Impacts of Ocean Warming and Acidification on Multiple Life Stages of Corals at the Houtman Abrolhos Islands

By
Taryn Foster
B.Sc. (Hons)

This thesis is submitted to the University of Western Australia, School of Earth and Environment, for the degree of Doctor of Philosophy

August 2016
Declaration of Authorship

The work contained herein is entirely my own, except where otherwise stated. For all work that has been published or prepared for publication with other authors, I have the permission of all co-authors to include this work in my thesis and my contributions as well as those of my co-authors are clearly indicated.

Student Signature: ……………………………………………………………………………………

Coordinating Supervisor Signature: ……………………………………………………………….
Statement of Candidate Contribution and Publications Arising from this Thesis

This thesis is presented as a series of papers in accordance with the standards set by the University of Western Australia. My contributions, as well as those of my co-authors are outlined below.

CHAPTER 2
Contributions: TF, JS, JF and MM designed the experiment; TF and JS conducted the experiment; TF, JS and JF conducted carbonate chemistry laboratory work; TF and CR conducted Micro CT laboratory work; TF analysed the data and wrote the manuscript; MM and JF read and commented on the manuscript.

CHAPTER 3
Contributions: TF designed the experiment with JG, CC, JF and MM; TF and CC conducted the experiment; TF analysed the data and wrote the manuscript; JG, CC, JF and MM read and commented on the manuscript.

CHAPTER 4
Foster T, Gilmour JP (in review) Seeing red: Coral larvae are attracted to healthy looking reefs. Marine Ecology Progress Series.
Contributions: TF and JG designed the experiment; TF conducted the experiment and laboratory work; TF analysed the data and wrote the manuscript; JG reviewed and commented on the manuscript.

CHAPTER 5
Contributions: TF designed the experiment with PC, MM and JF; TF conducted the experiment; TF and PC conducted X-ray and scanning electron microscopy work; TF analysed the data and wrote the paper; PC, MM and JF read and commented on the manuscript.

CHAPTER 6
Contributions: TF and PC designed the experiment; TF conducted the experiment; TF and PC conducted laboratory work; TF wrote the manuscript; PC reviewed and commented on the manuscript.
Table of Contents

Abstract ........................................................................................................................................... v
List of Figures .................................................................................................................................... vii
List of Tables ..................................................................................................................................... ix
Acknowledgements ............................................................................................................................. x

Chapter 1: General Introduction ....................................................................................................... 1
  1.1 The Importance of Coral Reefs ................................................................................................. 1
  1.2 Climate Change ....................................................................................................................... 2
  1.3 Ocean Acidification .................................................................................................................. 6
  1.4 The Effects of Elevated Temperature and pCO₂ on the Early Life Stages ......................... 8
  1.5 The Houtman Abrolhos Islands ............................................................................................. 10
  1.6 Aims and Thesis Structure ...................................................................................................... 11

Chapter 2: Reduced Calcification in Western Australian Corals during Anomally High Summer Water Temperatures .................................................................................................................. 13
  2.1 Abstract .................................................................................................................................. 13
  2.2 Introduction ............................................................................................................................. 14
  2.3 Methods ................................................................................................................................ 17
    2.3.1 Background ....................................................................................................................... 17
    2.3.2 Calcification Rates ............................................................................................................ 19
    2.3.3 Survival ............................................................................................................................ 22
    2.3.4 Environmental Parameters ............................................................................................. 22
    2.3.5 Data Analysis ................................................................................................................... 23
  2.4 Results .................................................................................................................................... 24
    2.4.1 Environmental Parameters ............................................................................................. 24
    2.4.2 Calcification and Survival ............................................................................................... 27
  2.5 Discussion ............................................................................................................................... 33

Chapter 3: Effect of Ocean Warming and Acidification on the Early Life Stages of Sub-tropical Acropora spicifera ................................................................................................................. 39
  3.1 Abstract .................................................................................................................................. 39
  3.2 Introduction ............................................................................................................................. 40
  3.3 Methods ................................................................................................................................ 43
    3.3.1 Collection Methods ........................................................................................................... 43
    3.3.2 Experimental Set-up .......................................................................................................... 43
    3.3.3 Larval Settlement and Post-settlement Survival ............................................................... 45
    3.3.4 Skeletal Weight ................................................................................................................ 46
    3.3.5 Data Analysis ................................................................................................................... 46
  3.4 Results .................................................................................................................................... 46
  3.5 Discussion ............................................................................................................................... 50

Chapter 4: Seeing red: Coral larvae are attracted to healthy looking reefs .................................... 57
  4.1 Abstract .................................................................................................................................. 57
  4.2 Introduction ............................................................................................................................. 58
  4.3 Methods ................................................................................................................................ 59
    4.3.1 Experimental Set-up .......................................................................................................... 59
    4.3.2 Fluorescence Emission Spectra ......................................................................................... 60
    4.3.3 Data Analysis ................................................................................................................... 60
  4.4 Results .................................................................................................................................... 60
    4.4.1 Fluorescence Emission Spectra ......................................................................................... 60
    4.4.2 Settlement ......................................................................................................................... 61
  4.5 Discussion ............................................................................................................................... 63
Abstract

Anthropogenic CO$_2$ is continuing to rise and consequently oceanic conditions are changing. Under a ‘business as usual’ scenario (RCP 8.5), the global sea surface temperature is projected to increase by ~3°C by 2100. Similarly ocean $p$CO$_2$ is set to increase by ~500 µatm (to ~930 µatm). These changes will have implications for marine biota, particularly calcifying organisms, such as corals. Thus in recent years there has been increased interest and a proliferation of studies on the effects of temperature and $p$CO$_2$ on corals. However, despite the fact that these changes are occurring simultaneously, there are relatively few studies that have investigated the combined impact of temperature and $p$CO$_2$. Further, the impact of these stressors on multiple life history stages is a particularly new field and the few experiments conducted to date have focused exclusively on tropical corals.

The sub-tropical Houtman Abrolhos Islands in Western Australia have been relatively isolated from many of the pressures affecting coral reefs around the world. However in the summer of 2011 the Western Australian (WA) coastline experienced a ‘marine heat wave’ and the first widespread mass coral bleaching event was recorded at the Abrolhos and at many other reefs along the WA coast. The first experimental chapter of this thesis was an in situ study monitoring the calcification rates of adult corals following the 2011 bleaching event. Corals showed abnormal seasonal and morphological patterns in their calcification, with higher calcification and survival rates in winter compared to summer and higher calcification rates in massive compared to branching morphologies. The most likely cause of the abnormal patterns in calcification, was the warmer than average summer water temperatures for the duration of the monitoring period (2012 and 2013), as well as ongoing recovery from the 2011 bleaching event. Reduced calcification rates (particularly in the temperature-sensitive branching morphologies) were likely a response to thermal stress, albeit at a sub-lethal and sub-bleaching level.

The following chapters in this thesis relate to laboratory experiments focused on the early life history stages of a dominant reef-building species at the Abrolhos Islands (*Acropora spicifera*). In these experiments the temperature and $p$CO$_2$ were manipulated to meet predictions for the
RCP 8.5 emission scenario for 2100. Elevated temperature had no effect on larval settlement, post-settlement survival or skeletal deposition, except to partially mitigate the effects of high $p\text{CO}_2$ on calcification. This indicated that sub-tropical $A.\text{spicifera}$ larvae and recruits might be able to withstand moderate temperature elevations. This apparent resilience to temperature increases may be because corals in the sub-tropics are typically exposed to lower temperatures and a wider range of temperatures than corals in the tropics. Additionally, coral larvae often need to survive long dispersal distances and a fluctuating thermal environment, which may also require some resilience to temperature changes in the early life history stages. While temperature did not affect settlement rates, larvae showed a high degree of photosensitivity, with a preference for red compared to white settlement substrates. This response suggests that settlement rates might be indirectly affected by temperature if crustose coralline algae (CCA) substrates bleach prior to spawning.

Acidification also had no effect on settlement and post-settlement survival, but had a strong negative effect on skeletal formation. This was evident in both the reduced size (surface area, volume, diameter, height) and weight of the high $p\text{CO}_2$ recruits as well as their malformed and porous skeletal structure. Neither temperature nor $p\text{CO}_2$ had any effect on the skeletal mineralogy of 1-month old recruits, with corals across all treatments having entirely aragonitic skeletons. This body of work highlights that responses to elevated temperature and $p\text{CO}_2$ may vary not only with latitude and species but also between life stages. If emissions continue along their current trajectory, the impacts on juvenile calcification are likely to be severe with negative implications for their survival into adulthood. It is encouraging however that the larvae and recruits of this dominant reef building species appear to be resilient to predicted temperature increases. While further work is required to validate the ‘sub-tropical refuge’ hypothesis explored in this thesis, the findings of this work suggest that the Abrolhos may be a viable temperature refuge as water heats up in the tropics and could become a key location in facilitating a range expansion southwards.
List of Figures

**Figure 1.1.** Atmospheric CO$_2$ concentrations history over the industrial era (right) and from year 0 to 1750 (left) from air enclosed in ice cores (green dots) and direct atmospheric measurements (blue line) (Fig. 6.11 in IPCC 2013). .................................................................3

**Figure 1.2.** Carbon dioxide concentrations following the four RCPs and their extensions to the year 2300 (reproduced from Box 1.1, Fig. 2, IPCC 2013). ......................................................................................3

**Figure 1.3.** (A) Projected changes in annual, globally averaged, surface ocean temperature until 2060, with shading indicating the 90% range of anomalies (reproduced from Fig. 11.19, IPCC 2013). (B) Changes with depth in annual mean temperature in the ocean relative to 1986-2005 for 2081-2100 (reproduced from Fig. 12.12, IPCC 2013).................................4

**Figure 1.4.** Map of the Houtman Abrolhos Islands off the Western Australian coastline. The study site at Basile Island is marked with a red dot. Imagery sourced from Esri and Landgate. ..................................................................................................................10

**Figure 2.1.** Study locations; Coral Bay, the Houtman Abrolhos Islands and Marmion Reef, covering 10° of latitude along the coast of Western Australia. .................................................................18

**Figure 2.2.** Surface area to dry weight regressions for *A. pulchra* and *P. damicornis*. ............21

**Figure 2.3.** Left axis: mean monthly SST anomalies at study sites (50 km resolution, NOAA, Coral Reef Watch, 2013), compared with long-term monthly averages over the past 10 years (2001-2010). Right axis: background *in situ* water temperatures prior to the temperature anomalies commencing in 2011, based on longer term averages from 2008 to 2010 for Coral Bay, 1990 to 1994 for Abrolhos (Pearce et al. 1999) and 2006 to 2010 for Marmion (dotted line). *In situ* water temperature through the period of temperature anomalies at the study sites (solid line). ..................................................................................26

**Figure 2.4.** Calcification rates (mean ± S.E, mg CaCO$_3$ cm$^{-2}$ d$^{-1}$) for corals at Coral Bay, Houtman Abrolhos Islands and Marmion Reef. *Experiment carried out at the Houtman Abrolhos Islands for first two seasons only. .................................................................28

**Figure 2.5.** Percentage survival (± S.E) corrected to 6-month time intervals at Coral Bay, Houtman Abrolhos Islands and Marmion Reef. *Experiment carried out at the Houtman Abrolhos Islands for first two seasons only. .................................................................29

**Figure 2.6.** Relative importance of variables contributing to variation in calcification rate within each species, as indicated by the sum of weighted AICc for each variable for all possible models. .........................................................................................32

**Figure 3.1.** Experimental set-up with four temperature-pCO$_2$ treatment sump tanks (above), each with four replicate larval tanks (below). Photo credit: Tim Campbell .......................45

**Figure 3.2.** Mean (±SE) percent settlement of *A. spicifera* under treatment conditions at one week post-fertilization (n = 4 per treatment).................................................................48

**Figure 3.3.** Mean (±SE) percent post-settlement survival of *A. spicifera* after being maintained under treatment conditions for 1 month (n = 4 per treatment). .........................48

**Figure 3.4.** Mean (±SE) skeletal weight (bars on the left y axis) and calcification rates (circles on the right y axis) of *A. spicifera* after 1 month under treatment conditions (n = 5 per treatment). .........................................................................................49

**Figure 4.1.** Fluorescence emission spectra of the synthetic settlement surfaces (red and white) and bleached and unbleached CCA .................................................................61

**Figure 4.2.** Coral settlement (% mean ± SE) on Crustose Coralline Algae (CCA) versus the synthetic substrate when the background colour was either red or white, under control, elevated temperature (High T), elevated pCO$_2$ (High pCO$_2$) and elevated temperature and pCO$_2$ (High T + pCO$_2$) conditions. ..................................................................................62

**Figure 4.3.** Settlement on the red synthetic surface typically occurred near or surrounding the CCA chip. A: Planulae ‘searching’ the surface of a CCA chip; B: settlement in the red background treatment, with newly settled larvae on and adjacent to the CCA chip; C: CCA chip with much of the surface occupied by 1-month old recruits; D: 1-month old recruits settled around CCA chips on the synthetic substrate when it appeared red; E: the CCA chip and background colour have been removed to show a typical ring formation of 1-month old
recruits surrounding the CCA chip when the background colour was red (dotted line indicates where CCA used to be).

**Figure 5.1.** Variation in seawater pH (A), total alkalinity (B), pCO$_2$ (C) and aragonite saturation state (D) in each treatment for the duration of the experiment. Measurements of pH were taken every second day and TA was measured every week. Mean weekly TA measurements were used to determine high frequency measures of pCO$_2$ and aragonite saturation state.

**Figure 5.2.** Variation in pH with time of day for each treatment.

**Figure 5.3.** Top-down and side view 3D X-ray images, from reconstructed scans, identifying the key skeletal structures discussed in this study. Scale bar is 500 µm.

**Figure 5.4.** Examples of manual measurements of diameter, corallite wall thickness (A, B), tertiary septa length : width (C, D), height and basal plate thickness (E, F). A, C and E show 3D reconstructions of skeletons and the location of the single ortho slice depicted in B, D and F. Scale bars are 500 µm.

**Figure 5.5.** X-ray microscopy and SEM images of 1-month old coral skeletons under temperature-pCO$_2$ treatments. Treatments include; ‘Control’ (A-D), ‘High T’ (E-H), ‘High pCO$_2$’ (I-L) and ‘High T + pCO$_2$’ (M-P) conditions. 3D X-ray images: A, E, I and M show a top-down view; B, F, J and N show a side view, all 3D X-ray images have a scale bar of 500 µm. SEM images: C, G, K and O show the top of the corallite wall; D, H, L and P show a tertiary septum, all SEM images have a scale bar of 10 µm. All of the four images shown horizontally per treatment are of a single, representative individual. See Figures 5.7 to 5.10 for images of the other individuals from each treatment.

**Figure 5.6.** Fractures and deformed skeletal structures in high pCO$_2$ treated corals. A-D highlight fractures in the septa (A, B) and corallite wall (C, D). E and F show small sections of missing septa and synapticulae. G and H display gross deformities, with large sections of the skeleton missing or malformed.

**Figure 5.7.** All remaining individuals from the ‘Control’ (i.e. excluding the individual shown in Figure 5.5). Optical microscope photographs (A, F, K, P). 3D X-ray top-down (B, G, L, Q) and side view (C, H, M, R) images and SEM corallite wall (D, I, N, S) and tertiary septum (E, J, O, T) images are given for each individual. Individuals are presented horizontally (A-E, F-J, K-O and P-T). Scale bars are 500 µm for all optical and 3D X-ray images and 10 µm for all SEM images.

**Figure 5.8.** All remaining individuals from the ‘High T’ treatment (i.e. excluding the individual shown in Figure 5.5). Optical microscope photographs (A, F, K, P). 3D X-ray top-down (B, G, L, Q) and side view (C, H, M, R) images and SEM corallite wall (D, I, N, S) and tertiary septum (E, J, O, T) images are given for each individual. Individuals are presented horizontally (A-E, F-J, K-O and P-T). Scale bars are 500 µm for all optical and 3D X-ray images and 10 µm for all SEM images.

**Figure 5.9.** All remaining individuals from the ‘High pCO$_2$’ treatment (i.e. excluding the individual shown in Figure 5.5). Optical microscope photographs (A, F, K, P). 3D X-ray top-down (B, G, L, Q) and side view (C, H, M, R) images and SEM corallite wall (D, I, N, S) and tertiary septum (E, J, O, T) images are given for each individual. Individuals are presented horizontally (A-E, F-J, K-O and P-T). Scale bars are 500 µm for all optical and 3D X-ray images and 10 µm for all SEM images.

**Figure 5.10.** All remaining individuals from the ‘High T + pCO$_2$’ treatment (i.e. excluding the individual shown in Figure 5.5). Optical microscope photographs (A, F, K, P). 3D X-ray top-down (B, G, L, Q) and side view (C, H, M, R) images and SEM corallite wall (D, I, N, S) and tertiary septum (E, J, O, T) images are given for each individual. Individuals are presented horizontally (A-E, F-J, K-O and P-T). Scale bars are 500 µm for all optical and 3D X-ray images and 10 µm for all SEM images.

**Figure 5.11.** Quantitative output from X-ray microscopy scans of 1-month old coral skeletons under the four temperature-pCO$_2$ treatments (mean ± SE). Measurements include; (A) surface area : volume, (B) diameter, (C) height, (D) basal plate thickness (E) corallite wall thickness and (F) tertiary septa length : width. Factors (Temperature, pCO$_2$ or their interaction, Temperature * pCO$_2$) significantly contributing to differences between
treatments are indicated by ★ at the top of the graph (n = 5 individuals per treatment). See

Figure 6.1. (A) One month old living Acropora spicifera recruit, (B) a typical Acropora spicifera recruit skeleton with organic material removed and (C) crushed skeletal material showing a typical ~60 µm² scan area grid analysed by Raman spectroscopy. Scale bars for A and B = 500 µm and scale bar for C = 40 µm.

Figure 6.2. XRD patterns for Acropora spicifera coral recruit skeletons grown under (a) control, (b) high temperature, (c) high pCO₂ and (d) high temperature + pCO₂ conditions. Aragonite standard peaks occur at 26.2° and 27.2° (green bars), and the calcite standard peak occurs at 29.4° (yellow bar).

Figure 6.3. Specific Raman shift of (a) a calcite standard and (b) a biogenic aragonite standard and skeletal material from (c) control, (d) high temperature, (e) high pCO₂ and (f) high temperature + pCO₂ treated Acropora spicifera coral recruits. The ~205 peak specific to aragonite is highlighted in green and the ~281 peak specific to calcite is highlighted in yellow.

List of Tables

Table 2.1. Species collected from each site..................................................................................18
Table 2.2. Sample periods defining the seasons referred to for the duration of the study from July 2011 to June 2013..................................................................................19
Table 2.3. Seasonal average (mean ± SD) for temperature (as measured by hand held pH meter on field trips) and seawater chemistry parameters at Coral Bay, the Abrolhos Islands and Marmion Reef..................................................................................................................24
Table 2.4. Best GLM models for predicting coral calcification within species.......................31
Table 3.1. Physical and chemical conditions for the duration of the five-week experiment (mean ± SD)..................................................................................................................................44
Table 3.2. Two-way analysis of variance (ANOVA) of settlement, post-settlement survival, and skeletal weight of Acropora spicifera recruits under four temperature-pCO₂ conditions........47
Table 5.1. Two-way ANOVAs testing for significant effects of temperature, pCO₂ and interactions between the two factors (temperature * pCO₂) on X-ray microscopy measurements of juvenile coral skeletons. † indicates significant effect (p < 0.05). .........82
Acknowledgements

I would like to thank my advisory committee: Malcolm McCulloch, Peta Clode, James Falter, James Gilmour and Mike van Keulen. Thank you for giving me the freedom to develop my independence as a researcher and pursue my interests, thank you for the many valuable comments on my manuscripts that have helped me to develop both my writing skills and my ideas, and most of all thank you for your guidance, support, and passion in introducing me to the world of science and research.

I would also like to thank the anonymous reviewers of the published and reviewed work from this thesis, who provided insights that have helped to develop some of the ideas presented here.

This work was funded by the Australian Research Council Centre of Excellence for Coral Reef Studies (CE140100020). I was supported by an Australian Postgraduate Award and UWA-AIMS top up scholarship.

I would like to thank the Batavia Coast Maritime Institute in Geraldton for access to their facilities. In particular I thank the wonderful staff at BCMI, including Suresh Job, Juan Gutierrez, Colin Johnson, Sarah Graham, Kim Morris, Dan Hoare and Soazig Laumaille for their technical assistance and ‘can do’ approach to everything.

I thank the Centre for Microscopy, Characterisation and Analysis (UWA) for access and training in their world-class microscopy facilities. In particular, thank you to Tamara Abel, John Murphy, Thomas Becker, Aaron Dodd and Jeremy Shaw for dedicating their time to my training.

I would also like to thank the following people from the Australian Institute of Marine Science: Andrew Negri and Andrew Heyward for enthusiastically teaching me their coral culturing techniques, Ben Radford for helping me analyse my field data and Victor Beltram for supplying cultured zooxanthellae for my larval experiments.

A big thank you to the people of the Abrolhos Islands, particularly the fishing and pearling community of Basile Island who welcomed and included me during my two-month stay there. I would especially like to thank the Basile family for their generosity and support at the Abrolhos Islands and for helping me to make the ‘field lab’ a reality. A special thank you to Andrew Basile whose McGyver-style solutions to the problems encountered at this remote location never ceased to amaze me.

I would like to extend a special thank you to the friends who have volunteered their time, energy and insights into this work: Jane Melvin, Miin Chua, Courtney Wood, Kaija Antipas, Zoe Snedden, Abby Mitchell, Leo Montoya Ruiz, Liza Roger, Kirsty Brooks, Claire Ross, Frazer McGregor, Pete Scarpuzza, Michael Holcomb, Sharyn Hickey, Asha McNeil, Arielle Fontaine and Lucy Georgiou. Thank you to Jessie Short and Sana Dandan who have been there through thick and thin, both in the field and in the office.

Lastly to mum, dad, Shell and Rob: thank you for your unwavering support and belief in me. It gave me the resilience and endurance to get on with the job when things didn’t work out according to my master plan. To mum and dad thank you for being my star volunteers in Geraldton and at the Abrolhos and thank you for letting me turn your home into a ‘one stop shop’ for anyone doing research in the Midwest or even passing through the region. Your tremendous generosity and hospitality made this project possible and I could not have done it without you.
Chapter 1: General Introduction

1.1 The Importance of Coral Reefs

Coral reefs are ecosystems of exceptional biodiversity, containing 34% of all described marine species, despite only occupying ~0.2% of the world’s ocean (Reaka-Kudla 1997; 2001). As well as supporting many thousands of marine species, coral reefs provide a barrier that protects coastlines and other important coastal ecosystems such as mangrove and seagrass habitats. Approximately 10% of the world’s population live within 100 km of coral reefs and of these ~75% are from the poorest developing nations (Donner and Potere 2007). Many millions of people rely on reef systems as a primary source of protein (Salvat 1992) and micronutrients (including vitamin A, zinc, iron and calcium) (Kawarazuka and Béné 2011), highlighting the importance of coral reefs to human health. The structural complexity and diversity of coral reef habitats has also allowed many organisms to become highly adapted with a variety of specialized mechanisms to prevent overgrowth and predation or to catch prey. Some of these mechanisms involve bioactive compounds, which have become the centre of a rapidly expanding marine biotechnology industry including the development of painkillers and anti-viral drugs and the isolation of anti-cancer and anti-HIV agents (Bruckner 2002; Amador et al. 2003; Ramessar et al. 2014).

The two main profit-generating industries that rely directly on coral reefs are reef-related tourism and commercial fishing. Tourism in general is one of the world’s largest industries, with an estimated 277 million jobs supported by travel and tourism in 2014, almost 10% of the world’s total employment (World Travel and Tourism Council, 2015). In Australia, the economic contribution of the Great Barrier Reef (GBR) was over $7 billion in 2013, with $6.4 billion of this generated from tourism and $192 million from commercial fishing (Deloitte Access Economics, 2013). Despite the immense social, ecological and economic value of coral reefs, they are facing a range of unprecedented human pressures and it is estimated that one third of reef-building corals species are threatened with extinction (Carpenter et al. 2008). Major
local threats include destructive fishing practices, overfishing, coastal development and reduced water quality due to agricultural runoff and dredging. In the Caribbean, overfishing of the major groups of herbivorous fish is one of the major causes of the declines in coral cover from 55% in the 1970s to 10% today (Gardner et al. 2003; Côté et al. 2005). Inshore Caribbean ecosystems shifted from coral-dominated to algal-dominated following the loss of grazing herbivores. The GBR has also seen major declines in coral cover in the past 30 years from 28% to 13%. One of the three major reasons for this decline is crown of thorns starfish outbreaks, a local disturbance thought to be influenced by poor water quality from agricultural runoff (De'ath et al. 2012). However the two biggest threats to coral reefs worldwide are global disturbances. As a result of anthropogenically produced CO$_2$, climate change and ocean acidification are driving rapid global changes in ocean temperature and chemistry, changes that will have consequences for all marine organisms, with coral reefs thought to be among the most sensitive (Orr et al. 2005; Hoegh-Guldberg et al. 2007; Veron et al. 2009).

1.2 Climate Change

The concentration of atmospheric carbon dioxide (CO$_2$) is currently 390 ppm (equivalent to a partial pressure or $p$CO$_2$ of 390 µatm), 40% higher than in 1750 (Figure 1.1; IPCC 2013) and higher than the Earth has experienced for at least the last 800,000 years (Lüthi et al. 2008). Under the IPCC 2013 high emissions scenario (RCP 8.5) $p$CO$_2$ is set to reach over 900 ppm by 2100, while under the medium-high (RCP 6) and medium low (RCP 4.5), it is set to reach ~670 ppm and ~540 ppm respectively (Meinshausen et al. 2011). Even under the lowest emissions scenario (RCP 2.6), where $p$CO$_2$ has started to decline by 2050, it is still projected to take another two centuries, until 2300, to be reduced to below current levels (Figure 1.2).
Figure 1.1. Atmospheric CO$_2$ concentrations history over the industrial era (right) and from year 0 to 1750 (left) from air enclosed in ice cores (green dots) and direct atmospheric measurements (blue line) (Fig 6.11 in IPCC 2013).

Figure 1.2. Carbon dioxide concentrations following the four RCPs and their extensions to the year 2300 (reproduced from Box 1.1, Fig. 2, IPCC 2013).
Increasing CO$_2$ has driven increases in oceanic temperatures by trapping extra heat in the Earth’s atmosphere via the ‘Greenhouse Effect’. The global Sea Surface Temperature (SST) trend showed warming at a rate of 0.091°C per decade from 1950 to 1999 and then increased to 0.099°C per decade from 2000 to 2014 (Karl et al. 2015). Global surface ocean temperatures are projected to continue to increase by 0.5°C to 1.5°C by 2060 (Figure 1.3A) and by 1°C to more than 3°C by 2100 depending on emissions scenarios (IPCC 2013) (Figure 1.3B).

**Figure 1.3.** (A) Projected changes in annual, globally averaged, surface ocean temperature until 2060, with shading indicating the 90% range of anomalies (reproduced from Fig. 11.19, IPCC 2013). (B) Changes with depth in annual mean temperature in the ocean relative to 1986-2005 for 2081-2100 (reproduced from Fig. 12.12, IPCC 2013).
Corals are among the most sensitive animals to increasing ocean temperatures. Even an elevation in temperature of just 1-2°C above the average summer maximum, if sustained over a period of weeks, can cause coral bleaching (Hoegh-Guldberg 1999). Coral bleaching is the expulsion of symbiotic algae from the tissues, which results in the pale or ‘bleached’ appearance of the coral. The algal symbionts or zooxanthellae, provide up to 100% of the coral’s energetic needs (Muscatine et al. 1981). Thus corals are considered to be in a high state of stress when they are bleached, and consequently mass bleaching events often result in high rates of mortality and even community shifts (Goreau et al. 2000; Smith et al. 2008; Depczynski et al. 2013). There are also indirect impacts of thermal stress and bleaching on coral health. Warmer water temperatures have been shown to have a strong positive correlation with infectious coral disease outbreaks. Both pathogen virulence and coral host susceptibility are thought to increase with elevated water temperatures, indicating that warmer water and bleaching could increase the effects of infectious diseases on coral reefs (Selig et al. 2006). The prevalence of mass bleaching events has increased in recent years (Hoegh-Guldberg 1999; 2005). Before the 1970s no mass bleaching events had been recorded, however between 1979 and 2009 seven major mass bleaching events were recorded (Veron et al. 2009), causing much concern about the future of coral reefs in an era of rapid climate change. Elevated temperature can also cause sub-bleaching stress responses, where corals appear healthy but key functions, such as calcification, become impaired. Reduced calcification rates due to warmer than average temperatures, have been recorded on the GBR (Lough and Barnes 2000; De'ath et al. 2009; D'Olivo et al. 2013), in the Red Sea (Cantin et al. 2010) and at the Thai-Malay Peninsula (Tanzil et al. 2013).

In response to increasing temperatures, some coral species appear to be expanding or shifting their geographical ranges to higher latitudes and to temperatures more optimal for coral growth (Precht and Aronson 2004; Yamano and Sugihara 2011). These changes in distribution could cause modifications to sub-tropical and temperate ecosystems (Yamano and Sugihara 2011), while declines in species diversity may be seen at tropical reefs (Greenstein and Pandolfi 2008). However there are many uncertainties around how a range shift would occur. Factors such as species differences in thermal tolerance and ability to migrate, as well as capacities to cope with
reduced aragonite saturation states at high latitude locations, could affect the ability of many species to successfully shift or expand their ranges in response to changing temperatures.

Western Australia (WA) has an ideal coastline for a potential mass southward migration, with a wide latitudinal range (−13°S to 34°S), the poleward flowing Leeuwin Current bringing warm, oligotrophic waters as far south as 30°S, and geographical connections between tropical and temperate locations at the sub-tropical Houtman Abrolhos Islands. Further, tropical coral reefs have been observed in the fossil record at what are presently temperate reefs in WA. For example at Rottnest Island off Perth (32°S), there is evidence of significant branching and plate Acropora reefs approximately 125,000 years ago (Szabo 1979). Because adult corals are sessile organisms, such large-scale latitudinal changes in distribution are only possible during the larval stage of the lifecycle, highlighting one of the many reasons why the early life stages will be of critical importance in determining how corals will cope with climate change.

### 1.3 Ocean Acidification

Range shifts or expansions may provide a buffer against increasing water temperatures, however they will not aid in protecting corals from ocean acidification. The ocean acts as a sink for atmospheric CO₂, absorbing approximately a quarter to one third of the total anthropogenic CO₂ emissions (Le Quéré et al. 2010; IPCC 2013). This uptake of CO₂ by the oceans has caused major changes to the carbonate chemistry of seawater, through a series of four reactions:

\[
\text{CO}_2^{\text{atmos}} \leftrightarrow \text{CO}_2^{\text{aq}} \quad (1.1)
\]
\[
\text{CO}_2^{\text{aq}} + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \quad (1.2)
\]
\[
\text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^- \quad (1.3)
\]
\[
\text{HCO}_3^- \leftrightarrow \text{H}^+ + \text{CO}_3^{2-} \quad (1.4)
\]

The ocean absorbs carbon dioxide gas, forming aqueous CO₂ (equation 1.1). This combines with water to produce carbonic acid (H₂CO₃) (equation 1.2), which dissociates producing hydrogen ions (H⁺) and bicarbonate ions (HCO₃⁻) (equation 1.3). Under normal conditions more
than 99.99% of hydrogen ions will join with carbonate ions (CO$_3^{2-}$) (IPCC 2013) (equation 1.4). The overall result of elevated CO$_2$ is that there are higher concentrations of hydrogen ions and lower concentrations of carbonate ions. The significance of these changes is that increasing H$^+$ concentrations are reducing the pH of seawater (i.e. acidification), while the reduced availability of CO$_3^{2-}$ is reducing the saturation state of calcium carbonate (CaCO$_3$). Many marine organisms use CaCO$_3$, in its aragonite and/or calcite form, to build support and protective structures such as shells and skeletons. With $p$CO$_2$ set to rise to >900 ppm by the end of the century under the ‘business as usual’ scenario (RCP8.5), and corresponding pH reductions of 0.31 units projected (IPCC 2013), calcification will likely become increasingly difficult. Reef-building or scleractinian corals build their skeletons from aragonite, a more soluble form of CaCO$_3$ than calcite. The fossil record shows that some species of ancient corals could produce calcitic skeletons (Stolarski et al. 2007) and modern corals have also been shown to switch to producing calcite under manipulated seawater magnesium to calcium ratios (Ries et al. 2006). However the ability of modern corals to rapidly produce their aragonite skeletons is thought to be one of the major reasons for their widespread success over the past 240 million years (Veron 1995; Ries et al. 2006).

There have been many studies reporting reduced calcification under elevated $p$CO$_2$ conditions, both in aquarium experiments (Langdon and Atkinson 2005; Fine and Tchernov 2007; Muehllehner and Edmunds 2008; Anthony et al. 2008) and in situ at CO$_2$ vents (Fabricius et al. 2011). At manipulated $p$CO$_2$ conditions of ~700 µatm, calcification has been reported to decline by 40 to 80% (Langdon and Atkinson 2005; Muehllehner and Edmunds 2008). Since increases in temperature and $p$CO$_2$ are occurring simultaneously, it is important to determine what their combined impact will be. In situ, rates of coral calcification are thought to have declined by 14-30% worldwide in recent years, with elevated temperature and ocean acidification thought to be the major causes (De'ath et al. 2009; Manzello 2010). However, laboratory studies measuring calcification under both elevated temperature and $p$CO$_2$ have yielded conflicting results, with elevated temperature shown to both mitigate (Langdon and Atkinson 2005; Muehllehner and Edmunds 2008; McCulloch et al. 2012) and further depress (Reynaud et al. 2003; Rodolfo-
Metalpa et al. 2011) calcification under high $p$CO$_2$. Elevated temperature probably increases calcification under high $p$CO$_2$ until physiological temperature thresholds are reached and then acts to further depress calcification. Further research is required to investigate the combined impacts of temperature and acidification not only on a variety of species and morphologies of coral, but also on corals from a range of different latitudes and in different life stages of their life cycle.

1.4 The Effects of Elevated Temperature and $p$CO$_2$ on the Early Life Stages

Sexual reproduction can produce new genotypes, thereby increasing a coral’s adaptive potential (van Oppen and Gates 2006; Harrison 2011). Furthermore, migration to more optimal environments is usually only possible in the planktonic larval stage of a coral’s lifecycle. Both evolutionary changes and range shifts will be important for corals to keep up with the current rate of environmental change. It is therefore crucial to know how climate change and ocean acidification are expected to impact the early life history stages. The past decade has seen significant advances in this field. Acidification is known to reduce fertilization by reducing sperm flagella motility (Morita et al. 2010) and fertilization is further inhibited at the low sperm concentrations likely experienced in situ (Albright et al. 2010; Albright and Mason 2013). Larval duration and settlement rates do not appear to be directly impacted by high $p$CO$_2$, however settlement rates can be indirectly affected by disrupting the settlement cues associated with their crustose coralline algae (CCA) settlement substrate (Albright and Langdon 2011; Webster et al. 2013b). Both elevated temperature and elevated $p$CO$_2$ can bleach CCA (Anthony et al. 2008; Webster et al. 2011) and cause changes to the microbial communities on the CCA surface, thereby disrupting coral settlement cues (Webster et al. 2011; 2013a). Early post-settlement calcification is particularly vulnerable to the effects of acidification, with the majority of studies reporting that growth or calcification is significantly reduced by high $p$CO$_2$ (Albright et al. 2008; Cohen et al. 2009; Suwa et al. 2009; de Putron et al. 2011; Drenkard et al. 2013). Elevated temperature alone has been shown to cause reduced larval survival, larval duration and settlement as well as embryonic aberrations and reduced post-settlement survival
However, there are very few studies investigating the impacts of both elevated temperature and elevated $pCO_2$ on corals in the early life stages. A combination of elevated temperature and $pCO_2$ appears to have a compounding effect on fertilization success (Albright and Mason 2013) and skeletal weight (Anlauf et al. 2011) but no effect on larval survivorship and metamorphosis (Chua et al. 2013a). However, it should be noted that the study on survivorship and metamorphosis by Chua et al (2013a), did not apply a very low pH treatment.

Nearly all of the studies that have been conducted to date have been in tropical locations, where corals are already living close to their upper thermal tolerance limits (Berkelmans and Willis 1999). High latitude reefs situated at the fringes of coral distribution have been shown to be hotspots for genetic diversity and evolutionary potential (Budd and Pandolfi 2010). Additionally, some sub-tropical coral reefs provide the geographical ‘stepping stones’ between tropical and temperate reefs required for poleward migration and may also become temporary refuges for corals if tropical locations become too warm. However, there is probably a limit to how far corals would be able to expand their ranges regardless of temperature increases, due to variability in saturation state with latitude. Saturation states are lower at sub-polar and polar locations compared to tropical and temperate regions (Fabry et al. 2009). Some studies have even reported undersaturation of aragonite in northern polar seas (Yamamoto-Kawai et al. 2009; Bates et al. 2009). A recent study has also shown that ocean acidification may already have reduced coral calcification at tropical coral reefs, further highlighting that carbonate chemistry is also an important consideration in assessing the viability of range shifts. Thus, to determine the viability of a range shift or refuge at sub-tropical high latitude reefs, it is critical to know how corals in these regions will be affected by projected changes to both ocean temperature and carbonate chemistry.
1.5 The Houtman Abrolhos Islands

The sub-tropical Houtman Abrolhos Islands (~28-29°S) are situated off the Western Australian coastline, approximately 30-60 nautical miles (~60-100 km) west and northwest of Geraldton (Figure 1.4). The Islands lie in the path of the warm, southward flowing Leeuwin Current and provide a connection between the more tropical Ningaloo Reef (~23°S) and temperate reefs to the south such as Rottnest Island and Marmion Reef (~31°S). Although the Abrolhos are a high latitude reef system, the water temperature ranges from 19°C to 24°C (Pearce et al. 1999), thus they have a unique mix of tropical coral and temperate macroalgal species. The Islands are also the highest latitude reef system in WA known to undergo mass coral spawning (Babcock et al. 1994). The Abrolhos therefore provide an ideal location to study the effects of warmer water temperatures and acidification on corals in the sub-tropics, and in particular, the impacts of these stressors on corals during the early life stages.

Figure 1.4. Map of the Houtman Abrolhos Islands off the Western Australian coastline. The study site at Basile Island is marked with a red dot. Imagery sourced from Esri and Landgate.
1.6 Aims and Thesis Structure

This thesis has been written as a series of papers, with five experimental chapters as papers, each with their own set of specific aims and outcomes. The broad aim of the thesis was to investigate the impacts of increasing oceanic temperatures and ocean acidification on various life history stages of corals at the sub-tropical Houtman Abrolhos Islands, with particular emphasis on calcification. Outlined below are the specific aims of each of the five sections.

1. Chapter 2: Reduced calcification in Western Australian corals during anomalously high summer water temperatures

The aim of this study was to investigate the impacts of high summer temperatures on the calcification and survival rates of a range of coral species and morphologies at latitudes ranging from tropical to temperate over two summer and two winter seasons, following the 2011 marine heat wave event.

2. Chapter 3: Effect of ocean warming and acidification on the early life stages of sub-tropical Acropora spicifera

This chapter aimed to investigate the impacts of elevated temperature and $pCO_2$ on the early life stages of the coral *Acropora spicifera* from the Abrolhos Islands, including 1) larval settlement, 2) post-settlement survival and 3) early skeletal growth (measured as changes in skeletal weights). More specifically, this chapter aimed to determine whether sub-tropical larvae and recruits respond differently to these stressors compared to larvae and new recruits from tropical locations.

3. Chapter 4: Seeing red: Coral larvae are attracted to healthy looking reefs

This chapter also investigated larval settlement rates under various temperature-$pCO_2$ regimes, but larvae were offered a red or a white synthetic settlement substrate as well as small chips of CCA for settlement. This experiment aimed to test how larvae respond to both spectral and chemical settlement cues under elevated temperature and $pCO_2$. 
4. **Chapter 5: Ocean acidification causes structural deformities in juvenile coral skeletons**

This study further investigated the impacts of elevated temperature and $p\text{CO}_2$ on skeletal formation in newly settled corals. Instead of employing the commonly used bulk method of weighing the skeletons (as was done in Chapter 3), 3D X-ray microscopy and Scanning Electron Microscopy (SEM) were used to closely examine and quantify changes to the architecture of the developing skeletons under different temperature-$p\text{CO}_2$ regimes. This chapter also aimed to develop a range of new morphological metrics to study changes to individual elements of the skeleton as well as the overall structure under acidification.

5. **Chapter 6: Skeletal mineralogy of coral recruits under high temperature and $p\text{CO}_2$**

In this chapter Raman spectroscopy and X-ray diffraction were used to determine whether the skeletal mineralogy of new recruits could change with changing carbonate chemistry. The specific aim of this chapter was to determine whether newly recruited corals were able to produce calcite (the less soluble crystal form of CaCO$_3$) instead of or as well as aragonite, under high $p\text{CO}_2$ and/or temperature conditions.
Chapter 2: Reduced Calcification in Western Australian Corals during Anomalously High Summer Water Temperatures


2.1 Abstract

Here we report the seasonal response of calcification rates over two years, for three coral species (Acropora pulchra, Pocillopora damicornis and Goniastrea aspera), to anomalously warm summer water temperatures (2012 and 2013), following the most severe marine heat wave on record (2010-2011). The study sites at Coral Bay (Ningaloo Reef), the Houtman Abrolhos Islands and Marmion Reef (Perth) spanned over 10° of latitude and ranged from tropical to temperate habitats. Calcification rates were determined using repeated measurements of buoyant weight normalized to surface area and rates were monitored over two winters and two summers from 2011 to 2013. Average growth rates followed predicted latitudinal trends, with the fastest growth in the tropics (Coral Bay) and slowest growth rates in the temperate zone (Marmion). However, calcification rates did not show the expected seasonality (higher in summer, lower in winter), even at high latitude locations. Instead, there was either little difference between summer and winter growth or growth was slower in the summer (on average, a reduction of ~40% across locations and species). Additionally, differences in species growth rates did not follow expected trends, with usually fast growing branching corals not calcifying much faster than the normally slower growing massive corals (A. pulchra, grew ~40% faster than G. aspera, which grew ~6% faster than P. damicornis across all latitudes and seasons). Survival rates were also reduced in the summer months, while among species P. damicornis had the lowest survival and G. aspera the highest. We conclude that high temperature stress through the summers of 2011 to 2013 was the most likely cause for the lack of seasonality in calcification rates, the similarity in calcification rates among species and the increased mortality of susceptible species in the summer months. The effect of prolonged elevated temperature anomalies (1.5 to 3°C) on the growth and survival of colonies over consecutive years was often
greater than the fundamental influences of season and species, highlighting the extent to which climate change could now be re-structuring the life histories of corals on Western Australian reefs.

2.2 Introduction

Coral growth rates typically vary with season, latitude and taxa (Shinn 1966; Crossland 1981; Harriott 1999; Lough and Barnes 2000; Lough 2008). Lough and Barnes (2000) observed that extension and calcification rates decreased with increasing latitude, which was directly related to decreasing water temperatures down latitudinal gradients. Similarly, higher growth rates generally occur in summer months, particularly at high latitude locations. For example, Shinn (1966) reported a decrease in linear extension of *Acropora cervicornis* in the winter months in Florida (25°N), while at the Houtman Abrolhos Islands (28°S), growth rates for *Acropora formosa* were 5 times higher in summer than in winter (Crossland 1981). A shorter growing season was also reported for corals at high latitude locations on the Great Barrier Reef, with greatly reduced growth in winter (Isern et al. 1996). Similarly, Kinsey (1985) noted that seasonal differences between summer and winter net community calcification rates reported from the northern Hawaiian Islands (French Frigate Shoals, 24°N) (Atkinson and Grigg 1984), the Houtman Abrolhos Islands (Smith 1981) and across the Great Barrier Reef generally increased with increasing latitude and ranged from a factor of 2 to 4. Even in the tropics, where temperature and daylight hours vary comparatively less throughout the year, community calcification rates were 30 to 40% higher in summer than in winter (Kinsey 1979). Seasonality appears to be greatest at high latitude reefs, consistent with the presence of more pronounced seasonal changes in both temperature and light (Isern et al. 1996; Howe and Marshall 2002). Warmer water temperatures increase the metabolism of the zooxanthellae, stimulating calcification (Eckert et al. 1988), while higher light availability, enhances calcification by providing photosynthates for energy as well as triggering calcium uptake (Al-Horani et al. 2003). Among coral taxa, branching species typically grow much faster than massive species (Harriott 1999). The fast growing but more fragile branching corals are generally the most
dominant in terms of coral cover, but are less able to withstand mechanical damage than the slow growing but more robust morphologies, which dominate high energy areas (Adey 1978). Branching species such as acroporids and pocilloporids grow at rates of approximately 100-150 mm/year and 50 mm/year respectively, while massive morphologies such as Montastrea and Porites grow around 5-10 mm/year (Dullo 2005). However, elevated water temperatures in summer months can potentially change the typical pattern of variation in growth between seasons and across coral taxa. Very high temperatures in summer months can stress corals, reducing growth and survival (Shinn 1966; Yap and Gomez 1984). Furthermore, it is often the faster growing, branching morphologies, such as the acroporids and pocilloporids that are most susceptible to temperature stress, while the massive morphologies such at the faviids and poritids appear to be more resilient (Marshall and Baird 2000).

The optimal temperatures for coral calcification are constrained within their thermal limits, with growth and survival typically reduced when maximum summer temperatures exceed long-term averages (Clausen and Roth 1975; Jokiel and Coles 1977; Marshall and Clode 2004). Growth rates generally increase with temperature only until maximum summer temperatures reach long-term averages, beyond which corals become stressed (Marshall and Clode 2004). Coral bleaching usually occurs after prolonged exposure to small increases (0.5-1.5°C), or short exposure to large increases (3-4°C) in water temperature above the average summer maximum (Jokiel and Coles 1977; Glynn and D'croz 1990). Bleaching and the subsequent loss of photosynthetic capacity, reduces metabolic energy availability for processes such as calcification (Grottoli et al. 2004), while prolonged temperature stress can result in partial or whole-colony mortality (Baird and Marshall 2002). Several studies have reported suppressed growth between one and four years following a bleaching event (Goreau and Macfarlane 1990; Leder et al. 1991; Suzuki et al. 2003; Omata et al. 2006). On reefs under high local stress, recovery time was shown to take at least eight years based on coral growth banding (Carilli et al. 2009).
Considerably less is known about the impacts of thermal stress on calcification rates before the bleaching threshold has been reached. However, there have been a number of recent studies reporting reduced calcification in situ, in response to increased water temperatures. For example, Cantin et al. (2010) reported a 5% decrease in growth of Diploastrea heliopora for every 0.2°C increase in sea surface temperature (SST) above the summer maximum of 30.5°C, in the central Red Sea. Overall, a 30% reduction in coral growth was observed after the 1998 mass bleaching event, in corals showing no obvious signs of stress (Cantin et al. 2010). Additionally, it has been suggested that reduced calcification rates in the massive coral Porites, which are among the most resistant corals to temperature, were due to increasing temperature stress on the GBR in recent years (Cooper et al. 2008; De'ath et al. 2009; D'Olivo et al. 2013). Similarly, around the Thai-Malay Peninsula in Southeast Asia, Porites growth rates showed region wide declines, with a significant link to increases in SST, however within region variability suggested that variables aside from temperature were also at play (Tanzil et al. 2013). The in situ studies as well as laboratory temperature studies outlined above, indicate that calcification rates are reduced by temperature stress long before the bleaching threshold is reached.

Coral reefs around the world are increasingly impacted by warming oceans, leading to mortality and mass bleaching, as well as reduced growth and reproduction (Szmant and Gassman 1990; Hoegh-Guldberg 1999; Baker et al. 2008). There have been comparatively few mass bleaching events reported along the west coast of Australia (excluding the severe mass bleaching in 1998 of the offshore atoll Scott Reef). For example, most of the coral reefs that occur in the coastal waters of Western Australia escaped the effects of temperature anomalies that caused mass bleaching on most of the world’s coral reefs during the 1997-1998 El Niño event (Wilkinson 1998; 2000). However in recent years, there have been apparent increases in the frequency of elevated temperature anomalies on WA reefs. Most notably, in the summer of 2011 a marine heat wave occurred along the coast of Western Australia (Pearce et al. 2011; Feng et al. 2013; Pearce and Feng 2013). Tropical species were reported far outside of their usual ranges, mass-mortals of fish, lobster and abalone were observed, there were resulting shifts in benthic community structure and the first widespread coral bleaching event was recorded for locations
spanning 1200 km down the WA coastline; from Barrow Island (20°S) to Rottnest Island (32°S) and at most reef systems in between. (Pearce et al. 2011; Moore et al. 2012; Depczynski et al. 2013). Decreases in coral cover following the bleaching event ranged from ~20-85% (Moore et al. 2012). Corals in these regions were likely still recovering from the heat wave, when in the summer of 2012 water temperatures along WA were again anomalously high and locations in the mid and southwest regions were on bleaching alert level 2 for 3-4 months (NOAA Coral Reef Watch, 2013). Then in the summer of 2013, the Ningaloo region was again on bleaching alert level 2 from February to April and all other WA bleaching stations were on bleaching warnings or alerts for that period. Patchy bleaching was observed in the northern sections of Ningaloo reef and the Abrolhos Islands in 2013 (Ningaloo, Ningaloo Atlas, 2013; Abrolhos, authors’ pers. obs). The initial aims of this study were to examine seasonal survival and calcification rates of three coral species with contrasting life histories and morphologies, from habitats spanning tropical to temperate and to provide baseline coral calcification data for WA corals. However, summer temperatures were anomalously high for the duration of the study from 2011 to 2013. Instead, we investigated the impacts of abnormally high summer seawater temperatures on rates of coral calcification and survival in species with contrasting susceptibilities to temperature stress (Marshall and Baird, 2000), at sites spanning 10° of latitude across WA.

2.3 Methods

2.3.1 Background

Study species included the branching corals *Acropora pulchra* and *Pocillopora damicornis* as well as the massive coral *Goniastrea aspera* (Table 2.1). Due to cooler winter temperatures, *A. pulchra* is not found at the highest latitude site (Marmion Reef) and could therefore not be included at this location. Study sites were located within three distinct biogeographic regions along 1200 km of the Western Australian coastline, spanning tropical to temperate reefs. Coral Bay (23°09’S) is located within the tropical Ningaloo Reef, the Houtman Abrolhos Islands (28°52’S) exist in a region of biogeographic overlap between tropical and temperate reef
environments and Marmion Reef (31°48’S) is in temperate waters (Figure 2.1). At all reefs, study sites were located in comparable back-reef habitats, in approximately 4 m water depth. Coral calcification rates were monitored over two years with surveys conducted around months in which water temperatures were warmest (summer) and coolest (winter, Table 2.2).

Table 2.1. Species collected from each site

<table>
<thead>
<tr>
<th>Location</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coral Bay</td>
<td>Acropora pulchra</td>
</tr>
<tr>
<td></td>
<td>Pocillopora damicornis</td>
</tr>
<tr>
<td></td>
<td>Goniastrea aspera</td>
</tr>
<tr>
<td>Houtman Abrolhos Islands</td>
<td>Acropora pulchra</td>
</tr>
<tr>
<td></td>
<td>Pocillopora damicornis</td>
</tr>
<tr>
<td></td>
<td>Goniastrea aspera</td>
</tr>
<tr>
<td>Marmion Reef</td>
<td>Pocillopora damicornis</td>
</tr>
<tr>
<td></td>
<td>Goniastrea aspera</td>
</tr>
</tbody>
</table>

Figure 2.1. Study locations; Coral Bay, the Houtman Abrolhos Islands and Marmion Reef, covering 10° of latitude along the coast of Western Australia.
Table 2.2. Sample periods defining the seasons referred to for the duration of the study from July 2011 to June 2013.

<table>
<thead>
<tr>
<th>Location</th>
<th>Season</th>
<th>Dates</th>
<th>Days between weighing dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coral Bay</td>
<td>Winter 2011</td>
<td>July to October 2011</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Summer 2012</td>
<td>October 2011 to May 2012</td>
<td>237</td>
</tr>
<tr>
<td></td>
<td>Winter 2012</td>
<td>May to November 2012</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>Summer 2013</td>
<td>November 2012 to June 2013</td>
<td>209</td>
</tr>
<tr>
<td>*Houtman Abrolhos Islands</td>
<td>Winter 2011</td>
<td>July to November 2011</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>Summer 2012</td>
<td>November 2011 to April 2012</td>
<td>177</td>
</tr>
<tr>
<td>Marmion Reef</td>
<td>Winter 2011</td>
<td>July to November 2011</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>Summer 2012</td>
<td>November 2011 to May 2012</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>Winter 2012</td>
<td>May to October 2012</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td>Summer 2013</td>
<td>October 2012 to April 2013</td>
<td>177</td>
</tr>
</tbody>
</table>

*Abrolhos Islands were monitored for only the first year from July 2011 to April 2012.

2.3.2 Calcification Rates

At each site, growth experiments were conducted using coral fragments 10 cm in length (branching) or diameter (massive), cut from replicate colonies of each species. There were 16 replicate colonies of each species at each site and one fragment was cut from each colony. The buoyant weight of each fragment was measured (Jokiel et al. 1978; Davies 1989) and then glued to acrylic tiles using underwater epoxy (Z-Spar™ Splash Zone Compound A-788). Samples were then re-weighed to determine the combined weight of the coral and tile. Sample tiles were randomly attached to two larger HDPE sample plates each holding eight tiles (n = 16) and deployed at the same sites from which colonies were originally sampled. After the initial deployment, samples were weighed before and after the cool and warm water periods at each location, in order to determine the change in buoyant weight per day in winter and summer (Table 2.2). To determine the mass of the coral in air from the buoyant weight in seawater, the following equation was used:

\[ M_{air} = \frac{M_{sw} \rho_{CaCO_3}}{\rho_{CaCO_3} - \rho_{sw}} \]  

(2.1)
Where $M_{\text{air}}$ is the mass of skeletal CaCO$_3$, $M_{\text{sw}}$ is the mass of the sample in seawater (minus the combined weight of the tile and epoxy) and $\rho_{\text{CaCO3}}$ and $\rho_{\text{sw}}$ are the densities of the coral and seawater respectively (Jokiel et al. 1978). $\rho_{\text{sw}}$ was calculated from the temperature and salinity of the seawater and $\rho_{\text{CaCO3}}$ is the density of aragonite (2.93 g cm$^{-3}$).

Dry weights were normalized to surface areas to determine calcification rates in g cm$^{-2}$ d$^{-1}$. Surface areas of the massive $G. \text{aspera}$ were determined in the field at each weighing, using the aluminum foil technique (Marsh 1970), but with the surface area being quantified directly by flattening the foil on a 1 cm grid, photographing it, then analyzing the digitized images in image analysis software, ImageJ. The surface area of both $A. \text{pulchra}$ and $P. \text{damicornis}$ samples were calculated retrospectively using a surface area to dry weight regression for a range of sizes for each species (Figure 2.2). There was a strongly linear relationship between surface area and dry weight for both $A. \text{pulchra}$ ($R^2 = 0.98$) and $P. \text{damicornis}$ ($R^2 = 0.97$). Surface areas for the $A. \text{pulchra}$ regression were determined by measuring the diameter and height of each branch, which were used to calculate the surface area of the coral as a series of cylinders (Naumann et al. 2009). For the more complex morphology of $P. \text{damicornis}$, micro computed tomography (CT) was used (Skyscan 1176 In Vivo X-ray MicroCT) to obtain precise surface area measurements. The source voltage and current were 40 kV and 511 $\mu$A respectively, no filter was used and the resolution was set to a pixel size of 35 $\mu$m. In total, 283 projections were created at rotation steps of 0.7°, which were then reconstructed using the software NRecon. Surface areas were determined using the analysis software CTan. A cut cube of $\text{Porites}$ was used to calibrate the relationship between x-ray attenuation and surface area. In CTan, the Hounsfield Unit value (corresponding to X-ray attenuation values), that most closely matched the known surface area value of the reference $\text{Porites}$ cube, was then used to determine subsequent surface area.
Figure 2.2. Surface area to dry weight regressions for *A. pulchra* and *P. damicornis*.

MicroCT was not employed to determine surface area for *G. aspera* and *A. pulchra* for the following reasons. It is preferable to determine the actual surface area in the field on each field trip, rather than back calculating from regressions. The foil technique was easily applied to the massive *G. aspera* to quickly determine the surface area while the samples were alive, without causing significant stress from exposure. However for the branching species, this was not an option as exposure time would have been too long, causing stress to the samples. Geometry was chosen instead of MicroCT for *A. pulchra* because many of the samples, after two years of growth, were far too large to be scanned by the Skyscan MicroCT and a comparison of coral surface area measurement techniques by Naumann et al. (2009), reported that simple geometry produced relatively similar results to MicroCT for corals with staghorn morphology.

Using the surface area and calculated skeletal mass in air, the calcification rate in mg cm$^{-2}$ d$^{-1}$ was calculated according to:

$$\dot{g}_{\text{net}} = \frac{2(M_f - M_i)}{\tau(S_f + S_i)}$$

(2.2)

Where $M_f$ is the final mass, $M_i$ is the initial mass, $\tau$ is number of days between weighing dates, $S_f$ is the final surface area and $S_i$ is the initial surface area.
2.3.3 Survival

Survival was determined by revisiting sites and counting mortality on weighing trips.

Differences in the number of days between weighing trips (Table 2.2) were accounted for by measuring survival at adjusted 6-month time intervals, according to the following equation (Ebert 1999):

\[ \text{Surv}_{t_2} = (\text{Surv}_{t_1})^{t_2/t_1} \]  

(2.3)

Where \( \text{Surv}_{t_2} \) is the survival corrected to a 6-month time interval, \( \text{Surv}_{t_1} \) is the observed survival, \( t_2 \) is the number of days in 6 months (182.5 d) and \( t_1 \) is the number of days between trips.

Standard errors were applied using the profile likelihood method (Ebert 1999).

2.3.4 Environmental Parameters

Environmental parameters were measured at all sites throughout the study to determine the extent of variation among regions and their influence on rates of coral calcification. Water temperature was logged hourly (Onset HOBO U22 Water Temp Pro v2) for the duration of the study. Both high-resolution temperature logger data and coarse resolution 50 km satellite SST data (NOAA, Coral Reef Watch, 2013) were analysed. The 50 km SST data (which extends as far back as 2001) were included to give an indication of longer-term averages, as long-term fine resolution data were unavailable for these locations. Monthly SST anomalies from 2011 through to 2013 were calculated based on the 2001-2010 monthly averages (50 km SST data). There were slight differences between the logger and satellite datasets for each location. These disparities are mostly due to logger deployment at a 4 m depth compared to satellite surface measurements, as well as the 50 km pixels encompassing more of the warm Leeuwin Current offshore. However, the seasonal signals detected in the 50 km SST anomalies were reflected in the high-resolution logger data. Total alkalinity samples as well as pH and salinity measurements were taken during each field trip to determine whether seawater chemistry varied
between locations. Seawater pH was measured daily during field trips using a Schott handylab pH 12 pH meter. Total alkalinity (TA) samples were also taken daily at the study sites and were measured using linear array spectrometers (Yao and Byrne 1998) and a Metrohm 865 Dosimat Plus titrator. Salinity was determined using a refractometer. These parameters were then entered into the program CO₂SYS (Lewis et al. 1998) to calculate partial pressure of carbon dioxide ($p\text{CO}_2$) and aragonite saturation state ($\Omega_{ar}$).

### 2.3.5 Data Analysis

The variance in calcification rate by latitude, season, species and temperature anomaly was investigated using hierarchical partitioning, a modified version of generalized linear modelling (Chevan and Sutherland 1991; Mac Nally 2000). Hierarchical partitioning was used to calculate goodness of fit measures for all combinations of model independent variables, including latitude, season, species and temperature anomaly. Using the hierarchical partitioning algorithm (Chevan and Sutherland 1991), the independent contribution of each variable over all model combinations was calculated.

Generalized Linear Mixed Models (GLMM; Burnham and Anderson 2002) were then used to determine the effects of latitude, season, and temperature anomaly on coral calcification rates within a species. Model fits for all possible combinations of these variables were compared using Akaike Information Criterion corrected (AICc). Selection of the best models was based on being within 2 AICc of the minimum AICc. To determine the importance of each variable on coral calcification, the weighted AICc values were summed across all possible models. The statistical software R was used for model fitting.

To test for significant differences between latitude, season and species a three-way ANOVA with Tukey’s Honest Significant Difference (HSD) post-hoc comparisons was employed. To look at differences within a location, season or species, one-way ANOVA’s were used. Tukey’s HSD post-hoc tests were conducted on the one-way ANOVA’s if there were more than two variables. All analyses were conducted using the statistical package SPSS version 22.
2.4 Results

2.4.1 Environmental Parameters

There were no major seasonal or regional variations in seawater carbonate chemistry (Table 2.3). Seawater chemistry varied little between seasons and locations for the duration of the study, with mean daytime pH ranging between 8.09-8.18, mostly within 2 standard deviations of each other. The only exception was a mean daytime pH of 7.94 at the Abrolhos during the late summer of 2012. A similar lack of variation was observed in \( pCO_2 \) and \( \Omega_{ar} \). However, because pH measurements and total alkalinity samples were only taken during the day, there could be a diurnal bias in the carbonate chemistry data. Similarly, there was little variation in salinity with season and latitude (Table 2.3).

Table 2.3. Seasonal average (mean ± SD) for temperature (as measured by hand held pH meter on field trips) and seawater chemistry parameters at Coral Bay, the Abrolhos Islands and Marmion Reef.

<table>
<thead>
<tr>
<th>Location and Season</th>
<th>n</th>
<th>Temperature (°C)</th>
<th>Salinity</th>
<th>TA (µmol kg(^{-1}))</th>
<th>pH(_4)</th>
<th>( pCO_2 ) (µatm)</th>
<th>( \Omega_{ar} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coral Bay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter 2011</td>
<td>9</td>
<td>22.7 ± 0.7</td>
<td>35.2</td>
<td>2269 ± 22</td>
<td>8.14 ± 0.03</td>
<td>302 ± 24</td>
<td>3.66 ± 0.17</td>
</tr>
<tr>
<td>Summer 2012</td>
<td>5</td>
<td>26.2 ± 1.1</td>
<td>35.2</td>
<td>2275 ± 29</td>
<td>8.12 ± 0.03</td>
<td>314 ± 26</td>
<td>3.98 ± 0.30</td>
</tr>
<tr>
<td>Summer 2013</td>
<td>8</td>
<td>23.5 ± 0.5</td>
<td>35.2</td>
<td>2265 ± 9</td>
<td>8.09 ± 0.03</td>
<td>344 ± 33</td>
<td>3.46 ± 0.19</td>
</tr>
<tr>
<td><strong>Abrolhos Islands</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter 2011</td>
<td>8</td>
<td>21.1 ± 1.5</td>
<td>35.4</td>
<td>2212 ± 46</td>
<td>8.12 ± 0.08</td>
<td>319 ± 74</td>
<td>3.30 ± 0.53</td>
</tr>
<tr>
<td>Summer 2012</td>
<td>15</td>
<td>24.9 ± 1.3</td>
<td>35.6</td>
<td>2234 ± 52</td>
<td>7.94 ± 0.06</td>
<td>523 ± 79</td>
<td>2.74 ± 0.32</td>
</tr>
<tr>
<td><strong>Marmion Reef</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter 2011</td>
<td>3</td>
<td>18.2 ± 2.5</td>
<td>35.5</td>
<td>2307 ± 12</td>
<td>8.17 ± 0.07</td>
<td>283 ± 55</td>
<td>3.47 ± 0.74</td>
</tr>
<tr>
<td>Summer 2012</td>
<td>2</td>
<td>22.8 ± 3.0</td>
<td>36.0</td>
<td>2328 ± 20</td>
<td>8.16 ± 0.15</td>
<td>303 ± 127</td>
<td>3.98 ± 1.38</td>
</tr>
<tr>
<td>Winter 2012</td>
<td>3</td>
<td>19.5 ± 0.8</td>
<td>35.5</td>
<td>2302 ± 16</td>
<td>8.18 ± 0.03</td>
<td>272 ± 25</td>
<td>3.63 ± 0.19</td>
</tr>
<tr>
<td>Summer 2013</td>
<td>3</td>
<td>23.5 ± 0.6</td>
<td>36.3</td>
<td>2331 ± 17</td>
<td>8.12 ± 0.03</td>
<td>323 ± 27</td>
<td>3.79 ± 0.25</td>
</tr>
</tbody>
</table>

TA: total alkalinity; \( pCO_2 \): partial pressure of carbon dioxide; \( \Omega_{ar} \): aragonite saturation state.
In situ temperature logger data showed predictable latitudinal differences with mean monthly water temperatures ranging from 22 to 28°C at Coral Bay, 20 to 25°C at the Abrolhos and 17 to 24°C at Marmion. However seasonally, the mean monthly water temperatures in situ were 1 to 3°C higher than during preceding years for two or more summer months during 2012 and 2013 at all study sites (Figure 2.3). Similarly, monthly SST anomalies showed a seasonal signal due to normal temperatures in winter and anomalously high temperatures in summer for the duration of the two-year study. Through 2011 to 2013, summer seawater temperatures were higher than their climatological monthly averages at all of the study sites (Figure 2.3). In the summer of 2011, monthly SST anomalies were between +2 and +4°C for one to four months for all sites. Although the growth experiments were only established after the 2011 heat wave event, temperature data for that period have been included due to its potential impact on the subsequent growth monitored during the study period. Temperatures were also anomalously high during the following summers of 2012 and 2013. Monthly average SST anomalies for 2012 and 2013 were +1 to +3°C for 2 or more months. The highest anomalies occurred in the early summer months of January and February at all locations.
**Figure 2.3.** Left axis: mean monthly SST anomalies at study sites (50 km resolution, NOAA, Coral Reef Watch, 2013), compared with long-term monthly averages over the past 10 years (2001-2010). Right axis: background *in situ* water temperatures prior to the temperature anomalies commencing in 2011, based on longer term averages from 2008 to 2010 for Coral Bay, 1990 to 1994 for Abrolhos (Pearce et al. 1999) and 2006 to 2010 for Marmion (dotted line). *In situ* water temperature through the period of temperature anomalies at the study sites (solid line).
2.4.2 Calcification and Survival

2.4.2.1 Latitude

Hierarchical partitioning showed that the overall variation in coral calcification rates were mostly attributed to temperature anomaly (32%) and latitude (29%), while season (20%) and species (18%) contributed less. For all species, the latitude accounted for a relatively large proportion of the variation in calcification rate, due largely to the reduced growth rate at the highest latitude reef. However the latitudinal effect could not be separated from that of temperature anomaly and together these two factors accounted for over half the variation in growth rate.

Overall, calcification rates followed predicted trends with regard to latitude, with Coral Bay having significantly higher calcification rates than the Abrolhos Islands ($p = 0.020$), which had significantly higher rates than Marmion ($p < 0.001$). Unexpectedly, growth rates at Coral Bay, the most tropical reef, were not consistently higher than the Abrolhos Islands, which are located in the overlapping tropical-temperate zone. For example, winter growth in 2011 at the Abrolhos was significantly higher than at Coral Bay for $A. pulchra$ ($p < 0.001$), but not significantly different for $P. damicornis$ and $G. aspera$ (Figure 2.4). In contrast, summer 2012 growth in Coral Bay was significantly higher than the Abrolhos for both $P. damicornis$ ($p = 0.018$) and $G. aspera$ ($p < 0.001$), however $A. pulchra$ had very similar growth rates at the Abrolhos ($p = 0.713$). The most temperate Marmion Reef however, showed the expected reductions in growth rates compared to both Coral Bay and the Abrolhos. In the winter of 2011, $P. damicornis$ at Marmion grew at approximately two thirds of the rates of those at the Abrolhos and Coral Bay (0.55 compared to 0.84 and 0.93 mg cm$^{-2}$ d$^{-1}$ respectively) and $G. aspera$ showed similar differences (0.73, 1.05 and 1.23 mg cm$^{-2}$ d$^{-1}$ for Marmion, Abrolhos and Coral Bay respectively), while the growth rates recorded at Coral Bay for $G. aspera$ for the following three seasons, were approximately five times the rates at Marmion (Figure 2.4). No latitudinal trends were observed with respect to survival (Figure 2.5).
Figure 2.4. Calcification rates (mean ± S.E, mg CaCO$_3$ cm$^{-2}$ d$^{-1}$) for corals at Coral Bay, Houtman Abrolhos Islands and Marmion Reef. *Experiment carried out at the Houtman Abrolhos Islands for first two seasons only.
Figure 2.5. Percentage survival (± S.E) corrected to 6-month time intervals at Coral Bay, Houtman Abrolhos Islands and Marmion Reef. *Experiment carried out at the Houtman Abrolhos Islands for first two seasons only.