussed on climate change and sediment/light or herbicide interactions. All experiments were conducted at the AIMS SeaSim facility under highly controlled and replicated conditions, and supported by several Masters and PhD students.

Many of the experiments are already published in the peer review literature, a detailed list of all experiments and their main findings is provided in Table 2.1. The data analysis and write up of some experiments is still ongoing. For instance, the PhD student conducting the main experiments on the effect of sedimentation and climate change (Christopher Brunner) still has one year to finalise his studies (see his report in Chapter 2.2).

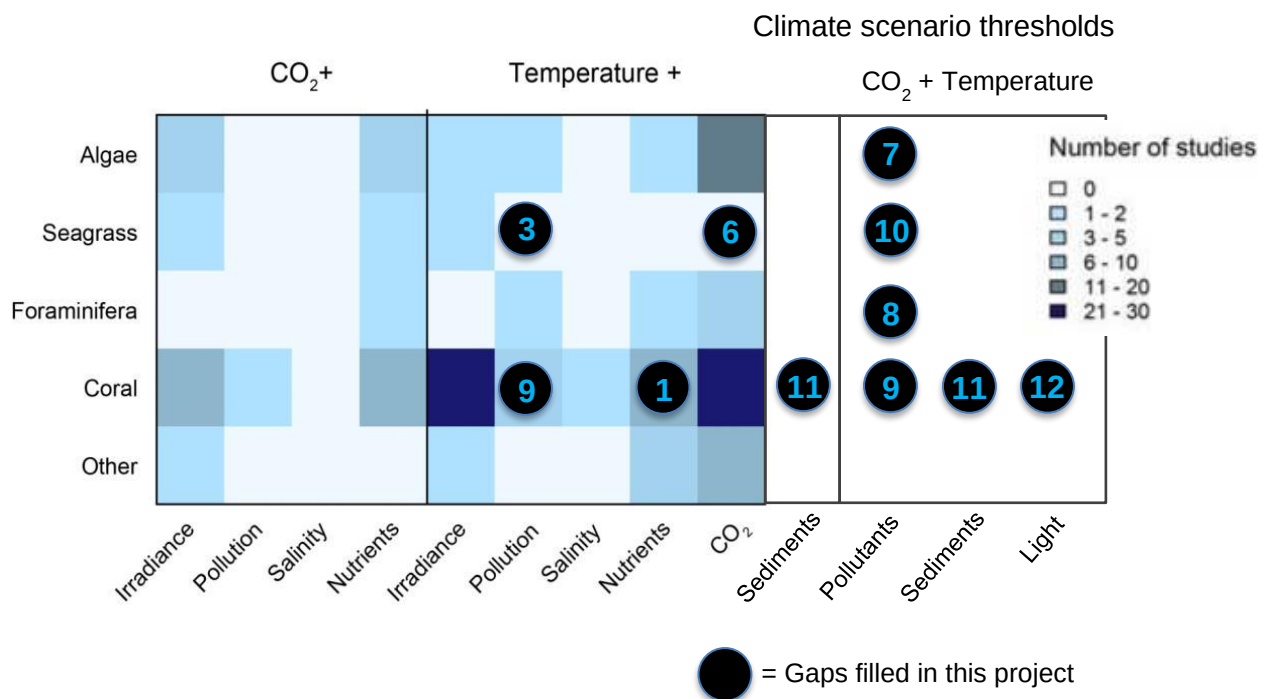


Figure 2.1: Gap analysis for experimental cumulative impact studies. Numbers in each circle refers to corresponding Research Project listed in Table 2.1. Climate scenarios (on the right) were added here to illustrate experiments conducted as part of this project.

Table 2.1: Outcomes of project experimental work in 2.1.6 and 5.2. Climate refers to combined OW and OW stress mimicking future climate predictions.

Research Project	Organism	Pressure	Main finding	Reference
1	Corals (juvenile)	Temperature x Nutrients	Gamete fertilization, larval survivorship and larval settlement significantly reduced as temperature increased, but only fertilization was further affected by simultaneous nutrient enrichment. Combined high temperatures and nutrient enrichment affected fertilization in an additive manner, whereas embryo abnormalities increased synergistically.	Humanes, Noonan et al. (2016)
2	Corals (larvae)	Temperature x Sediments + Nutrients	Fertilization success was reduced 80% by suspended sediments, and up to 24% by temperature, while the addition of nutrients to suspended sediments had no further impact. Larval survivorship was unaffected by any of these treatments. However, settlement success of larvae developing from treatment-exposed embryos was negatively affected by all three stressors (e.g. up to 55% by suspended sediments). Strongest negative effects observed under simultaneous temperature and sediment exposures.	Humanes, Ricardo et al. (2017b)
3	Seagrass	Temperature x Herbicide	The majority of the combined stresses caused sub-additive (antagonistic) effects. However, both stressors caused negative responses and the sum of the responses was greater than that caused by temperature or diuron alone.	Wilkinson, Collier et al. (2017)
4	Corals (juvenile)	Sediments + Nutrients	Individual and combined effects strongly depend on the coral species	Humanes et al. (2016)
5	Seagrass	Temperature only	The thermal optimum (T_{opt}) for gross photosynthesis of <i>Z. muelleri</i> , which is more commonly distributed in sub-tropical to temperate regions, was 31°C. The T_{opt} for photosynthesis of the tropical species, <i>H. uninervis</i> and <i>C. serrulata</i> , was considerably higher (35°C on average). All seagrasses were tested up to 43°C. This suggests that seagrass species are adapted to water temperature within their distributional range	Collier, Ow et al. (2017)
6	Seagrass	Temperature x OA x Light	Results depend on species, but temp stress in combination with CO ₂ worst. Response is highly light dependent.	Collier, Langlois et al. (2018)

Research Project	Organism	Pressure	Main finding	Reference
7	<i>Halimeda</i>	Climate x Herbicide	Interactive and additive effects. See chapter 2.4.	Marques, Flores et al. (2020b)
8	Forams	Climate x Herbicide	Data analysis pending	Marquez et al. (in prep)
9	Corals	Climate x Herbicide	Both had negative effects on coral photosynthesis singly. In combination the outcomes were worse.	Flores et al., (in prep, Chapter 2.3)
10	Seagrass	Climate x Herbicide	Data analysis pending	Ontario-Gomera et al. (in prep)
11	Corals (juvenile)	Climate x Sediment	Juvenile coral more susceptible to sedimentation under CC	Brunner et al. (in press, Chapter 2.2)
12	Corals (juvenile)	Climate x Light	Data analysis pending	Brunner et al., (in prep)

The response and the intensity of results depends on the parameter chosen and organism (Table 2.1). Temperature over a certain threshold and climate change scenarios simulation predicted conditions in 2050 or 2100 had negative effects on most responses (e.g. photosynthesis, calcification in most organisms. Similarly, increased levels of sediments or herbicides reduced photosynthesis or growth of organisms ranging from coral to foraminifera and seagrass. In most cases the combined response to climate change and local stressors were larger than individual effects. In other words, increased temperatures or future predicted climate made organisms more vulnerable to sediment or herbicides. Examples on this are given in Figure 2.2 and are found in the examples given in the report chapters or in the publications. The response of the seagrass *Halimeda ovalis* to diuron followed a typical dose response curve (Figure 2.2a (Wilkinson et al., 2017)). However, with increasing temperatures the curve shifted, leading to changes in EC₅₀ values. Similar functions can describe the reduction in fertilisation success for a coral species (Figure 2.2b, (Humanes et al., 2017b)). Increasing temperature ‘left shifts’ this curve, meaning that lower amounts of sediments lead to a 50% reduction in fertilisation success. Similarly, oxygen production of a coral species was reduced under much lower diuron concentrations when the corals were exposed to future climate scenarios (Figure 2.2c and Chapter 2.3). Further examples can be found in Chapter 2.2 (Example 1: Cumulative sedimentation, temperature and acidification pressure decreases coral post-settlement survival), Chapter 2.3 (Example 2: Combined effects of climate change and the herbicide diuron on the coral *Acropora millepora*) and Chapter 2.4 (Example 3: Acclimation history modulates effect size of calcareous algae (*Halimeda opuntia*) to herbicide exposure under future climate scenarios).

These findings have clear management implications. Ecologically relevant thresholds or guideline values for sediments and herbicides will need to be lowered in the future to provide the same protection. This is congruent with our analysis using species sensitivity distributions

presented in Chapter 3. These 'climate adjusted thresholds' pose hidden costs of climate change for management.

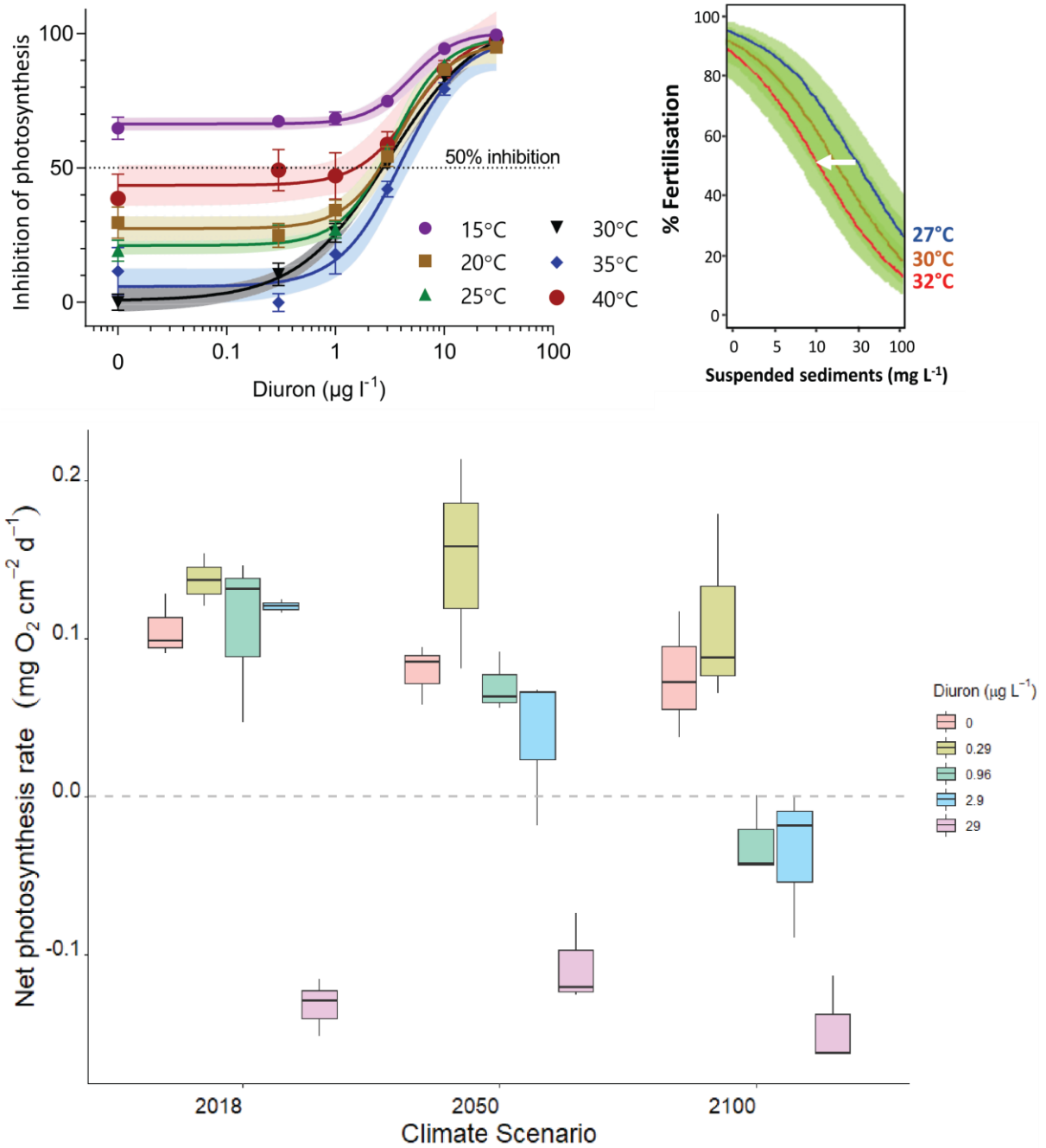


Figure 2.2: Examples of shifting sensitivity of coral reef organisms to herbicides or sediment under temperature increase of climate change scenarios, (a) the effect of diuron on the photosynthesis of the seagrass *Halophila ovalis* under 6 different temperatures (Wilkinson et al., 2017), (b) the effect sediment load on fertilisation the coral *Acropora tenuis* (Humanes et al., 2017b) under 3 different temperature and (c) the effect of diuron on the coral *Acropora millepora* under 3 climate scenarios (Chapter 2.3).

2.2 Example 1: Cumulative sedimentation, temperature and acidification pressure decreases coral post-settlement survival

Author: Christopher Brunner

Contributing authors: Andrew Negri, Gerard Ricardo, Mia Hoogenboom and Sven Uthicke

In press, Science of the Total Environment

2.2.1 Summary

Nearshore corals are under pressure from global climate change combined with local stressors, such as poor water quality. In this study we experimentally investigated the influence of climate change on sedimentation thresholds for coral recruits. *Acropora millepora* recruits were raised under current and future climate scenarios [up to a temperature of 29.9°C (+1.2°C above ambient) and pCO₂: 950 ppm] as well as being exposed to sediment deposition events at five and ten weeks of age (experiment 1) or only at ten weeks (experiment 2). Results show that both future climate conditions and sediment deposition lowered recruit survival. The recruits were twice as sensitive to sediment deposition stress when raised under future climate scenarios at all ages tested. Increased recruit mortality under 'medium' and 'high' OW and OA conditions were likely linked to a reduced capacity to remove sediment under these scenarios. The ability of larger recruits to more rapidly clear sediments from their tissues enhanced their survival during deposition events. The timing of sediment exposure following coral settlement was also important, as only ten weeks old recruits grown in current climate were able to survive the highest tested deposition levels (80 mg cm⁻²). This study is the first to quantify the extent to which sediment deposition thresholds for recruit survival are affected by realistic future climate conditions. Quantifying the thresholds for stress on sensitive life stages such as post-settlement survival, which is seen as bottleneck in the population growth of scleractinian corals, is critical to improve sustainable local and global management efforts. Water quality guideline values for sediments on coral reefs will need to be adjusted to account for extra risk to recruits as the climate changes.

2.2.2 Introduction

Coral mass bleaching and mortality events are becoming more frequent and severe as the climate changes (Hoegh-Guldberg, Poloczanska et al., 2017; Hughes, Kerry et al., 2017b). Both global change and local stressors such as declining water quality are well documented pressures causing the 'coral crisis' (Carilli, Norris et al., 2010; Hoegh-Guldberg, Mumby et al., 2007), but understanding the contributions of multiple drivers on the decline of coral is in its infancy (Hughes, Barnes et al., 2017a). To predict the resilience of coral reefs in future climate regimes, experimental and monitoring studies often focus on ocean warming (OW), ocean acidification (OA), or their interaction (Foster, Gilmour et al., 2015; Hoegh-Guldberg et al., 2007). However, with over 39% of the human population living within 100 km of the ocean (Cesar, Burke et al., 2003), coastal development and agricultural land use are responsible for intensifying local pressures, including poor water quality, on near-shore coral reefs (Doney, Ruckelshaus et al., 2012; Magris, Grech et al., 2018; Wolanski & De'ath, 2005). By 2050, local pressures may account for 28% of the predicted decline in coral cover under a high emissions scenario, or up to 66% of coral loss under a low emissions scenario (Wolff, Mumby et al., 2018b). Recent reviews have revealed that combinations of poor water quality with OW or OA

have more severe effects on corals than individual pressure alone and that the effects are most often additive or synergistic (Ban, Graham et al., 2014; Uthicke et al., 2016). However, few studies have effectively quantified how the impact thresholds for water quality on corals will be affected in future climate regimes, information critical for development of sustainable management strategies (Doney et al., 2012; Magris et al., 2018; Uthicke et al., 2016).

Increased sediment loads through natural resuspension events or human activities (i.e. dredging operations or river runoff from agriculture, mining and urbanisation) is a pantropical problem for coral reefs (GBRMPA, 2018b; Jones, Bessell-Browne et al., 2016). Sediments harm corals through a variety of exposure pathways depending on whether the particles are suspended (light attenuation and shifts in the spectral profile resulting in the disturbance of autotrophic feeding) or deposited (smothering and disturbance of autotrophic and heterotrophic feeding) (Anthony & Fabricius, 2000; Jones et al., 2016). Coral post-settlement survival represents a critical bottleneck in the coral lifecycle (Ritson-Williams, Arnold et al., 2009). This early life-stage can be particularly sensitive to deposited sediments which can easily smother coral recruits due to their initially small size (< 1 mm) (Jones, Ricardo et al., 2015; Moeller, Nietzer et al., 2016).

Although additive and synergistic effects of sediment interactions have been identified for adult colonies (OW and sedimentation, sedimentation and pollution) (Ban et al., 2014; Bessell-Browne, Negri et al., 2017b), and for recruits (OW and sedimentation) (Foster, Falter et al., 2016; Fourny & Figueiredo, 2017), no studies investigated the combined effects of sedimentation on coral recruits under future climate scenarios (OW and OA). Here, we exposed coral recruits that had been raised since settlement in three climate regimes (OW and OA combined) to sediment deposition events in two long-term experiments. Sedimentation was simulated once or twice within the first ten weeks after coral settlement to explore the impacts and potential interactions of climate and sediment stress on coral recruit survival, growth and sediment removal capability.

2.2.3 Materials and Methods

Survival and polyp growth of *Acropora millepora* recruits were quantified in two sediment-climate exposure experiments. In both experiments, *A. millepora* recruits grown in three climate scenarios were exposed to six environmentally relevant sediment deposition levels at either five and ten weeks (experiment 1), or only at ten weeks after settlement (experiment 2). Following every sediment exposure, the recruits were given a four-week recovery phase after which responses were photographically documented (Figure 2.2.1). These experiments were carried out at the National Sea Simulator (SeaSim) located at the Australian Institute of Marine Science (AIMS) between November 2017 and February 2018.

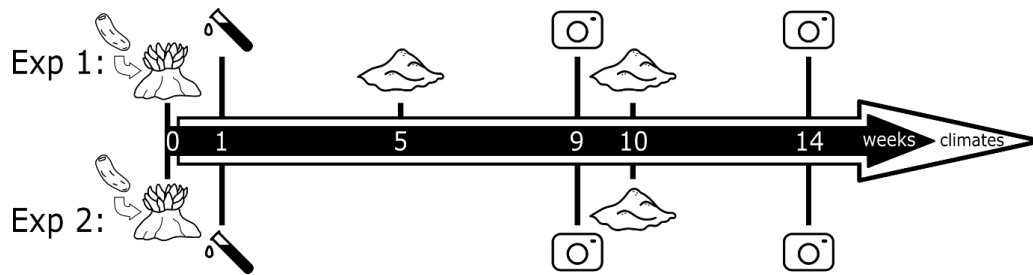


Figure 2.2.1: Timeline for experiment 1 (top) and experiment 2 (bottom). The figure illustrated the settlement of *A. millepora* larvae and when the recruits were inoculated with cultured *Symbiodiniaceae*, when they were smothered for three days with six environmentally relevant sediment loads, and when responses were photographically captured. Exposure to three climate scenarios (4 replicate tanks per climate) occurred just after settlement until the end of the study. The symbol at week 5 and 10 indicates the sedimentation events.

2.2.3.1 Spawning and larvae rearing

In November 2017, gravid *A. millepora* colonies were collected from 2 to 6 m water depth near Falcon Island on the central Great Barrier Reef (18°45'57.0"S 146°31'57.0"E) and transported to the National Sea Simulator at the Australian Institute of Marine Science, Townsville. After collection, the coral colonies were maintained in outdoor flow-through aquaria in unfiltered seawater under ~50% shading. Egg and sperm bundles from eight colonies were collected and cross-fertilised as per Negri and Heyward (2000). After two hours (>90% of cleavage), the embryos were rinsed twice in 50 L filtered seawater (FSW, 0.04 μm) and transferred into 420 L flow-through larval rearing aquaria with circular water flow. The embryos were left to develop for 18 h after which gentle aeration was introduced.

2.2.3.2 Coral larvae settlement

Disc-shaped experimental substrates (2 cm diameter x 1 cm height) for recruit exposures were manufactured from polyvinyl chloride (PVC) and sanded on the upper surface to accelerate biofilm formation as per Lee, Walford et al. (2009) and Ricardo, Jones et al. (2017). In order to initiate settlement and metamorphosis of *A. millepora* larvae, the discs were conditioned for two months in an indoor flow-through aquarium [daily light integral (DLI): 4.0 mol photons $\text{m}^{-2} \text{d}^{-1}$ and photosynthetic active radiation (PAR) 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$] together with known coral larvae settlement inducers, the crustose coralline algae (CCA) *Porolithon onkodes* and *Titanoderma prototypum* (Harrington, Fabricius et al., 2004; Heyward & Negri, 1999).

Following coral spawning, daily assays were performed to assess when the coral larvae were competent to settle. In each well of a six-well tissue culture plate ($n = 3$), settlement of ten larvae was tested in 10 mL FSW (0.04 μm) along with one 25 mm^2 chip of live *P. onkodes* (Harrington et al., 2004). Once the assays indicated a settlement response of at least 90% (7 days after spawning), all coral larvae were presented with the conditioned discs in static tanks (120 discs per 50 L at approximately 2 larvae mL^{-1}). From these discs, 576 discs were selected that had an average of six uniformly settled recruits across the disc surface (i.e. no settlement close to other recruits or close to the disc rim). The selected discs were randomly distributed between 12 FSW-filled (0.04 μm) climate-controlled 50 L indoor flow-through aquaria (48 discs per aquarium, 24 discs per experiment). One week after settlement, the coral recruits were inoculated with cultured Symbiodiniaceae [*Cladocopium goreau*i, ID: SCF055-01.10 isolated from *Acropora tenuis* from Nelly Bay, Magnetic Island and formerly known as *Symbiodinium*

clade C1 (Bongaerts, Carmichael et al., 2015; González-Pech, Bhattacharya et al., 2019)], according to the methodology described in Chakravarti, Negri et al. (2019).

2.2.3.3 Climate treatments

Three climate scenarios were tested in this study: hereafter referred to as ‘current’, ‘medium’ and ‘high’ (see Table 2.2.1). Each scenario was based on a different combination of temperature and carbon dioxide partial pressure (pCO₂). The temperature of the current climate scenario was based on the historic daily mean reef temperature at a typical mid-shelf coral reef in the central Great Barrier Reef (GBR) (Davies Reef, 18°49'52.7"S 147°38'07.8"E). Temperature data (4 m water depth) from 1991 to 2012 (Australian Institute of Marine Science, 2020) were used to calculate the historic average water temperature for every day of the planned experimental period. At the typical spawning time of *A. millepora* in November (Harrison, 2010), the calculated historic daily average temperature was 26.2°C, which increased to 28.7 °C until the end of the experiment in February. The mean pCO₂ used for the current climate treatment (450 ± 50 ppm) was based on latest mean sea surface pCO₂ monitoring data of inshore reefs by AIMS and Uthicke, Furnas et al. (2014).

The temperature of the medium and high climate scenarios were based on degree heating weeks (DHW), a measure for accumulated heat stress at a given location and time (Kayanne, 2016). In the GBR, elevated temperatures over extended periods have shown that more than 8 DHW cause extensive coral mortality (70 - >90%) (Hughes et al., 2017b; Kayanne, 2016). To generate a maximum of 8 DHW by the end of this experiment, a temperature of 1.2°C above the calculated historic daily mean reef temperature was selected for the high climate treatment. For the medium climate treatment, a temperature increase of 0.6°C, half that of the high climate treatment, was applied. The pCO₂ concentrations used for the medium and high climate scenarios were based on the representative concentration pathway model ‘RCP8.5’ that predicts pCO₂ levels of 680 ppm and 940 ppm for the years 2050 and 2100 (Collins, Knutti et al., 2013b; IPCC, 2014c; Meinshausen, Smith et al., 2011b). Immediately after settled recruits were transferred into the climate-controlled aquaria, the climate conditions were ramped over a period of one week to reach these three climate scenarios (four replicate tanks per climate) (Table 2.2.1).

Table 2.2.1: Climate and sediment treatments. The current temperature was based on the historic daily mean reef temperature at Davies Reef which increased from 26.2 to 28.7°C between November and February. In the high climate treatment, the increased temperature compared to the current climate was based to reach 8 Degree Heating Weeks (DHW) by the end of the experiment. pCO₂ ± SE was based on monitoring data (Uthicke et al., 2014) and on RCP8.5 (IPCC, 2014c). Sediment levels resembled conditions in calm inshore reefs, near river runoff and ≥2.5 km from dredging operations (Babcock & Davies, 1991; Babcock & Smith, 2000; Jones et al., 2016; Nichols, Diaz et al., 1990; Rogers, 1990; Tebbett, Goatley et al., 2017).

Climate	Temp (°C)	pCO ₂ (ppm)	Sediment (mg cm ⁻²)					
			Calm inshore reef			River plume		Dredging
Current climate	26.2 – 28.7	410 ± 50	0	5	10	20	40	80
Medium climate	Current + 0.6	680 ± 50	0	5	10	20	40	80
High climate	Current + 1.2	940 ± 50	0	5	10	20	40	80

The three climate regimes were created by manipulating the temperature and pCO₂ of filtered seawater (0.04 µm). The set temperatures were created by blending streams of 22 °C and 36 °C FSW with heat exchangers, which was followed by the pCO₂ manipulation. CO₂ gas was diluted in the passing through temperature-controlled seawater with a contactor. The water was then distributed between four 50 L replica aquaria for each of the three climate regimes. The aquaria were randomly positioned across the temperature-controlled laboratory to avoid room effects. Temperature and pH sensor data were automatically monitored and logged every 20 seconds by a SIMATIC WinCC SCADA system (Siemens AG, Munich, Germany). The temperature within the aquaria was additionally monitored with calibrated HOBO temperature-light data loggers (UA-002-64, Onset Computer Corporation, Adelaide, Australia). The pH was manually checked with a calibrated pH electrode (PHC201, HACH, Loveland, USA) once a week. Before and after the sediment deposition experiments, water samples were collected and preserved with mercury chloride for the determination of total alkalinity (TA) and dissolved inorganic carbon (DIC) using a Vindta 3C (Marianda, Kiel, Germany). The pCO₂ and the saturation state of calcite and aragonite (Ω_{Calc} and Ω_{Arag}) were calculated from the pH, TA, salinity and temperature data with the R package Seacarb v3.2.13 (Gattuso, 2020).

For the maintenance of accurate climate regimes, the flow passing through the aquaria was set to three turnovers per day. Water movement within every aquarium was created by a stream pump (Turbelle nanostream 6015, Tunze Aquarientechnik GmbH, Penzberg, Germany). A diurnal light cycle was applied (12:12 hour day and night) including a three-hour linear ramping time after sunrise and before sunset using one Hydra FiftyTwo HD LED (Aquaria Illumination, Allentown, USA) per aquarium. This resulted in a DLI of 4 mol m⁻² d⁻¹ which together with a calibrated light spectrum (JAZ spectrometer, Ocean Insight, Florida, USA) resembled conditions found at 4 m water depth in a typical inshore reef according to measurements at Middle Reef, Magnetic Island. Since the coral recruits were kept in 0.04 µm FSW, they were fed daily with 10 mL newly hatched *Artemia* sp. per 50 L flow-through aquarium.

2.2.3.4 Sediment treatments

The effects of sediment deposition on coral recruits developed under three climate regimes were tested with two simulated sediment deposition events. Based on measurements in calm inshore reefs, near river runoff and ≥2.5 km from dredging operations (Babcock & Smith, 2000; Jones et al., 2016; Nichols et al., 1990; Rogers, 1990; Tebbett et al., 2017), six sedimentation intensities ranging from 0 to 80 mg cm⁻² were simulated (Table 2.2.1). Coarse silt sediment (Median particle size: 53 – 75 µm) was used for the simulated depositions, which equates to the most common particle size (~60 µm) in close proximity (125 to 200 m) to dredging operations (Jones et al., 2016). The sediment was collected (Middle Reef, 19°11'45.8"S 146°48'50.2"E), dried and sieved by Duckworth, Giofre et al. (2017). The dried sediment and unfiltered seawater were used to create a sediment mixture, which was conditioned for two days in an aerated aquarium prior to the exposure to the coral recruits. Sediment samples were snap-frozen before and after exposure of the coral recruits and stored at -80°C for Total Organic Content (TOC) analysis.

Sediment depositions were simulated by enclosing four discs with settled recruits per sediment treatment with a transparent PVC tube (Ø = 96mm) (Figure 2.2.2a). Five sediment slurries and one control were created using the conditioned sediment mixture, FSW (0.04 µm) and a

nephelometer (Turbimax CUS31, Endress & Hauser, Reinach, Switzerland). The six sediment deposition treatments were generated by respectively pouring 100 mL of these well-mixed slurries into the transparent PVC tubes, and allowing the sediment to settle for 12 hours (Table 2.2.1, Figure 2.2.2b & c). Following the 12 hours, the upper part of the PVC tubes was removed to allow water circulation, with a 35 mm tall transparent PVC rim left around the discs (≥ 10 mm distance to discs) so that the sediment would not be immediately carried away by the current (Figure 2.2.2). Three days following the initiation of the sedimentation event, the transparent PVC rims were removed and the sediment remaining on the discs carefully collected by individually placing the discs in glass jars and gently spraying the sediment off with FSW. The cleaned discs were then placed back into the aquaria. The collected sediment of every disc was filtered through pre-weighed $0.4 \mu\text{m}$ polycarbonate filters, dried at 60°C for ≥ 24 h, and weighed (± 0.0001 g) to validate the amount of deposited sediment.

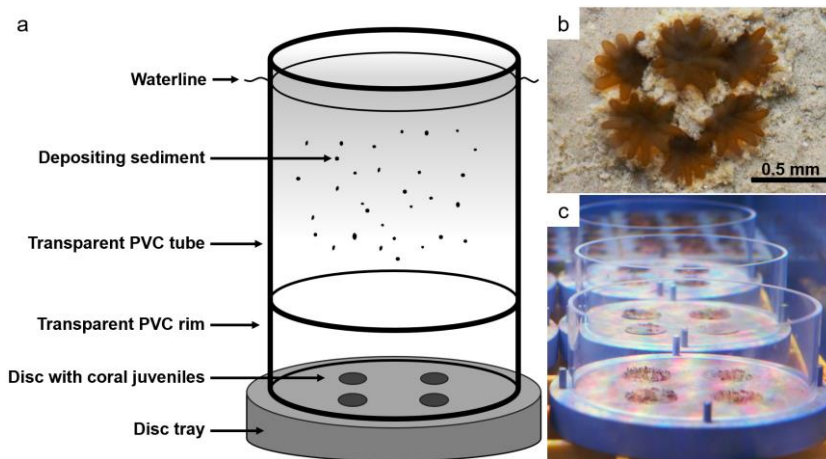


Figure 2.2.2: Experiment setup (a) setup during sediment application with a removeable transparent PVC tube guiding the sediment from the water surface to the discs with coral recruits, (b) ten weeks old coral recruit 4 h after being covered by 40 mg cm^{-2} sediment and (c) four discs with coral recruits placed in a disc tray. Sediment deposited on the entire surface surrounded by a transparent PVC rim.

2.2.3.5 Sampling

Coral survival and size (number of polyps) were documented fortnightly with high resolution images (Nikon D810 with Nikon AF-S 60mm f/2.8G ED macro lens, Tokyo, Japan). Four Ikelite DS161 strobes (Indianapolis, USA) were used to achieve an even light distribution, and the orientation of discs relative to the camera was kept constant for all images. The images of each disc were stacked to a time series and analysed to record recruit survival and number of polyps (as a proxy for recruit growth) using the software ImageJ Fiji (Version 1.52u). Coral recruits were excluded from the dataset if they fused with neighbouring recruits during settlement or throughout the experiment. Recruits were classified as alive when they featured at least one living polyp. Polyp growth was determined by calculating the number of polyps present at each time point minus 1, since all non-fused recruits have one polyp at settlement. Sediment removal was photographically documented one hour after the sediment was applied (STYLUS TG-3 Tough, Olympus, Tokyo, Japan). For the polyp growth and sediment removal analyses, only live recruits were investigated. Due to mortality following a first sediment exposure, 20% less recruits were included in the polyp growth and sediment removal analyses at a second sediment exposure (week 14 in experiment 1). The sediment level of 80 mg cm^{-2} was excluded from analyses of polyp growth because less than 3 recruits were alive in each of the treatment

groups at this sediment level. Overall, the fate of 2,582 *A. millepora* recruits were recorded over the duration of the experiment (n = 43 to 118 per sediment-climate treatment in each experiment).

2.2.3.6 Data analysis

Statistical analyses were performed using R (version 3.6.3) (R Core Team, 2020). Initial data exploration was conducted following the protocols in Zuur, Ieno et al. (2010) and subsets of the data were created based on experiment type and age. For binomial data (alive or not, sediment cleared or not), the response of recruits was summarised for every settlement disc. These subsets were separately analysed using Generalized Linear Mixed-Effects Models (GLMMs) with the package glmmTMB (version 1.0.1) (Brooks, Kristensen et al., 2017) and confidence intervals for the fitted models were calculated and plotted using R. For the polyp growth data (polyp counts of surviving corals) a Poisson model was applied and for the survivorship (percentage of survival) and sediment removal data (probability of sediment removal) a Binomial model was used. To account for varying numbers of recruits in each treatment, the response variable (i.e. survival) was weighted by combining it with the total number of recruits using the R core command 'cbind'. Sediment loads were treated as a continuous factor and climate scenarios were used as categorical fixed factors. The 'aquaria', 'disc trays', and 'discs' were incorporated as random factors. Overdispersion and model validation were assessed using simulation-based approaches with the package DHARMA (version 0.2.7) (Hartig, 2020). Using an Analysis of Deviance [Type II Wald chi-square test (χ^2)], statistically significant differences between the treatments were identified using the ANOVA and summary functions of the package car (version 3.0.7) (Fox & Weisberg, 2020). Lethal concentrations (LC₅₀) and effect concentrations (EC₅₀) that describe the sediment deposition levels that cause a 50% change in response from control (current climate at 0 mg cm⁻²) were also estimated from the GLMMs by calculating at what sediment load (intercept with x-axis) a 50% decrease in the control occurs (Blasco, Chapman et al., 2016). For each climate scenario, separate LC₅₀ were generated for the survival analysis and separate EC₅₀ for the sediment removal analysis.

2.2.4 Results

2.2.4.1 Coral recruit survival

Coral recruits raised under three climate scenarios were exposed to increasing levels of sediment deposition stress in two experiments: 1) two deposition events at five and ten weeks and 2) one deposition event at ten weeks following coral settlement. Recruit survival decreased significantly with increasing sediment load as well as with intensifying climate regime in both experiments, and these trends were observed for both ages of *A. millepora* recruits (Figure 2.2.3, Table 2.2.2). For example, sediment deposition alone (≥ 40 mg cm⁻²) significantly decreased the survival of recruits ($\chi^2 < 0.001$) in all climate regimes (Figure 2.2.3). In sediment-free conditions (0 mg cm⁻²), the high climate treatments also decreased the survival in comparison to current climate (Figure 2.2.3). For example, at week 9 in experiment 1, coral survival in 0 mg cm⁻² was 16.6% lower in the 2100 climate scenario in comparison to the same sediment treatment in the current climate regime (Figure 2.2.3). There was a sediment-climate interaction on coral recruit survival following the exposure of recruits to sediment for the first time in each experiment (week 9 in experiment 1 and week 14 in experiment 2) (Table 2.2.2). However, there was no interaction following a subsequent sediment exposure (week 14) in

experiment 1 (Table 2.2.2). In every case, the survival of recruits was lowest when the highest sediment deposition was combined with the high climate conditions.

The sediment deposition levels that caused 50% mortality (LC_{50}) decreased under future climate scenarios in both experiments (Figure 2.2.3, Table 2.2.3). A comparison of LC_{50} s suggests that coral recruits raised under high climate conditions were approximately twice as sensitive to sediment deposition than those raised under the current climate regime. Recruits raised under medium conditions exhibited intermediate sensitivity and this overall pattern was consistent in both experiments and for both age classes of recruits (Table 2.2.3). The sediment deposition LC_{50} s were similar (within a climate scenario) regardless of recruit age or whether there were one or two deposition events (Table 2.2.3). At the highest tested sediment deposition (80 mg cm^{-2}), the only recruits that survived were those raised under current climate conditions and exposed to sediment at ten weeks of age: $30 \pm 9\%$ (mean $\pm$ SE, Figure 2.2.3).

Table 2.2.2: Analysis of Deviance results for *Acropora millepora* recruit survival following continuous climate stress and exposure to sediment deposition at different ages. The data are shown following a four-week recovery phase after the sediment exposures. Interactions are shown with an * and significant results ($p < 0.05$) are in bold.

Experiment	Age	χ^2			n	
	Weeks	Sediment	Climate	Sediment * Climate	Number of discs per treatment	Number of recruits per disc
1	5	1 st sediment deposition				
	9	< 0.001	0.008	0.002	16	1 – 15
	10	2 nd sediment deposition				
	14	< 0.001	0.010	0.230	16	1 – 15
2	5	No sediment deposition				
	9		< 0.001		16	1 – 17
	10	1 st sediment deposition				
	14	< 0.001	0.001	0.003	16	1 – 17

Table 2.2.3: Sediment depositions that cause a 50% change in response ($\pm$ SE) of *Acropora millepora* recruits for survivorship (lethal concentration: LC_{50} , Figure 3) and sediment removal (effect concentrations: EC_{50} , Figure 5). The calculation of the lethal and effect concentrations for all climates and ages were based on the respective control (current climate at 0 mg cm^{-2}). Sediment removal capability was recorded one hour after the sediment deposition (experiment 1: week 5 and 10, experiment 2: week 10). Survival was quantified four weeks after the exposure to sedimentation (experiment 1: week 9 and 14, experiment 2: week 14).

	Experiment 1		Experiment 2	
	Survival	Sediment removal	Survival	Sediment removal
Climate	LC_{50} [mg cm^{-2}]	EC_{50} [mg cm^{-2}]	LC_{50} [mg cm^{-2}]	EC_{50} [mg cm^{-2}]
Current	1 st sediment deposition			
	45 ± 13	37 ± 11		

Medium	42 ± 13	31 ± 10		
High	24 ± 12	19 ± 10		
	2 nd sediment deposition		1 st sediment deposition	
Current	37 ± 10	>80	51 ± 6	51 ± 10
Medium	31 ± 9	71 ± 24	31 ± 5	32 ± 8
High	14 ± 9	61 ± 20	23 ± 6	41 ± 9

2.2.4.2 Polyp size

Acropora millepora recruits that survived until the end of the experiment were larger (had more live polyps) with increased sediment load (Figure 2.2.4). This effect was the same under all three climate treatments (no climate effect or interaction, Table 2.2.4). In experiment 1, corals surviving highest sediment deposition (40 mg cm⁻²) for the first time had 1.7-times more polyps than those that were not exposed to sediments (0 mg cm⁻², Figure 2.2.4). After the second deposition, the sediment-exposed survivors had 2.4-times more polyps in the 40 mg cm⁻² treatment. A similar trend of 1.3-times more polyps of survivors at higher sediment exposures was observed in experiment 2 (after a single exposure at 10 weeks) (Figure 2.2.4).

2.2.4.3 Sediment removal

The probability that recruits were able to completely remove sediments within an hour from their tissues decreased with increasing sediment loads. Sediment removal by recruits exposed to sedimentation for the first time was significantly impacted by the climate scenario and sediment load, independent of the recruit age (Table 2.2.5). Sediment deposition levels able to be cleared by 50% of recruits (EC₅₀) were the greatest for recruits grown under current climate conditions in each experiment (Table 2.2.2). The lowest sediment clearing probabilities were observed in the high climate scenario, with values at least 1.2-times lower compared to sediment removal in the current climate. Survivors of the first sedimentation showed a much greater ability to remove sediment during a subsequent deposition with EC₅₀ values doubling in all climates at the second deposition (Figure 2.2.5, Table 2.2.2).

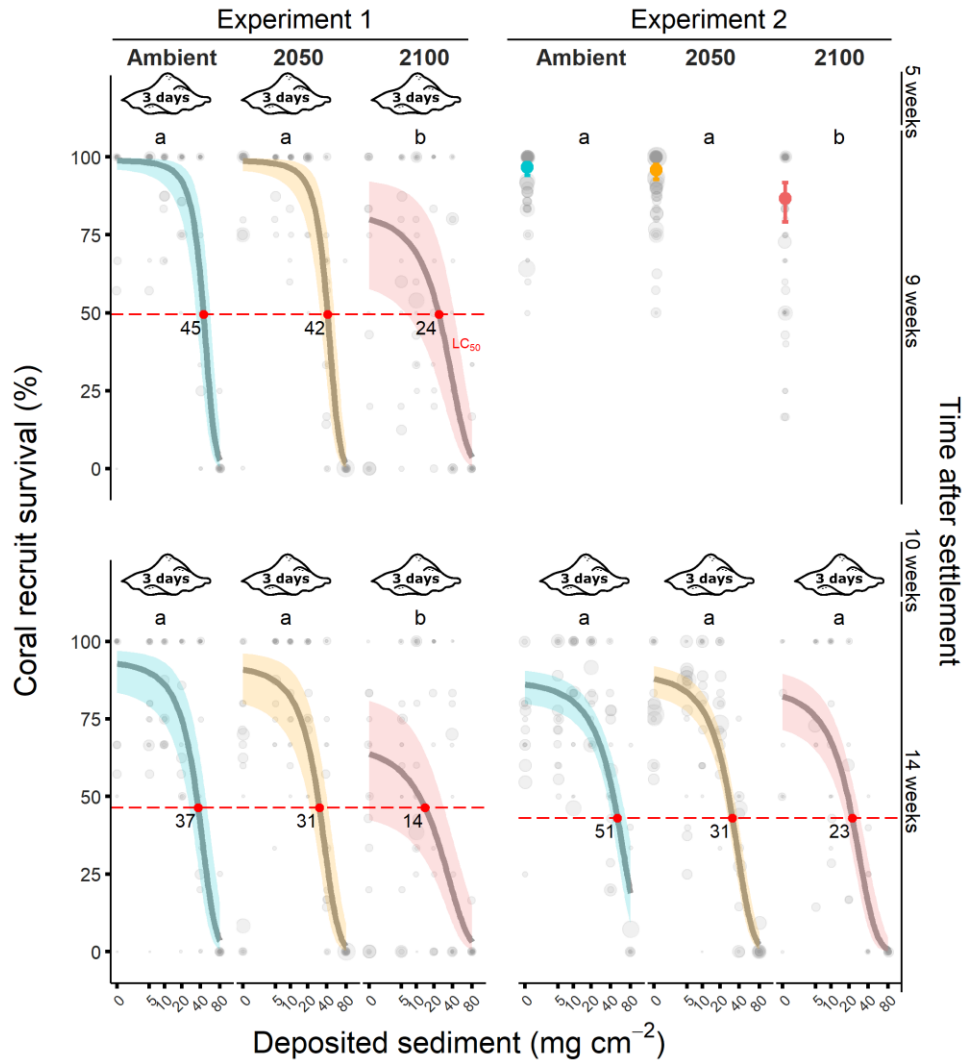


Figure 2.2.3: *Acropora millepora* recruit survival following continuous climate stress and exposure to three-day long sediment deposition events at different ages. Experiment 1: survival of coral recruits since settlement, which were exposed to six different sediment deposition intensities at five and ten weeks of age. Experiment 2: coral recruit exposure to sedimentation after ten weeks only. Ribbons and error bars show 95% confidence intervals of predicted mean sediment effects under each climate treatment. Grey bubbles are percentage survival of corals settled on the same settlement disc. The greater the bubble diameter, the more coral recruits were present per settlement disc. On the 16 settlement discs per climate-sediment treatment combination, numbers of recruits ranged from 1 to 17. Red dotted lines with annotation (sediment mg cm^{-2}) illustrate the Lethal Concentration of 50% (LC_{50}), which is based on the control (Current climate, 0 mg cm^{-2}) in each week. Different lowercase letters indicate significant differences between the climates in each week (Table 2.2.2).

Table 2.2.4: Analysis of Deviance results for *Acropora millepora* recruit size (number of polyps) following continuous climate stress and exposure to sediment deposition at different ages. The data are shown following a four-week recovery phase following the sediment exposures. Interactions are shown with an * and significant results ($p < 0.05$) are in bold.

Experiment	Age	χ^2			n	
	Weeks	Sediment	Climate	Sediment * Climate	Number of discs per treatment	Number of recruits per disc
1	5	1 st sediment deposition				
	9	< 0.001	0.093	0.080	16	1 – 10
	10	2 nd sediment deposition				
	14	< 0.001	0.428	0.651	16	1 – 9
2	5	No sediment deposition				
	9		0.083		16	1 – 17
	10	1 st sediment deposition				
	14	< 0.001	0.536	0.501	16	1 – 14

Table 2.2.5: Analysis of Deviance results for the probability that *Acropora millepora* recruits are sediment-free one hour following the exposure to sediment deposition. Interactions are indicated with an * and significant results ($p < 0.05$) are in bold.

Experiment	Age	Event	χ^2			n	
	Weeks		Sediment	Climate	Sediment * Climate	Number of discs per treatment	Number of recruits per disc
1	5	1 st sediment deposition	< 0.001	0.008	0.730	16	1 – 10
	10	2 nd sediment deposition	< 0.001	0.121	0.131	16	1 – 9
2	5	No sediment deposition					
	10	1 st sediment deposition	< 0.001	0.008	0.179	16	1 – 14

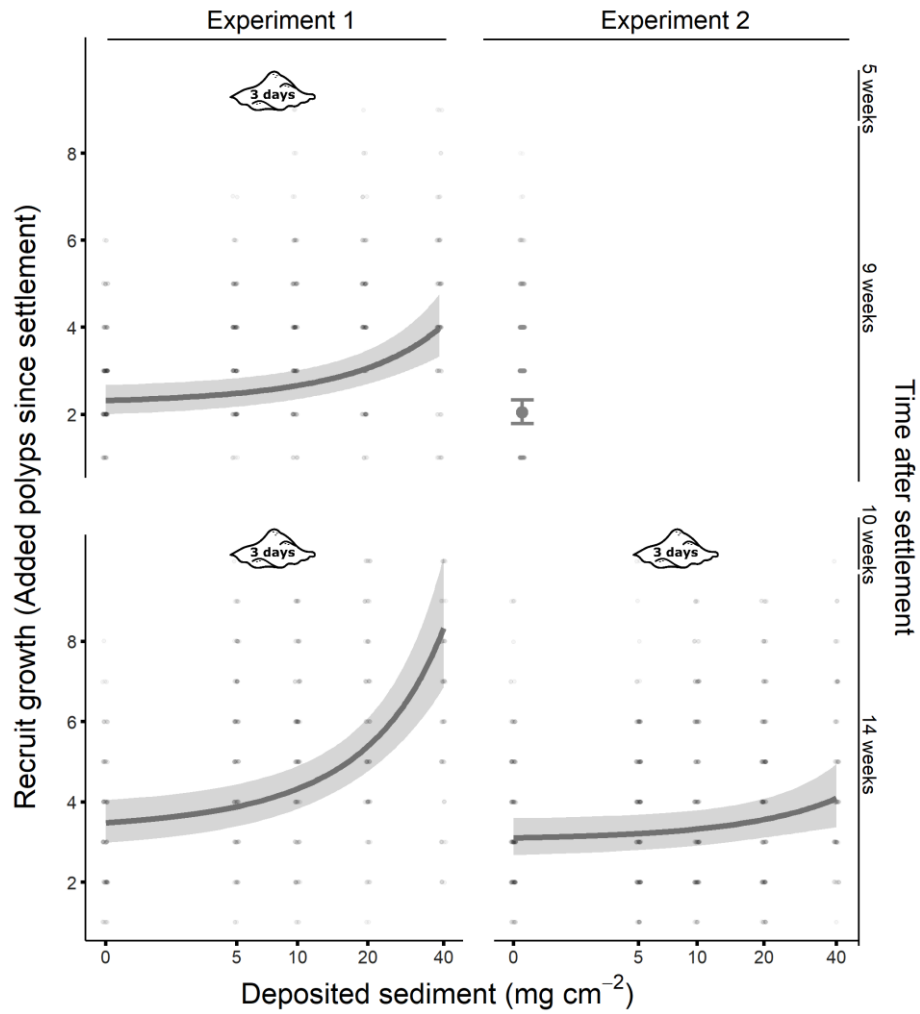


Figure 2.2.4: Relationship of sediment load and recruit size (number of polyps added since settlement) of surviving *Acropora millepora* recruits. Data shown following continuous climate stress and exposure to three-day long sediment deposition events at different ages. Experiment 1: coral recruits smothered five and ten weeks after settlement. Experiment 2: coral recruits were only exposed to sedimentation after ten weeks. Ribbons and error bars show 95% confidence intervals. Grey points are polyp growth raw data.

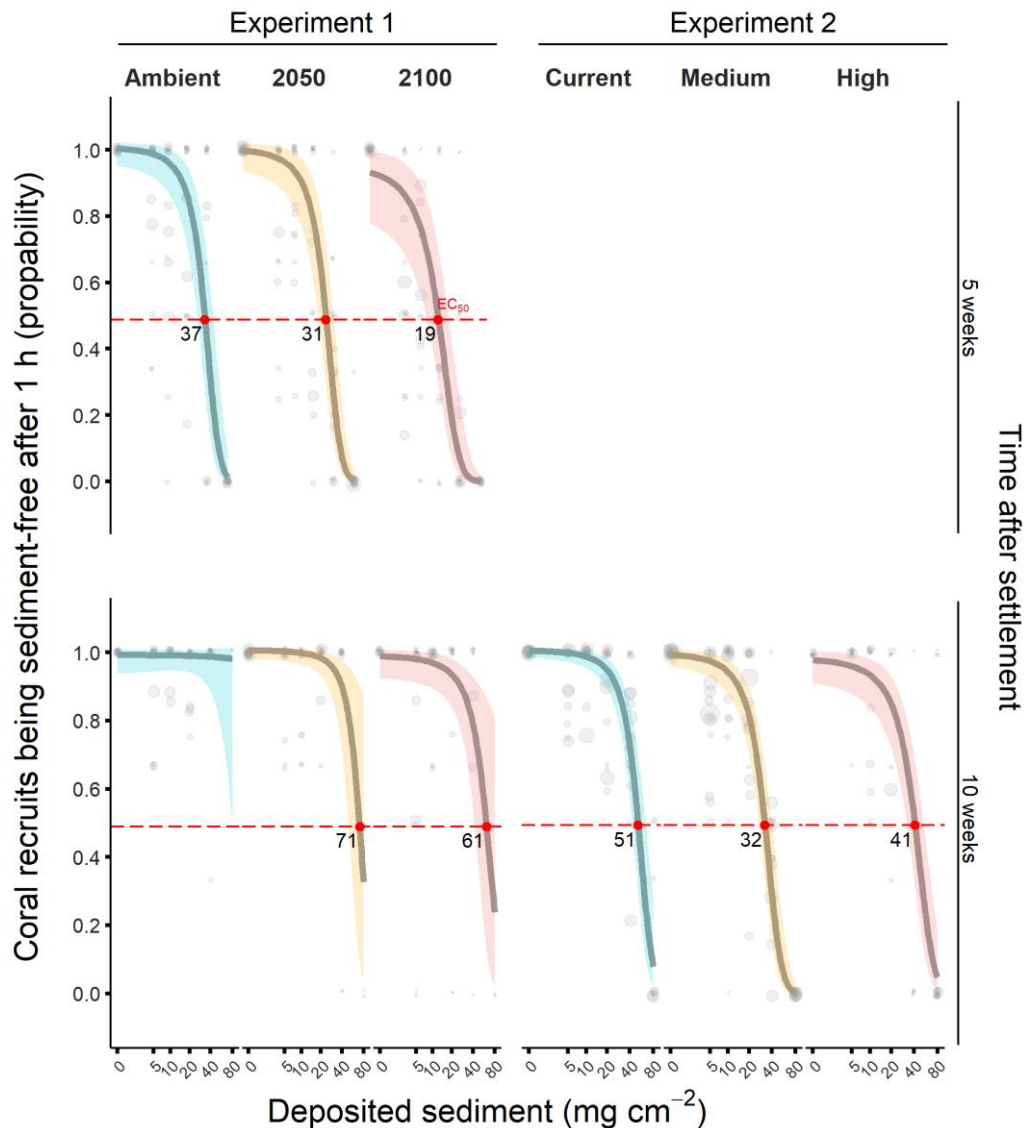


Figure 2.2.5: Probability that *Acropora millepora* recruits grown in three climate scenarios are clear of sediment one hour following the exposure to different sediment loads. Experiment 1: coral recruits smothered with sediment five and ten weeks after settlement. Experiment 2: coral recruit exposure to sedimentation after ten weeks only. Ribbons and error bars show 95% confidence intervals. Grey bubbles are percentage of recruits on each disc that had successfully removed sediment after 1 hour. The greater the bubble diameter, the more coral recruits were present per settlement disc. Red dotted lines with annotation (sediment mg cm⁻²) illustrate the Absolute Effect Concentration of 50% (EC₅₀), which is based on a probability of 0.5.

2.2.5 Discussion

Acropora millepora recruits were more vulnerable to sediment deposition stress when raised under future climate scenarios. This same general trend was apparent at both recruit ages tested, and whether recruits were exposed to sediment depositions once (experiment 2) or twice (experiment 1). In high climate regimes, recruits were approximately twice as sensitive to sediment deposition than those raised under the current climate scenario. A reduced ability of recruits to clear sediments when raised under future climate scenarios may have led to the higher sedimentation-related mortality under 'medium' and 'high' OA and OW conditions. However, the specific nature of the climate and the timing of sedimentation also influenced recruit survival. Although approximately twice as many polyps were found on survivors of an

elevated sediment level (40 mg cm^{-2}), recruit survival in the greatest tested sediment load (80 mg cm^{-2}) was only observed for corals raised in current climate and which were not exposed to sedimentation until ten weeks of age. This study is the first to demonstrate that sediment deposition thresholds for recruit survival are affected by realistic future climate conditions. Consequently, as was suggested for other contaminants (e.g. Negri, Smith et al. (2020) for copper and the herbicide diuron) and in other chapters in this report (Chapter 2.3 and Chapter 2.4), the water quality guidelines values for sediments on coral reefs will need to be adjusted to account for extra risk to recruits as the climate changes.

2.2.5.1 Effects of future climate scenarios on recruits

Pressures related to future climate scenarios can affect the survival of recruits over the first months post-settlement. In the present study the highest climate regime (temperature: $+1.2^\circ\text{C}$ to 29.9°C , pCO_2 : 950 ppm) decreased recruit survival by 17% nine weeks after settlement, and by 32% fourteen weeks after settlement in comparison to current climate conditions, even in the absence of sediments. Previous studies illustrated that elevated temperature alone can lead to a $>20\%$ decrease in survival of *P. astreoides* recruits [$+4^\circ\text{C}$ above ambient, to 30°C ; Fourney and Figueiredo (2017)]. Coral mortality following thermal stress events was reported for adult corals, as it is linked to both acute oxidative stress in the host and symbiont as well as energy limitation in heavily bleached colonies (Lough & van Oppen, 2018). The effects of OA on the health of coral recruits may also have contributed to mortality as calcification becomes more difficult at low aragonite saturation levels, potentially drawing upon the recruit's limited energy reserves (Albright, Mason et al., 2008). Other studies have also reported negative synergistic interactions of OW and OA on calcification in *Porites panamensis* recruits ($+1^\circ\text{C}$ to 29.6°C and pH reduced by -0.2 to -0.25) (Anlauf, D'Croz et al., 2011). There is no clear consensus about cumulative effects of OW and OA on adult corals. For example, Anderson, Cantin et al. (2019) showed that temperature stress ($+2.5^\circ\text{C}$ to 31°C) had a greater impact on the survival of *Acropora* spp. than OA alone or in combination with OW. However, other studies found that for adult corals the combined exposure to OA and OW generally leads to additive impacts on calcification and survival (McCulloch, Falter et al., 2012; Reynaud, Leclercq et al., 2003; Rodolfo-Metalpa, Houlbrèque et al., 2011). Further studies are therefore required to elucidate the conditions under which future OA and OW cause corals, and particularly coral recruits, to be more susceptible to additional stressors.

2.2.5.2 Effects of sediments on recruits

Corals are able to actively remove sediments from their tissues via ciliary activity, hydrostatic expansion, tentacle movement and mucus production (Bessell-Browne, Fisher et al., 2017a; Hubbard & Pocock, 1972; Ricardo, Jones et al., 2016; Stafford-Smith & Ormond, 1992). Despite this diversity of sediment-removal mechanisms, the sediment deposition events in the current study caused concentration-dependent effects on survival in recruits, even when raised in current climate conditions. For example, only half of the five-week-old recruits were able to survive the single sediment deposition pulse of $45 \text{ mg cm}^{-2} \text{ day}^{-1}$ ($=\text{LD}_{50}$). Other studies have also reported mortality in *Leptastrea purpurea* and *Acropora hyacinthus* recruits of different ages at lower sediment depositions repeated every 3 days over several weeks (Moeller, Nietzer et al., 2017). Five-week old recruits in this study were able to remove half the deposited sediment at $37 \text{ mg cm}^2 \text{ day}^{-1}$ ($=\text{EC}_{50}$) after one hour, a concentration very similar to the LD_{50} for the same recruits, indicating that sediment deposition which overcomes early removal processes is the main driver of mortality. Recruits which survived high sediment depositions

were on average larger (more polyps), indicating greater mortality of small recruits with less polyps and a greater capacity of the larger recruits to clear sediments. Indeed, the older, larger recruits exposed to sediments for the first time at ten weeks were more resistant to sediment deposition (higher LD₅₀ and EC₅₀ for sediment clearance) than those exposed at five weeks. The greater vulnerability of early recruits to sediments was also reported for *L. purpurea* and *A. hyacinthus* (Moeller et al., 2017), highlighting the importance of rapid recruits growth to survive sediment deposition events. This ‘escape by size’ from sediment deposition effects also has important implications for managing the timing of anthropogenic activities that increase sediment loads in seawater adjacent to coral reefs.

2.2.5.3 Effects of future climate scenarios on sediment deposition thresholds for coral recruits

In the present study, the recruits raised under future climate scenarios were more vulnerable to sediment deposition stress. Indeed, the lethal sediment deposition levels (LC₅₀) for recruits showed consistent decreases by about 50% under high future climate conditions for both five- and ten-week old recruits and after one or two deposition events. There was also a general trend of reduced sediment clearance under future climate scenarios, potentially explaining this increasing vulnerability. No other studies have tested the influence of realistic future climate scenarios on thresholds for recruit survival under sediment deposition. However, one other study found increased mortality in *Porites astreoides* recruits exposed to 30 mg cm⁻² of fine port sediment when temperatures were increased by 4°C to 30°C, and concluded that the effects of sediments and temperature stress were additive (Fourney & Figueiredo, 2017). This finding is consistent with our findings, but further studies using different combinations of stressors in a multitude of environments is required to understand potential interactions between climate and sediment impacts more broadly.

The recruits we raised under future climate conditions exhibited signs of stress, even in the absence of sediment deposition (0 mg cm⁻²), which is particularly evident in the high future climate scenarios. For example, the ten-week old recruits in the high scenario had a 29% reduction in survival at 0 mg cm⁻² compared with current climate conditions. When facing their first exposure to sediments, the LD₅₀ dropped from 51 mg cm⁻² in current conditions to 23 mg cm⁻² for recruits raised under the high climate scenario. The ability of recruits to immediately clear deposited sediment decreased significantly in future climate change scenarios and smothering of these polyps for longer durations is the likely cause of increased mortality. This result is consistent with a previous study in which the thermally bleached adult corals *A. millepora* (branching), *Porites* spp. (massive) and *Turbinaria reniformis* (plating) showed a 3 to 4-fold lower sediment clearance compared to corals not affected by temperature (Bessell-Browne et al., 2017a). The only *A. millepora* recruits to survive (30 ± 9% SE) in the greatest sediment load tested here (80 mg cm⁻²) were raised in current climate conditions and not exposed to sediment until ten weeks old (experiment 2 in Figure 2.2.3). This response underscores the influence of age (and size) as well as pressure from future climate conditions on the vulnerability of coral recruits.

2.2.5.4 Ecological relevance and application

The future climate scenarios applied in the current experiments were realistic with the high scenario representing the likely increase in pCO₂ by 2100 under RCP8.5 (IPCC, 2014b), while the temperature increase of +1.2°C for ten weeks represents 8 degree heating weeks, already

observed on coral reefs during bleaching events (Hughes et al., 2017b). The study examined the effects of a single inshore reef-relevant sediment type and size class and the effect thresholds may not be valid for all sediments. Recent studies have reported the composition and diameter of sediments has a major influence on thresholds for coral fertilisation (Ricardo, Jones et al., 2018) but not on coral larval settlement (Ricardo et al., 2017). The sediment type and size is also likely to affect coral recruit mortality (Jones et al., 2015), with Fourney and Figueiredo (2017) reporting little effect of $\geq 30 \text{ mg cm}^{-2}$ depositions of coarse reef sediment (average diameter: 500 – 2000 μm) on small *P. astreoides* recruits ($\sim 1000 \mu\text{m}$ diameter), whereas $\geq 60 \text{ mg cm}^{-2}$ of their applied fine port sediment (average diameter: 63 – 500 μm) showed significant effects on recruit survival. More studies with a diversity of species and sediment types are needed to address this issue, but it is important that they also apply sufficient levels of sediment stress to derive LC_{50} and EC_{50} values which can then be compared between studies and climate conditions, as well as being directly applied in risk assessments and to derive water quality guideline values (Simpson, Batley et al., 2013).

In summary, our experiments have shown that cumulative climate and sedimentation stress are having detrimental impacts on the survival and sediment clearance capability of coral recruits. The climate and timing of sediment exposure following coral settlement are important factors, as only ten weeks old recruits grown in current climate were able to survive deposition levels of (80 mg cm^{-2}) frequently observed near dredging works. Since post-settlement survival is seen as bottleneck in the lifecycle of scleractinian corals (Jones et al., 2015; Randall, Negri et al., 2020; Ritson-Williams et al., 2009), our results stress the importance of sustainable water quality management efforts for both dredging and land-based runoff, particularly in times of changing climate.

2.3 Example 2: Combined effects of climate change and the herbicide diuron on the coral *Acropora millepora*

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Manuscript in preparation

2.3.1 Summary

The Great Barrier Reef (GBR) is threatened by climate change and local pressures including contaminants in nearshore habitats. This study investigated the combined effects of a GBR-relevant contaminant, the herbicide diuron, under three climate scenarios on the coral *Acropora millepora*. All physiological responses tested (effective quantum yield ($\Delta F/F_m'$), photosynthesis, daily calcification rate) were negatively affected with increasing concentrations of diuron. Interactive effects between diuron and climate were observed for all responses; however, climate had no significant effect on $\Delta F/F_m'$ or daily calcification rates. On the contrary, photosynthesis was negatively affected as the climate scenarios were adjusted from ambient (28.1°C, $p\text{CO}_2 = 397 \mu\text{atm}$) to RCP8.5 2050 (29.1°C, $p\text{CO}_2 = 680 \mu\text{atm}$) and 2100 (30.2°C, $p\text{CO}_2 = 858 \mu\text{atm}$) with EC50 values declining from 19.4 to 10.6 and 2.6 $\mu\text{g L}^{-1}$ diuron in turn. These results highlight the likelihood that water quality guideline values will need to be adjusted as the climate changes.

2.3.2 Introduction

Climate change, including ocean warming (OW) and ocean acidification (OA), is a major threat to coral reefs worldwide (Doney, Fabry et al., 2009; Hoegh-Guldberg et al., 2007; Hughes, Anderson et al., 2018a). Since the pre-industrial period, the global average temperature has increased by about 1°C (IPCC, 2014c; Lough, Anderson et al., 2018) with a projected increase of 1-3°C by 2100 (Collins et al., 2013b). The present-day atmospheric CO_2 ($p\text{CO}_2$) is approximately 400 μatm , with a projected rise to 750-900 μatm by the end of the century (Meinshausen, Smith et al., 2011a). About a quarter of the CO_2 emitted from anthropogenic sources enters the ocean to produce carbonic acid (Ganadell, Le Que´re´ et al., 2007; Hoegh-Guldberg et al., 2007), which leads to a reduction in available carbonate ions to marine calcifying organisms.

Many studies have investigated the effects of both OW and OA on marine calcifying organisms, including tropical reef-building corals (Brierley & Kingsford, 2009; Edmunds, Brown et al., 2012; Hoegh-Guldberg et al., 2017). One response of corals due to OW is bleaching which is the breakdown in the symbiotic relationship between the coral host and algal symbiont (Goreau & Hayes, 1994), and typically occurs when seawater temperatures are ~1.5°C above their long-term monthly summer averages for several weeks (Hughes et al., 2018a). Bleaching events are becoming more frequent and severe and can negatively affect reproduction (Baird & Marshall, 2002) and cause disease (Bruno, Selig et al., 2007), with prolonged and severe bleaching often leading to large-scale coral mortality events (Hughes et al., 2017b). OA can also affect corals in a variety of ways, including reduced calcification (Langdon, 2002; Schneider & Erez, 2006) and reduced net productivity (Anthony, Kline et al., 2008) as well as

having direct and indirect impacts on recruitment (Albright et al., 2008; Anthony, Maynard et al., 2010).

In addition to the risks posed by climate change, inshore coral reefs face a suite of local pressures, including poor water quality caused by coastal development and agricultural activities. Along with increased sediment and nutrient loads, pesticides, including herbicides, contaminate nearshore ecosystems globally (Bao, Maruya et al., 2012), including the Great Barrier Reef (GBR) (O'Brien, Lewis et al., 2016; RWQIP, 2018b). During the summer wet season, flood plumes can transport large amounts of agricultural herbicides ($> 17,000$ kg) to the GBR (Brodie, Lewis et al., 2017b). However, due to frequent application and persistence of these herbicides, they have been detected year-round (Gallen, Thai et al., 2019; Mercurio, Mueller et al., 2016). Photosystem II (PSII) herbicides, including diuron, are the most frequently detected herbicides in the GBR lagoon (Gallen et al., 2019; Lewis, Brodie et al., 2009; Warne, Smith et al., 2020) and act by blocking the plastoquinone binding site of photosystem II, which disrupts electron transport flow and inhibits photosynthesis, in turn limiting carbon fixation (Oettmeier, 1992). While concentrations of PSII herbicides are highest in freshwater and estuarine ecosystems (RWQIP, 2018a) concentrations of diuron of up to $0.778 \mu\text{g L}^{-1}$ (average measured concentration over one-month passive sampler deployment) have recently been detected around a nearshore island of the GBR (Gallen et al., 2019), exceeding the proposed PC99 water quality guideline value (WQGV) for diuron ($0.43 \mu\text{g L}^{-1}$; protection concentration for 99% of species) (Warne, Batley et al., 2018a) and indicating exposure to nearshore species. Several studies have investigated the effects of diuron on tropical marine phototrophs, including seagrass (Flores, Collier et al., 2013; Wilkinson et al., 2017), microalgae (Magnusson, Heimann et al., 2008), coral (Jones & Kerswell, 2003; Negri, Flores et al., 2011) and foraminifera (van Dam, Negri et al., 2012a). Due to its likely exposure and the extensive toxicity dataset for diuron (Warne, King et al., 2018b) this PSII herbicide is commonly used as a reference toxicant for toxicity bioassays (Thomas, Flores et al., 2020a; Thomas, Flores et al., 2020b; Wilkinson, Collier et al., 2015).

PSII herbicide concentrations in tropical waters are typically highest over the summer wet season (Gallen et al., 2019; Smith et al., 2012); therefore, it is likely that nearshore tropical species (including corals) will be co-exposed to herbicides and high seasonal water temperatures. Studies have already shown that diuron toxicity to corals and their symbionts (Negri et al., 2011) foraminifera (van Dam et al., 2012a) and seagrass (Wilkinson et al., 2017) occurs at lower concentrations as temperatures increase. However, this past research has primarily focussed on the effects of PSII herbicides on chlorophyll a fluorescence endpoints, such as inhibition of effective quantum yield ($\Delta F/F_m'$), which is proportional to photosynthetic efficiency but may not represent whole-organism responses in symbiotic corals (Ralph, Smith et al., 2007). Furthermore, apart from a single study with calcifying tropical marine algae *Halimeda* sp., Marques et al. (2020b), previous investigations on PSII herbicide toxicity to tropical species only tested the influence of OW applicable to current heatwave conditions (Negri et al., 2011) and have not considered the potential influence of combinations of OW and OA relevant to future climate change scenarios.

This is the first study to investigate the influence of future climate change conditions (2050 and 2100 RCP 8.5 scenario predictions (IPCC, 2014c; Meinshausen et al., 2011a)) on the toxicity of the GBR-relevant herbicide diuron to a model coral species *Acropora millepora*. The measured responses included photosynthetic efficiency ($\Delta F/F_m'$), net photosynthesis rates,

net calcification rates (as a proxy for coral growth) and survival. Holistic experimental approaches such as this are well suited to inform regulators on the potential need to adjust water quality guideline values for contaminants as the climate changes (Negri et al., 2020).

2.3.3 Materials and Methods

2.3.3.1 Sample collection

Four adult colonies (30-40 cm diameter) of *Acropora millepora* were collected from Backnumbers Reef (18°29.264 S, 147°09.174 E), GBR under Great Barrier Reef Marine Park Authority (GBRMPA) permit G12/35236.1. Colonies were transported to the National Sea Simulator (SeaSim) at the Australian Institute of Marine Science (AIMS) in Townsville and maintained in 1700 L flow-through tanks. Terminal portions of *A. millepora* branches (~2 cm height) were fragmented and affixed on marked glass tiles and maintained in flow-through filtered seawater at $27 \pm 1^\circ\text{C}$ under $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ for at least one week prior to experimentation. Coral fragments were fed daily with *Artemia* nauplii ($0.5 \text{ nauplii mL}^{-1}$) during the healing period; however, corals were not fed during the duration of the two-week experiment due to possible effects on water quality within the experimental chambers.

2.3.3.2 Experimental setup

Acropora millepora were exposed to three climate change scenarios (OW + OA): $28.1 \pm 0.3^\circ\text{C}$ and $397 \pm 64 \text{ ppm CO}_2$, $29.1 \pm 0.3^\circ\text{C}$ and $680 \pm 90 \text{ ppm CO}_2$, $30.2 \pm 0.3^\circ\text{C}$ and $858 \pm 149 \text{ ppm CO}_2$ (details are given in Table 1), and six concentrations of diuron (measured: 0, 0.29, 0.96, 2.9, 9.6 and $29 \mu\text{g L}^{-1}$, Appendix 2, Table A1), including a solvent control, in an orthogonal design. Corals ($n=4$ replicate coral fragments per chamber) were placed in custom 3 L acrylic chambers (15 cm diameter x 19 cm height, working volume 2.5 L) in temperature-controlled water baths with the appropriate concentration of $p\text{CO}_2$ gently bubbling directly in each chamber. Due to a limited number of experimental chambers, there were no seawater controls (only solvent controls) and there was a 7-day staggered start for corals at $29 \mu\text{g L}^{-1}$ diuron to maximise number of diuron treatments. $p\text{CO}_2$ dosing was achieved via mass flow controllers (Aalborg GFC17) delivering a precise flow of CO_2 to a series of membrane contactors (3M Liqui-Cel Extra-Flow 2.5X8). Analytical grade diuron (> 98% purity) was purchased from Sigma-Aldrich (NSW, Australia) and stock solutions (10 mg L^{-1}) were prepared in filtered seawater ($0.4 \mu\text{m}$) using ethanol as a solvent carrier (< 0.03% v/v). Header tanks (60 L) were filled daily with filtered seawater ($1 \mu\text{m}$) and spiked with the appropriate volume of diuron stock to achieve target diuron concentrations. Diuron was delivered from the header tanks to the experimental chambers using peristaltic pumps (Masterflex L/S and Ismatec IPC 12) for a turnover rate of 1-1.5 times per day. Three independent replicate chambers were used for each diuron concentration and each climate change scenario (a total of 54 chambers). Chambers were randomised within their respective water baths to minimise potential systematic effects of chamber position.

Corals were illuminated over 13 h cycles (Aqua Illumination LED Hydra) with ramping up of light intensity for the first three hours to approximately $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ then down to darkness over the last three hours (equivalent to $7 \text{ mol m}^{-2} \text{d}^{-1}$ daily light integral, DLI). Irradiance was measured with a Licor LI-250A meter with LI-190R quantum sensor. Physico-chemical parameters (pH, salinity and dissolved oxygen) were measured at least four times during the duration of the 15-d experiment (Table 2.3.1). Water temperature and $p\text{CO}_2$ levels were

controlled by a programmable logic controller (PLC) and measured every 10 minutes (Table 2.3.1). Salinity was measured via a handheld meter (Horiba LAQUAact PC110), pH was measured with a multimeter (HQ40d equipped with Intellical PHC301 pH electrode (Hach, USA)) and oxygen concentration was determined with a handheld meter (HQ30d equipped with Intellical LDO101 oxygen probe (Hach, USA)).

2.3.3.3 Chemical analysis

Analytical samples (two concentrations per climate scenario) were taken at initiation and termination of experiment. Aliquots (1 mL) were transferred into 1.5 mL liquid chromatography amber glass vials and spiked with a diuron surrogate standard (diuron-d6; stock solution of 1 $\mu\text{g mL}^{-1}$), of which the final concentration of the surrogate standard was 10 ng mL^{-1} . Diuron concentrations were determined by HPLC-MS/MS (SCIEX Triple QuadTM 6500 QTRAP[®] mass spectrometer Shimadzu Nexera X2 μHPLC system) at the Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland (Mercurio, Mueller et al., 2015; Thomas et al., 2020a). The Sciex software package Analyst Software 1.6 was used for data acquisition and MultiQuant 3.0 was used for quantification. The measured concentrations were calculated based on the linear relationship between nominal and the time weighted average (time = 0 and 15 d) of measured concentrations.

2.3.3.4 Seawater carbonate system parameters

Dissolved inorganic carbon (DIC) and total alkalinity (A_T) were determined on a Vindta 3C system (Marianda, Kiel, Germany) from seawater samples taken at the end of the experiment (Table 2.3.1). DIC and A_T were used to calculate carbonate system parameters (calcite saturation state, Ω_{Ca} , and aragonite saturation state, Ω_{arag}) according to (Mehrbach, Culberson et al., 1973) with modifications from (Dickson & Millero, 1987) using CO2sys software (Pierrot, Lewis et al., 2006).

2.3.3.5 Chlorophyll fluorescence

Effective quantum yield ($\Delta F/F_m'$) measurements were taken just prior to the start of exposure and at 7 and 14 days exposure using a pulse amplitude modulation fluorometer (mini PAM, WALZ, Germany; settings: ML = 5, G = 2, D = 2, SI = 8). Two measurements per coral were obtained using a 6-mm fibre optic probe perpendicular to the surface of the coral. Minimum fluorescence was determined by applying a pulse-modulated red measuring light (650 nm, 0.15 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$). A short pulse (800 ms) of saturating actinic light ($>3000 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) was applied to measure light adapted maximum fluorescence. Effective quantum yield was calculated based on F and F_m' (Genty, Briantais et al., 1989): $\Delta F/F_m' = (F_m' - F)/F_m'$.

2.3.3.6 Net photosynthesis and calcification rates

Coral ($n=3$ replicate corals per treatment) sed for incubations were chosen at random. Oxygen production and respiration rates, and light and dark calcification rates were determined in corals after 12-15 d exposure for the 0, 0.29, 0.96 and 2.9 $\mu\text{g L}^{-1}$ diuron treatments with three replicate coral fragments per treatment. Corals exposed to 9.6 $\mu\text{g L}^{-1}$ diuron died after 9 days of exposure, irrespective of the climate scenario, therefore rates of oxygen production, respiration and calcification could not be determined for these individuals. Since there was a

staggered start for the corals in the 29 $\mu\text{g L}^{-1}$ diuron treatments, these physiological processes were measured after 7-8 d exposure.

To assess net photosynthesis and calcification rates, corals were incubated in the light and dark with respective $p\text{CO}_2$ seawater and diuron treatments in 50 mL clear acrylic chambers for 3 h under experimental light conditions and 4 h dark incubations. Incubation chambers were placed on custom underwater stirrer plates under the same light and water temperature as the experimental chambers. After each incubation, dissolved oxygen concentration (DO) was immediately measured in each incubation chamber including two blank incubations per treatment as per Strahl et al., (2015). Following dark incubations, corals were snap frozen in liquid nitrogen for future determination of surface area and volume. Surface area of *A. millepora* colonies were determined via the wax dipping method (Stimson & Kinzie, 1991) and volume of coral was determined using water displacement as per Strahl, Stolz et al. (2015). Oxygen production and respiration rates (in $\text{mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) were normalized to surface area:

$$\text{Oxygen production and respiration rates} = \frac{\Delta\text{DO} \times V_{\text{chamber}} - V_{\text{coral}}}{\text{SA} \times T \times 1000}$$

where ΔDO is the difference in DO of blank incubations and incubations with coral (in mg L^{-1}); V_{chamber} is the volume of the incubation chamber (in mL); V_{coral} is the displacement volume of the coral (in mL); SA is the surface area of the coral (in cm^2); and T is the incubation duration (in hours).

Incubation water samples were collected immediately in 50 mL conical centrifuge tubes and fixed with mercuric chloride and analysed for A_T to determine calcification rates by the alkalinity anomaly technique (Chisholm and Gattuso, 1991). A_T was measured on a Metrohm 855 robotic titrosampler (Metrohm, Herisau, Switzerland) by gran titration using 0.5 mol L^{-1} hydrochloric acid using multiple seawater standards and certified reference material (CRM Batch 164 and 174, A.G. Dickson, Scripps Institution of Oceanography, San Diego, CA, USA) as described in Uthicke and Fabricius (2012). Light and dark calcification rates were normalized to surface area:

$$\text{Calcification (in } \mu\text{mol CaCO}_3 \text{ cm}^{-2} \text{ h}^{-1}\text{)} = \frac{\frac{\Delta\text{TA}}{2} \times (V_{\text{chamber}} - V_{\text{coral}}) \times 1.028}{\text{SA} \times T \times 1000}$$

where ΔTA (in $\mu\text{mol kg SW}^{-1}$); V_{chamber} is the volume of the incubation chamber (in mL); V_{coral} is the displacement volume of the coral (in mL); 1.028 is the density of seawater (in kg L^{-1}); SA is the surface area of the coral (in cm^2); and T is the incubation duration (in hours) (Cohen, Krueger et al., 2017).

Oxygen production and respiration rates were used to calculate daily net photosynthesis and light and dark calcification rates for daily net calcification rates using 13 h of daylight and 11 h of darkness.

2.3.3.7 Bleaching and mortality

Coral fragments were photographed at initiation and termination of the experiment using a high-resolution digital camera (Nikon D810 with a 60-mm lens and a Nikon Speedlight SB-910 flash). Bleaching was assessed via changes in coral colour as per Bessell-Browne et al. (2017b) using ImageJ2 (Rueden, Schindelin et al., 2017). A random 0.5 cm^2 square surface

area representative of the colour of the coral was taken to assess bleaching. Partial mortality was also quantified from these images using ImageJ2.

2.3.3.8 Data analysis

Generalized linear mixed models (GLMM) were fitted for each of the coral responses ($\Delta F/F_m'$, net photosynthesis, daily net calcification) with 'Climate' and 'Diuron' as the main fixed effects and 'Fragment' as the categorical random factor in R 4.0.3 using the package glmmTMB (Brooks et al., 2017; RStudio, 2018). Chlorophyll fluorescence data was modelled as a beta distribution using a logit link function. Dunnett's test was used to test for comparisons against the control (2018 climate scenario, 0 $\mu\text{g L}^{-1}$ diuron). Linear regression models were plotted in GraphPad Prism (V7).

Concentrations that inhibited $\Delta F/F_m'$, net photosynthesis and calcification by 50% (EC50) were estimated in R using the package bayesNEC (Fisher, Ricardo et al., 2019). This package uses a model averaging approach based on DIC weighted averaged predictions across a potential candidate model set composed of a range of functional NEC models adapted from Fox (2010), along with several more commonly used concentration-response relationship models. In all cases model fit was assessed visually through chain mixing, R hat values and an assessment of the number of divergent transitions. Where models showed poor model fit, they were excluded from model averaged estimates of endpoints. EC10 and EC50 values were calculated using WAIC weighted model averaged estimates of all successfully fitted models.

$\Delta F/F_m'$ was modelled as a function of log diuron using Bayesian non-linear models, with the response modelled as a beta distribution. Due to multiple 0 $\Delta F/F_m'$ values for the higher diuron concentrations and since beta distribution are defined on the interval > 0 and < 1 , $\Delta F/F_m'$ was transformed as per Smithson and Verkuilen (2006) whereby if y assumes extremes 0 and 1, a useful transformation is: $(y * (n - 1) + 0.5) / n$ where n is the sample size. The final models from bayesnec used for $\Delta F/F_m'$ 2018 and 2050 included: nec4param, nechorme4, neclin, neclinhorme, ecxlin, ecx4param, ecxwb1, ecxwb2. Final models for $\Delta F/F_m'$ 2100 were neclin, neclinhorme, ecxlin, ecx4param, ecxwb1, ecxwb2. All models used for $\Delta F/F_m'$ were weighted using pseudo Bayesian Model Averaging method 'pseudobma' via the loo package (Vehtari, Gabry et al., 2020; Vehtari, Gelman et al., 2017) with the BB option set to TRUE (i.e. using Bayesian bootstrapping). Both net photosynthesis and daily calcification rates were modelled as a function of log diuron with the response modelled as a gaussian distribution. The final models used for net photosynthesis for 2018 were: nec4param, nechorme4, neclin, neclinhorme, ecxlin, ecxwb1, ecxwb2 while the final models for 2100 were neclin, neclinhorme, ecxlin, ecx4param, ecxwb1, ecxwb2. Final models for 2050 were the same as 2018 with the inclusion of ecx4param while the models used for 2100 excluded nec4param and nechorme4. The final models used for net calcification for 2018 and 2050 included nechorme4, neclin, neclinhorme, ecxlin, ecx4param, ecxwb1, ecxwb2 while the final models for 2100 included nec4param. EC50 values for each climate scenario were interpolated between the mean response value for 2018, 0 $\mu\text{g L}^{-1}$ diuron and the mean response value at 2100, 29 $\mu\text{g L}^{-1}$ diuron.

2.3.4 Results

Physico-chemical and carbonate chemistry parameters in the treatment chambers over the experimental period are provided in Table 2.3.1 and were in the range of the target treatment

levels. Measured concentrations of each diuron treatment at each climate scenario were similar (Appendix 2, Table A1).

2.3.4.1 Coral bleaching and survival

After 14 days exposure no bleaching (no visible colour change) was observed in live corals in all treatments (data not shown); however, all corals exposed to 9.6 and 29 $\mu\text{g L}^{-1}$ diuron [several times the guideline value] in all climate change scenarios died (complete tissue loss) after 9 days exposure. One coral exposed to 2.9 $\mu\text{g L}^{-1}$ diuron in the 2100 climate scenario died and another coral in the same treatment showed 14% partial mortality (data not shown).

Table 2.3.1: Measured physico-chemical parameters and seawater carbonate chemistry (measured and calculated) during the experimental period. Parameters (mean $\pm$ SD) include pH, temperature, salinity and dissolved oxygen (DO). Carbonate chemistry (mean $\pm$ SD) includes partial pressure of CO_2 ($p\text{CO}_2$), dissolved inorganic carbon (DIC), total alkalinity (TA), calcite saturation state (Ω_{Ca}) and aragonite saturation state (Ω_{arag}) of each climate change condition throughout the duration of the experiment. n denotes sample size.

	n	2018	2050	2100
pH (NIST)	96 – 99	8.11 $\pm$ 0.03	7.94 $\pm$ 0.02	7.84 $\pm$ 0.05
Temperature ($^{\circ}\text{C}$)	92 – 93	28.1 $\pm$ 0.3	29.1 $\pm$ 0.3	30.2 $\pm$ 0.3
Salinity	32 – 41	34.6 $\pm$ 0.5	34.6 $\pm$ 0.5	34.7 $\pm$ 0.4
DO (mg L^{-1})	36	7.66 $\pm$ 0.1	7.50 $\pm$ 0.2	7.40 $\pm$ 0.1
$p\text{CO}_2$ (μatm)	6	397 $\pm$ 64	680 $\pm$ 90	858 $\pm$ 149
DIC ($\mu\text{mol kg}^{-1}$ SW)	6	2039 $\pm$ 14	2136 $\pm$ 21	2137 $\pm$ 12
TA ($\mu\text{mol kg}^{-1}$ SW)	6	2291 $\pm$ 45	2349 $\pm$ 18	2309 $\pm$ 18
Ω_{Ca}	6	4.05 $\pm$ 0.6	3.91 $\pm$ 0.5	3.36 $\pm$ 0.2
Ω_{arag}	6	2.70 $\pm$ 0.4	2.61 $\pm$ 0.3	2.25 $\pm$ 0.1

2.3.4.2 Effective quantum yield

Control corals (no herbicide) from all climate scenarios exhibited similar $\Delta F/F_m'$ after 7 days (2018: 0.67 $\pm$ 0.03 (SD); 2050: 0.67 $\pm$ 0.03; 2100: 0.68 $\pm$ 0.03; Appendix 2 Table A2). These measurements were repeated after 14 d for concentrations up to 2.9 $\mu\text{g L}^{-1}$ and there was no significant difference in $\Delta F/F_m'$ between day 7 and day 14 measurements for each treatment (pairwise t-test, $p = 0.678$). $\Delta F/F_m'$ was negatively affected by diuron but not climate scenario (Table 2.3.2) and $\Delta F/F_m'$ decreased with increasing diuron concentrations (Fig. 2.3.1). The modelled concentrations that inhibited $\Delta F/F_m'$ by 50% (EC50, in $\mu\text{g L}^{-1}$) for each climate (2018, 2050, 2100) were 2.06, 2.02 and 2.03, respectively (Fig. 2.3.1; Table 2.3.3). Despite a significant interaction term likely due to completely suppressed $\Delta F/F_m'$ at 29 $\mu\text{g L}^{-1}$ in the 2100 scenario, there were no significant differences in these EC50 values, with the probabilities of the 2050 and 2100 EC50 values being different than the 2018 EC50 estimated as 0.615 and 0.539, respectively (Appendix 2 Fig. A1.A).

Table 2.3.2: Generalized linear mixed model summary results analysing the effects of climate change scenario, diuron and the interaction of the two to physiological endpoints of *Acropora millepora*. SS – sum of squares, DF denotes degrees of freedom, F – F-statistic. Statistically significant ($\alpha = 0.05$) effects are in bold.

Endpoint	Factor	χ^2	DF	p
$\Delta F/F_m'$	Climate	3.41	2	0.18
	Diuron	7443.13	5	<0.0001
	Interaction	53.75	10	<0.0001
Net Photosynthesis	Climate	62.84	2	<0.0001
	Diuron	395.69	4	<0.0001
	Interaction	29.38	8	<0.001
Daily Calcification	Climate	4.26	2	0.12
	Diuron	116.78	4	<0.0001
	Interaction	25.08	8	0.0015

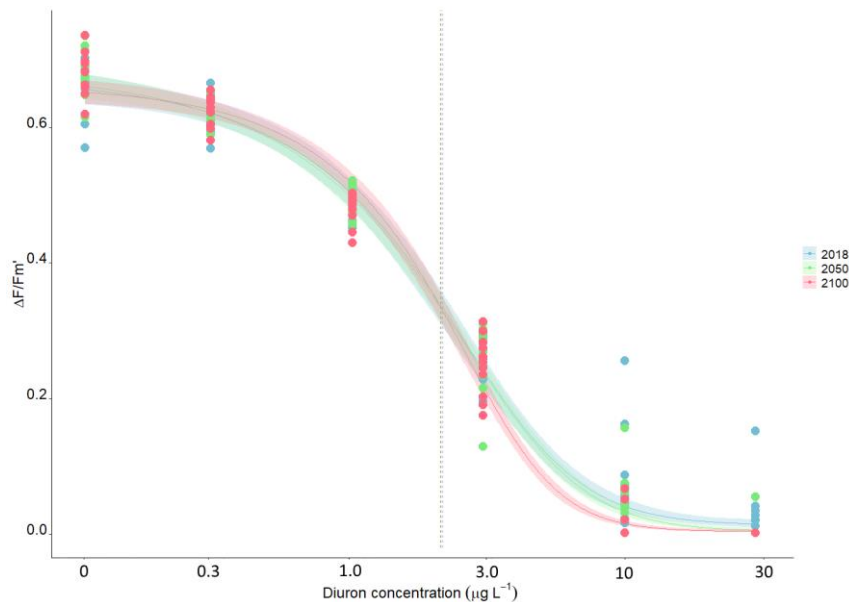


Figure 2.3.1: Concentration-response relationships of the effects of varying concentrations of diuron on $\Delta F/F_m'$ of *A. millepora*. Bayesian non-linear beta model fits of $\Delta F/F_m'$ (solid lines) and 95% confidence intervals (shaded areas) to derive EC50 values (dashed lines).

Table 2.3.3. Summary of EC50 values (95% CI) for $\Delta F/F_m'$, net photosynthesis and net calcification of *A. millepora* under three climate change scenarios and varying concentrations of diuron. NC denotes not calculable.

	$\Delta F/F_m'$	Net photosynthesis	Daily calcification
2018	2.06 (1.86-2.24)	19.4 (10.6-29.1)	20.6 (1.76-NC)
2050	2.02 (1.88-2.17)	10.6 (5.75-23.7)	42.2 (4.91-NC)
2100	2.03 (1.89-2.16)	2.56 (1.39-5.75)	9.08 (2.40-NC)

2.3.4.3 Net photosynthesis and calcification rates

Net photosynthesis rates were influenced by both climate change scenario and diuron, and an interaction was observed (Table 2.3.2; Figure 2.3.2). Photosynthesis was not affected at $0.96 \mu\text{g L}^{-1}$ diuron under 2018 conditions; however, the same concentration reduced photosynthesis by 35% and 100% under 2050 and 2100 climate conditions, respectively (Figure 2.3.2). Negative net photosynthesis rates were observed for corals exposed to $\geq 0.96 \mu\text{g L}^{-1}$ diuron and under 2100 conditions while this did not occur until $29 \mu\text{g L}^{-1}$ diuron for corals under 2018 and 2050 conditions (Figure 2.3.2). Diuron significantly reduced net photosynthesis to levels lower than control (2018, $0 \mu\text{g L}^{-1}$) at lower concentrations as the climate scenarios changed: $\geq 29 \mu\text{g L}^{-1}$ (2018), $\geq 2.9 \mu\text{g L}^{-1}$ (2050), $\geq 0.29 \mu\text{g L}^{-1}$ (2100) (Appendix 2, Table A2). Oxygen production (from light incubation) was the main contributor to changes in net photosynthesis rates as dark respiration rates were comparatively consistent across treatments (Appendix 2, Fig. A2), and was thus not further analysed. The approximate diuron concentrations that inhibited net photosynthesis by 50% (EC₅₀, in $\mu\text{g L}^{-1}$) for each climate (2018, 2050, 2100) were: 19.4, 10.6 and 2.56, respectively (Table 2.3.3). The probability of the net photosynthesis EC₅₀ values for the 2050 and 2100 scenarios being less than the 2018 EC₅₀ were 0.861 and 0.997, respectively (Appendix 2, Fig. A1.B).

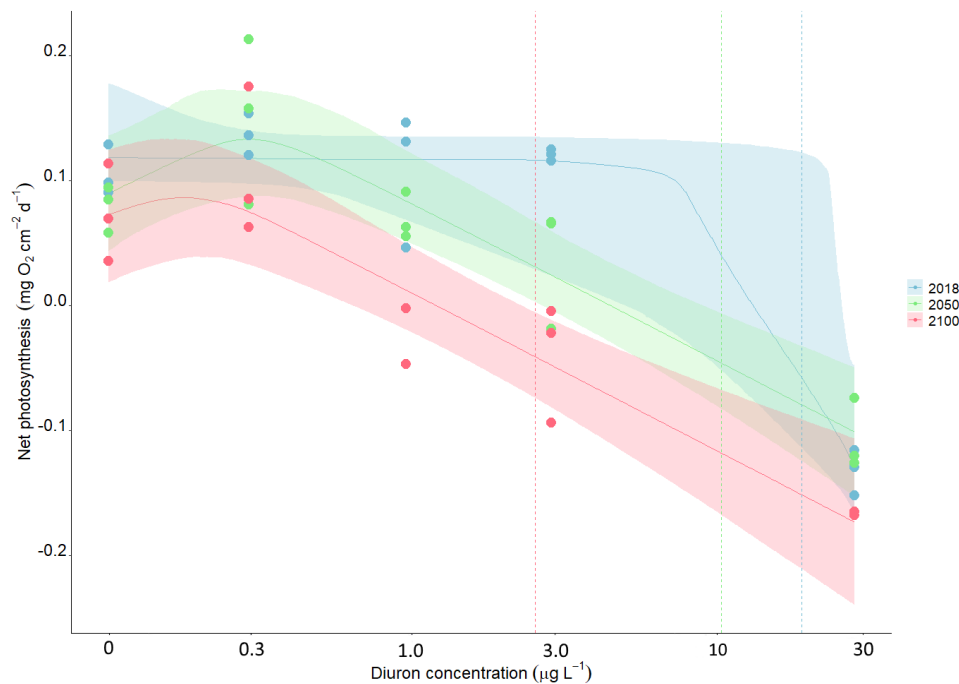


Figure 2.3.2: Concentration-response relationships of net photosynthesis rates (in $\text{mg O}_2 \text{ cm}^{-2} \text{ d}^{-1}$) of *A. millepora* under three climate change scenarios and four elevated concentrations of diuron. Bayesian non-linear gaussian model fits of net photosynthesis (solid lines) and 95% confidence intervals (shaded areas) to derive EC₅₀ values (dashed lines). Percent inhibition of net photosynthesis is relative to control corals (2018, $0 \mu\text{g L}^{-1}$ diuron) after 7-15 days exposure.

Daily calcification rates were affected by diuron but not by climate scenario and there was a significant interaction between these factors (Table 2.3.2; Fig. 2.3.3). Net calcification rates were negatively correlated with diuron; however, substantial increases in calcification rates were observed for corals exposed to $0.29 \mu\text{g L}^{-1}$ diuron from all climate change scenarios (Figure 2.3.3). This corresponded to an average increase of 2.4-fold (range: 0.8-4.5) compared to controls ($0 \mu\text{g L}^{-1}$ diuron). Negative daily calcification rates were observed for corals at $\geq 2.9 \mu\text{g L}^{-1}$ diuron (Fig. 2.3.3) though there was high variability in calcification rates of corals under all climate change scenarios, potentially explaining the apparent interaction (Fig. 2.3.3; Appendix 2 Table A2). Most of the positive calcification across treatments occurred in illuminated corals (Appendix 2 Fig. A3). The approximate diuron concentrations that inhibited daily calcification by 50% (EC50, in $\mu\text{g L}^{-1}$) for each climate (2018, 2050, 2100) were 20.6, 42.2 and 9.08, respectively (Table 2.3.3). However, the probabilities that daily calcification EC50 values for the 2050 and 2100 scenarios were different to the 2018 EC50 were low: 0.373 and 0.693, respectively (Appendix 2 Fig. A1.C).

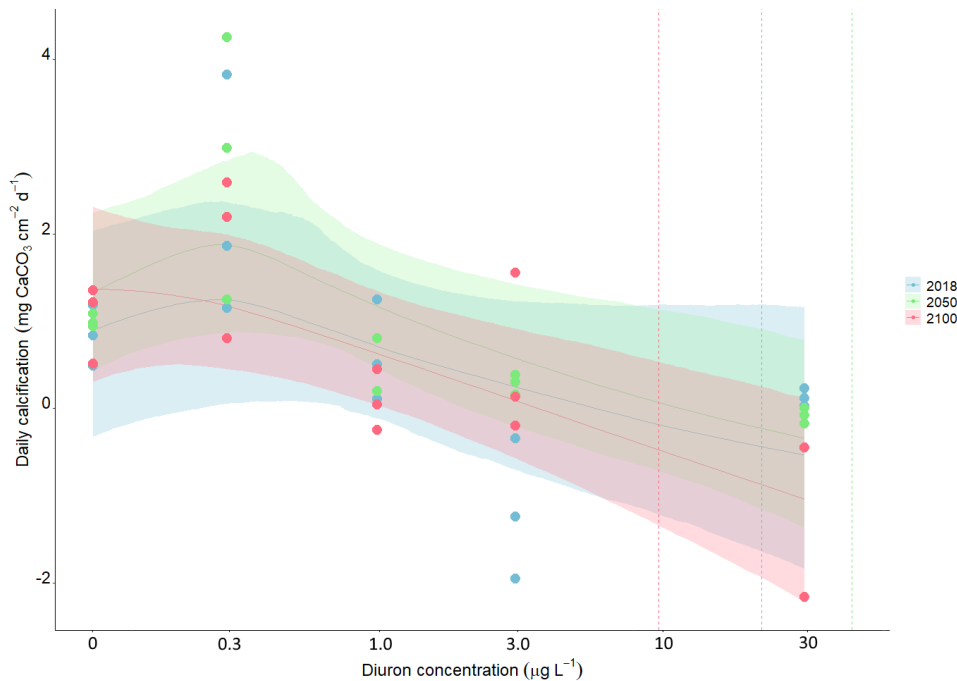


Figure 2.3.3: Concentration-response relationship of the effects of different diuron concentrations on daily calcification rates ($\mu\text{mol CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$) of *A. millepora*. Bayesian non-linear gaussian model fits of daily calcification (solid lines) and 95% confidence intervals (shaded areas) to derive EC50 (dashed lines). Percent inhibition of daily calcification is relative to control corals (no herbicide) after 7-15 days exposure.

2.3.4.4 Relationships between physiological parameters

$\Delta F/Fm'$ was positively correlated with oxygen production under all climate scenarios (Figure 2.3.4, Table 2.3.4). There were significant linear relationships between light calcification rate and oxygen production rate and between daily calcification and net photosynthesis rates for the 2050 and 2100 climate scenarios (Figure 2.3.4, Table 2.3.4).

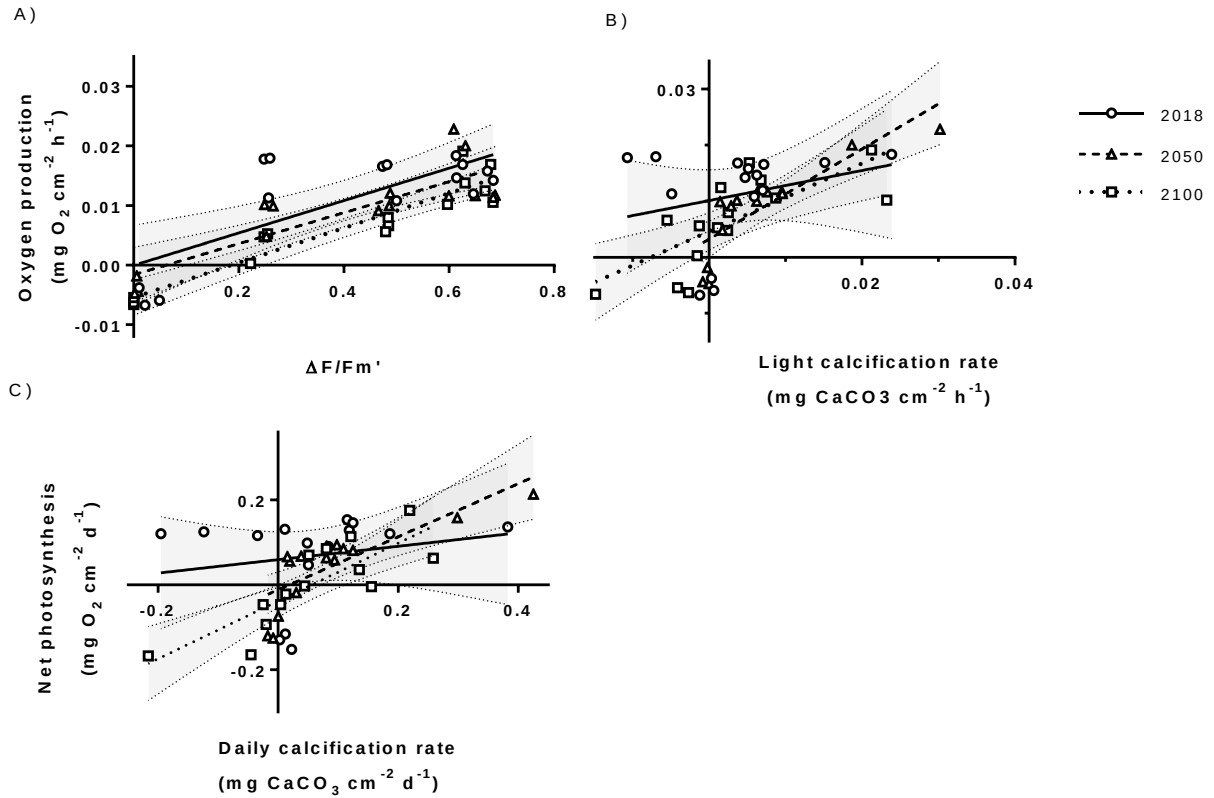


Figure 2.3.4: Regression models ($\pm 95\%$ confidence intervals) of (A) effective quantum yield ($\Delta F/Fm'$) and oxygen production, (B) light calcification rate and oxygen production and (C) daily calcification rate and net photosynthesis for *Acropora millepora* under three climate change scenarios and elevated diuron concentrations. Goodness of fit values are in Table 2.3.4.

Table 2.3.4: Summary results of regression models from Figure 2.3.4A) oxygen production versus effective quantum yield, 2.3.4B) light calcification rate versus oxygen production and 2.3.4C) daily calcifications and net photosynthesis rates production for all climate scenarios. Statistically significant ($\alpha = 0.05$) effects are in bold.

	r^2	F-statistic (1,13)	p-value
Effective quantum yield vs oxygen production			
2018	0.57	17.15	0.0012
2050	0.72	33.93	<0.001
2100	0.89	101.1	<0.001
Light calcification rate vs oxygen production			
2018	0.066	0.91	0.36
2050	0.72	32.64	<0.001
2100	0.53	14.7	0.0021
Daily calcification rate vs net photosynthesis			
2018	0.039	0.53	0.48
2050	0.64	22.88	0.0004
2100	0.68	25.35	0.0003

2.3.5 Discussion

The herbicide diuron negatively affected effective quantum yield ($\Delta F/F_m'$), net photosynthesis and calcification in *Acropora millepora* branches and the effects on net photosynthesis were exacerbated by future climate conditions (OW and OA). The effects of diuron was concentration-dependent and no corals survived at $\geq 9.6 \mu\text{g L}^{-1}$ diuron for the full two-week exposures in any of the climate scenarios. Net photosynthesis EC50s significantly decreased with increased $p\text{CO}_2$ and temperature. In contrast, $\Delta F/F_m'$ EC50 values were similar under all climate scenarios and while daily calcification EC50 at 2100 was lower than that of 2018 and 2050, there was a high variation in response as shown by the wide confidence intervals of the modelled EC50 values. The increased negative effects of diuron under future climate conditions on net photosynthesis, but not on $\Delta F/F_m'$ or calcification, demonstrates the value of assessing multiple toxicity endpoints and highlights the likelihood that water quality guideline values may need to be adjusted as the climate changes.

2.3.5.1 Future climate scenarios tested did not affect coral physiology in the absence of diuron

Elevating OW and OA from ambient (28.1°C , $p\text{CO}_2 = 397 \mu\text{atm}$) to RCP8.5 2050 (29.1°C , $p\text{CO}_2 = 680 \mu\text{atm}$) and 2100 (30.2°C , $p\text{CO}_2 = 858 \mu\text{atm}$) conditions had little or no effect on $\Delta F/F_m'$, net photosynthesis and calcification in *Acropora millepora* branches in the absence of the herbicide diuron. Previous experimental evidence has shown each of these parameters can be affected by OW and OA, but the responses are species-specific and vary considerably with experimental conditions selected (Ban et al., 2014; Chan & Connolly, 2012; Kornder, Riegl et al., 2018). Thermal stress from OW alone can cause oxidative damage to PSII in symbionts

which leads to reduced $\Delta F/Fm'$, limiting electron transport and reducing oxygen production, and when combined with thermal effects on respiration, reduces net photosynthesis (Coles & Jokiel, 1977; Lesser, 2011). Calcification rates were higher in the light than in the dark due to light-enhanced calcification (Goreau, 1959; Kawaguti & Sakumoto, 1948), but thermal stress can suppress light-enhanced calcification due to effects on photosynthesis (stress on PSII and loss of symbionts) (Langdon & Atkinson, 2005). OA, on the other hand, can affect calcification due to reduced dissolved inorganic carbon (Langdon & Atkinson, 2005), but its direct effects on PSII and $\Delta F/Fm'$ and net photosynthesis are less clear and can be either enhanced or suppressed (Noonan & Fabricius, 2016). The effects of OA and OW have generally been shown to be exacerbated by experimental combinations of these pressures (Anthony et al., 2008), but usually the conditions tested have been more severe than those applied in the present study, likely explaining the lack of effect observed on $\Delta F/Fm'$, net photosynthesis and calcification in *A. millepora* branches (in the absence of diuron) here. Furthermore, the exposure duration was relatively brief in the current study, and corals may have had adequate energy resources to continue to calcify in the short-term under increased pCO_2 conditions.

2.3.5.2 Effects of diuron alone on *Acropora millepora*

Diuron had clear concentration-dependent effects on $\Delta F/Fm'$, exhibiting an EC_{50} (2.02 - 2.06 $\mu g L^{-1}$) similar to those reported in other coral studies (2.3 – 6 $\mu g L^{-1}$) (Jones & Kerswell, 2003; Jones, Muller et al., 2003; Negri et al., 2011) and reinforcing the sensitivity of this photophysiological endpoint. Diuron also affected net photosynthesis and calcification rates with higher EC_{50} s predicted; however, the EC_{50} for calcification had wide confidence intervals and should be considered preliminary. Similar concentrations or chronic exposures can lead to coral bleaching (Cantin, Negri et al., 2007; Jones et al., 2003; Negri, Vollhardt et al., 2005), decreased lipid content, reproductive output and mortality (Cantin et al., 2007). Reduced calcification in corals due to herbicide exposure has been reported previously in *Pocillopora damicornis* colonies (Vandermeulen, Davis et al., 1972), with suppression of light-enhanced calcification the likely mechanism. As a proxy for growth, inhibition of calcification should be investigated further as an ecologically relevant endpoint for the effects of contaminants on corals.

2.3.5.3 The influence of future climate scenarios on diuron toxicity

This is the first study to investigate the effects of both the global pressure of climate change (OW and OA) and the local pressure of the herbicide diuron on corals. $\Delta F/Fm'$ was negatively affected by diuron across all climate scenarios and inhibition of $\Delta F/Fm'$ was nearly identical at low diuron concentrations between scenarios. While interactive effects of climate and diuron were observed for $\Delta F/Fm'$, this significant interaction was most likely due to the near complete suppression of $\Delta F/Fm'$ at the two highest diuron concentrations, and similar EC_{50} values were estimated for all three climate scenarios.

Diuron acts by blocking electron transport in Photosystem II which under illumination causes an increase in chlorophyll fluorescence and a reduction in $\Delta F/Fm'$ (Genty, Briantais et al., 1989), and this correlated with reduced oxygen production in the current study (Figure 2.3.4A). The $\Delta F/Fm'$ EC_{50} was lower than that of net photosynthesis under 2018 climate scenario. However, net photosynthesis was far more suppressed by diuron under the 2100 scenario and similar EC_{50} s for $\Delta F/Fm'$ and net photosynthesis indicated close to a 1:1 link between the reduction of electron transport and net photosynthesis by diuron under these conditions.

Indeed, there were strong negative and interactive effects of both future climate scenarios and diuron on net photosynthesis. This influence of OW and OA on suppressing net photosynthesis in the presence of diuron was primarily due to the net effects on oxygen production and/or respiration under illuminated conditions (Appendix 2 Fig. A2), indicating light also plays a role, but it is uncertain whether the influence affects oxygen production, respiration or both.

Diuron concentrations $\geq 2.9 \mu\text{g L}^{-1}$ reduced daily net calcification to below zero (= net dissolution) in all climate scenarios. This is likely to be driven by suppression of light-enhanced calcification as calcification was only affected in illuminated corals (Appendix 2 Figure A3) and the effects on light calcification rate and oxygen production were well correlated (Figure 2.3.5B). The diuron EC50s for net calcification were generally higher than for $\Delta F/Fm'$ and net photosynthesis, indicating that despite the herbicide reducing photosynthesis, the corals maintained an intracellular pool of available dissolved inorganic carbon required for calcification (Langdon & Atkinson, 2005) over the short exposure period. While the 2100 EC50 was lower than that of the 2018 scenario, indicating possible exacerbation of future climate scenarios on calcification, the data were too inconsistent and confidence intervals too wide to support this conclusion. Additionally, there was a limitation of sample size in the study and an increase in sample size may have better defined the trend and/or influence of climate on calcification. The thermal stress applied in the future climate scenarios (up to $+2\text{C}^\circ$ for less than two weeks) was below the bleaching threshold for *A. millepora* in all the diuron/climate treatments. However, thermal bleaching of *A. millepora* at higher temperatures ($+5\text{C}^\circ$) is enhanced by diuron exposure (Negri et al., 2011) and reduced calcification rates have been observed in bleached corals (Goreau, 1959; Goreau & Goreau, 1959; Kawaguti & Sakumoto, 1948). These types of interactions are more likely to be evident over prolonged co-exposures to PSII herbicides during heatwaves or under future climate conditions.

2.3.5.4 Enhanced calcification at low diuron concentrations

Enhanced calcification, and to a lesser extent net photosynthesis, were observed for corals exposed to $0.29 \mu\text{g L}^{-1}$ diuron across all climate scenarios. The phenomenon that low concentrations of a chemical can induce a beneficial effect while damaging effects are observed at higher concentrations (a stimulatory hormetic-like response) has been intensively studied (Calabrese & Blain, 2005; Duke, Cedergreen et al., 2006). A review of 5600 concentration-response relationships by Calabrese and Blain (2005) found that the maximum stimulatory response was usually less than two-fold greater than the control. In this study, calcification rates in corals at $0.29 \mu\text{g L}^{-1}$ diuron were an average of 2.4-fold higher than the rates of control corals which is within the range found in the review. Additionally, Roubeix, Mazzella et al. (2011) found moderate concentrations of diuron ($< 5 \mu\text{g L}^{-1}$) stimulated growth of several species of diatoms. Other PSII herbicides have been found to have stimulatory effects, including simazine ($10\text{-}16 \mu\text{g L}^{-1}$) which increased protein content in rye (Ries, Chmiel et al., 1967) and barley (Pulver & Ries, 1973). These stimulatory responses in calcification and photosynthesis may be linked since light-enhanced calcification is largely photosynthesis dependent (Vandermeulen et al., 1972). Furthermore, diuron is a phenylurea herbicide, and previous studies have shown that carbon (^{14}C) in urea is incorporated into skeletal carbonate of corals (Crossland & Barnes, 1974). Bisc  r  , Ferrier-Pag  s et al. (2018) observed enhanced photosynthetic and calcification rates in coral with urea enrichment. Urease, which is found in both coral host and algal symbionts (Barnes & Crossland, 1976) catalyses the hydrolysis of urea to produce inorganic carbon and ammonia (Krajewska, 2009). Ammonia then neutralizes

the protons formed during calcification and shifts the equilibrium towards the precipitation of CaCO_3 , enhancing calcification (Biscéré et al., 2018). Further research is needed to understand the mechanism underlying the observation that low concentrations of diuron can enhance calcification by an average of more than two-fold across all climate treatments.

2.3.5.5 Conclusion

The increased effects of diuron under future climate conditions on net photosynthesis but not on $\Delta F/Fm'$ or calcification demonstrates the value of assessing multiple toxicity endpoints. Many previous studies on the effects of herbicides such as diuron on corals have relied on $\Delta F/Fm'$ inhibition as a toxicity endpoint (Jones & Kerswell, 2003; Jones et al., 2003; Negri et al., 2011); however, additional stressors such as OA and OW can also influence other important physiological functions. Measuring whole-organism responses, such as net photosynthesis, is needed to reveal the potential enhanced toxicity of diuron under future climate scenarios. While climate-enhanced effects of diuron on calcification were not significant, the links between oxygen production and light calcification indicates that over prolonged exposures skeletal growth in corals may be more vulnerable to climate change in the presence of contaminants like diuron that suppress photosynthesis. This study supports the management of contaminants to improve coral reef resilience to climate change (RWQIP, 2018b) and adjustments to water quality guideline values to account for future climate conditions should also be considered (Negri et al., 2020).

2.4 Example 3: Acclimation history modulates effect size of calcareous algae (*Halimeda opuntia*) to herbicide exposure under future climate scenarios

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2.4.1 Summary

Tropical marine habitat-builders such as calcifying green algae can be susceptible to climate change (warming and acidification). This study evaluated the cumulative effects of ocean warming (OW), ocean acidification (OA) and the herbicide diuron on the calcifying green algae *Halimeda opuntia*. We also assessed the influence of acclimation history to experimental climate change conditions on physiological responses. *H. opuntia* were exposed for 15 days to orthogonal combinations of three climate scenarios [ambient (28°C, $p\text{CO}_2$ = 378 ppm), 2050 (29°C, $p\text{CO}_2$ = 567 ppm) and 2100 (30°C, $p\text{CO}_2$ = 721ppm)] and to six diuron concentrations (up to 29 $\mu\text{g L}^{-1}$). Half of the *H. opuntia* had been acclimated for eight months to the climate scenarios in a mesocosm approach, while the remaining half were not pre-acclimated, as is current practice in most experiments. Climate effects on quantum yield ($\Delta F/F_m$), photosynthesis and calcification in future climate scenarios were significantly stronger (by 24, 46 and 26%, respectively) in non-acclimated algae, suggesting experimental bias may exaggerate effects in organisms not appropriately acclimated to future-climate conditions. Thus, full analysis were done on acclimated plants only. Interactive effects of future climate scenarios and diuron were observed for $\Delta F/F_m'$, while the detrimental effects of climate and diuron on net photosynthesis and total antioxidant capacity (TAC) were additive. Calcification-related enzymes were negatively affected only by diuron, with inhibition of Ca-ATPase and upregulation of carbonic anhydrase. The combined and consistent physiological and biochemical evidence of negative impacts (across six indicators) of both herbicide and future-climate conditions on the health of *H. opuntia* highlights the need to address both climate change and water quality. Guideline values for contaminants may also need to be lowered considering climate adjusted thresholds. Importantly, this study highlights the value of applying substantial future climate acclimation periods in experimental studies to avoid exaggerated organism responses to OW and OA.

2.4.2 Introduction

Coral reefs are simultaneously exposed to multiple global and local pressures (Ban et al., 2014; Wolff et al., 2018b). At a global scale, near future scenarios predict the co-occurrence of rising sea temperatures and increased CO_2 partial pressure ($p\text{CO}_2$) in response to high atmospheric CO_2 emissions (IPCC, 2014a). Climate change represents the greatest threat to coral reefs and is already contributing to increased frequency and severity of global bleaching events

(Hughes et al., 2017a), with a diversity of coral reef taxa living very close to their thermal limits (Negri et al., 2020). Many field and laboratory experiments have evaluated ecophysiological effects of ocean warming (OW) and/or ocean acidification (OA) on key coral reef species, usually reporting negative outcomes (Collier et al., 2018; Fonseca, Marangoni et al., 2017; Jiang, Guo et al., 2019; Scherner, Pereira et al., 2016). Poor water quality, including elevated sediments, nutrients and contaminants, represents one of the most common local pressures threatening the health of coral reefs worldwide (Norström, Nyström et al., 2016). Understanding the combined effects of local and global pressures can help regulators and management agencies prioritise which water quality parameters need to be managed to help protect sensitive ecosystems as the climate change (Hughes et al., 2017a; MacNeil, Mellin et al., 2019). In addition, quantifying the responses of habitat-building marine species, such as corals and calcifying algae, to cumulative pressures can lead to the development of cumulative risk maps and help quantify climate-adjustments for water quality guideline values (Halpern et al., 2015; Negri et al., 2020; Signore del, Hendriks et al., 2016; Uthicke et al., 2016).

A mounting body of research has shown that herbicide contamination decreases reef species resilience to thermal stress (Negri & Hoogenboom, 2011; van Dam et al., 2012a; Wilkinson et al., 2017). Diuron is one of the most frequently detected herbicides in the Great Barrier Reef (Lewis et al., 2009), and its toxic effects on coral reef phototrophs, such as seagrasses, corals, crustose coralline algae and symbiont-bearing foraminifera is well documented (Negri, Flores et al., 2015; Negri et al., 2011; van Dam et al., 2012a; van Dam, Negri et al., 2012b; Wilkinson et al., 2017). However, nothing is known about the effects of herbicides on reef-building green algae such the abundant calcifying Chlorophytes (Verbruggen, De Clerck et al., 2006).

Halimeda spp. are calcifying Chlorophytes, abundant over tropical shallow-water environments (Verbruggen et al., 2006). They are ecologically important habitat-builders and play a major role on sediment production and on the reef carbonate budget (Castro-Sanguino, Bozec et al., 2020). Also, these fast growing algae offer significant ecological contributions as a carbon-sink species and to overall primary productivity in tropical marine habitats (Hillis-Colinvaux, 1980; Rees, Opdyke et al., 2006). There have been several studies that investigated the effects of OA on *Halimeda* spp. (Hofmann, Heiden et al., 2014; Meyer, Vogel et al., 2015; Peach, Koch et al., 2017; Scherner et al., 2016; Vogel, Fabricius et al., 2015a; Vogel, Meyer et al., 2015b), detecting both negative and neutral effects on photosynthesis and/or calcification. However, little is known about the effect of OA and temperature combined, which is the most likely scenario arising from increasing atmospheric CO₂ emissions (Campbell, Fisch et al., 2016; Sinutok, Hill et al., 2012). Considering their ecological importance, it is pivotal to evaluate the effects of both global (climate) and local (contamination) pressures on *Halimeda* spp.

Research on the co-occurrence of multiple stressors is crucial for ecosystem management (Halpern et al., 2015; Uthicke et al., 2016); however, most of the literature on multiple stressors do not account for potential acclimation or adaptation to OW and/or OA, and whether this acclimation can increase species resilience to the effects of climate change and reduced water quality (Byrne, Foo et al., 2020). Long-term exposure to climate change-related parameters may lead to acclimation (Kenkel, Moya et al., 2017; Vogel et al., 2015a), cross- or transgenerational effects (Diaz, Lardies et al., 2018; Karelitz, Lamare et al., 2019) or adaptation (Gibbin, Chakravarti et al., 2016; Torda, Donelson et al., 2017). Zhao, Yang et al. (2018), for example, found that transgenerational acclimation alleviates OA effects on clam calcification. Nevertheless, there is insufficient information to conclude whether acclimation

would minimize the expected negative biological consequences of climate change in combination with water quality pressures, and there have been recent calls to include appropriate acclimation periods in future studies (Cornwall, Comeau et al., 2020; Cornwall, Diaz-Pulido et al., 2019).

The present study evaluated whether extended acclimation to future climate conditions affects the response of *Halimeda opuntia* to the herbicide diuron under climate change conditions. Firstly, we evaluated whether an eight-month acclimation period to future climate scenarios influences the physiological responses of *H. opuntia*, to OA and OW. Subsequently, we tested the combined effects of diuron contamination, OW and OA [ambient, 2050 and 2100 scenarios, temperature and $p\text{CO}_2$ conditions under RCP 8.5 (IPCC, 2014a)], on the physiological and biochemical responses of *Halimeda opuntia* previously acclimated (eight months) to ambient or two future climate scenarios. Assessing biological effects of stress using biomarkers over different levels of biological organization (e.g. physiological, cellular and molecular scales) provides a holistic and integrated evaluation of organismal health (Dias, Madeira et al., 2020; Marques, Abrantes et al., 2020a). Thus, we examined ecologically relevant endpoints from organismal (photosynthesis and calcification) and biochemical biological organization levels (oxidative stress parameters, ion-regulation and calcification related enzymes) in the present study.

2.4.3 Materials and Methods

2.4.3.1 Sample collection and acclimation

Halimeda opuntia collected from Fantome Island (Queensland, Australia, 18°41.126'S, 146°30.731'E), were distributed in nine acclimation tanks (working volume 250 L) in the National Sea Simulator (SeaSim), at the Australian Institute of Marine Science (AIMS, Townsville). Three fully independent replicate tanks simulated water temperature and $p\text{CO}_2$ levels projected for 2050, another three for 2100 projected conditions from RCP 8.5 (IPCC, 2014a), and the last three were held at ambient conditions. Temperatures in mesocosms followed an annual trend based on long term averages from Davies Reef (Uthicke, Patel et al., 2020). These flow through tanks were shallow (78 x 142 x 28 cm), covered with natural carbonate sediment from the reef and housed multiple organisms (corals, sponges, echinoderms, seagrasses, foraminifera, fishes and other taxa) in a mesocosm approach. Temperature and $p\text{CO}_2$ targets for each experimental condition are provided in Table 2.4.1, and a detailed system description is given in Uthicke et al. (2020).

Table 2.4.1: Temperature and $p\text{CO}_2$ treatments applied to each climate change scenario. Seasonal cycles and consequent determination of current temperatures are based on the 15 years average (ranging from 23.4°C during winter to 28.3°C during summer) for Davies Reef (Queensland, Australia). Details in Uthicke et al. (2020).

	2018	Year 2050	Year 2100
Temperature	Current	Current + 1°C	Current + 2°C
$p\text{CO}_2$	410 ± 50 ppm	685 ± 50 ppm	940 ± 50 ppm

After eight months (from May 2017 to January 2018) of acclimatisation to the conditions of mesocosm tanks, newly formed thalli of *H. opuntia* (~5 cm each) were collected and haphazardly allocated in experimental treatments (Figure 2.4.1) described below.

2.4.3.2 Experimental design

To assess differences between acclimated and not acclimated organisms, six randomly selected thalli of *H. opuntia* from the 2018 mesocosm tanks ('present day') were placed in experimental chambers kept under ambient conditions. Thus, although we regard these as non-acclimated to future climates, they are acclimated to tank conditions. Experimental chambers kept under 2050 condition received three thalli from 2018 mesocosm tanks (non-acclimated) and three thalli from 2050 mesocosm tanks (algae acclimated to 2050 temperature and $p\text{CO}_2$ conditions, Table 2.4.1). Similarly, each replicate 2100 condition chamber received three thalli from 2018 mesocosm tanks (non-acclimated) and three thalli from 2100 mesocosm tanks (algae acclimated to 2100 temperature and $p\text{CO}_2$ conditions, Table 2.4.1) (Figure 2.4.1).

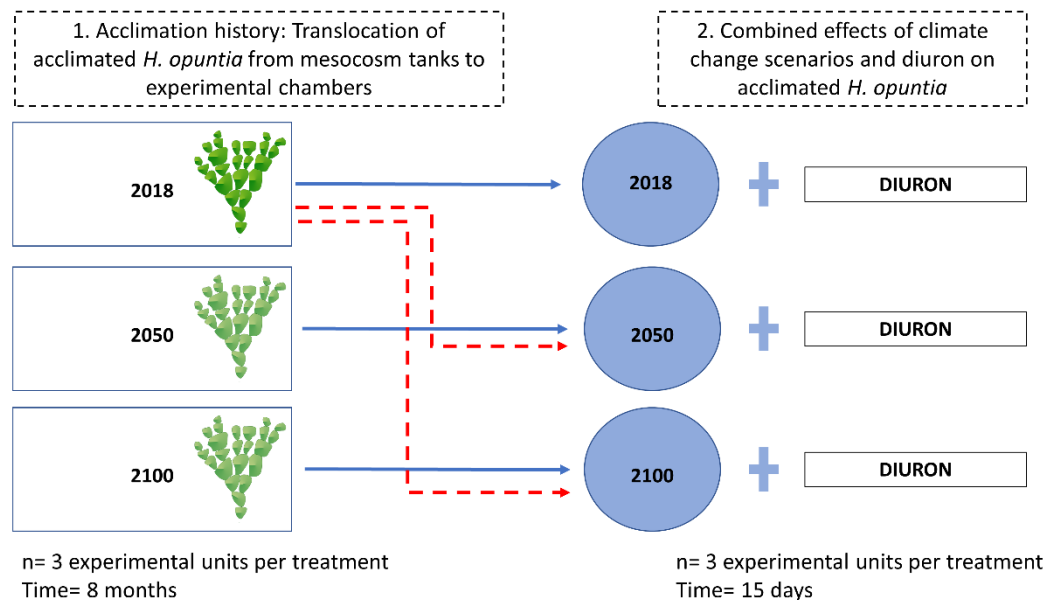


Figure 2.4.1: (1) Translocation of acclimated (blue line) and non-acclimated (dotted line) *Halimeda opuntia* from mesocosm tanks to experimental chambers and (2) simultaneous exposure to climate scenarios and diuron.

Halimeda opuntia were exposed for 15 days to three climate change scenarios (Table 2.4.1) and six diuron concentrations, combined in an orthogonal design within 3 L treatment chambers (Figure 2.4.1). Custom built acrylic chambers (working volume: 2.5 L; 54 total chambers, n=3 replicates per treatment combination) were kept in a flow-through system and distributed into three water baths. Water baths were used to maintain water temperatures similar to the mesocosm tanks (~28, 29 and 30°C for the summer conditions in January 2018). Water movement, as well as $p\text{CO}_2$ levels, for each climate scenario (mean $p\text{CO}_2$: 397, 680 and 858 μatm) were achieved by gentle bubbling of air- CO_2 mixtures. Air- CO_2 mixtures were set to the respective climate scenario, and preliminary tests showed that given the slow water exchange, the partial pressure of carbon dioxide ($p\text{CO}_2$) in the water inside the chambers maintained at equilibrium with the supplied air (see Table 2.4.3). The $p\text{CO}_2$ and injection rates

were digitally controlled by a programmable logic controller (PLC; Siemens S7 1511-1 PN) using mass flow controllers (Aalborg, USA) and Telaire CO₂ sensors in a feedback loop system. Seawater temperature was monitored and controlled by a supervisory control and data acquisition (SCADA) computer system.

Six diuron concentrations (nominal concentrations: solvent control (0), 0.3, 1, 3, 10 and 30 µg L⁻¹ diuron) were delivered by flow-through to each climate scenario in a factorial design. Diuron stock solutions [Sigma-Aldrich (> 98% purity), 10 mg L⁻¹] were prepared in filtered seawater (0.4 µm) using ethanol as a solvent carrier (final concentration < 0.03% v/v). Header tanks (60 L) were filled daily with filtered seawater (0.5 µm) and dosed with appropriate diuron stock to achieve the target diuron concentrations. Seawater from the header tanks was delivered to experimental chambers using peristaltic pumps (Masterflex L/S and Ismatec IPC 12). Turnover rate was 1.5 x d⁻¹ and chambers were randomized in the water baths to minimise position and tank effects. Light levels during exposure was maintained at ~150 µmol m⁻² s⁻¹ over a 12:12 h light:dark cycle. Filtered (0.2 µm) water was collected at the beginning and the end of the experiment for diuron analysis by HPLC-MS/MS (Mercurio et al., 2015).

Table 2.4.2: Information on the physiological and biochemical endpoints used.

Response variable	Description
Effective quantum yield ($\Delta F/F_m'$)	Reduction in the effective quantum yield ($\Delta F/F_m'$) corresponds to reduction in the efficiency of photochemical energy conversion within Photosystem II under a given light intensity (Genty et al., 1989).
Net photosynthesis rate	Reduction in the net oxygen production under a given light intensity.
Net calcification rate	Reduction in net calcification rate, measured by the alkalinity anomaly technique, corresponds to reduction in the calcium carbonate precipitation by the organism and therefore reduction in growth rates.
Total antioxidant capacity (TAC)	Increased TAC, in general, indicates that the organism is investing on antioxidant defences and is a common regulatory response to environmental stressors. Reduced TAC, in general, indicates an intense use of antioxidants related to environmental stress, and/or disbalance between requirement and production (Lushchak, 2011; Marangoni et al., 2019).
Ca-ATPase	Reduction in Ca-ATPase results in disruption in Ca homeostasis and consequent altered cellular functions; indicates direct inhibition (resulting from oxidative stress or binding by competitors, like xenobiotics) (Tellis et al., 2014).
Carbonic anhydrase (CA)	Upregulation in CA activity is related to compensatory mechanisms to keep carbon-concentration mechanisms and acid-base balance. Reduction suggests inhibition by binding (Bertucci et al., 2013; Supuran, 2016).

Seawater temperature, pH, salinity and dissolved oxygen (DO) measurements were taken daily from at least one random chamber of each treatment (HQ40d equipped with Intellical

PHC301 pH electrode (Hach, USA) for pH; Horiba LAQUAact PC110 for salinity, and HQ30d equipped with Intellical LDO101 oxygen probe (Hach, USA) for dissolved oxygen (DO)), while temperature was continuously logged at 10-min intervals (Onset HOBO temperature logger, Onset Computer Corp., MA, USA). Dissolved inorganic carbon (T_DIC) and total alkalinity (TA) concentrations were analysed (VINDTA 3C system) from seawater samples taken three times during the experiment. Multiple in-house seawater standards and certified reference material (A. G. Dickson, Scripps Institute of Oceanography, Dixon, Batch 159 and 164) were used to validate the accuracy of results. These data were used to calculate carbonate system parameters [$p\text{CO}_2$, calcite saturation state (Ω_{Ca}), and aragonite saturation state (Ω_{arag})] according to Mehrbach et al. (1973) and modifications by Dickson and Millero (1987), using CO2SYS software (Lewis & Wallace, 1998).

After 15 days under experimental conditions, *H. opuntia* were assessed for ecologically relevant physiological biomarkers (chlorophyll α fluorescence, oxygen production and calcification rates – see below), and snap frozen in liquid nitrogen for further analysis of biochemical biomarkers (See Table 2.4.2 for details).

2.4.3.3 Chlorophyll α fluorescence

$\Delta F/F_m'$ was measured from each individual using an Imaging-PAM fluorometer (I-PAM, Walz GmbH, Germany) after a 5 min light adaptation. Data-MAXI software (Imaging Win, Walz GmbH, Germany) was used to select 3-6 areas of interest (AOI) and the average of AOIs was used as final value for each *H. opuntia* individualized fragment.

2.4.3.4 Quantification of oxygen production and calcification rates

Incubations were run in 50 mL clear acrylic chambers placed on stirrer plates submersed in a water bath under the same temperature as the experimental chambers. One *H. opuntia* fragment from each chamber was incubated for 1 h in the light (under $\sim 150 \mu\text{mol m}^{-2} \text{s}^{-1}$) and subsequently 1 h in the dark. Two blanks (no *H. opuntia*) were run in parallel for every six samples. DO was measured before and after light and dark incubations to determine oxygen production and respiration rates. Water samples were collected for total alkalinity (AT) and fixed with mercuric chloride to determine light and dark calcification rates by the Alkalinity Anomaly technique (Chisholm & Gattuso, 1991) before and after incubations. AT was determined on a Metrohm 855 robotic titrosampler (Metrohm, Switzerland). Oxygen production and respiration were used to calculate net photosynthesis rate (expressed in $\text{mg O}_2 \text{h}^{-1} \text{g fresh wet weight}^{-1}$), while light and dark calcification rates were used to calculate net calcification rates. Rates were normalized to fresh wet weight (FW). Daily net calcification was calculated considering 12 h of light and 12 h of darkness and expressed as $\mu\text{M C d}^{-1} \text{gFW}^{-1}$.

2.4.3.5 Biochemical biomarkers

Aliquots (100-200 mg) of cold-ground *H. opuntia* were sonicated (ultrasound sonication, Cole-palmer ultrasonic processor model cp130) with homogenization buffer (1.5 mL) and centrifuged (10000 g, 20 min, 4°C). For each biochemical analysis, a different homogenization buffer was used (see Supplementary material). Total protein content was determined using a commercial kit (BioRad, DC Protein Assay Kit) and BSA as standard. Protein levels were used for biomarker normalization.

Total antioxidant capacity (TAC) was determined using OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit (Cell Biolabs Inc., San Diego, CA, USA), which is based on the reduction of copper (II) to copper (I) by antioxidants and consequent proportional color production. Absorbance was measured at 490 nm in a microplate reader (BioTek SynergyHT) and compared to uric acid standard curves. Results were normalized to the total protein content in the homogenate and expressed as μM Copper Reducing Equivalents (CRE)/mg protein.

Ca-ATPase activity was determined following the method described by Vajreswari et al. (1983), with some modifications (see Supplementary material). Briefly, homogenates were incubated for 30 min (30°C) in a working solution containing 189 mM NaCl, 5 mM CaCl_2 , 20 mM Tris-HCl and 1 mM Oubain at pH 7.6. Reaction was started by adding 2 mM ATP. Inorganic phosphate (Pi), released by the enzyme activity in the reaction medium, was measured at 620 nm (BioTek SynergyHT) using a Pi determination commercial kit (MAK308, Sigma-Aldrich). Results were expressed as $\text{nmol Pi} \times \text{h}^{-1} \times \text{mg protein}^{-1}$.

Carbonic anhydrase (CA) activity was measured using the delta pH method (Henry, 1991) with modifications by Haglund et al. (1992) and Marangoni et al., (2017b). Briefly, 30 μL of the homogenized sample was added to 2 mL of working buffer solution (225 mM mannitol, 75 mM sucrose, 10 mM NaH_2PO_4 , 10 mM Tris-base). Substrate (1 mL of deionized water saturated with CO_2) was added to the reaction medium, and the pH was measured every 5 s for up to 30 s. Blank measurements were taken using only working solution. Data were expressed as enzyme units $\times \text{mg protein}^{-1}$.

2.4.3.6 Data analysis

Acclimation

Firstly, in order to compare responses of eight months climate-acclimated and non-acclimated algae, we tested the effects of climate change grouping the scenarios by the duration of exposure to future climate conditions. Generalized linear mixed models (GLMM) were fitted using *chamber* as a random factor, *Acclimation* as a fixed factor [3 levels: control/ambient, acclimated, and non-acclimated under climate change conditions]. Because the response to diuron was not of interest for us in these initial tests, we only used *Diuron* as covariate. This was possible after initial tests showed that there was no significant *Acclimation* $\times$ *Diuron* interaction, and thus we removed the interactive term from the models. When appropriate (significant effect of fixed factor), pairwise differences were inferred by comparisons *a posteriori* using the least-square means method and Bonferroni corrections.

Cumulative effects of OW, OA and diuron

The combined effects of climate change scenarios and diuron on biological responses (of only acclimated algae) were then tested using GLMMs with *chamber* as a random factor, *Scenarios* as a fixed factor (3 levels: 2018, 2050 acclimated, 2100 acclimated), and *Diuron* as a numeric factor. The interaction between *Scenario* and *Diuron* was also assessed. When applicable, a *a posteriori* pairwise comparisons were performed.

When significant diuron effects were observed, concentration-response curves were fitted using four-parameter logistic curves in GraphPad Prism v7. Diuron concentrations inhibiting each endpoint by 10% and 50% (EC_{10} and EC_{50}) were determined from each curve. Analyses

were performed in R (v. 3.6.0; R Core Team, 2019) using the packages glmmTMB (v. 0.2.3), performance (v. 0.4.2), emmeans (v. 1.4), ggstatsplot (v. 0.0.12) and ggeffects (v. 0.11.09).

2.4.4 Results

Physicochemical parameters of seawater were homogeneous between chambers from the same climate change treatment thorough the experiment. Daily measurements (mean $\pm$ SE) of pH, temperature, salinity and DO, and carbonate system parameters are provided in Table 3. Nominal concentrations of 0, 0.3, 1, 3, 10 and 30 $\mu\text{g L}^{-1}$ diuron corresponded to measured concentrations of 0 (below detection limit), 0.29, 0.96, 2.9, 9.67 and 29.1 $\mu\text{g L}^{-1}$ diuron.

Table 2.4.3: Water physicochemical and carbonate system parameters (measured and calculated) for each climate change scenario (mean $\pm$ standard deviation).

Scenario	Water quality (n=80)				Carbonate system (n=3)			
	Measured parameters				Measured parameters		Calculated parameters	
	Temperature (°C)	pH (NIST)	salinity	DO (mg l ⁻¹)	TA (mmol kgSW ⁻¹)	T_DIC (mmol kgSW ⁻¹)	ρCO_2	Ω_{arag}
Ambient	28.26 $\pm$ 0.14	8.2 $\pm$ 0.7	34.69 $\pm$ 0.05	7.69 $\pm$ 0.02	2242 $\pm$ 10	1931 $\pm$ 16	378 $\pm$ 19	3.46 $\pm$ 0.10
2050	29.12 $\pm$ 0.17	8.0 $\pm$ 0.4	34.76 $\pm$ 0.02	7.59 $\pm$ 0.05	2256 $\pm$ 38	1989 $\pm$ 43	567 $\pm$ 24	2.70 $\pm$ 0.12
2100	29.95 $\pm$ 0.09	7.9 $\pm$ 0.5	34.76 $\pm$ 0.03	7.50 $\pm$ 0.03	2280 $\pm$ 11	2074 $\pm$ 5	721 $\pm$ 50	2.48 $\pm$ 0.14

2.4.4.1 Acclimation to future climate scenarios

Initial data analysis was conducted on the physiological responses of *H. opuntia* to elucidate if climate-acclimation duration affects the outcome of the experiments. The 'Acclimation' factor was significant for all three analyses. $\Delta F/F_m'$ and oxygen production was significantly lower in *H. opuntia* exposed to future climate scenarios for 15 days. Inhibition compared to the control (2018) conditions was stronger in non-acclimated (24 and 46%, for $\Delta F/F_m'$ and oxygen production respectively, Figure 2.4.2) than in acclimated algae (3 and 21%). Post hoc comparisons confirmed that non-acclimated and acclimated algae responded differently in both photosynthesis-related parameters (Table 2.4.4).

In contrast to photosynthesis, calcification was enhanced under future climate scenarios (Figure 2.4.2). However, effects of future OA and OW scenarios on calcification were also greater for non-acclimated (26%) than for acclimated (19%) *H. opuntia*. Although due to the high variance in this parameter the contrast between non-acclimated and acclimated was not statistically significant, the non-acclimated response was different to the control and the acclimated was not (Table 2.4.4). These results demonstrate that exposure to future climate scenarios without acclimation can result in exaggerated responses to OA and OW conditions (Figure 2.4.2).

Considering these differences and that long acclimation to future climate conditions represents more realistic biological scenarios, the combined effects of diuron and climate scenarios were considered only on climate-acclimated algae.

Table 2.4.4: ANOVA and post hoc tests results (p values) from models analysing main effects of climate acclimation history [fixed factor Acclimation, 3 levels: Control (ambient conditions of temperature and $p\text{CO}_2$), Acclimated before experiment, Non-acclimated to climate conditions before experiment]. Results for Diuron not shown here because for this analysis it was only used as a co-variate, and Chamber used as random factor. Significant ($\alpha=0.05$) differences are in bold. Reduction (-) and increasing (+) relative to control are in brackets (estimated from least square means).

Response	Acclimation	Pairwise comparisons of interest		
		Control x Long term	Control x Short term	Long term x Short term
$\Delta F/F_m'$	<0.0001	1.000 (-2.9%)	0.002 (-24%)	<0.0001
Production	<0.0001	0.009 (-21.1%)	<0.0001 (-46.3%)	0.002
Calcification	0.021	0.130 (+19.3%)	0.022 (+26.2%)	1.000

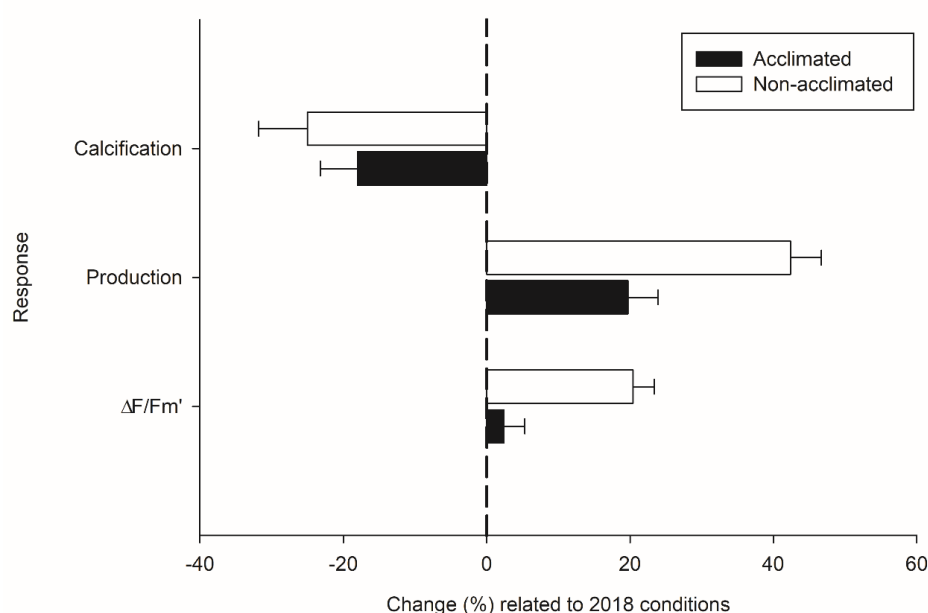


Figure 2.4.2: Average (+SE) inhibition of each parameter, by the end of the 15-day experiment, compared to 2018 conditions. Inhibition calculated from Wilkinson et al. (2017). Black bars indicate responses of eight-month acclimated algae, and white bars indicate responses of algae not previously acclimated to future climate change conditions.

2.4.4.2 Cumulative effects of climate change and diuron

Halimeda opuntia acclimated for 8 months to climate change conditions were exposed for 15 days to six different diuron concentrations under three climate conditions, and a range of response variables were measured. GLMM models assessing the combined effects of climate change scenarios and diuron indicated a significant interaction between these stressors on $\Delta F/Fm'$ of acclimatized *H. opuntia* (Table 2.4.5). Exposure to diuron reduced $\Delta F/Fm'$ in a typical concentration-dependent manner (Figure 2.4.3) with 50% inhibition (EC_{50}) reached at $6.19 \mu g L^{-1}$ (4.68-8.41 CI) under ambient conditions (Figure S1). In general, the reduction in $\Delta F/Fm'$ caused by diuron was attenuated (steeper negative slope under present-day scenarios) under future climate scenarios, when compared to the reduction observed under ambient conditions (2018) (Figure 2.4.3). The likely cause of this difference in slopes (i.e., the significant interaction) is that high $\Delta F/Fm'$ values around 0.6 at zero diuron concentration only occur in the present-day conditions, whereas the 'baseline' photosynthetic efficiency is reduced in future climate scenarios.

Table 2.4.5: Summary of results from GLMM analysing the combined effect of Scenarios (categorical variable, 3 levels: 2018, 2050 and 2100) and diuron (numerical variable) on the ecophysiological endpoints for climate-acclimated *Halimeda opuntia*. Statistically significant ($\alpha=0.05$) effects are in bold. Comparisons *a posteriori* (least-square method), applied to categorical terms with statistically significant main effects.

Endpoint	Factor	X ²	p	Post hoc results
$\Delta F/F_m'$	Climate scenarios	3.65	0.1614	
	Diuron	79.37	<0.0001	
	Interaction	11.28	0.0036	
Net Photosynthesis	Climate scenarios	10.94	0.0042	2018>2100
	Diuron	18.07	<0.0001	
	Interaction	0.55	0.7578	
Daily Net Calcification rate	Climate scenarios	7.42	0.0245	2050>2018
	Diuron	7.70	0.0055	
	Interaction	3.77	0.1522	
TAC	Climate scenarios	9.00	0.0111	2018>2100
	Diuron	104.73	<0.0001	
	Interaction	0.96	0.6191	
Ca-ATPase	Climate scenarios	4.99	0.0823	
	Diuron	6.21	0.0127	
	Interaction	0.72	0.6961	
Carbonic anhydrase	Climate scenarios	0.25	0.8833	
	Diuron	6.19	0.0129	
	Interaction	0.90	0.6376	

Future climate scenarios and diuron exposure individually had negative effects on net photosynthesis (Table 2.4.5, Figures 2.4.4a and b). The lowest net photosynthesis rate (57.4% inhibition, compared to ambient/no diuron) occurred for the combination of 30 $\mu\text{g L}^{-1}$ diuron and 2100 future climate change scenario and this response, coupled with an absence of interaction (Table 2.4.5), indicates a clear additive effect of these pressures on photosynthesis.

Climate change scenario and diuron alone significantly affected daily net calcification rates (Table 2.4.5). Net calcification rates increased under future climate conditions, with the strongest effect (28.5% increase) under the 2050 scenario (Figure 2.4.5a, Table 2.4.5). Net calcification was reduced by a maximum of 27.7% across climate scenarios in the presence of higher diuron concentrations (Figure 2.4.5b). The absence of interaction between climate and diuron (Table 2.4.5) indicates additivity of responses, but in this case the pressures had the opposite effects on the response.

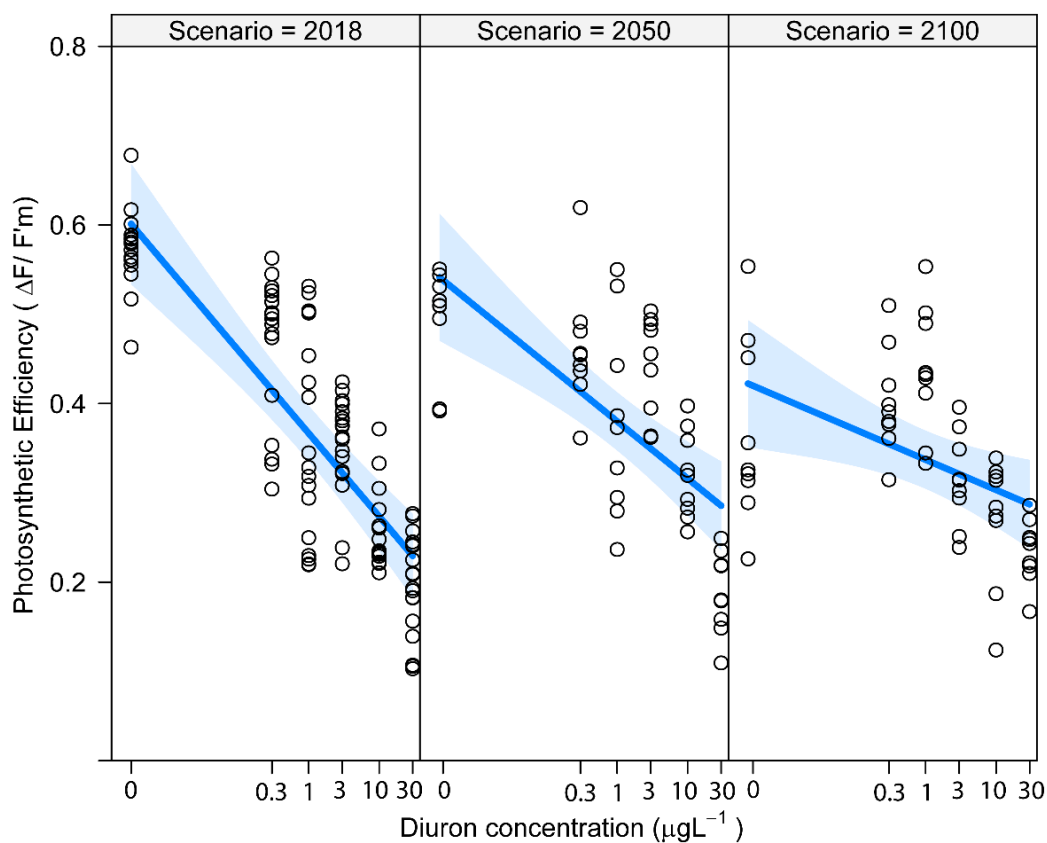


Figure 2.4.3: Interactive effects of climate scenarios and diuron on photosynthetic efficiency. Predicted values (mean $\pm$ SE) in blue, partial residuals in black.

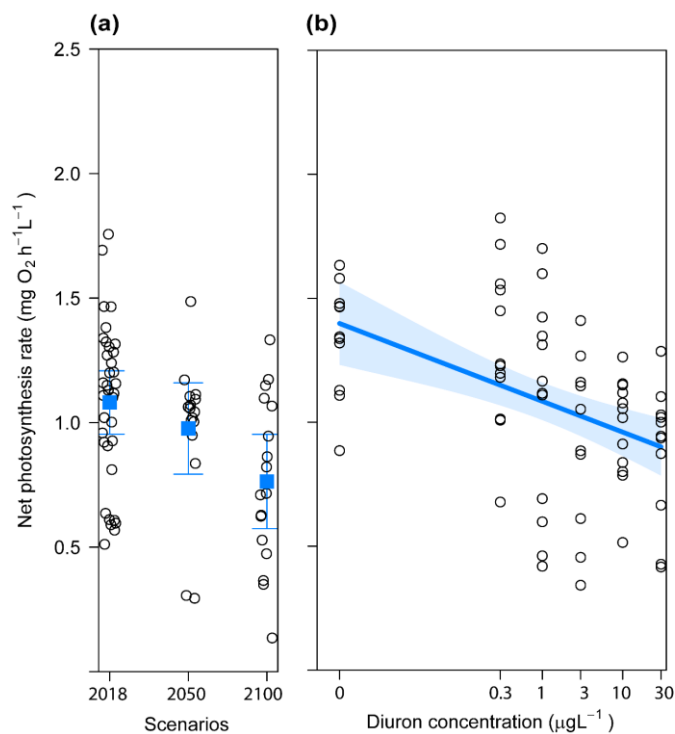


Figure 2.4.4: Main effects on net photosynthesis rate; Predicted values (mean $\pm$ SE) in blue for (a) climate change scenarios; (b) diuron concentration. Partial residuals in black.

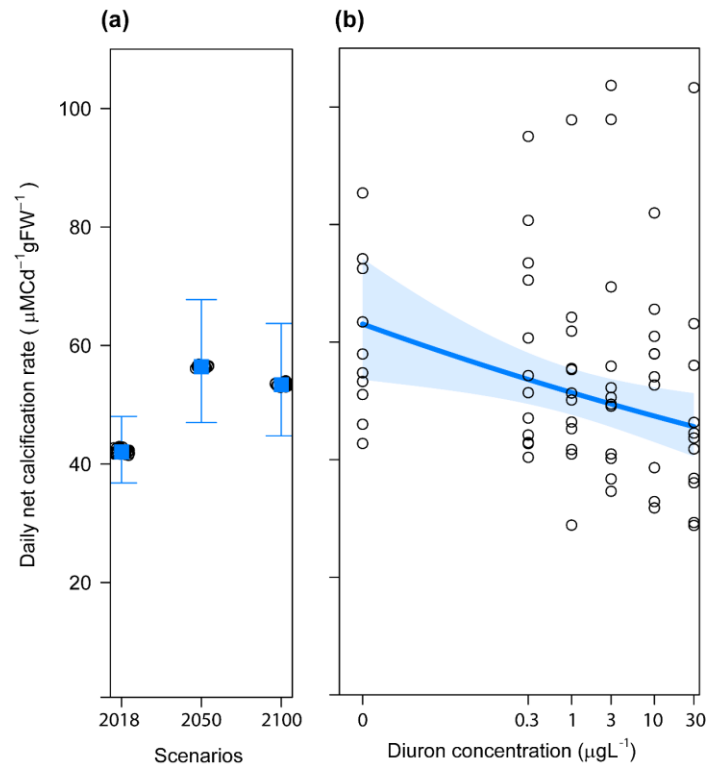


Figure 2.4.5: Main effects on daily net calcification rates; Predicted values (mean $\pm$ SE) in blue for (a) climate change scenarios; (b) diuron concentration. Partial residuals in black.

2.4.4.3 Biochemical biomarkers

Several biochemical biomarkers were assessed in addition to physiological parameters. Both climate scenarios and diuron had a significant effect on TAC, which was reduced by 29.6% under 2100 scenario and by 65.36% following exposure to the highest diuron concentration (Table 2.4.5, Figure 2.4.6). Maximum TAC reduction (66%) occurred at $30 \mu\text{g L}^{-1}$ diuron combined to the 2100 scenario and the absence of interaction (Table 2.4.5) again indicates these pressures combined in an additive way.

Diuron significantly inhibited Ca-ATPase activity (Table 2.4.5), reaching up to 29.7% inhibition under concentrations higher than $10 \mu\text{g L}^{-1}$ (Figure 2.4.7a). Conversely, CA activity significantly increased in response to diuron (Table 5). Under $30 \mu\text{g L}^{-1}$ diuron, CA activity increased 53.2% (Figure 2.4.7b). Climate scenarios did not significantly affect Ca-ATPase and CA, and neither exhibited a significant climate $\times$ diuron interaction.

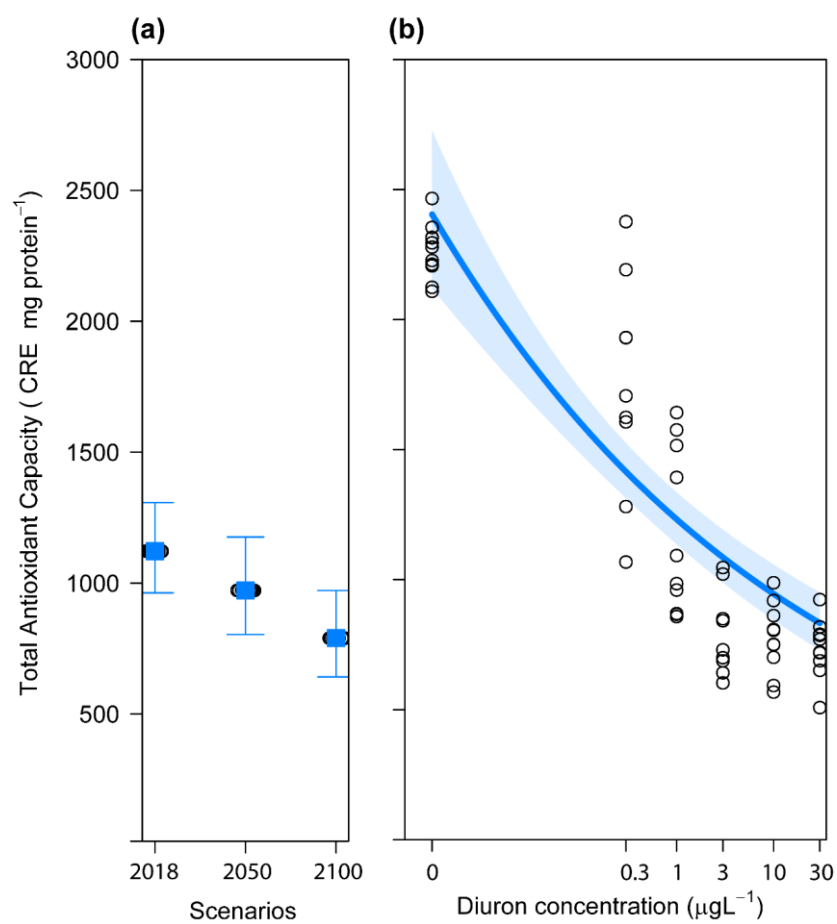


Figure 2.4.6: Main effects on TAC. Predicted values (mean $\pm$ SE) in blue for (a) climate change scenarios; (b) diuron concentration. Partial residuals in black.

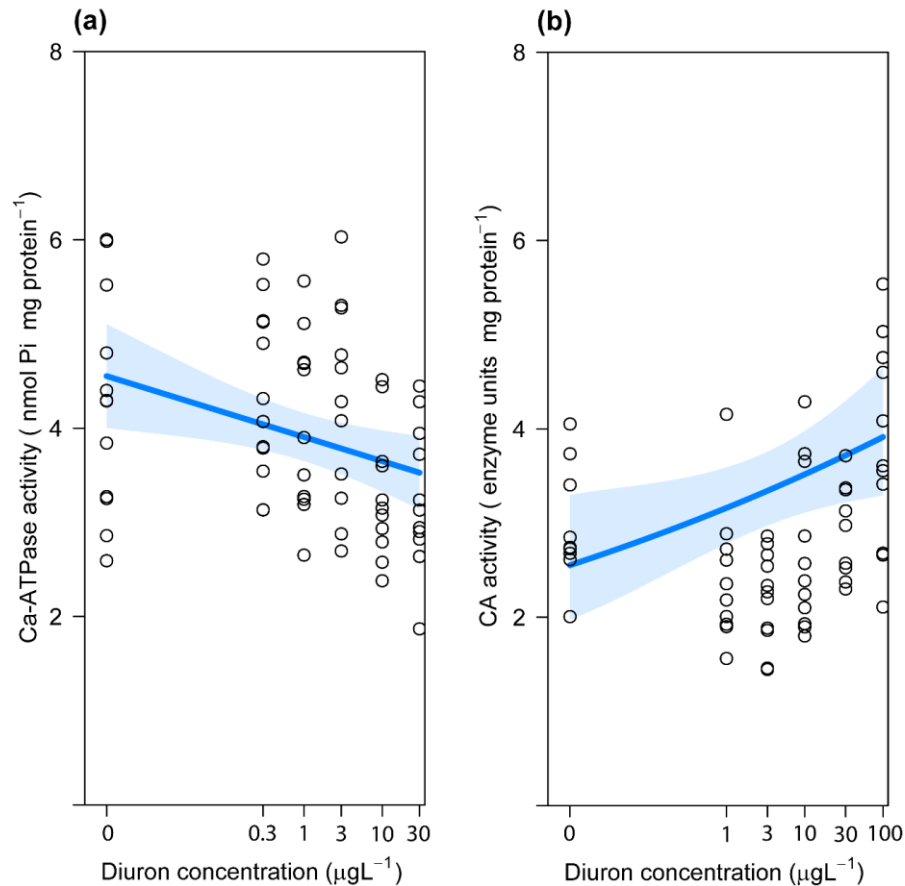


Figure 2.4.7: Main effects of diuron on (a) Ca-ATPase activity and (b) CA activity; Predicted values (mean $\pm$ se) in blue. Partial residuals in black.

2.4.5 Discussion

The simultaneous effects of warming, acidification and herbicide exposure on a calcifying alga were investigated following extended acclimation to three climate scenarios and addressing both biochemical and physiological responses.

2.4.5.1 Acclimation history

The responses of $\Delta F/F_m'$ and net photosynthesis in *H. opuntia* when exposed to the future climate scenarios after 8 months acclimation were over 20% lower than for non-acclimated algae. This result highlights the problem of experimental designs which ignore long-term climate acclimation that are occurring over decadal time scales as the climate changes.

Acclimation history to temperature and light stress can modulate some physiological responses in *Symbiodinium* isolates (Rogers, Marcovich et al., 2010) and corals (Brown, Downs et al., 2002; Edmunds, 2009) exposed to high irradiance and warming. However, since these works focus on the impacts of heat waves and high irradiance events rather than global climate change, they do not consider long term acclimation to increased temperature. An evaluation of climate acclimation durations applied in multi-stressor experiments revealed none of the 45 studies reviewed by Uthicke et al. (2016) had pre-acclimated tropical marine benthic coral reef organisms to OA or OW prior to exposure to local stressors (i.e. light, nutrients, contaminants). Indeed, long-term acclimation to future climate scenarios prior to

evaluating cumulative stress has rarely been assessed (Byrne et al., 2020; Cornwall et al., 2020; Cornwall et al., 2019; Karelitz et al., 2019). While most studies continue to expose reef organisms to relatively sudden OA/OW 'shock', some experiments do consider a gradual change or ramp (from 72 h to four weeks) in OA and/or OW levels (e.g. (Anderson et al., 2019; Brown, Bender-Champ et al., 2019; Kamenos, Burdett et al., 2013; Scherner, Bastos et al., 2018; Wright, Mera et al., 2019)). For example, Zande, Achlatis et al. (2020) exposed coral colonies to OW and OA treatments by gradually increasing treatment levels for over four weeks. However, while ramping to 2100 climate conditions over days to a month might avoid an acute climate shock, this duration of ramped exposure does not represent acclimation over a significant lifespan of many coral reef species. Previous experimental studies evaluating OW and OA, alone or in combination, only acclimatize *Halimeda* fragments to aquarium conditions (48 h to 2 months), and rarely to the tested climate conditions (Campbell et al., 2016; Hofmann et al., 2014; Meyer et al., 2015; Peach et al., 2017; Price, Hamilton et al., 2011; Vogel et al., 2015b), and results from the current study indicate that failing to adequately acclimate organisms may lead to the exaggeration of some physiological responses in response to secondary stressors.

Some research on the evolutionary aspects of potential adaptation or acclimatization of marine species to OA and OW has only recently commenced (Byrne et al., 2020; Comeau, Cornwall et al., 2019; Uthicke et al., 2020). Still, little is known about the potential for calcareous algae to acclimatize or adapt to climate change (Clark, Poore et al., 2013; Zou & Gao, 2013). However, Cornwall et al. (2020) recently demonstrated that some species of coralline red algae can become more tolerant to OA after six generations of exposure. Growth was affected in recruits initially exposed to OA, but this difference disappeared by the seventh generation. That study also revealed that pre-exposure to high pH variability did not accelerate tolerance, contrary to former hypotheses (Cornwall et al., 2020; Kapsenberg & Cyronak, 2019). Furthermore, Vogel et al. (2015a) found that several *Halimeda* species can acclimatize to future OA conditions and thrive around natural CO₂ seeps (mean pH_{Total} = 7.8, pCO₂ = 862 µatm), exhibiting similar net calcification and photosynthesis to algae in control areas. On the other hand, some laboratory experiments have demonstrated that acclimation to OA (based on calcification parameters) does not occur within one year for multiple calcifying coral reef species (including *Halimeda*) (Comeau et al., 2019).

Reviews by Cornwall et al. (2019) and McCoy and Kamenos (2015) recommend future research on calcifying algae to prioritize longer term experiments and consider long acclimation to OA and OW. Recent studies on sea urchin using the same mesocosm system as described in the current study also suggested that effects of OA and OW on sea urchins in short term experiments without acclimation may have exaggerated potential climate effects (Uthicke et al., 2020). Ideally, we suggest investigations into cumulative effects of climate change and additional stressors would include substantial climate acclimation or even multi-generational adaptation periods.

2.4.5.2 Climate change scenario effects on organismal response and biochemistry

After eight months acclimation to the three climate scenarios in a mesocosm, photosynthetic efficiency ($\Delta F/F_m'$) of *H. opuntia* was significantly reduced in the future climate scenarios, indicating incomplete acclimation of the photosynthetic apparatus. Net photosynthesis also remained suppressed under future climate scenarios in comparison to algae maintained in

'present day' conditions. Conversely, net calcification rate was highest under the 2050 scenario, indicating the potential links between photosynthesis and calcification under conditions of OA and OW are not straightforward.

The cumulative effects (and direction of effects) of climate scenarios and diuron on the measured responses of *H. opuntia* are summarised in Table 6. Diuron was the main factor driving the ecophysiological responses and this interacted with the climate change treatments to affect $\Delta F/Fm'$, while additive effects (no interaction, but both main effects significant and in the same direction) were observed for net photosynthesis and TAC. Diuron also affected each of the biochemical responses, while future climate conditions only affected TAC in *H. opuntia*.

Table 2.4.6: Summary and direction of effects (based on general linear mixed models (GLMM) given in Table 2.4.5). Black symbols indicate statistically significant effect ($p < 0.05$); Open symbols indicate no effect ($p > 0.05$). Upward and downward arrows indicate increase or decrease in response to the stressor (OW+OA or diuron). Circles indicate no consistent pattern of response to the stressor.

Endpoint	Summary of GLMM		
	Climate scenario	Diuron	Interaction
$\Delta F/Fm'$	↓	↓	●
Net photosynthesis rate	↓	↓	↓
Net calcification rate	↑	↓	○
TAC	↓	↓	↓
Ca-ATPase	↓	↓	○
CA	○	↑	○

Few studies have investigated the effect of both increased temperature and OA on *Halimeda* (Campbell et al., 2016; Sinutok, Hill et al., 2011). Campbell et al. (2016) found that OW (+3°C) mitigates the negative effects of OA (-0.4 pH units) on calcification in *Halimeda spp.*, consistent with the modest upregulation on net calcification in our study. Sinutok et al. (2012) found both photosynthesis and calcification in *Halimeda spp.* reduced under 4-week exposure to OW (+4°C) combined to OA (-0.4 pH units). Meyer et al. (2015) and Vogel et al. (2015a) on the other hand found reduced calcification but photosynthesis was unaffected under elevated pCO_2 of 996-1073 μatm (compared to controls). These differences in experimental outcomes from experimental OA/OW exposures may be resulting from different pCO_2 levels, temperature levels, different experimental conditions (including acclimation) or from species-specific traits.

Few studies evaluated biochemical responses of calcifying algae to environmental stressors (Hofmann et al., 2014; Hofmann, Straub et al., 2013; Scherner et al., 2016). Of the three biochemical parameters measured in the current study, only total antioxidant capacity (TAC) was affected by OW+OA conditions in acclimated *H. opuntia*. Oxidative stress parameters are widely used to assess organismal fitness and environmental health (Downs, Fauth et al., 2002; Downs, Fauth et al., 2005; Lesser, 2006; Monserrat, Martínez et al., 2007). For corals, hydrocorals and symbiont-bearing foraminifera, oxidative stress parameters were shown to be

effective biomarkers of stress by OW, OA or pollution (Downs, Mueller et al., 2000; Luz, Zebral et al., 2018; Prazeres, Martins et al., 2012; Stuhr, Reymond et al., 2017). *Halimeda* species boast impressive antioxidant protection ((Novoa, Mara de Oliveira Silva et al., 2017) and references therein), and have been investigated as a source of natural antioxidants (Novoa, Andrade-Wartha et al., 2011; Saad-Allah & Nessim, 2016), but no previous work has assessed oxidative responses of calcifying algae to climate change or pollution.

In the present work, an 8-month acclimation to 2100 OA and OW conditions led to reduced TAC levels in *H. opuntia*, but levels were unaltered under 2050 conditions. This may result from a strong baseline of antioxidant capacity, which confers *H. opuntia* resilience to the formation of reactive oxygen species (ROS) by mild environmental change (Gazali, Nurjanah et al., 2019; Saad-Allah & Nessim, 2016). The higher temperatures and lower pH in the 2100 scenarios may induce oxidative stress (Liao, Yang et al., 2019; Stuhr et al., 2017) which partially overwhelms this antioxidant capacity, accounting for the observed effects on TAC. Considering that oxidative stress parameters are potential monitoring tools (Marangoni, Dalmolin et al., 2019) and that *Halimeda* has high antioxidant levels (Novoa et al., 2011; Saad-Allah & Nessim, 2016), it is suggested that investigating the antioxidant capacity of green algae could clarify the biochemical mechanisms behind *Halimeda*'s resilience to OA and OW.

While photosynthesis and calcification in *H. opuntia* were both affected under future climate conditions, carbonic anhydrase (CA) the enzyme which acts on both processes (CO₂ fixation and biocalcification, as well as acid-base balance (Bertucci, Moya et al., 2013) was unaffected (Table 2.4.6). In contrast, Hofmann et al. (2014) and Scherner et al. (2016) found increased CA activity on *Halimeda* spp. exposed more extreme OA at *p*CO₂ conditions. The lack of response by CA may be due to the modest acidification conditions applied here, or to a potential antagonism between the influence of OA and OW. Thermal stress can inhibit CA in *Symbiodinium* microalgae but not in its Zoanthid host (Graham, Parekh et al., 2015), and can also downregulate CA expression in corals (Bertucci et al., 2013). Little is known of the combined effects of OW and OA on CA activity from calcifying species and further studies on its response to OA and OW alone may elucidate its role and potential as a maker for climate stress in calcifying green algae.

Ion-transporters, such as Ca-ATPase, are purportedly involved in calcification process (Al-Horani, Al-Moghrabi et al., 2003; Hofmann, Koch et al., 2016) and have been applied as a cellular biomarker of stress from climate change and/or pollution on corals (Fonseca et al., 2017), hydrocorals (Marangoni, Marques et al., 2017), and foraminifera (Marques et al., 2020a; Prazeres et al., 2012; Prazeres, Uthicke et al., 2015). While calcification was affected by future climate scenarios, there was not corresponding effect on Ca-ATPase activity, again highlighting the complex pathways between biochemical responses and physiological effects.

2.4.5.3 Diuron exposure

The responses of most parameters in *H. opuntia* under diuron exposures were far stronger than responses affected by the future climate conditions applied. It is known that water quality may modify ecosystem resilience (MacNeil et al., 2019), and herbicides are contaminants of global relevance frequently detected in the Great Barrier Reef (Lewis et al., 2009). Therefore, the magnitude of the response found for diuron in this study is concerning.

The concentration of diuron which inhibited $\Delta F/Fm'$ by 50% (EC_{50}) of $6.2 \mu\text{g L}^{-1}$ was similar to diuron EC_{50} s for other tropical calcifiers such as corals and crustose coralline algae (Negri et al., 2011). This reduction in $\Delta F/Fm'$ by diuron indicates direct blocking of PS II reaction centres (Jones et al., 2003), which in turn explains the reduction of net photosynthesis, also observed at elevated diuron concentrations (Ralph et al., 2007). Both photosynthesis and the activity of carbonic anhydrase are important processes for calcification (Wizemann, Meyer et al., 2014), with CA involved in conversion of carbon species and pH gradients favouring CaCO_3 precipitation. Photosynthesis in macroalgae is also postulated to facilitate calcification (Beer de & Larkum, 2001; McNicholl, Koch et al., 2019). It was therefore not surprising the strong effect of diuron on *H. opuntia* photosynthesis resulted in concomitant reduction in both calcification and Ca-ATPase activity, as well as an up-regulation of CA. Photoinhibition by diuron also leads to the production of ROS and oxidative stress (Almeida, Gomes et al., 2019; Nestler, Groh et al., 2012; Rutherford & Krieger-Liszky, 2001). However, it is unclear whether ROS, generated by herbicide exposure, directly inhibited the enzyme (Moreau, Castilho et al., 1998) or that a lack of energy/ATP (caused by reduced production) affected the active ion transport.

Decreasing levels of TAC during diuron exposure may indicate the employment of non-enzymatic antioxidants to prevent oxidative damage, or direct binding to antioxidants. A possible mechanism behind the depletion of non-enzymatic antioxidants is the application of Glutathione reductase (GSH) on the biotransformation system, as it is expected that diuron would induce the action of detoxification enzymes (e.g. Glutathione-S-Transferase (GST), which uses GSH as substrate) (Lushchak, 2011; Marques, Silva de Assis et al., 2014; Torres, Pires et al., 2002). The decrease in TAC under diuron exposure are strongly indicative of increased ROS formation, which triggers many biochemical effects, including ATPase inhibition (Lushchak, 2011; Tellis, Lauer et al., 2014). *H. opuntia* may be investing energy to prevent oxidative stress, in a context of decreased photosynthesis and potential energy shortage. Increased CA activity is suggested to result from increasing CO_2 supply to compensate for reduced photosynthetic efficiency (Campbell et al., 2016; Price et al., 2011). It is difficult to detangle the relationship of CA with photosynthesis and calcification as this response may also be a compensation to maintain calcification levels (Hofmann et al., 2013).

2.4.5.4 Combined effects of diuron and future climate scenarios

Net photosynthesis, net calcification and TAC demonstrated significant negative individual effects of diuron and future climate scenarios on *H. opuntia* (Table 2.4.6). The lack of statistically significant interactions between these factors suggests that diuron effects do not change across climate scenarios. However, given that both climate condition and herbicide reduced photosynthesis and TAC significantly, the combined effects of these parameters are considered additive for *H. opuntia*, increasing the known number of tropical reef species that can be affected by climate change more severely in the presence of herbicides (Negri et al., 2011; Uthicke et al., 2016; van Dam et al., 2012a).

Interactive effects were observed for $\Delta F/Fm'$, with algae becoming less sensitive to moderate ($3 - 10 \mu\text{g L}^{-1}$) concentrations of diuron under the 2050 climate scenario. While we expect toxicity thresholds for whole organism effects (i.e. growth, reproduction and survival) to increase at temperatures above acclimation (Negri et al., 2020), some biochemical physiological processes can become optimal near the upper limit of their thermal range. For

example, similar reductions in the sensitivity of photosynthetic efficiency to diuron at moderately elevated temperatures have been observed for corals (Negri et al., 2011) and seagrass (Wilkinson et al., 2017). This may be resulting from the influence of temperature on the toxicokinetic of diuron in the algae (only +0.86°C above ambient in the 2050 scenario), but it is unclear whether elevated $p\text{CO}_2$ in the 2050 scenario could also play a role.

Algae from both climate future scenarios responded to diuron with reduced TAC. This indicates that, under future scenarios of OW and OA, more antioxidant defences are required by *H. opuntia* to deal with diuron exposure. The observed effects of climate scenarios and diuron on TAC (no interaction) suggests an additive response, with TAC depletion greatest under diuron exposure in future climate scenarios. While physiological and biochemical responses indicated that photosynthesis, calcification and the antioxidant defence system of this calcifying algae were all affected by diuron (and the effects were often greatest in the 2010 scenario), no algal death or necrosis was observed during the 15-day co-exposure. So, although climate change will likely reduce the tolerance of *H. opuntia* to herbicide contamination by additive and interactive pressures, this species was able to survive the high herbicide concentrations over this duration under future climate conditions.

Populations of *Halimeda* are commonly observed at potentially stressful locations like carbon dioxide seeps (Vogel et al., 2015a), but there is no field data that assesses the health of this alga across both $p\text{CO}_2$ and water contamination gradients. Diuron concentrations approaching $1 \mu\text{g L}^{-1}$ can be found in nearshore waters of the Great Barrier Reef (Lewis et al., 2012, 2009). Across all climate scenarios this concentration of diuron reduced F/F_m' by 19-37%, net photosynthesis by 7- 54%, calcification by 7 – 46% and TAC by 25 to 64%. While each of these responses to diuron are sub-lethal, reduced calcification due to herbicide exposure over prolonged periods could have flow-on effects ecological effects as *Halimeda* sp. are considered key habitat forming species on coral reefs, shoals and banks in tropical ecosystems. Increased calcification under the 2050 climate scenario may offer temporary protection to *H. opuntia* against the effects of herbicide; however, inhibition of calcification by diuron increased under the 2100 climate scenario. Recent studies emphasize the relevance of considering multiple stressors scenarios on climate change research (Ban et al., 2014; Wolff et al., 2018b). The observed effects of both herbicide and future-climate conditions on the health of *H. opuntia* highlights the need to address both climate change and water quality.

This study was the first to demonstrate that key calcifying green algae are vulnerable to herbicides, and that its physiological and biochemical responses were modified under acclimation to future climate scenarios. Furthermore, the combined and consistent physiological and biochemical evidence of negative impacts (across six indicators) of both herbicide exposures and future climate conditions on the health of *H. opuntia* indicates that the maintenance of healthy populations is best served by reducing carbon emissions and improving water quality. The experiments also established that *H. opuntia* can partially acclimate to extended high OA/OW conditions, and the current standard practise of not acclimating organisms in marine climate change experiments would lead to erroneous results. To improve ecological relevance and minimise exaggerated responses, future laboratory studies should also apply appropriate acclimation periods.

3. ADJUSTING TROPICAL MARINE WATER QUALITY GUIDELINE VALUES FOR ELEVATED OCEAN TEMPERATURES

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3.1 Summary

Increased frequency of summer heatwaves and poor water quality are two of the most prevalent and severe pressures facing coral reefs. While these pressures often co-occur, their potential risks to tropical marine species are usually considered independently. Here we extended the application of multi-substance-PAF (ms-PAF) to a non-chemical stressor – elevated sea surface temperature. We then applied this method to calculate climate-adjusted water quality guideline values (GV) for two reference toxicants: copper and the herbicide diuron for tropical marine species. Firstly, we developed a species sensitivity distribution (SSD) for thermal stress based on published experimental data for 41 tropical benthic marine species, using methods adapted from water quality GV derivation (Figure 3.1). This enabled quantitative predictions of community effects as temperatures exceeded acclimation values.

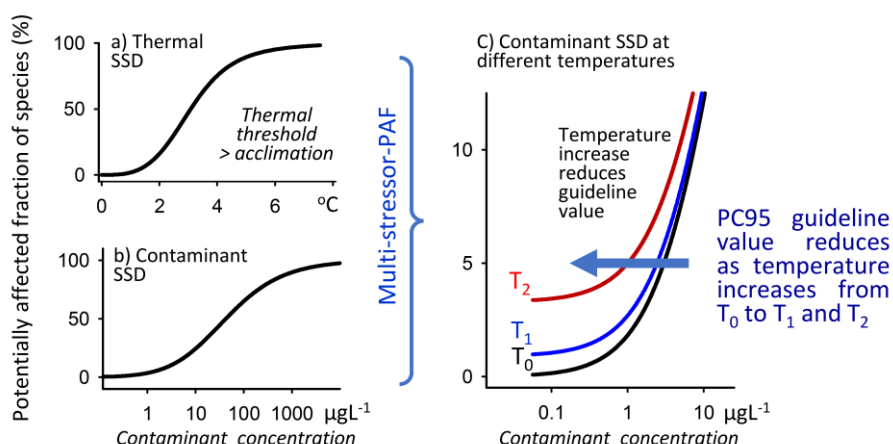


Figure 3.1: Combining the effects of contaminant and thermal stress using ms-PAF to adjust water quality guideline values for contaminants, (a) species sensitivity distribution (SSD) for thermal stress, (b) SSD for contaminants such as the herbicide diuron and the metal copper and (c) the potentially affected fraction of species affected by the contaminant as the temperature increases.

The resulting protective temperature values (PTx) were similar to temperatures known to initiate coral bleaching and are therefore relevant for application in multi-stressor risk

assessments. The extended ms-PAF method enabled the adjustment of current water quality GVs to account for thermal stress events. This approach could be applied to other ecosystems and other non-contaminant stressors (e.g. sediment, low salinity, anoxia and ocean acidification), offering an alternative approach for deriving environmental GVs, reporting and assessing the risk posed by multiple stressors (Figure 3.1).

3.2 Introduction

Tropical marine ecosystems are under increasing pressure from ocean warming, and stressful warming events can occur simultaneously with poor water quality to accelerate the decline of coral reefs (Ban et al., 2014; Hughes et al., 2017a). Maintaining contaminant concentrations below water quality guideline values (GV) is one objective of water quality management programs, intended to improve the resilience and recovery of reefs to thermal bleaching of corals (Brodie, Baird et al., 2017a; Reef 2050, 2018). However, current GVs are derived from single-chemical ecotoxicological experiments performed under optimal conditions and do not account for additional stressors that can affect the toxicity of contaminants (Heugens, Hendriks et al., 2001; Kwok, Leung et al., 2007; Lau, Yung et al., 2014; Zhou, Wang et al., 2014). There are many studies that demonstrate the influence of temperature on the sensitivity of marine ectotherms to contaminants, and increases in metal toxicity above a thermal optimum is a common example (Cairns, Heath et al., 1975; Heugens et al., 2001; Noyes, McElwee et al., 2009; Zhou et al., 2014). As temperatures in tropical coral reef waters have already increased by around 1°C since the industrial revolution (Lough et al., 2018), and further 2 – 3°C increases are predicted by 2100 (Lough et al., 2018; Magnan, Colombier et al., 2016), it is crucial to consider adjustments to water quality GVs (“climate-adjusted-thresholds”) for heatwave conditions that will become more frequent and longer in duration as global temperatures increase (Oliver, Donat et al., 2018).

Understanding the effects of temperature on marine water quality GVs requires data representative of marine communities (rather than single species), but there are currently no empirical datasets that quantify the combined risk of thermal stress and any contaminant to marine communities. Contaminant GVs are usually based on species sensitivity distributions (SSD) (Posthuma, Suter II et al., 2002); that describe the relationship between the potentially affected fraction (PAF) of multiple species relative to contaminant concentration. SSDs developed from species and toxicity test conditions relevant to different climates often show differences in sensitivity to a range of contaminants (Bejarano, Gardiner et al., 2017; Kwok et al., 2007; Raymundo, Rocha et al., 2019). For example, Zhou et al. (2014) modelled the influence of temperature on SSDs for three metals over 10 and 15°C temperature ranges, and showed the concentration protective of 90% of species reduced by up to an order of magnitude for warmer climate species, suggesting a 10-fold safety factor to protect communities across wide temperature ranges. However, those SSDs were each developed from optimal temperature tests and do not address the case where organisms are exposed to contaminants outside their normal thermal ranges.

Generating SSDs that account for specific contaminants at multiple temperatures is presently limited by inadequate experimental data (Zhou et al., 2014). An alternative approach is to estimate the PAF of the combined effects from individual SSDs of chemicals with an SSD derived for thermal effects, using the multi-substance potentially affected fraction (ms-PAF) method (Traas, Van de Meent et al., 2002) (re-named here “multi-stressor-PAF” to indicate

that chemical and non-chemical stressors are being combined). This method is mainly used to predict the combined effects of chemical mixtures from individual chemical SSDs by applying well-established models of joint action (de Zwart & Posthuma, 2005; Plackett & Hewlett, 1952), such as the independent action (IA) model (also known as the response addition model), applied where the chemicals do not have the same mode of action (de Zwart & Posthuma, 2005). Originally intended to address mixture toxicity for single species, their application to predict the ecological risk of complex chemical mixtures by applying these to multiple SSDs has been proposed (Traas et al., 2002), critically reviewed (Backhaus & Faust, 2012), and applied to mixtures of contaminants with up to 10 different modes of action (Parkerton, Bok et al., 2018). While limited by assumptions that are not always met, ms-PAF rarely overestimates combined effects and represents the most used and validated “bottom-up” approach to predict the effects of multiple contaminants on communities from single contaminant data (Parkerton et al., 2018; Traas et al., 2002). To extend this application to include thermal stress on marine communities, SSDs are needed that relate the PAF of a marine assemblage to temperature stress (there are already many aquatic SSDs for individual contaminants (Posthuma, van Gils et al., 2019)). Like metals and many contaminants, thermal stress acts on multiple pathways simultaneously (Cairns et al., 1975) and these will differ between species; therefore, temperature stress need not be treated differently to most contaminants with respect to deriving SSDs. So far, thermal SSDs have been described for freshwater fish (Leuven, Hendriks et al., 2011) and molluscs (Verbrugge, Schipper et al., 2012), as well as mostly temperate coastal species across a 15°C range (from 5 to 20°C) (de Vries, Tamis et al., 2008). However, thermal SSDs for tropical marine species are likely to be distinctive as they are exposed to narrower temperature ranges, leading to low temperature stress thresholds above species optima for many taxa (Gaston, 2009; Somero, 2005).

Here, we developed the first thermal SSD for tropical species based on temperature stress thresholds of 41 species across 12 phyla. This allowed the derivation of temperatures below which 99, 95, 90 and 80% of tropical marine species respectively should be protected. We then explore the use of the ms-PAF IA model to combine temperature and chemical SSDs to derive climate-adjusted water quality GVs.

3.3 Materials and Methods

3.3.1 SSD for thermal stress

Since SSDs represent a community response to stress and thermal stress acts on multiple pathways, the development of a thermal SSD for marine communities requires temperature stress data for multiple species across diverse phyla (de Vries et al., 2008). A literature search was conducted to identify studies that had quantified the effects of elevated temperatures on benthic marine species associated with tropical coral reefs. The Web of Science and Google Scholar were used to search for literature published after 1980 using the following terms: (aquatic OR marine OR estuar* OR coral) AND (temperature OR thermal OR temp*) AND (ecotoxic* OR toxic*) AND (tropical OR sub- tropical OR subtropical), and supplemented with papers cited in recent reviews (Ban et al., 2014; Uthicke et al., 2016). Only studies with species that occur in the tropics and on coral reef ecosystems were included, and only tests performed at 18°C or higher, which has been identified as the minimum temperature for coral reef growth (Kleypas, McManus et al., 1999), were considered. In order to apply ms-PAF to estimate the joint effects of multiple stressors, SSDs need to be derived from consistent endpoint metrics

(e.g. LC₅₀) and exposure durations (either acute or chronic) (Traas et al., 2002). The contaminant SSDs (below) were derived using Australian and New Zealand water quality guideline (ANZ) methods (Hobbs, Warne et al., 2005; Warne et al., 2018a), so the thermal SSD was derived using the same methods. Temperature stress data were eligible for consideration if the source included adequate descriptions of: (i) the temperatures applied and exposure period; (ii) replication; and (iii) the statistical method or model used to determine effect. Studies were only included if they: (i) reported ecologically-relevant endpoints (e.g. mortality, growth, reproduction) and (ii) were conducted over “chronic” exposure durations (as defined in Warne et al. (Warne et al., 2018a)). The data quality assessment process was formalised by answering 18 questions on how the data were generated (e.g. test organism, experimental, design and statistical analysis) based on the information provided in the articles (Hobbs et al., 2005; Warne et al., 2018a). Only “acceptable” quality experimental data (score ≥50% (Warne et al., 2018a)) were included in the thermal SSD to ensure reliable and consistent temperature stress values. Most of the publications reported multiple endpoints; however, the dataset was reduced to obtain a single threshold value to represent the sensitivity of each species in the SSD (Warne et al., 2018a).

Metrics for temperature stress have previously included the temperature at which 50% of organisms are killed (ET₅₀), or the time taken to reach 50% mortality (de Vries et al., 2008; Urban, 1994). For consistency with existing contaminant SSDs, only thermal effects data that caused a 10% ecologically relevant effect (ET₁₀) or no observed effect (NOET) for each species were used to create the thermal SSD in the present study. We defined the temperature threshold (TT_x) for each species as the maximum temperature above acclimation at which negative affects did not occur as follows:

$$TTx = Tx - Ta \quad (1)$$

Ta (°C) is the experimental acclimation temperature (even for short acclimation periods). Most experiments applied few temperature temperatures and Ta was assumed optimal. Tx (°C) is the highest temperature exceeding the Ta where there is no statistically significant effect (P > 0.05) on species x (i.e. NOET) or the effect is not greater than 10% (i.e. ET₁₀). If the NOET was equivalent to the acclimation temperature (Ta), the data were excluded, as this provided no information to identify a thermal effect threshold above acclimation.

For consistency with the copper and diuron SSDs (below), the thermal SSD was derived using the Burrlioz 2.0 software (CSIRO, 2019). This software applies the Burr type III statistical distribution that best fits the effect TTx data of multiple species, and calculates the potentially affected fraction of species in a community (PAF (%)) according to the following equation:

$$PAF (\%)_{Burr\ III} = \frac{100}{\left[1 + \left(\frac{b}{TTx}\right)^c\right]^k} \quad (2)$$

where b, c and k are location and shape parameters of the selected distribution. Burrlioz 2.0 calculated temperatures for four levels of ecosystem protection, i.e. the protective temperature (PT_x) for 80, 90, 95 and 99% of species in a community/ecosystem (PT80, PT90, PT95 and PT99, respectively). Alternative SSD calculation methods (e.g. gamma, lognormal, log-log) derived almost identical PT_x values (Table S2). This thermal SSD, derived from chronic data, will be relevant to extended periods of increased sea surface temperature (e.g. summer

heatwave conditions of several weeks), but not short-term temperature fluctuations (e.g. pulsed releases of heated water from industry) nor to the longer-term increases to basal sea surface temperatures associated with climate change.

3.3.2 SSD for copper and diuron

Ecotoxicity data to generate the SSD for copper (26 marine species from 8 phyla) were obtained from the Australian and New Zealand water quality guidelines (ANZG, 2018). These data were originally collated and used to derive the copper guideline values in 2000. Ecotoxicity data to generate the SSD for diuron (20 marine species that belonged to six phyla) were obtained from recently proposed water quality guidelines (Warne et al., 2018b). The SSDs for these contaminants were also derived using Burrlioz 2.0 (CSIRO, 2019).

3.3.3 *ms-PAF* to predict the joint effects of thermal stress and contaminants using the Independent Action model

The independent action (IA) model of joint action is used for stressors that have different modes of action but do not interact at the target site of toxic action (de Zwart & Posthuma, 2005; Traas et al., 2002). IA was chosen as the most appropriate model of joint action to estimate the combined effect of temperature stress on each of the two contaminants as the modes of action of all three stressors are likely to differ (including between species) and because IA is considered more conservative (generally predicts lower joint action) than the alternate concentration addition approach (Traas et al., 2002). Furthermore, IA was previously shown to be a good predictive model for single species responses to copper and diuron in combination with thermal stress (Uthicke et al., 2016; van Dam et al., 2012a; van Dam, Uthicke et al., 2015). Using the IA model of joint action, the “multiple stressor” potentially affected fraction of a community (*ms-PAF_{IA}*) was calculated by combining the PAF of each stressor (Traas et al., 2002) as follows:

$$ms-PAF_{IA} = PAF_A + PAF_B - (PAF_A \times PAF_B) \quad (3)$$

where *PAF_A* and *PAF_B* are the potentially affected fractions from two stressors with different modes of action that do not interact (e.g. temperature and copper or temperature and diuron), scaled from 0 (0% of species in the community affected) to 1 (100% of species in the community affected).

3.4 Results

3.4.1 Data contributing to the thermal SSD.

The literature search identified thermal stress data for 41 species belonging to 12 phyla which met the minimum data requirements to derive an SSD (Hobbs et al., 2005; Warne et al., 2018a). The species included tropical habitat building corals, crustose coralline algae, calcifying green algae, seagrasses, reef-associated invertebrates including foraminifera and sea urchins, as well as benthic diatoms and coral symbionts.

3.4.2 Thermal SSD and guideline values for thermal stress and contaminants

The thermal threshold (*TT_x*) values ranged from 1.0 – 6.3°C. The curve fit was not bimodal and there was no apparent relationship between taxa and threshold. For example, some coral

species had TTx values as low as 1 – 2°C above acclimation, while the survival of other coral species were only affected at 6°C above acclimation (Figure 3.2). There was no correlation between the acclimation temperature T_a and the TTx in this dataset ($R^2 = 0.0125$). The fit of the Burr III distribution to the data, combined with the amount and types of ecotoxicity data available, resulted in a set of ‘very high reliability’ (Warne et al., 2018a) PTx values (Table 3.1). These PTx values are the equivalent of PCx values for contaminants, such that at least 99% of species will be protected from adverse chronic effects provided the temperature was not greater than 0.83°C above the acclimation temperature and no contaminants were present (Table 3.1). Similarly, at least 95% of species would be protected from adverse chronic effects provided the temperature was not greater than 1.4°C above the acclimation temperature and no contaminants were present.

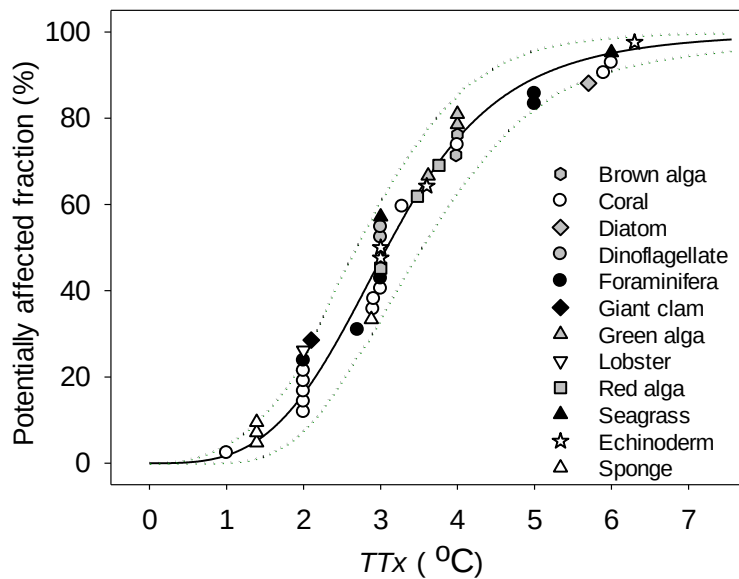


Figure 3.2: Species sensitivity distribution (SSD) of the thermal threshold data (TTx) used to derive protective temperature values (PTx). SSD was fitted with the Burr III function using Burrlioz 2.033 and replotted using SigmaPlot 13 (Systat Software Inc.). The dashed lines are the 95% confidence intervals for the SSD.

The diuron and copper SSDs generated ‘very high reliability’ PCx values (Table 3.2) based on the fit of the Burr III distribution to the data combined with the amount and type of ecotoxicity data available (Warne et al., 2018a). The PCx values for diuron were identical to those previously published by Warne et al. (2018a), while the PCx values for copper were very similar to those in the Australia and New Zealand national water quality guidelines (0.3, 1.3, 3, 8 $\mu\text{g L}^{-1}$ for Cu PC99, PC95, PC90, PC80 respectively (ANZG, 2018)). These minor differences in PCx values are due to updates in Burrlioz software since the copper GVs were originally derived in 2000 (the same data were used to derive the previous GVs and those generated in this study).

Table 3.1: The protective temperatures PTx (% CI range) for elevated temperature exposure in the absence (SSD, Figure 3.2) and in the presence of elevated concentrations of contaminants (Figure 3.3). Concentrations of copper and diuron equivalent to their PCx values were applied resulting in identical adjustments to PTx for each (or any) contaminant. Grey percentages in brackets indicate the % change in the PTx values compared to increased temperature alone (no contaminant present).

Thermal stress	PT99 (°C)	PT95 (°C)	PT90 (°C)	PT80 (°C)	PT50 (°C)
No contaminants	0.83 (0.50—1.4)	1.4 (1.0—1.8)	1.7 (1.4—2.1)	2.2 (1.8—2.5)	3.1 (2.6—3.5)
+PC99 Cu or diuron	-	1.3 (8%)	1.7 (2%)	2.1 (4%)	3.1 (1%)
+PC95 Cu or diuron	-	-	1.4 (18%)	2.0 (10%)	3.0 (3%)
+PC90 Cu or diuron	-	-	-	1.8 (20%)	2.9 (6%)
+PC80 Cu or diuron	-	-	-	-	2.7 (13%)

Table 3.2: Protective concentrations PCx (95% CI range) for copper and diuron exposures at the acclimation temperature (T_a) of each species. The table also includes the influence of elevated temperature on PCx values ($\mu\text{g L}^{-1}$) for copper and for diuron exposures. Grey percentages in brackets indicate the % change in the PCx values due to temperature exposure above the T_a . As temperatures increase to 1.5°C above acclimation no adjustment is possible for PC99 and PC95 as > 5% of species are predicted to be affected by temperature alone (Table 3.1).

Copper	PC99 ($\mu\text{g L}^{-1}$)	PC95 ($\mu\text{g L}^{-1}$)	PC90 ($\mu\text{g L}^{-1}$)	PC80 ($\mu\text{g L}^{-1}$)	PC50 ($\mu\text{g L}^{-1}$)
T_a	0.29 (0.024—2.2)	1.4 (0.44—5.4)	3.0 (1.2—9.4)	7.4 (3.0—20)	42 (20—108)
$T_a + 0.5^\circ\text{C}$	0.25 (14%)	1.4 (0%)	3.0 (0%)	7.4 (0%)	42 (0%)
$T_a + 1.0^\circ\text{C}$	-	0.90 (36%)	2.4 (20%)	6.6 (11%)	40 (5%)
$T_a + 1.5^\circ\text{C}$	-	-	1.0 (67%)	4.7 (36%)	35 (17%)
$T_a + 2.0^\circ\text{C}$	-	-	-	1.3 (82%)	25 (40%)
$T_a + 2.5^\circ\text{C}$	-	-	-	-	13 (69%)
Diuron	PC99 ($\mu\text{g L}^{-1}$)	PC95 ($\mu\text{g L}^{-1}$)	PC90 ($\mu\text{g L}^{-1}$)	PC80 ($\mu\text{g L}^{-1}$)	PC50 ($\mu\text{g L}^{-1}$)
T_a	0.43 (0.079—0.77)	0.67 (0.39—1.1)	0.86 (0.70—1.4)	1.20 (0.99—2.1)	2.4 (1.8—2.4)
$T_a + 1.0^\circ\text{C}$	-	0.59 (12%)	0.80 (7%)	1.13 (6%)	2.3 (4%)
$T_a + 1.5^\circ\text{C}$	-	-	0.61 (29%)	1.0 (17%)	2.2 (8%)
$T_a + 2.0^\circ\text{C}$	-	-	-	0.66 (45%)	1.9 (21%)
$T_a + 2.5^\circ\text{C}$	-	-	-	-	1.4 (42%)

3.4.3 Predicting the influence of thermal stress on diuron and copper guideline values using ms-PAF

The influence of increasing thermal stress on the copper SSD, predicted by the IA joint action model (Traas et al., 2002) was to shift the SSD vertically above the standard SSD based on toxicity data derived at acclimation temperatures (Figure 3.3a). This in turn affected PCx values. For example, at a temperature of 1°C above acclimation ($T_a + 1^{\circ}\text{C}$) the $PC95$ for copper decreased to $0.9\ \mu\text{g L}^{-1}$ (a reduction of 36%, Table 3.2) in order to still protect 99% of species. The shift in PCx was larger for low PCx values and at increasing temperatures above acclimation (TTx). As temperatures increase to 1.5°C above acclimation no adjustment to $PC99$ and $PC95$ values is possible as $> 5\%$ of species are predicted to be affected by temperature alone (Table 1). At 1.0°C above acclimation an adjustment of the copper $PC95$ of 36% is suggested but no adjustment of the $PC99$ is required as this threshold has already been reached at an increased temperature of 0.83°C (from the SSD and Table 3.1). The SSDs converged at high copper concentrations ($> 10\ \mu\text{g L}^{-1}$) as the metal started to have a greater influence on the percentage of affected species (up to $T_a + 2.5^{\circ}\text{C}$).

Increasing thermal stress was also predicted to shift the diuron SSD vertically above the SSD based on toxicity data derived at acclimation temperatures (Figure 3.3b). At $T_a + 1^{\circ}\text{C}$ the $PC95$ for diuron decreased to $0.59\ \mu\text{g L}^{-1}$ (a reduction of 12%, Table 3.2). The greatest predicted influence of temperature on diuron GVs occurs as the PCx decreases (i.e. greater proportional effect at $PC95$ than $PC90$) and at higher temperatures. For example, the $PC80$ for diuron would need to be reduced from 1.20 to $0.66\ \mu\text{g L}^{-1}$ at $T_a + 2^{\circ}\text{C}$ in order to still protect 80% of species from this combination of herbicide and thermal stress (Table 3.2).

3.4.4 Predicting the influence of contaminants on the thermal stress protection temperature values using ms-PAF

Low concentrations of copper and diuron caused minor shifts in the thermal SSD (Figure 3.4), and only a small predicted effect on PTx values (Table 3.1). Concentrations of copper and diuron equivalent to their PCx values were applied resulting in identical adjustments to PTx for each contaminant. The presence of any contaminant at its $PT95$ concentration would have the same influence of PTx . For example, $PT95$ reduced by the same amount from 1.4 to 1.3°C when co-exposed to the $PC99$ concentrations of copper ($0.29\ \mu\text{g L}^{-1}$) or diuron ($0.43\ \mu\text{g L}^{-1}$) (Table 3.1). The influence of contaminants on PTx increases at higher concentrations. For example, the $PT80$ for thermal stress was reduced from 2.2 to 1.8°C when co-exposed to the $PC90$ concentrations of copper ($3.0\ \mu\text{g L}^{-1}$) or diuron ($0.86\ \mu\text{g L}^{-1}$) (Table 3.1).

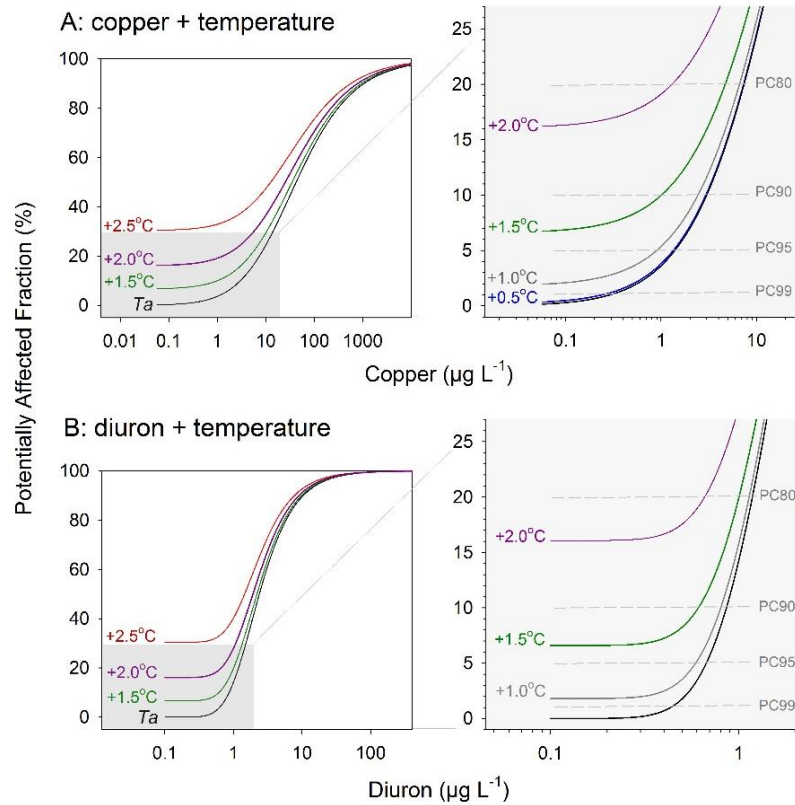


Figure 3.3: Species sensitivity distributions (SSDs) for copper (a) and diuron (b) at acclimation temperatures (T_a , black curve), and at temperatures greater than the acclimation temperature (TTx). The left plot shows full-scale SSDs while the right plot is an expanded view of grey areas in the left-hand plots. Adjustments to water quality guideline values (PC99 – PC50) are shown in Table 3.2.

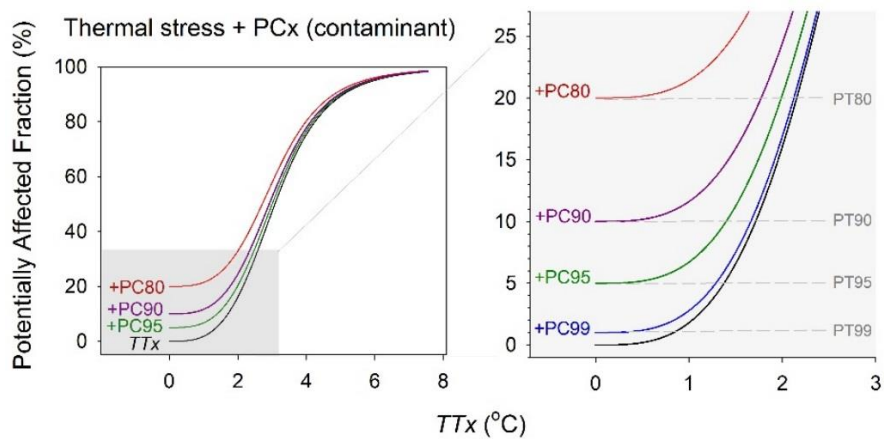


Figure 3.4: Species sensitivity distribution (SSD) for thermal stress at temperatures above acclimation (TTx) (black curve), and shifts in the thermal SSD at PC99, PC95, PC90 and PC80 concentrations for any contaminant (values for copper and diuron can be found in Table 3.2). The left plot shows full scale SSDs while the right plot is an expanded view of grey areas in the left-hand plots. Adjustments to suggested thermal stress guideline values (PT99 – PT50) as contaminant concentrations increase are shown in Table 3.1.

3.5 Discussion

3.5.1 Water temperature thresholds and temperature stress guideline values

The generation of a thermal stress SSD for tropical species from ET_{10} /NOET data allowed us to derive thermal limits (e.g. $PT_{95} = 1.4^{\circ}\text{C}$), below which a proportion (e.g. 95%) of the community would be protected from deleterious effects of thermal stress. This result is consistent with the hazardous temperature increase (HTI5) of $<2^{\circ}\text{C}$ (protective of 95% of species and equivalent to our PT_{95}) calculated by de Vries et al. (2008) for 50 temperate aquatic species based on modelled 10% mortality data. The PT_x values could also be compared with thresholds for thermal bleaching of corals where photosynthetic symbionts are lost (often leading to colony mortality) during periods of high sea surface temperatures (SST) (Berkelmans, 2009; Hoegh-Guldberg, 1999). The analysis of many years of bleaching and SST data has led to the development of bleaching threshold indices such as Degree Heating Weeks (DHW), a measure of thermal stress accumulated through time, calculated by integrating temperatures at least 1°C above the mean monthly maximum (Liu, Rauen Zahn et al., 2013). For example, 4 weeks of temperatures 1.5°C above the mean monthly summer maximum equals 6 DHW (e.g. $4 \times 1.5^{\circ}\text{C}$ -weeks), and values of between 4 and 8 DHW have been shown to lead to widespread coral bleaching and habitat destruction in the tropics (Hughes et al., 2017b). The accumulated thermal stress in corals, which begins at approximately $1 - 1.5^{\circ}\text{C}$ above the normal summer temperatures (following 4 – 6 week exposure) is consistent with the PT_{95} (1.4°C) derived from our thermal SSD. The current study treats thermal stress like contaminants that may have multiple toxic pathways (e.g. metals). In some cases, this can result in taxon-specific modes of action and polymodal SSDs (Posthuma et al., 2002); however, there was no apparent relationship between taxa and thermal sensitivity. While this dataset from 41 species across 12 phyla should represent a diverse array of sensitivities (and potentially thermal stress pathways) exhibited by a tropical marine community, future studies should aim to include an even broader range of species to improve confidence that the predicted proportion of species affected by temperature anomalies is a suitable representation of the community response. Importantly, the thermal SSD was generated from chronic exposure experiments that differ in duration between taxa and life stages as defined by the water quality guideline methods (Warne et al., 2018a). Therefore, the PT_x values derived here represent a complementary approach to quantifying thermal stress thresholds for coral reef communities, and we suggest that the PT_x values could be applied to a minimum of 2 week exposures (equivalent to the longer experimental exposures required by water quality GV derivation methods for chronic values (Warne et al., 2018a)).

3.5.2 Thermal SSD

Thermal stress thresholds (ET_{50}) have been shown to correlate with acclimation temperature (T_a) over wide temperature ranges, and are lower in species from tropical climates (de Vries et al., 2008; McErlean, Mihursky et al., 1969). This is consistent with the *climate variability hypothesis* based on the decrease in annual SST variation range from temperate areas to the tropics, which results in coral reef ectotherms exhibiting smaller thermal windows and living closer to their thermal maxima (Compton, Rijkenberg et al., 2007; Somero, 2005; Stevens, 1989). T_a and TT_x were not correlated for the tropical species dataset, likely due to a relatively narrow range of acclimation temperatures ($22^{\circ}\text{C} - 29^{\circ}\text{C}$), and this allowed us to employ TT_x values directly as the x-axis of the SSD, and a single thermal SSD could therefore be applied across the narrow T_a range assessed. This thermal SSD approach could be developed and

modified to suit different purposes. For example, selecting only data that applied summer acclimation temperatures (experiments where T_a = summer maximum) to derive TT_x would generate thermal SSDs more compatible with other metrics for stress on coral reefs such as degree heating weeks (see above). The use of LT_{50} data (temperature above acclimation causing 50% mortality), which are less conservative than the ET_{10} and NOET data used in the current study, would generate thermal SSDs that predict the percentage of species seriously impacted (e.g. killed) by elevated temperatures (similar to previous thermal SSDs for mostly temperate aquatic species (de Vries et al., 2008)). Furthermore, the use of thermal ranges from field data (Berkelmans, 2009; Collas, Buijse et al., 2018; Leuven et al., 2011; Verbrugge et al., 2012) could allow the generation of alternative thermal SSDs to assess the tolerance of coral reef communities to heatwaves and address changes to community composition that might occur with the predicted increased frequency of heat waves as the climate changes (Oliver et al., 2018).

3.5.3 Using ms-PAF to estimate the influence of thermal stress on water quality guidelines

Multisubstance-PAF has been used to predict the joint risk of multiple contaminants on aquatic communities from multiple SSDs (Backhaus & Faust, 2012; Parkerton et al., 2018). Our study shows how the extension of ms-PAF to predict the simultaneous effects of chemical and physical stressors (in this case temperature) can be applied to adjust water quality GV to account for thermal stress. While the more direct approach of testing the toxicity of contaminants at many temperatures would be the ideal method to generate SSDs at thermally-stressful temperatures, such datasets are limited to only a few toxicants and individual taxa (see examples below). Other contaminant SSDs developed at different temperatures have so far been generated for species exposed within their normal thermal windows (e.g. tropical or temperate) (Bejarano et al., 2017; Kwok et al., 2007; Raymundo et al., 2019; Zhou et al., 2014), but do not generally account for the additional thermal stress as species encounter heatwave conditions. An advantage of the ms-PAF modelling approach is that it can draw upon the many existing contaminant SSDs, and accounts for the additional thermal stress by the development of a thermal stress SSD to predict the influence of elevated temperatures on toxicity.

Combining the thermal stress and contaminant SSDs using the IA joint action model resulted in reduced PC_x values for copper and diuron as temperature increased; for example, predicted PC_{90} values were reduced between 29% (diuron) and 67% (copper) at temperatures of $T_a + 1.5^\circ\text{C}$. This study illustrates that contaminant GVs are unlikely to provide their stated level of protection during heatwave conditions and will need to be decreased in order to do so. These thermal adjustments will become more important with the increasing frequency of heatwave events predicted under the ambitious goals of the Paris agreement (UNFCCC, 2015), and more realistic scenarios (Collins, Knutti et al., 2013a). These reductions in predicted concentrations of copper and diuron to provide a stated level of protection are not large because the temperature ranges being considered are relatively small (albeit ecologically relevant). As the temperatures increase to over 2.4°C above acclimation, the thermal stress SSD predicts that more than 50% of the tropical reef community would have reached the thermal threshold, so temperature itself would dominate the negative effects on the community.

3.5.4 Validating the ms-PAF approach to predict the influence of thermal stress on contaminant toxicity

The application of ms-PAF_{IA} for any combination of contaminants assumes that distinct responses of individual species to each stressor (e.g. one species may be very sensitive to one class of contaminant, while another is more sensitive to another contaminant class) is ameliorated by combining high quality SSDs with many diverse taxa represented (Traas et al., 2002) (e.g. 41 species across 12 phyla for the thermal SSD). There are few laboratory data so far that can be used to effectively validate the ms-PAF approach. SSDs for temperate and tropical aquatic species (generated using data within their respective normal temperature ranges) have shown that PC90 (HC10) values could differ by up to 16-fold (Kwok et al., 2007). Another recent study derived SSDs for three metals from multiple single temperature tests (performed at different temperatures) and showed reductions in PC90 values by almost 50% when temperatures were increased by 2°C intervals (Zhou et al., 2014). These studies and others that compare differences in sensitivity between temperate and tropical communities generally recommend that temperate water quality guideline values be divided by a 10-fold safety factor to derive guidelines for tropical species (for which there is generally little data) (Lau et al., 2014). More specific validation of the ms-PAF predictions can be made by comparison with single species copper and diuron toxicity tests on tropical marine species, conducted at different temperatures. For example, the EC₅₀s for larval settlement of two coral species to copper exposure were reduced by 19 and 27% (i.e. the corals became more sensitive) at $T_a + 3^\circ\text{C}$ (Negri & Hoogenboom, 2011). Analysis of the same dataset showed that the IA model of joint action was able to accurately predict (to within 30%) the combined effect of most combinations of copper and temperature (up to $T_a + 3^\circ\text{C}$) (Uthicke et al., 2016). The IA model was also able to predict ($<3^\circ\text{C}$ above T_a) the increase in diuron toxicity with elevated temperature in tropical coral symbionts, symbiont-bearing foraminifera and seagrass (van Dam et al., 2012a; van Dam et al., 2015; Wilkinson et al., 2017). These single-species studies indicate that the IA model can be suitable for predicting joint effects of stressor combinations that include temperature. Reviews of the effectiveness of both the IA and chemical addition models to predict the impacts of multiple chemicals on aquatic organisms have revealed good agreement with observed responses, and that cases of underestimation (due to interactions) are rare (Belden, Gilliom et al., 2007; Cedergreen, Christensen et al., 2008).

3.5.5 Implications for risk assessment

The ms-PAF approach of predicting the combined effects of thermal stress and contaminants on tropical reef communities has several related applications for regulation and monitoring programs. For example, to maintain 95% protection of all species, during a heatwave the PC95 for any contaminant could be adjusted as shown in Table 3.2, where T_a is equivalent to the normal monthly average temperature. A similar approach could be taken to quantify the influence of water quality on heat stress to coral reef communities. For example, the thermal stress SSD predicts 10% of the community could be affected (=PT90) over prolonged heating of $T_a + 1.7^\circ\text{C}$ above the historical summer SST (T_a), and ms-PAF can be used to predict how contaminants change the proportion of the community affected. In addition, more than one contaminant can be included along with thermal stress in the ms-PAF (de Zwart & Posthuma, 2005). Furthermore, the ms-PAF method could be applied to assess risk (spatially and temporally) by including thermal stress and contaminants as exposure layers in models and using SSDs and ms-PAF to model the potentially affected fractions of communities from individual locations and points in time. Extending this approach to temperate locations would

require the generation of thermal SSDs for temperate marine species. SSDs for ocean acidification (Azevedo, De Schryver et al., 2015; de Vries, Tamis et al., 2013), sediment exposures (Smit, Holthaus et al., 2008), anoxia (Elshout, Dionisio Pires et al., 2013), and salinity (Kefford, Marchant et al., 2011; Verbrugge et al., 2012) have already been developed for some freshwater and/or marine communities, offering further options for the ms-PAF approach to predict the hazards of multiple stressors in risk assessments and monitoring and reporting programs.

The generation of a thermal SSD for benthic marine organisms relevant to coral reefs presents an additional option for interpreting and quantifying the hazards posed by abnormally high sea surface temperatures that will become more frequent with predicted climate changes. Temperatures in tropical coral reef waters have already increased by nearly 1°C since the industrial revolution (Lough et al., 2018), and present trajectories point more towards further SST increases > 1.2°C by the end of the century (Magnan et al., 2016). Thus, the need for climate adjusted water quality guidelines is urgent. Understanding the combined hazards and risks posed by multi-contaminant mixtures is a developing field, with ms-PAF being one of the most widely explored “bottom-up” methods (Beyer, Petersen et al., 2014). At present the influence of heatwave events on the risks posed by contaminants are either addressed by applying conservative (e.g. 10-times) safety factors or ignored. The extension of ms-PAF to include thermal stress is a promising approach that, with further development and validation, could enable the adjustment of water quality GVs to account for heatwave events that will help manage and protect coral reefs from the increasing frequency and duration of such events.

4. Supporting Risk Analysis and Mapping

4.1 Exposure Maps

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4.1.1 Introduction

Collation and curation of exposure maps was an explicit objective of this project. Temporally and spatially explicit disturbance and environmental data was either generated for this project (e.g., River DIN and Light) or collated from different sources. When yearly data for the entire time-series was absent, long-term averages were obtained of available data (Table 4.1). Metrics of acute disturbances include measures of the magnitude (i.e. continuous variable) and frequency (i.e. binomial variable) of exposure which was determined based on specific thresholds. Crown of Thorns Seastar (CoTS) densities were collected along the coral cover transects (Marine Monitoring Program, MMP) and during AIMS manta-tow surveys (Long Term Monitoring Program, LTMP reefs). Data from each survey technique was combined by standardising transect CoTS densities to manta tow area and correcting for sampling bias due to higher detectability (by a factor of 9.3) on transects compared to manta tows.

4.1.2 Production and curation of Exposure Maps

To predict CoTS on reefs and years with no survey data, we used Inverse Distance Weighting analysis from the R-package *geosptdb* (Melo & Melo, 2015). Frequency of outbreaks was determined by CoTS densities above incipient outbreak levels of 0.22 individuals per tow (Moran & De'Ath, 1992). Exposure to cyclones and heat stress (measured as annual maxima Degree Heating Weeks- DHW) were obtained from GBR-wide spatial-temporal models at 1 km resolution (Matthews, Mellin et al., 2019). Frequency of storms and bleaching-risk events were derived from the cyclone and DHW exposure layers. However, because GBR-wide model predictions may not capture disturbance observed at the scale of transects, the frequency of these disturbances was complemented with observations from LTMP surveys to better represent true conditions on the reefs across space and time. For example, cyclone exposure measured as hours of damaging waves above 4m wave height captures mainly intense cyclones (Puotinen, Maynard et al., 2016). Frequency of storm impacts includes the presence of these cyclones and less intense storms that were not captured in cyclone layers but that were recorded as agents of coral mortality during surveys (see Osborne, Dolman et al., 2011 for methods). Frequency of bleaching or bleaching-risk mortality events was considered for DHW above 3 (Hughes, Kerry et al., 2018b) but because coral bleaching can be caused by other environmental stressors including those resulting from cyclones (Goreau, 1964; Van Woesik, De Vantier et al., 1995) our bleaching-risk metric was complemented by field data whenever bleaching was recorded as agent of coral mortality during surveys but DHW levels were below 3.

Table 4.1.1: List of acute disturbances and environmental variables collated as exposure maps. When yearly data for the long time-series (1992-2017) was absent, years of available data from which long-term averages were obtained are detailed in the description.

Predictors	Description	Source
Acute Events		
Heat Stress & Bleaching		
Heat Stress (DHW)	Annual maxima of Degree Heating Weeks (DHW)	Matthews et al., 2019
1 yr DHW	Lag of DHW	
Bleaching Risk	Occurrence of bleaching (in-situ) and DHW >3	derived from Matthews et al., 2019 and in-situ AIMS LTMP data
1 yr bleaching	Lag of bleaching risk	
3 yr bleaching	Cumulative bleaching risk over 3 yr	
5 yr bleaching	Cumulative bleaching risk over 5 yr	
Cyclones & Storms		
Cyclones	Annual hours exposed to waves above 4 m height (4m Wh)	updated from Matthews et al., 2019
1 yr cyclones	Lag of cyclones	
Storms		derived from Matthews et al., 2019 and in-situ AIMS LTMP data
5 yr storms	Cumulative storm events (cyclones + storms) over 5 yr	
CoTS		
Density	Densities of <i>Acanthaster cf. solaris</i> (ind. per manta tow)	derived from AIMS manta tow and LTMP/MMP surveys
1 yr density	Lag density of CoTS	
Outbreaks	Occurrence of CoTS densities > 0.22 per manta tow	derived from AIMS manta tow and LTMP/MMP surveys
1 yr outbreaks	Lag occurrence of CoTS outbreaks	
5 yr outbreaks	Cumulative occurrence of CoTS outbreak over 5 yr	
Total Acute Events		
	Annual occurrence of: Bleaching risk + Storm events + CoTS outbreaks	compiled from frequency metrics above
1 yr acute	Lag of total acute events	
5 yr acute	Cumulative acute events over 5 yr	
Water Quality		
Light (PAR)	Mol photons m ⁻² d ⁻¹ predicted at 8 m depth. Wet-season average (2002-2017)	derived from Canto et al., 2019
Chlorophyll a (Chla)	Mg m ⁻³ predicted at 5 m depth. Wet-season average (2010-2017)	eReefs (GBR4 model)

Fine Suspended Sediments	Kg m^{-3} predicted at 5 m depth. Wet-season average (2010-2017)	eReefs (GBR4 model)
Environmental DIN (DINe)	Mg m^{-3} predicted at 5 m depth. Wet-season average (2010-2017)	eReefs (GBR4 model)
Salinity Index	Cumulative exposure (PSU days^{-1}) to salinity <30 PSU. Wet-season average (2010-2017)	eReefs; index based on Berkelmans et al., 2012; Brinkman et al., 2012
River DIN	Mg m^{-3} of river DIN. Wet-season average (1992-2017)	updated from Wolff et al., 2018

Exposure to disturbances was assigned to each reef and year considering the date of benthic surveys. If the disturbance occurred post-survey for a given year, the disturbance of the previous year was assigned. The frequency of each acute disturbance and its cumulative lagged effects were evaluated using windows of 1, 3 and 5 years (Table 4.1.1). These time windows were chosen given the average temporal gap between consecutive surveys for a given reef and the availability of historical data for testing lagged effects. Cumulative effects of all disturbances combined (i.e. CoTS outbreaks + storms + bleaching) were also evaluated. The idea for this metric is to test the combined effects of recurrent acute disturbances and the critical role of recent history for the state and performance of coral reefs.

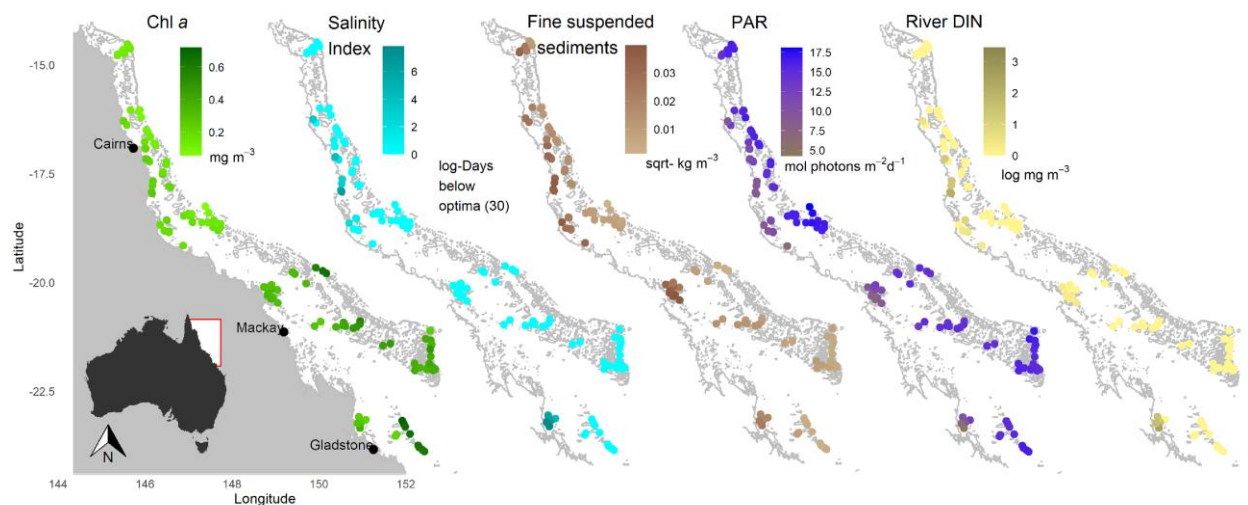


Figure 4.1.1. Examples of spatial variability in WQ metrics. Values represent reef-level overall means as treated in the models (except for River DIN for which yearly data for the whole time series was used). Data on Chlorophylla a (Chla), Salinity and fine suspended sediments were extracted from GBR4 eReefs models at depth (5m). PAR corresponds to Light at 8m depth based on Canto et al 2019. River DIN represents DIN concentration at individual reefs derived from modelled runoff influence of 22 rivers estimated to reach each reef

Because thresholds of water quality that correlate to negative impact on corals may depend on the time window evaluated (i.e. annual vs seasonal average) (De'ath & Fabricius, 2008) we focused on the WQ environment associated to the wet-season (i.e. Dec-April) to better assess the influence of catchment run-off and its importance in predicting coral reef state and performance at the scale of the GBR. The selected variables are known to have a direct and strong effect on coral health and have been targeted for management (Brodie et al., 2017b).

A full description of each variable is presented in Table 4.1.1, but overall, wet-season averages of Chlorophyll a (Chla), fine suspended sediments, DIN and salinity were extracted from GBR4-eReefs models (Steven, Baird et al., 2019a) and light availability was derived from Magno-Canto, McKinna et al. (2019). Salinity was represented as a cumulative index based on days of exposure to low (<30) salinity following Berkelmans, Jones et al. (2012) and Brinkman et al. (unpublished data). It has been argued that metrics of environmental DIN predictions are poor representatives of terrestrially-sourced DIN as DIN is entirely bio-available. Consequently, we explored an additional metric of nutrients that is likely to reflect better the direct contribution of river DIN in the discharge runoff. This river DIN metric which is based on the yearly (wet-season) discharge of 22 rivers (from 1990 to 2018) and the DIN concentrations at the river mouth (Wolff, da Silva et al., 2018a) is the only WQ metric here with yearly data throughout the whole time-series (Table 4.1.1).

Thus, we collated a set of exposure maps for 25 variables for a time period between 1992-2017, available on eAtlas <https://maps.eatlas.org.au/>. Most of these are listed in Table 4.1.1., and examples of those are shown in Figure 4.1.1.

4.2 The relative cumulative contributions of acute and chronic disturbances affecting the state and performance of corals on the Great Barrier Reef

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Manuscript submitted

4.2.1 Summary

Coral bleaching, cyclones, outbreaks of Crown-of-Thorns Seastar (CoTS), and poor water quality (WQ) threaten the health and resilience of the Great Barrier Reef (GBR). The contribution of acute vs. chronic disturbances to the state and performance of GBR reefs is poorly understood but critical for successful management. We evaluated the effect of 25 environmental variables on the dynamics of structurally-different coral populations in the Central and Southern GBR, based on long-term (25 years) coral monitoring and environmental data. The aim was to identify and compare the main environmental drivers for reef state (cover) and performance (based on observed vs expected rate of coral cover increase using a recently developed performance metric). In addition, we took a deeper look at the coral community by analysing coral cover and performance separately for coral growth forms. Low cover and low performance of Central GBR were best explained by prolonged exposure to CoTS outbreak conditions having the strongest effect on tabular *Acropora* (37% cover loss). Frequency and magnitude of storms contributed to more than 50% total cover loss affecting all growth forms. Degree Heating Weeks (DHW) values above 2.3 reduced total cover on Central GBR by 20% affecting all corals (except branching *Acropora*) but it did not affect performance. Until 2017, the Southern GBR was unaffected by bleaching-risk conditions (i.e. DHW>3). Here, low cover was mostly explained by the combined impacts of acute stressors mainly affecting tabular *Acropora* (38% cover loss) whereas low reef performance was attributed mostly to lagged

effects of CoTS outbreaks and cyclone intensity. Long-term averaged WQ metrics had low predictive power (< 4% explained variance) as the year to year variability in water quality parameters is not captured. Increasing the temporal resolution of the dataset increased the relative importance of WQ parameters as corals responded to variation in environmental conditions. Although this improvement was not consistent across coral types and regions, the performance metric appears more sensitive to water quality effects. River Dissolved Inorganic Nitrogen (DIN) was the only water quality variable incorporating yearly variability across the entire time-series and was the strongest WQ predictor. River DIN correlated negatively with performance of Central branching corals while in the Southern GBR the opposite was found for massive/submassive corals. Our results highlight complex reef dynamics and provide support for identifying monitoring needs and prioritizing local management interventions to increase the persistence and resilience of functional GBR reefs in the face of cumulative global disturbances.

4.2.2 Introduction

Understanding cumulative impacts from multiple stressors is critical for successful management of coral reefs. Although cumulative impacts have been identified as a key driver of reef health, there are still large knowledge gaps in our understanding of both the effects of individual stressors and the interplay of local and global stressors on the health and resilience of reef ecosystems (Ban et al., 2014; Harborne, Rogers et al., 2017). Coral cover (i.e. reef state) is the most common metric used to evaluate reef health, however reef resilience largely depends on reef recovery potential after disturbance (Osborne, Thompson et al., 2017). Measuring how reef recovery compares with the expected reef growth potential (i.e. expected in the absence of stressors) can provide information about changes in reef performance over time. Here, we calculated the performance of reefs based on the difference between observed and expected change in coral cover to assess the simultaneous impacts of acute and chronic stressors. The impact of multiple stressors on both reef state and performance has rarely been assessed together.

Both the frequency and magnitude of stressors are critical for evaluating impacts on ecosystem responses, but few studies consider both (Connell, Hughes et al., 1997; Hall, Miller et al., 2012; Mellin, Thompson et al., 2019b). Increased cyclone intensity and increased frequency of mass bleaching events due to heat stress are threatening the persistence of coral reef communities (Emanuel, 2005; Hughes et al., 2018a). Yet, the impacts of these acute stressors vary spatially and temporally on the GBR (Mellin et al., 2019b; Osborne et al., 2011) due to variations in their intensity, spatial extent, and frequency (e.g. Puotinen et al., 2016). Contrasting sensitivities of coral communities further contribute to variation in: impacts (Hughes et al., 2018b; Madin, Baird et al., 2014; Mellin et al., 2019b), coral recovery potential (Mellin, Matthews et al., 2019a; Ortiz, Wolff et al., 2018; Osborne et al., 2017) and the interactions among global-acute stressors (Ban et al., 2014; Vercelloni, Caley et al., 2017) and local human stressors (Fabricius, Okaji et al., 2010; Morgan, Perry et al., 2017; Oxenford & Vallès, 2016; Wooldridge & Done, 2009b).

It is widely accepted that poor water quality in the GBR Marine Park is linked to the increased loads of fine sediment, nutrients, and pesticides discharged from agricultural sources in the GBR catchment (Brodie & Fabricius, 2008; Kroon, Kuhnert et al., 2012). Specifically, terrestrially-sourced dissolved inorganic nitrogen (DIN) has been suggested to exacerbate frequency of Crown-of-Thorns Seastar (CoTS) outbreaks through increase of phytoplankton

as larval food (Fabricius et al., 2010; Wooldridge & Brodie, 2015), and to enhancement of bleaching susceptibility and disease prevalence in hard corals (Wiedenmann, D'Angelo et al., 2013; Wooldridge & Done, 2009b). River runoff reduces water quality (WQ) not only due to the direct effects of pollutants but also by affecting environmental parameters such as light, salinity, and chlorophyll, which are key drivers of reef health (De'ath & Fabricius, 2008; Fabricius, Logan et al., 2016; Fabricius, De'ath et al., 2013). There is empirical data linking poor WQ to decreasing coral health (Berkelmans et al., 2012; Humanes, Fink et al., 2017a; Humanes et al., 2017b) because WQ affects corals through different mechanisms (e.g. increasing coral disease, bleaching susceptibility, reducing coral recruitment, causing coral smothering). In combination poor water quality has been shown to result in reduced species richness and juvenile density, but not cover of corals on the GBR (Fabricius, Cooper et al., 2012). Consequently, measuring the contribution of WQ to the state and recovery of the GBR at a regional scale has proven challenging, with studies reporting contrasting conclusions regarding the relative importance of WQ on the GBR (Ceccarelli, Evans et al., 2019; Lam, Chaloupka et al., 2018; Matthews et al., 2019; Mellin et al., 2019a). Challenges for evaluating WQ include the availability of data at relevant spatial and temporal scales, the use of different metrics, and the fact that multiple WQ variables are highly correlated with each other. As a result, the effect of WQ on the GBR has often been assessed using proxies (Lam et al., 2018) or a single compound metric (Matthews et al., 2019; Mellin et al., 2019a). However, because impacts likely differ among taxonomic groups (De'ath & Fabricius, 2008; Fabricius et al., 2012) the evaluation of a compound single WQ variable may be of limited use to identify the most likely mechanisms of regional coral cover decline which is key to successful management.

Matthews et al. (2019); and Steven, Baird et al. (2019b) have improved the spatial and temporal resolution over which we can measure reef exposure to diverse environmental conditions. Here, we make use of these tools to improve our understanding of cumulative impacts of multiple stressors on the GBR. We combined i) 25 years of coral cover data evaluated at fine taxonomic resolution, ii) the exposure of 122 reefs to acute disturbances (using intensity and frequency metrics), and iii) multiple environmental drivers and indicators of water quality, to assess cumulative impacts on two regions of the GBR. Importantly, we evaluate reef health by comparing observed changes in reef state (i.e. coral cover) with Gompertz model predictions to assess reef performance, and we separately consider structurally different coral communities (i.e. *Acropora* tabular, *Acropora* branching, other branching corals, and massive/submassive/encrusting corals), to assess whether divergent responses to multiple stressors by different taxa are being masked, when evaluating regional reef health based on total coral cover alone. Our modelling approach considers two indicators of reef health, and many possible combinations of stressors including several WQ metrics, to identify and quantify the contribution of stressors that together have the best predictive value for the state and performance of GBR reefs, informing about more targeted management options.

4.2.3 Materials and Methods

4.2.3.1 Reef responses

The impact of multiple stressors on corals was evaluated using two metrics: 'reef state' i.e. observed coral cover, and 'reef performance', an index reflecting deviation from expected change in cover. Absolute percentage coral cover data were obtained for 122 reefs (335 reef sites) by the AIMS Long-Term Monitoring Program (LTMP) and Marine Monitoring Program

(MMP) between 1992 and 2017 (Figure 4.2.1). Coral cover was surveyed at 5-9 m depth from permanent photo- and video belt transects (see Abdo, Burgess et al., 2004 ; Jonker, Johns et al., 2008 for methods). Total coral cover and cover of four specific coral groupings, namely *Acropora* tabular, *Acropora* branching, other (non-*Acropora*) branching, and non-*Acropora* massive/submassive and encrusting species (MSE) were used for the study. A minor component of the coral community, such as free-living growth forms and foliose species with low abundance, were only included in total coral cover estimates.

“Performance” which was estimated for each coral group, is an index of reef functioning that evaluates the deviation of the observed reef state from the expected reef state based on a comparison of observed versus expected rate of coral cover increase in the absence of acute disturbances (Thompson, Costello et al., 2018). The observed coral cover in year x was used to calculate the expected coral cover in year $x+1$ by fitting Bayesian Gompertz equations models to all available time series from 1992 to 2001, but excluding reef data from periods with acute disturbances, as per Thompson and Dolman (2010). We chose pre-2002 years to parameterise the rates of cover increase because most of the GBR was affected by acute heat stress in 2002 (Osborne et al., 2017). Specific rates of coral cover increase for each coral group were used to obtain the group-specific expected coral cover over that time.

For total performance, a separate parameterisation of the Gompertz model was obtained differentiating the rates of coral cover increase for Acroporidae vs all other hard coral families, which were then combined for predicting the total coral cover increase (as per Osborne et al., 2017). The deviation of the observed coral cover (adjusted to 1 year interval since previous observation) from the expected cover was used to classify the performance of the reef (for total coral and each coral group) using a quantitative score system (0-1) based on the values of the upper and lower boundary of the expected cover distribution (Thompson et al., 2018): a score of 0 was applied if cover declined between surveys. Scores were scaled to between 0.1 when no change in cover was observed through to 0.4 when change was equal to the lower boundary of the predicted change. If cover change was within the upper and lower boundaries of the predicted change the score was scaled from 0.4 at the lower boundary through to 0.6 at the upper boundary. For all groups except MSE, if cover change was greater than the upper boundary of predicted change and less than double the upper boundary, scores were scaled from 0.6 at the upper boundary to 0.9 at double the upper boundary. For MSE, owing to their lower growth rates (Thompson et al., 2018), scores were scaled from 0.6 at the upper boundary to 0.9 at three-times the upper boundary. If change was greater than double (or triple for MSE) the upper boundary, a score of 1 was applied. For years where there was no survey, each reef was scored based on the mean score value of the previous year(s) (lag-1 or lag-2 if necessary). Only years not used in the parameterisation of expected cover (i.e. from 2002 onwards) and with no acute disturbance were used for modelling.

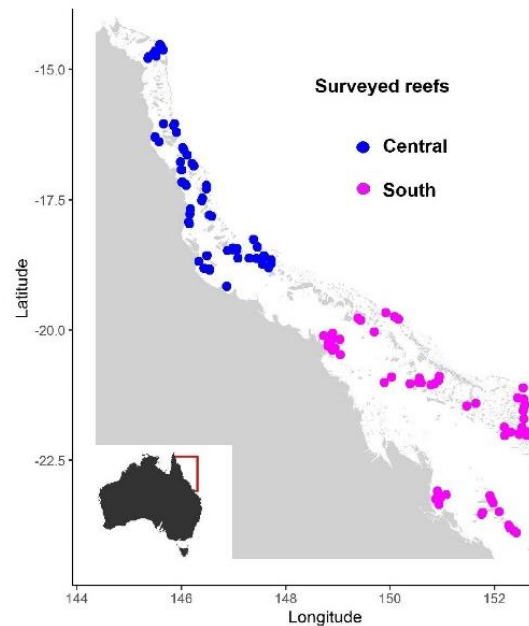


Figure 4.2.1: Reef locations surveyed by AIMS LTMP and MMP on the Central and Southern GBR regions.

4.2.3.2 Acute disturbance data

Temporally and spatially explicit acute disturbance and environmental data were either collated from existing sources or generated for this study (e.g. River DIN and Light). The long-term averages of available environmental data were used to compensate for the missing years (Table 4.1.1). Metrics of acute disturbances include measures of the magnitude (i.e. continuous variable) and frequency (i.e. binomial variable) of exposure which was determined from the magnitude metrics based on specific thresholds.

CoTS densities were collected along the coral cover transects (MMP) and during AIMS manta-tow surveys (LTMP reefs). Data from each survey technique was combined by standardising transect CoTS densities to manta tow area and correcting for sampling bias due to higher detectability (by a factor of 9.3) on transects compared to manta tows. To predict CoTS on reefs and years with no survey data, we used spatio-temporal Inverse Distance Weighting analysis with the function *idwST* from the R-package *geosptdb* which considers the value of a point from the weighted (with power value=2) averages of values of the nearest neighbours ($n=3$) in terms of the spatio-temporal locations (time factor=1) (Melo & Melo, 2015). Frequency of outbreaks was determined by counting an outbreak whenever CoTS densities were above defined “incipient outbreak” levels of 0.22 individuals per tow (Moran & De'Ath, 1992).

Exposure to cyclones and heat stress (measured as Degree Heating Weeks- DHW) were obtained from GBR-wide spatial-temporal models at 1 km resolution (Matthews et al. 2019). Frequency of storms and bleaching-risk events were derived from the cyclone and DHW exposure layers as presence/absence data. However, because GBR-wide model predictions may not capture disturbance observed at the scale of transects, frequency data was complemented with observations from the LTMP and MMP surveys to better represent true disturbances. For example, cyclone exposure was measured in hours of damaging waves where “damaging wave” conditions are defined as occurring when the average of the top one-

third of wave heights exceeded 4m wave (maximum wave heights ~10m) (Puotinen et al., 2016). This may miss swell that escapes the cyclone circulation. Therefore, smaller cyclones/storms are not included in the cyclone exposure data. 'Frequency of storm impacts' therefore includes the observation of storms as agents of coral mortality, attributable to both cyclones and less intense storms that were not captured in cyclone layers (see Osborne et al., 2011 for methods). Frequency of bleaching or bleaching-risk mortality events was considered for DHW above 3 (Hughes et al., 2018b), but because satellite-derived data may fail in capturing *in situ* conditions and coral bleaching can also be caused by other environmental stressors our bleaching-risk metric was complemented by field data whenever bleaching was recorded as agent of coral mortality during surveys even when DHW levels were below 3.

4.2.3.3 Spatial and zoning data

We consider the geographic location of reefs across the continental shelf (minimum distance to the coast) (as per Matthews et al., 2019) to account for inherent geomorphological characteristics that may affect the reef response to disturbances. Given that many reefs changed their management status due to the re-zoning in 2004, i.e., half-way through the time-series (GBRMPA, 2005), we categorised reefs as always open for fishing (OO), Open-Closed (OC), Closed-Open (CO), or Always closed (CC), to reflect those changes and to account for the relative importance of fishing management in explaining reef state and performance.

4.2.3.4 Statistical analyses

The relative importance of multiple stressors and the identification of the strongest predictors of the state and performance of the GBR were assessed using a full-subset modelling approach with generalised additive mixed models (GAMMS) (Fisher, Wilson et al., 2018). Reef responses were modelled independently for each coral group and region (North-Central - hereafter Central, and Southern GBR) due to the strong regional differences in disturbance patterns and water quality. Cover of each coral group category (i.e. reef state) was modelled as absolute proportional cover and both reef state and reef performance metrics were fitted with Beta distribution with each response variable bounded between >0 and <1 . We identified the final predictors for modelling based on their correlation coefficients among all variables considered. When values exceeded 0.7 the most meaningful variable of each pair was chosen based on ecological knowledge about their likely effects of coral population dynamics. Data transformations (e.g., log or square-root) were applied when necessary to improve the distribution of selected predictors for modelling. To account for spatial and temporal covariances among observations, a random term for sites (the lowest replication unit) nested within reefs, a smooth term of reef latitude coordinates (in kilometres using the Universal Transverse Mercator (UTM) coordinate system), and a smooth term of time (date of survey) were included in the model. This spatio-temporal structure was used as the null model, against which the full-subset models were built. Given the amount of data available for model fitting, the maximum number of fixed predictors was restricted to 5 in any one model with smoothed terms with $k=3$ to reduce overfitting. Further restrictions in multicollinearity (< 0.28) were applied during model runs for excluding collinear models (Fisher et al., 2018). Penalized-likelihood criteria estimates (AIC) using model weights were used to evaluate competing models by comparing model *delta* values ($\Delta AIC_c < 2$) (Burnham & Anderson, 2002).

Two outputs were derived from this approach: First, the variable importance score (i.e. relative importance) for each included predictor, as sum of model weight values across all the models where any given predictor was present (Fisher et al., 2018). Second, the identification of the

strongest predictors for explaining reef state and performance. The best predictors were evaluated from selected candidate models (within $\Delta AIC_c < 2$) based on the most parsimonious model (i.e. with the lowest number of variables), and the variable contribution to deviance explained among competing models (i.e. with similar number of predictors). By comparing a complete model set of possible acute disturbance metrics ($n = 17$) and environmental predictors ($n = 8$), we identified the combination of stressors that best explained the spatial variability in reef state and performance for structurally-different coral communities on the GBR. Final models were refitted using REML to get best fitted curves. All statistical analyses were performed in RStudio (RStudio, 2018) using the R packages *mgcv* (Wood, 2017) and *FSSgam* (Fisher et al., 2018).

Following the same approach described above to analyse variable importance scores, we ran a second set of models considering only the last 7 years of the dataset (i.e. 2010-2017). In this shorter time-series we were able to include the year to year variability of all environmental variables compared to using long-term averages. The intention with this set of models was to test the potential limitations in evaluating the relative importance of WQ metrics for long-term cumulative impact assessments, where year to year variability in environmental data was often lacking.

4.2.4 Results

4.2.4.1 Temporal trends of reef state and reef performance

Total coral cover averaged across all reefs of the Central and Southern GBR ranged between 18% and 41% from 1992 to 2017. In Central GBR (Figure 4.2.2a), cover declined after a peak in 1998. Massive, submassive and encrusting (MSE) species were the most dominant growth form but showed a slow decline over time. In contrast, the cover of non-*Acropora* branching corals (represented mainly by *Pocillopora* and *Porites* spp.) gradually increased. Total cover in the Southern GBR was slightly higher and increased from 1992 before declining dramatically between 2007 and 2010 (Figure 4.2.2b). Non-*Acropora* branching corals made a small contribution, while the relative contribution of Acroporids (tabular and branching forms) was high.

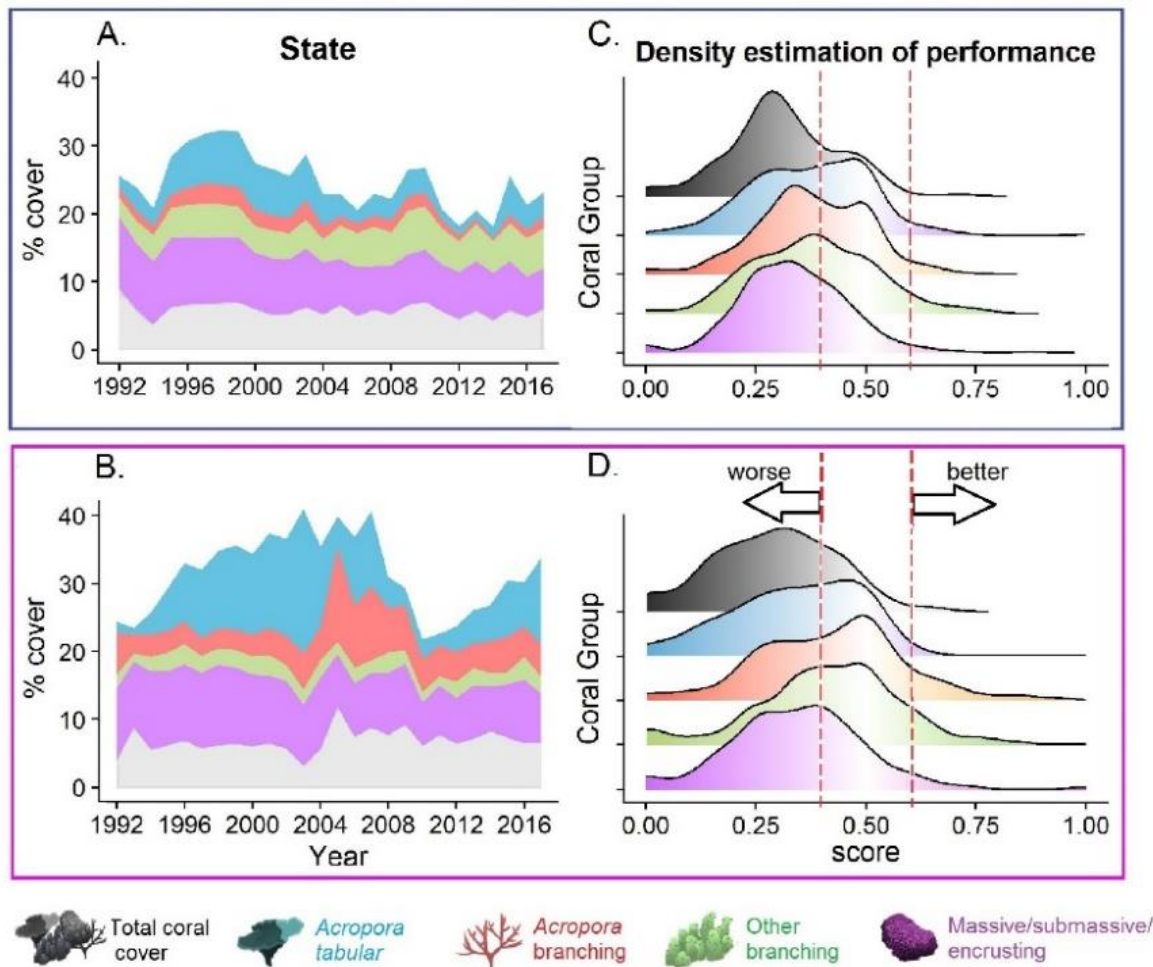


Figure 4.2.2: Averaged temporal trends of reef status (a and b based on % coral cover) and distribution of reef performance scores (c and d based on the expected rate of coral cover increase for the period 2002-2017 in the absence of acute disturbances) for each region (Central: a and c, South: b and d) and each coral group: *Acropora tabular* (blue), *Acropora branching* (red), other non-*Acropora* branching (green), massive-submassive-encrusting species (purple), all other growth forms (grey in a and b) and total coral cover (black in c and d).

In both regions, reef performance based on total coral cover showed a clear reduction from the expected rate of cover increase since 2002: the distribution of performance scores were predominantly below 0.5, which represents the rate of increase observed prior to 2002 (Figure 4.2.2). However, performances differed between coral groups and regions (Figure 4.2.2). Tabular *Acropora* performance was below expectations in both regions, whereas branching *Acropora* performance was below expectation in the Central region, but rates of cover increase were similar to expectation in the Southern region. Similarly, most other branching communities performed worse than expected in the Central region during periods of no acute disturbances compared to the Southern region (Figure 4.2.2c) whereas MSE communities performed similarly along the GBR with very few reefs exhibiting the expected rate of cover increase. Only in very few Southern reefs did the growth performance of branching corals (*Acropora* and non-*Acropora*) score better than expected (Figure 4.2.2d).

4.2.4.2 Relative importance of multiple disturbances on the state and performance of GBR reefs

Cover state and performance for total coral cover and for group-specific coral covers showed contrasting relative importance for the predictors (Figure 4.2.3). Metrics based on total coral cover in the Southern GBR failed to capture the importance of some acute disturbances and WQ variables that affect specific coral groups. Overall, both the magnitude and frequency of acute disturbances and their lagged effects were the most important pressures on reef state and performance and overshadowed the effects of the long-term mean environmental (water quality) conditions. For evaluating reef state, only in Central GBR did WQ (expressed as River DIN) show high relative importance for total cover and that of tabular acroporids and other branching corals. In the Southern GBR, WQ metrics were consistently scored with low relative importance with salinity showing the highest score (~0.7) for explaining the state of tabular *Acropora* and MSE (Figure 4.2.3). Different WQ metrics were identified as important when evaluating performance. In the Central GBR environmental DIN was the most important WQ variable for the performance of branching non-*Acropora* whereas salinity was more important for MSE. In Southern GBR, river DIN had the highest importance score for MSE only (Figure 4.2.3).

The second analysis focused on the data 2010-2017, when annual values for WQ variables were available. Analyses were limited by the short time period, and further restricted by dropping the many years with acute disturbances, making model selection ineffective. A comparison of the importance score between datasets (by subtracting to the score values with yearly data, the score obtained with averaged data) showed an increase in relative importance of several WQ variables when year-to-year WQ variability was modelled, compared to averaged WQ values. In light of our results from the complete data set showing importance scores greater than 0.8 as having some explanatory power in 'best' candidate models and given recent debate about the use of summed Akaike weights as metrics of variable importance (Galipaud, Gillingham et al., 2014; Giam & Olden, 2016) we interpret the results of the second analysis with caution and consider only a magnitude of change greater than 0.8 units as meaningful changes in variable importance scores due to increased temporal resolution in the data. WQ importance increased only when evaluating reef state and performance in Southern GBR (Figure 4.2.4). Only Chl-a, PAR and River DIN increased in relative importance when yearly variability was explicitly modelled compared to averaged values of WQ. No improvement in variable importance score was obtained when explaining variability of Central-GBR performance. Instead, a decline in the relative importance in PAR was observed for state (Figure 4.2.4).

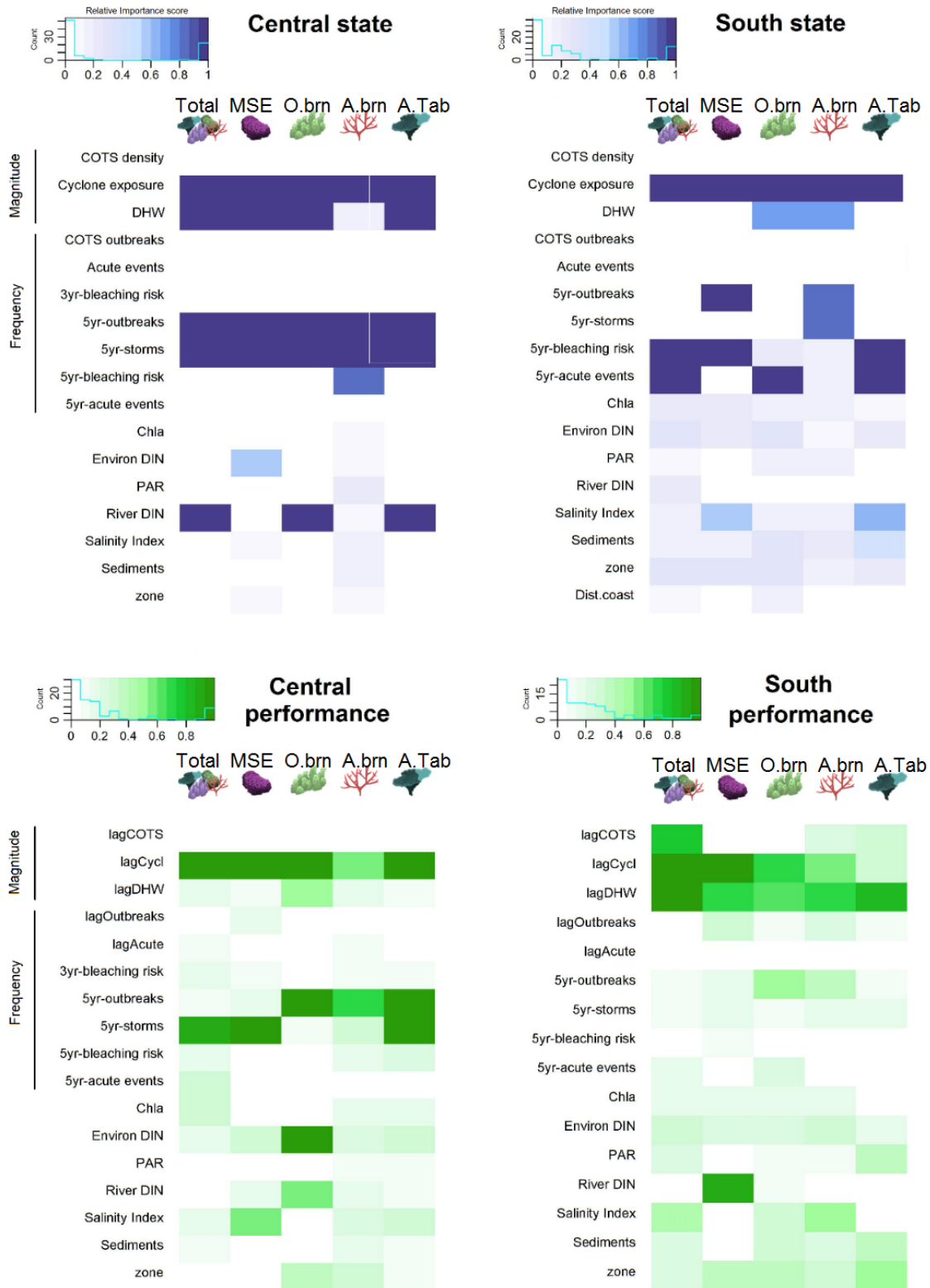


Figure 4.2.3: Variable importance score from GAMM full subset analyses to predict from the long-term data set (1992-2017) the state (blue) and performance (green) of total coral cover (Total) and that of specific growth forms (MSE: Massive-submassive-encrusting, O.brn: Other branching, A.brn: Acropora branching, A.Tab: Acropora Tabular) on Central (left) and Southern (right) GBR. Exposure to acute disturbances was evaluated using metrics of magnitude and frequency of stressors with lagged effects of 1, 3, 5 years. List of variables vary among panels as highly correlated variables were excluded separately from each data set. White boxes indicate the variable was not present in any of the top candidate 'best' models.

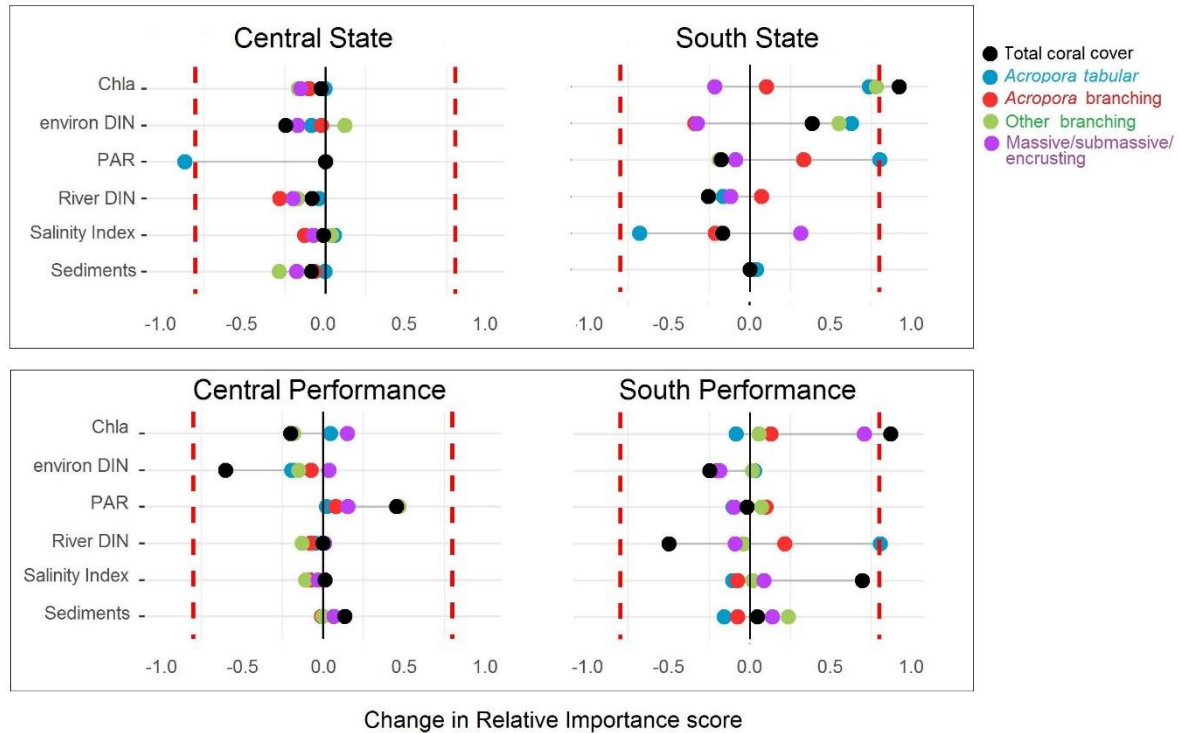


Figure 4.2.4: Change in variable importance score when comparing models with yearly (Yr) variability in WQ metrics vs averaged (Av) values (Change=Yr-Av) for the time series 2010-2017. Scores based on GAMMs with full-subsets approach for predicting the state (top) and performance (bottom) of coral cover (total and by groups) on Central (left) and Southern (right) GBR. Only changes beyond red dotted lines (>0.8) are considered meaningful.

4.2.4.3 Drivers of reef State in central GBR

Overall, there was high consistency in model outputs for Central GBR, as a single best model (with R^2 ranging from 0.46 to 0.68) was obtained for each but one response in coral cover (data not shown). Figure 4.2.5 displays the strongest predictors for the 'best' model, which included negative relationships to the same acute predictors. Only for branching *Acropora* ($R^2 = 0.33$) more than one candidate model was obtained with some candidate models including one WQ metric as a negative impact on branching *Acropora* cover but that WQ metric did not contribute to explained variance. Therefore, the most parsimonious model without WQ was selected (Figure 4.2.5).

Only a small proportion of the variability in total cover state was explained by acute disturbances in Central GBR (13% of variance explained) compared to the cover state of tabular *Acropora* (21%) and MSE (17%). However, observed cover of branching corals were least explained by the models (11% *Acropora*, 5% non-*Acropora*). This was not the case in the Southern GBR where the total coral cover had the greatest explanatory power among all groups (24% of variance explained) (Figure 4.2.5).

Prolonged CoTS outbreak conditions (i.e., number of years with CoTS densities >0.22 ind. per tow within 5yr periods, hereafter 5yrCoTS) was identified as the most important driver of low total coral cover, tabular *Acropora* and MSE species. A reef experiencing a single CoTS outbreak in the previous 5 years show 17% lower total coral cover (37% loss in tabular

Acropora) than a reef without any outbreaks in the previous 5 years (Figure 4.2.4). 5yrCoTS explained a lower proportion (19%) of the variability of branching *Acropora* (15% lower cover with a single year of outbreak) and did not affect the cover of branching non-*Acropora* corals. Storm impacts (both, the frequency of events [5yr-storms] and the exposure intensity [Cyclone exposure]) were the most important factors reducing cover for branching corals (*Acropora* and non-*Acropora*), MSE and total coral cover. Total coral cover declined on average 15% with each storm event which caused similar impacts among coral groups. Interestingly, only the intensity of cyclones (but not their frequency) negatively impacted the cover of tabular *Acropora*, which dropped by 50% after only few hours of cyclone exposure. Exposure to heat stress (i.e. DHW >2) was related to cover decline in tabular *Acropora*, MSE and branching non-*Acropora*, as reflected in the decline of total coral cover in response to DHW. At DHW below 2 there was no reduction in coral cover while a steep decline is shown above 2.3 DHW (Figure 4.2.4). In contrast, branching *Acropora* corals were unaffected by the frequency of bleaching or bleaching-risk events (i.e. DHW>3 over 5yr period).

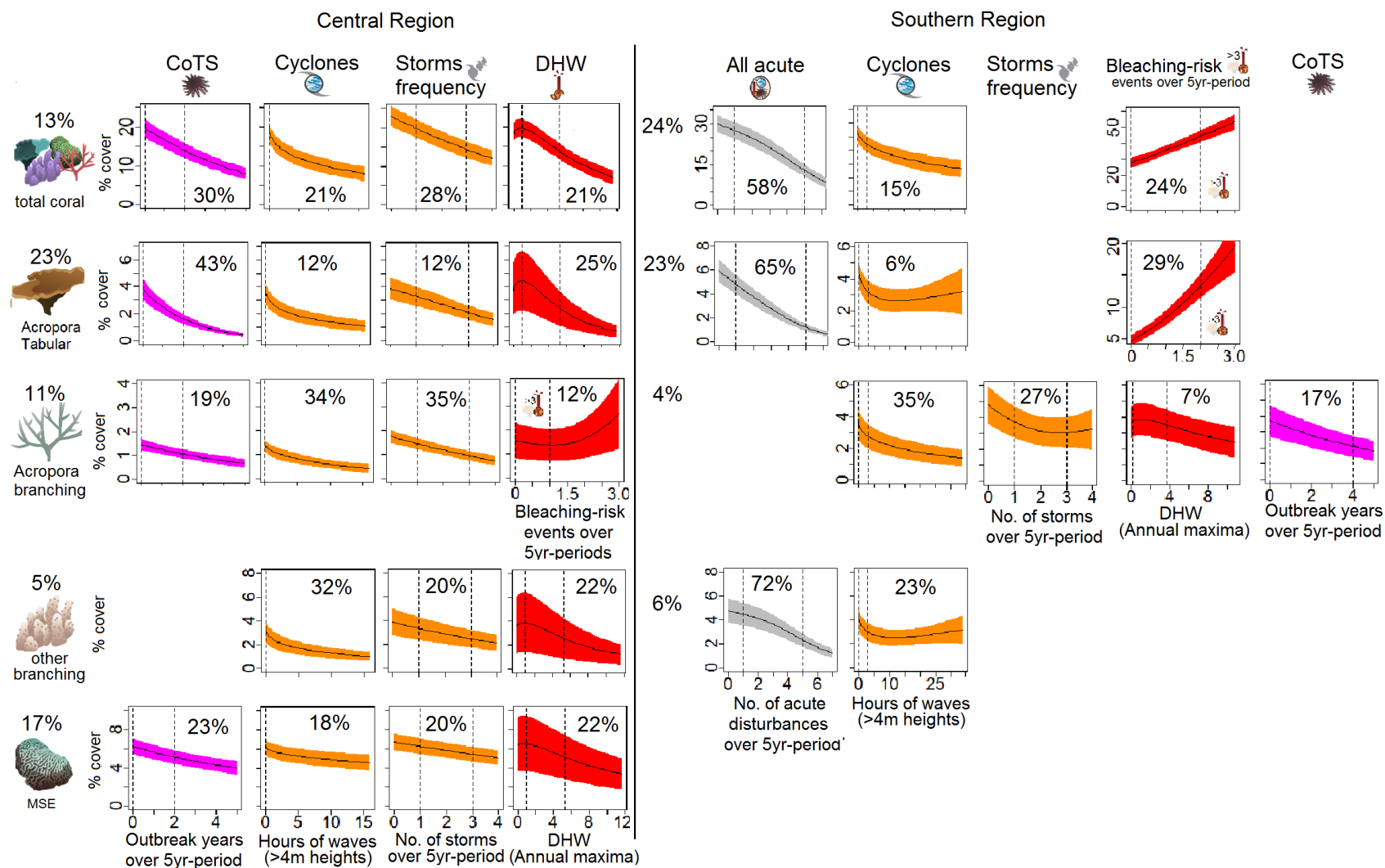


Figure 4.2.5: Predicted effects of acute stressors (columns) on reef status per coral category (rows) for the Central and Southern GBR (time series: 1992-2017). Percentages outside the plots indicate total variance explained by predictors. Percentages inside the plots indicate the relative contribution of each predictor to the total variance. Metrics other than those at the base of each column are indicated on each plot. Solid lines are the partial effects attributable to each of the variables based on GAMMs $\pm$ 95% confidence. Dashed vertical lines indicate 50 and 95 percentiles.

4.2.4.4 Drivers of reef State in southern GBR

For the Southern region, model outputs were less consistent. The most parsimonious model was selected per coral group (with R^2 ranging from 0.47 to 0.68) except for *Acropora* tabular ($R^2 = 0.51$) for which a single 'best' model was identified. A similar amount of variance was explained for total coral cover and the cover of tabular *Acropora* alone (24% and 23% respectively) with the same combination of stressors whereas the cover of branching *Acropora* and non-*Acropora*, and MSE were all poorly explained by the best predictors (4%, 6% and 1% of their variability explained, respectively) (Figure 4.2.5), instead, variability in their cover was mostly explained by reef location and time. Similar to Central GBR, the model of *Acropora*-branching and non-branching on Southern GBR resulted in candidate models differing in the presence of WQ metrics with no significant contribution to variance explained. Lack of temporal trends in the WQ data may explain the poor predictive power of WQ in the models as WQ variables increased in relative importance when yearly variability was explicitly modelled, compared to averaged values of WQ. This effect was greater for Chlorophyll a (Chla), DINE and PAR metrics, explaining the state of most coral groups except MSE (Figure 4.2.4).

Contrary to Central GBR, the cumulative frequency of all acute disturbance (i.e. total number of disturbance events over 5yr period- 5yrTCI) was by far the best predictor (contributing up to 72 % of explained variance) of cover state on Southern GBR. 5yrTCI explained most of the variability in total coral cover (58%), tabular *Acropora* (65%) and branching non-*Acropora* corals (72%). Total coral cover was 18% lower when at least 2 disturbances occurred within a 5yr period and up to 40% lower with 3 disturbances over the 5yr period. The cover of tabular *Acropora* was particularly sensitive to multiple acute events with estimates of 38% lower cover when 2 disturbances occurred within 5 years. Interestingly, 5yrCoTS (i.e. years with outbreak conditions over 5yr) alone was less important on Southern GBR contributing very little to the decline of branching *Acropora* corals alone. Similar to Central GBR, storm impacts (both, the frequency of events and the intensity of cyclones) were the most important drivers affecting negatively the cover of branching *Acropora*. However, only the intensity of cyclones (rather than the frequency of storms) explained the variability in total coral cover, tabular *Acropora* and branching non-*Acropora* corals with the last two exhibiting greater resistance to cyclones compared to Central GBR. Exposure to heat stress explained very little of the state in branching *Acropora* whereas the frequency of bleaching or bleaching-risk events were associated to greater total coral cover and that of tabular *Acropora* corals (Figure 4.2.5).

4.2.4.5 Drivers of reef Performance in central GBR

In Central GBR, model outputs of performance showed lower consistency than state models, with several competing models and lower predictive power (R^2 ranging from 0.22 to 0.29). Competing models differed mostly by the presence of WQ metrics for tabular *Acropora*. The performance of branching non-*Acropora* and tabular *Acropora* corals were the most informative metrics for explaining the impact of the selected multiple stressors (28% and 19% explained variability respectively) while the performance of total coral cover, MSE and branching *Acropora* were less well explained (17%, 10% and 6%, respectively).

In the absence of acute disturbances, the cover increase potential of the total coral community was hampered mainly by the lagged effect of intense cyclones (i.e. when cyclones occurred in the previous year) and the cumulative impact of all acute disturbances occurring over the previous five years (5yr-acute events). In other words, lag effects of strong cyclones hampered

recovery as an individual factor (measuring magnitude of exposure) or part of the combined acute disturbance history (measuring the frequency of impacts) (Figure 4.2.6). At the level of individual coral groups, previous prolonged CoTS outbreak conditions (5yr-outbreaks) were consistently the most important driver of low recovery during non-disturbance years for tabular *Acropora*, and branching corals (*Acropora* and non-*Acropora*). Interestingly, while the recovery of tabular *Acropora* was negatively affected by the lagged effects of intense cyclones, it was higher with greater past storm frequency. In contrast, performance of MSE was hindered by both the lagged effects of intense cyclones and the number of recurrent storms. While river DIN, environmental DIN, and salinity appeared in final models for branching non-*Acropora* (DIN metrics) and tabular *Acropora* (Salinity) (Figure 4.2.6), they explained only a small proportion of the variability in reef performance (<2%). However, PAR, Chla and sediments appeared in competing models for some coral groups, and may become important predictors once better temporal resolution for data will become available (Figure 4.2.4).

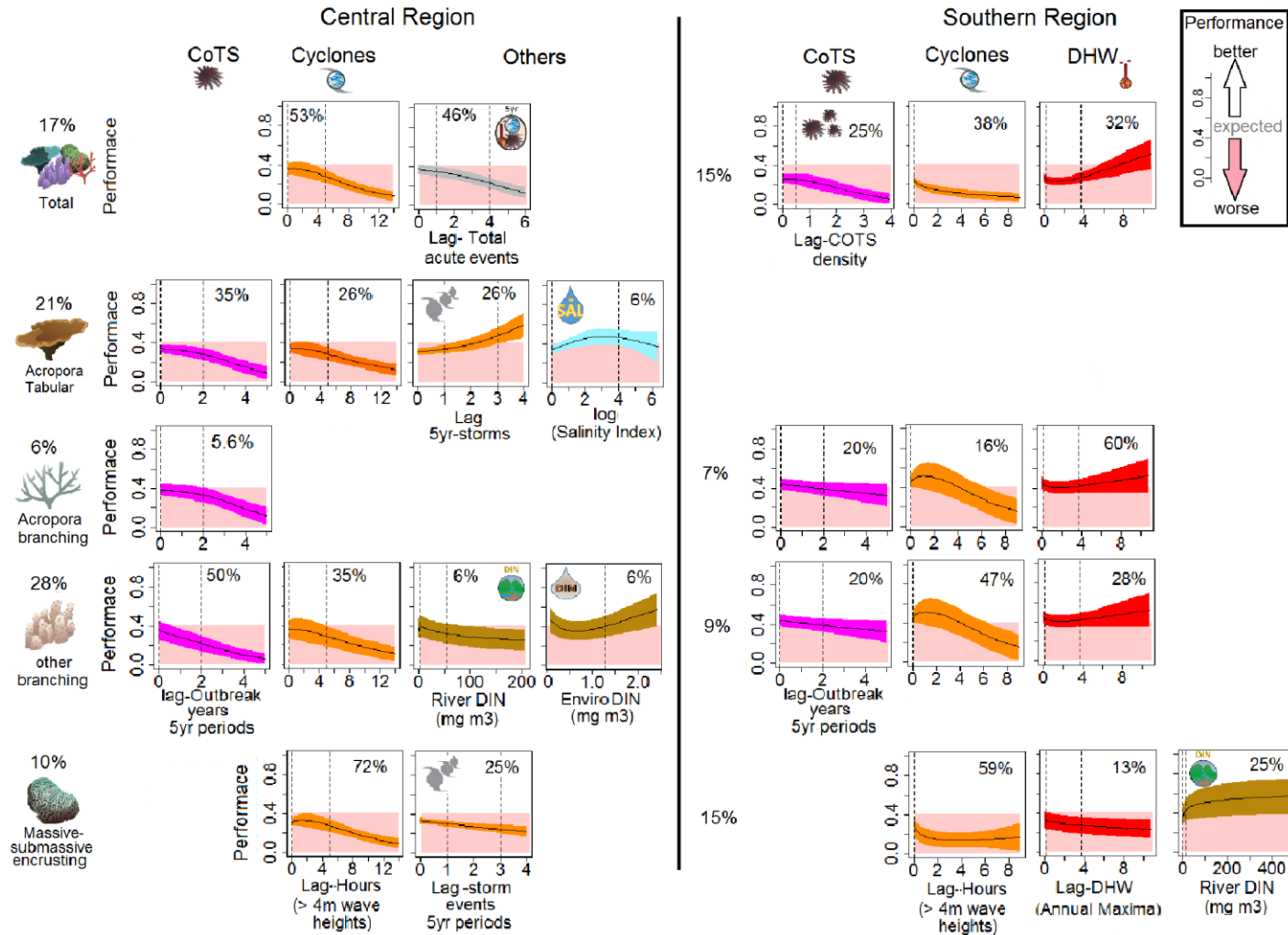


Figure 4.2.6: Predicted effects of acute and chronic stressors (columns) on reef performance for the period 2002-2017 per coral category (rows) for the Central and Southern GBR. Percentages outside plots indicate total variance explained by predictors. Inside plots, the relative contribution to total variance explained. Predictors other than those heading each column are indicated inside plots. Solid lines are fitted GAMM curves ± 95% confidence. Dashed vertical lines indicate 50 and 95 percentiles.

4.2.4.6 Drivers of reef Performance in southern GBR

Model outputs for coral performance in the South region had similar lower power to those for the Central GBR (R^2 ranging from 0.19 to 0.29), with multiple competing models (differing mainly in WQ metrics and lag-CoTS metrics, particularly for Acroporids). Contrary to Central GBR, the performance of total coral cover was as informative as that of MSE (15%), whereas the performance of branching *Acropora* and non-*Acropora*, and tabular *Acropora* was not well explained by the selected predictors (7%, 9% and <3%, respectively). The performance of total coral cover was negatively correlated to the lagged effects of intense cyclones and lagged CoTS densities, whereas it was positively correlated with the previous exposure to heat stress (lag-DHW). Similar results were obtained for the performance of branching corals (*Acropora* and non-*Acropora*) with lag-DHW being the most important predictor of positive performance of the fast-growing branching *Acropora*. Consequently, lagged exposure to DHW had a negative effect on the performance of slow-growing MSE corals. River DIN had a positive effect on performance of MSE (contributing to 25% of explained variance). This effect is likely attributed to the dominance of MSE corals in poor WQ environments such as inshore reefs where river influence is higher.

4.2.5 Discussion

Long-term monitoring data from the GBR combined from 2 major monitoring projects illustrate that overall, the rate of coral cover increase is worse than expected (i.e. reefs are underperforming). All coral groups in the Central GBR show predominantly low performance whereas in the Southern GBR all but MSE have predominantly performed as expected (i.e., 'performance score ~ 0.5) over the last decades. As a consequence, total coral cover declined slowly since the early 2000s on the Central GBR and dramatically nearly a decade later in the Southern region where some signs of recovery has been shown in recent years. Yet, coral cover has since declined as a consequence of the back-to-back massive bleaching event in 2016 and 2017 (Hughes et al., 2018b) and it is likely that reef performance will slow down given the third massive bleaching event in 2020 (pers. obs.).

Using two metrics of reef health: reef state and reef performance we showed that cover dynamics exhibited contrasting responses to the impact of multiple stressors. Overall, the prolonged exposure of reefs to multiple acute disturbances are the main drivers of reef state degradation and their legacy effects are responsible for low reef recovery. However, both the state and performance of reefs differ spatially and among components of the coral community. Some coral groups appeared to be unaffected (at least until 2018) by particular stressors that otherwise are known to be responsible for overall reef degradation on the GBR. Differential responses between Central and Southern regions and between measures of intensity vs frequency of disturbances highlight the complex dynamics of coral reef ecosystems and the importance of evaluating different exposure metrics on structurally-different coral communities to understand overall impacts on reefs. Our results may help understanding why some sectors of the GBR perform better than others in the face of repeated acute and chronic stressors.

Both the intensity and frequency of storms were identified as important predictors of reef state and performance along the GBR. However, while cyclone intensity was particularly important for predicting a reduction in cover state and low reef performance across all functional groups, our models suggest that the frequency of storm impacts in the Central region may be benefiting the performance of tabular *Acropora* corals. While coral losses after cyclones tend to be higher

on reefs dominated by fragile morphologies such as branching and plate corals (Foster, Foster et al., 2011; Madin & Connolly, 2006), these same morphologies show faster growth rates and can re-grow from small surviving fragments therefore have the capacity to recover rapidly. Acroporids in particular tend to show greater recovery after physical disturbance from cyclones (Adjeroud, Michonneau et al., 2009; Halford, Cheal et al., 2004).

Crown-of-Thorns-Seastars (CoTS) densities alone was a poor predictor of reef degradation. Instead, a cumulative metric of CoTS based on prolonged exposure to densities above incipient outbreak levels (i.e., years of CoTS densities greater than 0.22 per tow) was the best predictor of CoTS impact on reef state and performance. Despite that some Southern reefs experience more frequently CoTS outbreaks than in Central GBR (e.g. in the Swains region), CoTS alone were a weak predictor of reef state in the Southern GBR. The most likely explanation for this result is the fact that the metric of all acute disturbances combined was the strongest predictor and already incorporates frequency of CoTS outbreaks. Therefore, this result should not be interpreted as a lack of influence of CoTS outbreaks in the Southern GBR. Similarly, during periods of no disturbance, the legacy effect of CoTS contributes to overall reef degradation by slowing down performance in Southern GBR reefs. The contribution of CoTS as leading causes of coral loss and in undermining the recovery of *Acropora* populations on the GBR has been previously documented (De'ath, Fabricius et al., 2012; Osborne et al., 2011; Vercelloni et al., 2017). Here we provide further evidence of CoTS undermining the performance of branching non-*Acropora* corals and the cover status of non-branching coral communities (i.e. MSE which include *Montipora* spp). While *Acropora* and *Montipora* are the preferred prey of *Acanthaster* spp., their feeding preferences are variable and may depend on the abundance and distribution of prey (Pratchett, Caballes et al., 2017). Management efforts for understanding and preventing CoTS outbreaks and possibly targeted control on some reefs are important for improving overall reef resilience particularly when other factors are harder to control (climate change induce bleaching, ocean acidification and increased cyclone frequency and intensity).

Until 2018, the Southern GBR had experienced less widespread heating stress (95 percentile below 3.66 DHW) compared to northern and Central GBR (95 percentile below 5.29 DHW). The Southern GBR escaped the devastating impacts of heat stress that cause massive bleaching and coral mortality in the northern and Central GBR during 2016 and 2017 (Hughes et al., 2018b). Our model results support these patterns showing that 1) exposure to DHW above 2.3 was an important predictor of reduced coral cover in Central GBR where most reefs experienced DHW up to 5.29, 2) DHW was a poor predictor of regional reef degradation (low cover and low rates of cover increase) in the Southern GBR where most reefs experienced DHW below 3.7 during the same period, and 3) the frequency of bleaching and bleaching-risk events have no negative impact on corals in the Southern GBR. Our findings with impacts possible > 2.3 DHW are consistent with recent analysis of the GBR bleaching data from 2016 which show substantial mortality occurring at 3–4 °C-weeks (Hughes et al., 2018b). An apparent positive impact of bleaching-risk events on Southern GBR may indicate that our threshold of DHW is still below thermal threshold for corals in the Southern region or simply that very few reefs experienced levels much higher than this threshold. As heat stress was lower in the Southern GBR, the positive correlation with reef performance may be an indicator that the temperature optimum in Southern reefs is higher than the historic summer average, and that mild thermal stress may promote an increase in colony growth. Our threshold of 2.3 DHW for cover decline detected for different coral communities in Central GBR coincides with

that estimated by Ceccarelli et al. (2019) for total coral cover of inshore GBR reefs alone. Unfortunately, with the latest mass bleaching event in 2020 it is likely that the spatial patterns observed in our models with respect to the response of corals to DHW and bleaching-risk events will change.

WQ was relatively a poor predictor of reef health which is of no surprise given the strong predictive power detected for acute disturbances, and the poor temporal resolution in WQ data. Ceccarelli et al. (2019) obtained similar results while evaluating the effect of Chlorophyll *a*, turbidity and the frequency of exposure to highly turbid flood plumes (the “primary” water type) on total coral cover of inshore GBR reefs. In their study, negative effects of reduced WQ variables were still measurable, but the effect size was smaller than that of acute disturbances. While we assess one of the best available data for WQ metrics for the GBR (most measurements available e.g. through MMP are only for inshore reefs), these are still modelled products rather than in-situ observations which limit their accuracy and potential predictive power as some metrics may under-estimate real concentrations (Robson, Skerratt et al., 2020; Skerratt, Mongin et al., 2019). Yet, WQ metrics appeared in competing models with low predictive power, suggesting that most WQ metrics are more important for evaluating reef performance (i.e. rate of cover increase) than reef state (i.e. cover). Another recent modelling study identified the frequency of flood plumes as an important predictor of reef resilience (Mellin et al., 2019a). In addition, while we interpret the results of relative importance with caution, some variables (particularly Chl*a*, salinity and sediments) as predictors of reef performance are likely to change in relative importance when greater temporal resolution becomes available. Additional constraints for evaluating the power of WQ metrics apart from the temporal resolution of the data is the lack of strong spatial influence of WQ across the continental shelf. River DIN with yearly values throughout the long-time series had better predictive power compared to all other WQ metrics, particularly in Central GBR where its relative importance was comparable to that of acute stressors. Comparing models with and without yearly variability in WQ metrics suggest that power may increase for some metrics particularly for Southern GBR. Specifically, river DIN was a stronger predictor of the expected growth performance of MSE in the Southern region which may reflect the adaptation of MSE corals to poor WQ environments as it has been suggested for this type of communities in inshore reefs where the influence of river waters is greater (Roff, Clark et al., 2013; Wolff et al., 2018a).

Additionally, WQ can have indirect effects on coral health and performance not captured in the analysis. Although still under debate (Pratchett et al., 2017) WQ decline (increased inorganic nutrients) may increase phytoplankton food for CoTS larvae thus increasing numbers of surviving larvae for settlement (Fabricius et al., 2010), an effect which is further enhanced by elevated temperatures (Uthicke, Logan et al., 2015). Increased imbalances in dissolved inorganic nutrients may also increase bleaching susceptibility and coral disease risk (Wiedenmann et al., 2013), whereas corals adapted to turbid waters may exhibit higher bleaching tolerance (Sully & van Woessik, 2020). Negative Water quality impacts on coral reef communities were also demonstrated in smaller scale studies along water quality gradients (Fabricius & De'Ath, 2004). The reason our analysis is not able to capture/tease apart these effects is likely due to i) low temporal resolution of the WQ data as shown with our shorter term analyses, ii) spatial resolution not being adequate (i.e. data derived from 1 km and 4 km models may not be representative of environmental conditions experienced at the scale of surveys for biological data), and iii) the lack of interactive effects being explicitly tested in our models.

However, data presented here and in previous studies clearly indicate that WQ at least has a modulating effect on coral state and performance. Improved future monitoring designs should ensure that in addition to monitoring of biota sufficient data on abiotic parameters and WQ is collected to allow improved modelling in the future.

In summary, our models highlight the importance of lagged effects of cumulative acute impacts impairing reef recovery by slowing down coral cover increase and suggest that some reefs may be more resilient than others depending on how the coral community is structured and how it can cope with recurrent disturbance regimes. However, within the last few years reefs have experienced more intense and more frequent climate-related disturbances. The accelerated pace at which reefs are experiencing disturbance is impeding reef recovery and may lead to the loss of the ecosystem as we currently know it. Therefore, local actions to reduce WQ issues and controlling CoTS outbreaks will not suffice if global efforts to reduce global warming fail.

5. RECOMMENDATIONS AND CONCLUSION

This project consisted of three major sections: (i) hypothesis driven ecological experiments, (ii) the transfer and application of ecotoxicological approaches to climate related multi-species responses, and (iii) the development of exposure maps based on extensive compilation of observational and modelled data, in combination with observed and modelled responses of reef community states and performances for the Great Barrier Reef. Results of the three sections broaden our understanding of the cumulative impacts of climate change and water quality on coral reef ecosystems. An emerging picture is that climate change is the overwhelming driving factor for reef state and reef performance, together with other acute disturbances such as CoTS. Water quality plays an additional role, directly affecting specific organisms especially in areas explicitly exposed to river runoff. In addition, WQ is indirectly affecting reef state, e.g., through prolonged exposure to reduced light and increased turbidity, and the proliferation of CoTS larvae and primary and secondary outbreaks. A caveat is that relatively few long-term water quality time series and exposure data are available, greatly limiting our capacity to attribute change to water quality factors. Water quality also interacts with climate change in that combined stress of climate and local stress worsens the outcome for the organism, and retards reef recovery.

All sections have been communicated to stakeholders in workshops, direct updates and factsheets, and to the broader scientific community through multiple publications. Acceptance by the science community, as measured by feedback in discussions with other experts and citations of publications arising from this project is high.

In collaboration with eAtlas and as part of this project, experimental data, exposure maps and ecological modelling approaches are summarised as an interactive tool for managers and the public, intended as an educational tool explaining the background of cumulative impacts and the consequences to the reef. This tool is available at <https://eatlas.org.au/qbr/nesp-twq-5-2-cumulative-impacts>.

Based on our findings we conclude and recommend:

1. Local stressors must be taken into consideration when accessing the effect of climate change on coral reef communities.
2. Currently unabated climate change and ocean acidification will require to reduce ('climate adjust') thresholds for a variety of contaminants.
3. Although the effects of acute stressors (Climate, Cyclones, CoTS) overwhelm the influence of water quality parameters in the available pressure layers, modelling studies indicated that the effect of reef state and performance is also influenced by land runoff, thus improvement of WQ will assist reef condition.
4. Exposure maps and information on WQ on reefs and locations are limited, and information is often not available over long temporal scales needed to understand their influence on the reef. Better water quality data from within the GBR (not just the river mouth and inshore reefs) are necessary to attribute spatial and temporal changes in reef and seagrass communities to episodic and chronic changes in water quality. Improved water quality monitoring and development of new proxies for WQ should both be a priority for future monitoring projects.

5. The chronic local stressor of water quality is considered amenable to local management. In contrast, all of the acute '3 Cs' (Climate, Cyclones, CoTS) are extremely hard to manage. Out of these, only CoTS are (most likely) not a phenomenon requiring global collaborative management effort. Although CoTS are clearly also not trivial to manage, we argue that increased engagement in understanding and preventing CoTS outbreaks is needed in addition to WQ management and global negotiations to reduce and slow climate change.

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APPENDIX 1: PUBLISHED RESEARCH PAPERS

Table A1: Publications resulting from Projects 1.6, 2.1.6 and 5.2. Progress indicates publication status.

Paper	Reference	Progress	Number of citations (as at 10/06/2020)
1	Uthicke, S., Fabricius, K., De'ath, G., Negri, A., Smith, R., Warne, M., Noonan, S., Johansson, C., Gorsuch, H. and Anthony K (2016). Multiple and cumulative impacts on the GBR: assessment of current status and development of improved approaches for management. Final Report Project 1.6. Report to the National Environmental Science Program. Reef and Rainforest Research Centre Limited, Cairns (144pp.). https://nesptropical.edu.au/wp-content/uploads/2016/08/NESP-TWQ-1.6-FINAL-REPORTA.pdf	report online	11
2	Adams, M., Collier, C., Uthicke, S., Ow, YX., Langlois, L., O'Brien, K (2016). Model fit versus biological relevance: Evaluating photosynthesis-temperature models for three tropical seagrass species. <i>Scientific Reports</i>, 7, 39930, doi:10.1038/srep39930 https://www.nature.com/articles/srep39930	Published, peer reviewed	32
3	Anthony, KRN (2016). Coral reefs under climate change and ocean acidification - challenges and opportunities for management and policy. <i>Annu. Rev. Environ. Resour.</i>, 41, 59–81 (2016). https://www.annualreviews.org/doi/abs/10.1146/annurev-environ-110615-085610	Published, peer reviewed	57
4	Humanes, A., Noonan, S., Willis, B., Fabricius, K., Negri, A (2016). Cumulative effects of nutrient enrichment and elevated temperature compromise the early life history stages of the coral <i>Acropora tenuis</i>. <i>PLoS ONE</i>, 11: e0161616. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5004850/	Published, peer reviewed	27
5	Humanes, A., Ricardo, G., Willis, B., Fabricius, K., Negri, A. (2017). Cumulative effects of suspended sediments, organic nutrients and temperature stress on early life history stages of the coral <i>Acropora tenuis</i>. <i>Scientific Reports</i>, 7: 44101. https://www.nature.com/articles/srep44101	Published, peer reviewed	24
6	Wilkinson, A., Collier, C., Flores, F., Langlois, L., Ralph, P., Negri, A (2017). Combined effects of temperature and the herbicide diuron on Photosystem II activity of the tropical seagrass <i>Halophila ovalis</i>. <i>Scientific Reports</i>, 7: 45404. https://www.nature.com/articles/srep45404	Published, peer reviewed	15

7	Humanes, A., Fink, A., Willis, B., Fabricius, K., de Beer, D and Negri, A (2017). Effects of suspended sediments and nutrient enrichment on juvenile corals. <i>Marine Pollution Bulletin</i> , 125: 166-175 https://www.sciencedirect.com/science/article/abs/pii/S0025326X17306732	Published, peer reviewed	16
8	Rocker, M., Francis, D., Fabricius, K., Willis, B., Bay, L (2017). Variation in the health and biochemical condition of the coral <i>Acropora tenuis</i> along two water quality gradients on the Great Barrier Reef, Australia. <i>Marine Pollution Bulletin</i> , 119: 106-119. https://www.sciencedirect.com/science/article/abs/pii/S0025326X17302862	Published, peer reviewed	11
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11	Mellin, C., Matthews, S., Anthony, K., Brown, S., Caley, J., Johns, K., Osborne, K., Puotinen, M., Thompson, A and Wolff, N (2019) Spatial resilience of the Great Barrier Reef under cumulative disturbance impacts. <i>Global Change Biology</i> , 25:2431-2445 https://onlinelibrary.wiley.com/doi/abs/10.1111/gcb.14625	Published, peer reviewed	16
12	Negri, A., Smith, R., King, O., Frangos, J., Warne, M., Uthicke, S (2020) Adjusting tropical marine water quality guideline values for elevated ocean temperatures. <i>Environmental Science & Technology</i> , 54:1102-1110 https://pubs.acs.org/doi/abs/10.1021/acs.est.9b05961	Published, peer reviewed	1
13	Marques, J., Flores, F., Patel, F., Bianchini, A., Uthicke, S and Negri, A (in press) Acclimation history modulates effect size of calcareous algae (<i>Halimeda opuntia</i>) to herbicide exposure under future climate scenarios. https://www.sciencedirect.com/science/article/pii/S0048969720338304	Published, peer reviewed	0
Total citations			236

APPENDIX 2: ADDITIONAL DATA

Table A2: Measured concentrations of each diuron treatment at each climate scenario after 14 days exposure. BDL denotes below detection limit.

Nominal	2018	2050	2100
0	BDL	BDL	BDL
0.3	0.30	0.28	0.28
1	1.01	0.94	0.94
3	3.04	2.81	2.82
10	10.15	9.37	9.41
30	30.45	28.12	28.23

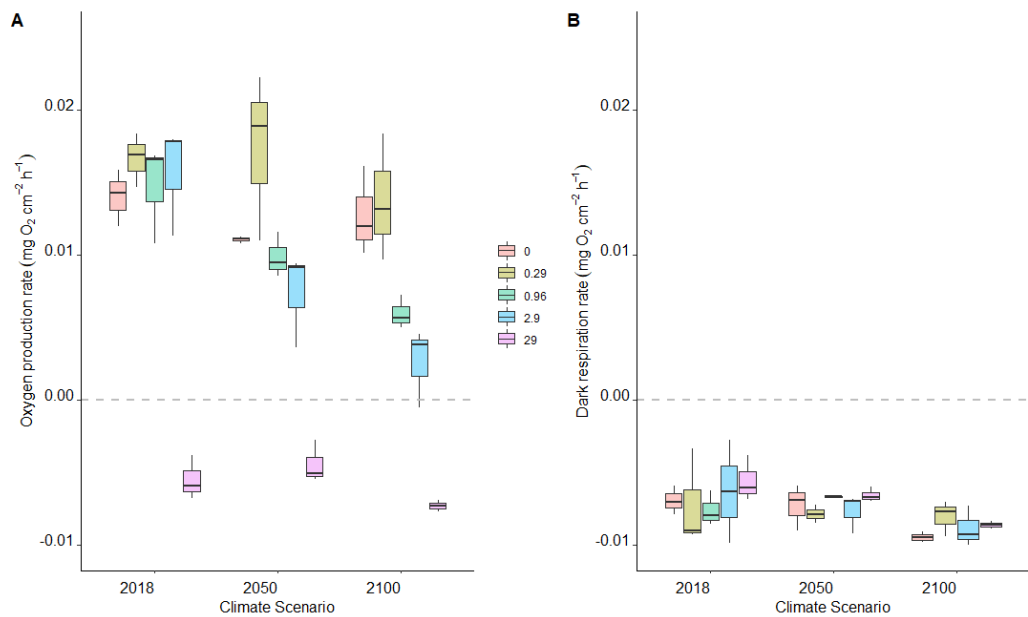


Figure A1: Boxplots of A) oxygen production (from light incubation) and B) dark respiration rates (from dark incubations) of *A. millepora* exposed to different concentrations of diuron under three climate scenarios.

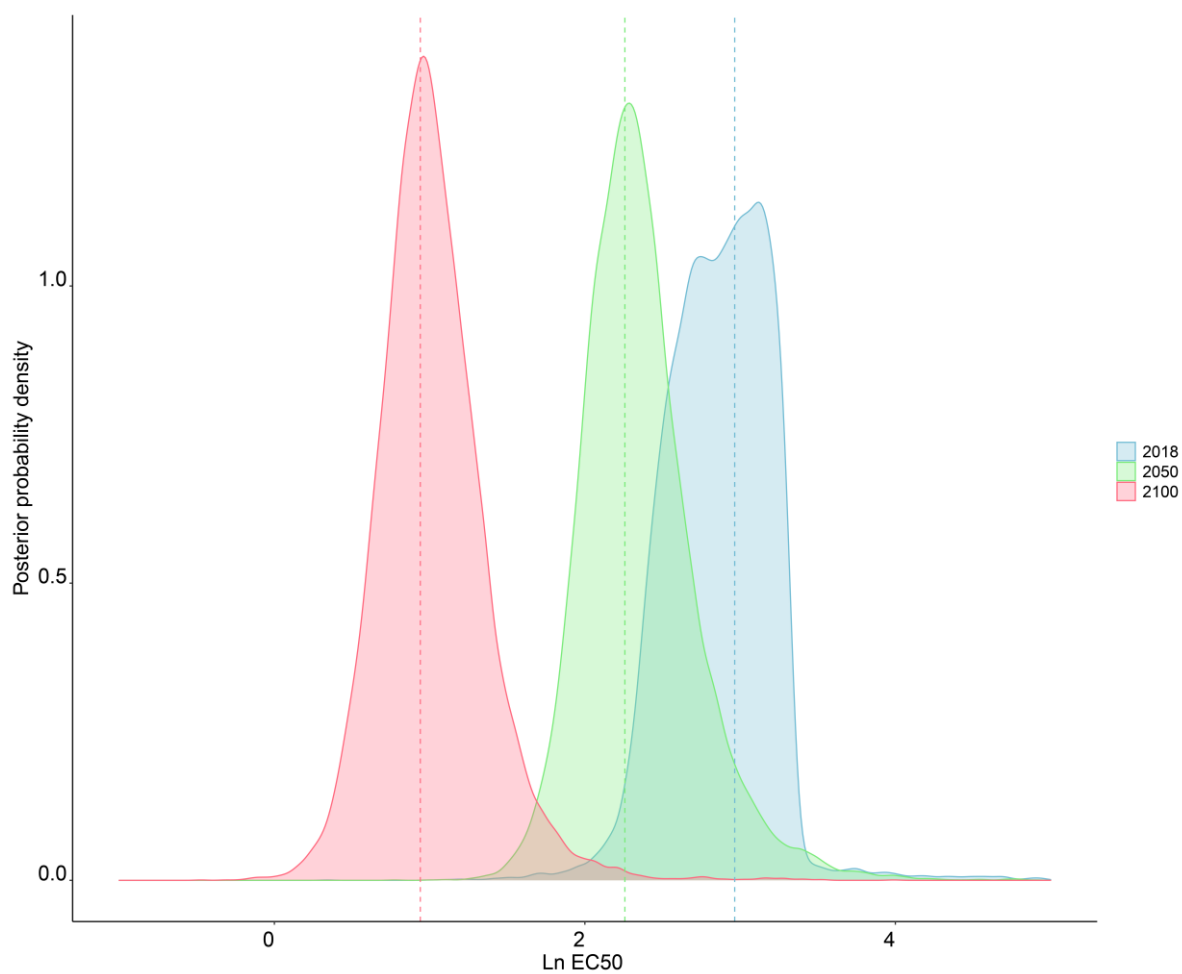


Figure A2: Probability density plots of net photosynthesis EC50s (Ln scale) from three climate scenarios.

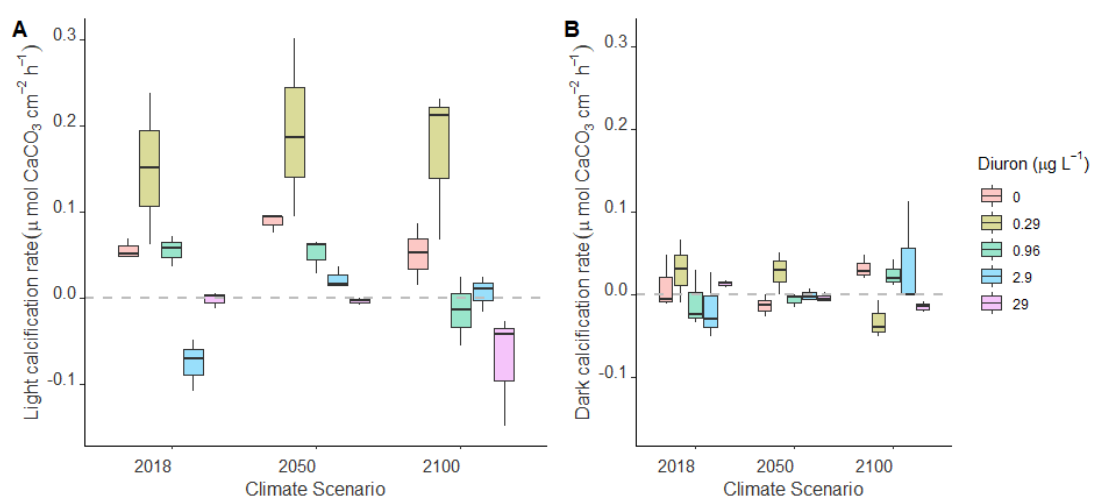


Figure A3: Boxplots of (A) light and (B) dark calcification rates ($\mu\text{mol CaCO}_3 \text{ cm}^{-2} \text{ h}^{-1}$) of corals exposed to three climate change scenarios and varying concentrations of diuron.

