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JAMES COOK
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The effects of ocean acidification and warming on the early life history stages of corals

Thesis submitted by

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For the degree of Doctor of Philosophy in Marine Biology

within the ARC Centre of Excellence for Coral Reef Studies and the
School of Marine and Tropical Biology

James Cook University

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Statement on the contribution of others

Some of the chapters of this thesis are also manuscripts that have been submitted for publication in peer-reviewed journals or are in preparation for submission. Several researchers have made contributions to these manuscripts and it is necessary to recognise their contribution.

Chapter 2 is a manuscript currently in review at 'Coral Reefs' and is co-authored by W. Leggat, A. Moya and A. H. Baird. Experiments for this manuscript were performed by CMC and AM. The statistical analyses were performed by CMC and AHB. AHB, AM and WL provided constructive comments in shaping the manuscript. All authors provided intellectual input into this manuscript.

Chapter 3 is a manuscript currently in review at PLOS one and is co-authored by W. Leggat, A. Moya and A. H. Baird. AHB and WL provided ideas and helped to design the study. Experiments for this manuscript were performed by CMC and AM. The statistical analyses were performed by CMC and AHB. AHB, AM and WL provided constructive comments in shaping the manuscript. All authors provided intellectual input into this manuscript.

Chapter 4 is a manuscript nearing submission and is co-authored by W. Leggat, A. Moya and A. H. Baird. Experiments for this manuscript were performed by CMC and AM. The statistical analyses were performed by CMC and AHB. AHB, AM and WL provided constructive comments in shaping the manuscript. All authors provided intellectual input into this manuscript.

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Abstract

The rapid increase in carbon dioxide (CO_2) in the atmosphere since the industrial revolution is heating the earth and decreased the ocean's pH level. Sea temperature is predicted to rise by between 2 to 4 °C and ocean pH is predicted to decrease 0.2-0.4 units by the end of this century. These changes, along with associated changes in the carbonate chemistry of seawater, are predicted to disrupt calcification and affect the physiology of many marine organisms. While the effects of ocean acidification (OA) on adult scleractinian corals have been extensively studied, until very recently, the effects of OA on the ecology of the early life history stages of corals remained largely unexplored. Similarly, while the effects of temperature on adults and early life history stages of corals are well known, the possible synergistic effects of temperature and OA have not been examined. It is important to assess the effect of OA on the early life stages of corals in order to predict the likely effects on population dynamics. In addition, such research will provide useful guidance for managing coral reefs. To address these critical knowledge gaps, I explored the effect of OA and temperature on fertilization, development, survivorship and metamorphosis using gametes and larvae of a number of abundant scleractinian corals from the Great Barrier Reef. In the first series of experiments, I used four treatment levels of $p\text{CO}_2$ corresponding to current levels of atmospheric CO_2 (approximately 380 ppm), and three projected values within this century (550, 750 and 1000 ppm), to test whether fertilization, embryonic development, larval survivorship or metamorphosis was affected by OA. None of these variables were consistently affected by $p\text{CO}_2$ suggesting that there will be no direct ecological effects of OA on the pre-settlement stages of reef corals, at least in the near future. In a second series of experiments, I tested the effect of OA in combination with elevated temperature on the

response variables mentioned above. We used four treatments: control, elevated temperature (+2°C), decreased pH (600-700 ppm) and a combination of elevated temperature and acidity. There were no consistent effects of OA on fertilization, development, survivorship or metamorphosis either alone, or acting synergistically with temperature. In contrast, temperature consistently increased rates of development, but otherwise had little effect. I conclude that temperature is more likely to affect the ecology of the early life history stages of corals in the near future, mostly by speeding up rates of development and therefore altering patterns of connectivity among reefs. In the third set of experiments, I compared the effects of ocean acidification on the larval metamorphosis of a spawning coral *Goniastrea retiformis* and a brooding coral *Leptastrea cf transversa* in Guam. Again, metamorphosis was not consistently affected by pCO_2 in either species. These results suggest that the mode of reproduction does not affect the larval response to pCO_2 and furthermore, there will be no direct effects of ocean acidification on settlement rates of reef corals, at least in the near future. In the final set of experiments, I tested the effect of ocean acidification on its own and in combination with elevated temperature on the growth of juveniles of *Acropora millepora* and *Acropora tenuis* using the treatment levels described above. Neither OA nor temperature had any significant effect on growth or the pattern of skeleton formation in these species. I concluded ocean acidification is not a threat to the early life history stages of corals in the foreseeable future.

Chapter 1: General Introduction

Coral reefs are one of the most important marine ecosystems as they support a high biodiversity of marine benthic and pelagic species (Sorokin 1993). In tropical coral reefs, scleractinian coral and calcareous algae share the role of building and cementing the massive carbonate framework that forms a habitat for coral reef organisms (Kleypas et al. 2006). A symbiotic relationship exists between the algae and coral host, allowing tight nutrient cycling which contributes to the high productivity on coral reefs despite many tropical reefs existing in a nutrient poor environment (Hatcher 1988). This high productivity supports high biodiversity, which benefits the human community by contributing to the economy in many tropical regions (Allen and Steene 1994). Each year, coral reefs provide nearly USD\$30 billion in net benefits of goods and services to world economies, including tourism, fisheries and coastal protection (Costanza et al. 1997; Bryant et al. 1998; Cesar et al. 2003). More than 100 countries are bordered by coral reefs and tens of millions of people depend on these reefs for part of their protein intake (Salvat 1992). One square kilometer of actively growing coral reef can provide the protein requirements of over 300 people if no other protein sources are available (Jennings and Polunin 1996). Despite the importance of coral reefs, they continue to deteriorate through a combination of direct and indirect human impacts, such as pollution and global climate change (Caldeira and Wickett 2003).

Global climate change is caused by the ongoing accumulation of CO_2 and other greenhouses gases such as methane, in the atmosphere (Bindoff et al. 2007; IPCC report, Fig. 1.1). Since the start of the industrial revolution (circa 1750) the atmospheric concentration of CO_2 has risen from a pre-industrial level of around 280 parts per million

(ppm) to today's concentration of approximately 393 ppm (Maona Loa Observatory, 2012, Table 1.1). This 37.5% rise in atmospheric CO_2 concentration is even more significant considering that almost 50% of total anthropogenic CO_2 emissions over the last 250 years have been absorbed by the world's oceans (Sabine et al. 2004). While this oceanic absorption has significantly buffered the rate of global warming, an often overlooked consequence is a decreasing pH level in the world's ocean, a process known as ocean acidification.

Ocean acidification (OA) has emerged as a serious threat to calcifying marine organism such as molluscs (Michaelidis et al. 2005), crustaceans (Kurihara et al. 2008), echinoderms (Kurihara and Shirayama 2004; Kurihara et al. 2004; Havenhand et al. 2008), corals (Gattuso et al. 1998; Kleypas et al. 2001; Langdon and Atkinson 2005; Jokiel et al. 2008), large calcareous algae (Kuffner et al. 2008) and some phytoplankton (Iglesias-Rodriguez et al. 2008). OA is also likely to affect the physiology of these organisms. In my thesis, I will focus on the effects of OA on the early life history of marine benthic organisms, in particular, scleractinian corals

Ocean acidification and ocean carbon chemistry

Global climate change driven by increased concentration of CO_2 in the atmosphere has created conditions that have already contributed to reef degradation, and will continue to do so in the future. These conditions include: rising sea surface temperatures (Carricart-Ganivet 2004; Crabbe 2007), increased CO_2 concentration in seawater (Feely et al. 2004; Fabry et al. 2008), sea level rise (Bindoff et al. 2007), increase in severity and frequency of tropical storms (Henderson-Sellers et al. 1998; Emanuel 2005) and increased frequency of

disease outbreaks (Jones et al. 2004; Boyett et al. 2007). Many authors predict that of these the most significant threat to coral reefs is increasing ocean acidity (Hoegh-Guldberg 2005; Hoegh-Guldberg et al. 2007). Oceans are estimated to have taken up approximately 50% of anthropogenic atmospheric CO_2 as the CO_2 cannot be dissipated by the ocean biological pump, where carbon is transferred from the atmosphere to deep ocean water and sediment thus stored (Sabine et al. 2004). The rate of CO_2 uptake by the ocean is slowing as the CO_2 stored in the deep ocean is saturating (Le Quere et al. 2007) causing the recently emitted CO_2 to be suspended in the pelagic ocean layer. The dissolved CO_2 in the surface layer will acidify the pelagic zone (Caldeira and Wickett 2003), creating an imbalance in carbon chemistry that is predicted to adversely affect the calcification process of marine benthic organisms by decreasing carbonate ion concentrations (Pörtner et al. 2005). Anthropogenic CO_2 can be buffered or neutralized by the dissolution of $CaCO_3$ from benthic sediments but this process requires thousands of years (Raven et al. 2005).

Carbon dioxide is continuously exchanged between the atmosphere and the ocean. When CO_2 is dissolved in the surface layers of the ocean it reacts with water to form carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions (Skirrow and Whitfield 1975). The more CO_2 that is absorbed in the ocean the more carbonic acid is produced, thereby decreasing the ocean's pH. In addition, these reactions reduce the availability of carbonate ions, which are required for calcification (refer to equation below). Such changes in ocean chemistry are predicted to have substantial direct and indirect effects on marine organisms especially those that calcify because they rely on the availability of carbonate ions for growth and development (Goreau and Bowen 1955; Langdon 2000; Cooper et al. 2008; De'ath et al. 2009; Pörtner 2009).

Calcification of Marine Organisms

Changes in ocean carbon chemistry are predicted to cause a decrease in calcification because carbonate ions (CO_3^{2-}) are a major component of the calcium carbonate ($CaCO_3$) skeletons of coral and other reef-building organisms (Kleypas and Hoegh-Guldberg 2006). Coral calcification occurs when the carbonate ions combine with calcium ions (Ca^{2+}) and when CO_2 admission to seawater exceeds the buffer capacity of the ocean. Carbonate ions become less available, thus preventing the production of the coral skeleton. When CO_2 combines with water to produce carbonic acid (H_2CO_3), protons (H^+) are released and combine with carbonate ions (CO_3^{2-}) to produce bicarbonate ions (HCO_3^-). Hydrogen ions (H^+) take up CO_3^{2-} to produce H_2CO_3, thus making CO_3^{2-} ions less available for marine organisms' calcification. The chemical reaction describing the entire process is given as:

$$CaCO_3 \rightarrow Ca^{2+} + CO_3^{2-} \quad \text{(calcification)}$$
$$\nearrow$$
$$2\,H^+ + CO_3^{2-} \leftrightarrow 2HCO_3^-$$

$$CO_2\,(g) \leftrightarrow CO_2\,(aq) + H_2O \leftrightarrow H_2CO^3 \leftrightarrow HCO_3^- + H^+$$
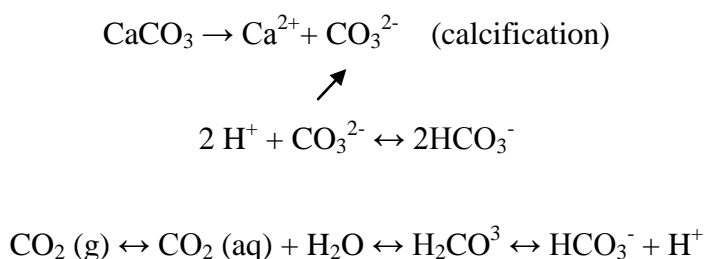
The calcification process involves the active transportation of ions from the surrounding seawater and through multiple coral cell layers to the sites of calcification at the calicoblastic layer (Allemand et al. 2004). Calcium ions (Ca^{2+}) are actively transported through cell layers from seawater in the ceolenteron to the calicoblastic tissue (Zocolla et al 1999; 2004). Metabolic CO_2 from respiration is utilized for bicarbonate production (Allemand et al. 2004). Carbonic anhydrase forms a family of enzymes that catalyse the rapid conversion of CO_2 to HCO_3^-; a reaction that occurs rather slowly in the absence of a catalyst. The enzyme speeds up the following equilibrium:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$
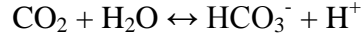
The bicarbonate ions are absorbed on a mucopolysaccharide in an organic membrane, where they combine with calcium to form the skeleton (Allemand et al. 2004). Although this process is not well understood, it is thought that the carbonate production will be reduced by excessive amounts of CO_2, thereby lowering the rate of calcification (Pörtner et al. 2005).

Aragonite saturation and calcification

While the dissolution of calcium carbonate is primarily driven by the availability of carbonate ions, it is also affected by other factors, such as the chemical structure of calcium carbonate. Calcium carbonate is commonly found in two forms: calcite and aragonite. The aragonite saturation state of (Ω_{arag}) is a measure of the thermodynamic potential for aragonite to form or dissolve; specifically it is the product of the concentrations of the reacting ions that form the mineral (Ca^{2+} and CO_3^{2-}), divided by the product of the concentrations of those ions when the mineral is at equilibrium (K_{sp}), that is, when the mineral is neither forming nor dissolving:

$$\Omega_{arag} = \frac{[Ca^+][CO^-]}{K_{sp}}$$

When $\Omega_{arag} > 1.0$, the formation of aragonite is favourable under ambient sea water temperature; when $\Omega < 1.0$, the dissolution of aragonite is favourable, it will cause decrease in the development of coral skeletons (Langdon and Atkinson 2005). Aragonite Ω of surface seawater is expected to decrease by 33% throughout the tropics from the present-

day values of 3-3.5 to 2-2.5 in 100 years; and the ratio of dissolved CO_2 gas to CO_3^{2-} will increase by a factor of 4 (Orr et al. 2005). Because coral reefs produce new $CaCO_3$ at a very slow rate it takes a long time to establish a mature coral colony (Goreau and Goreau 1959).

Mechanisms by which ocean acidification may affect the early life history of marine organisms

There are two main mechanisms by which ocean acidification may affect the physiology of the early life history stages of marine organisms; by affective calcification as described above, or via hypercapnia. Hypercapnia occurs when increased levels of CO_2 in water enter the organism by diffusion, equilibrates between all body compartments and acts predominantly through its acidifying effect on acid-base balance in body fluids. For example, *Sipunculus nudus* displays a range of reactions in acidified waters including metabolic depression (Pörtner and Reipschlager 1998), reduced rate of tissue acid-base regulation (Pörtner et al. 2005), and reduced rates of protein synthesis (Langenbuch and Pörtner 2002). These suppressions in metabolic activities can lead to cascading effects on an organism's life history, with potential effects on the early life history stages of marine organisms, such as a reduction in growth and survival of larval sea urchins (Shirayama and Thornton 2005 ; Kurihara 2008).

Responses of marine invertebrate larvae (MIL) to ocean acidification

Early life history stages of marine invertebrates are often more vulnerable to disturbances than adults (Yund et al. 1987; Kurihara and Shirayama 2004; Kurihara 2008). In most marine invertebrates, many important physiological processes (such as, fertilization, embryogenesis and dispersal, Fig. 1.2) occur in the planktonic stage. These early life

history stages are often perceived to be a bottleneck in the life of marine invertebrates with planktonic larvae because mortality during early life normally exceeds 90% (Gosselin and Qian 1997). Therefore, it is important to study the effects of climate change stressors on the early life history stages, as this will determine the success of dispersal and recruitment to new environments.

Ocean acidification reduces the fertilization success of some marine invertebrates. Exposure to CO_2 partial pressure from 1000ppm -10000ppm significantly decreases the swimming speed of sea urchin sperms (Havenhand et al. 2008) and fertilization success (Kurihara and Shirayama 2004; Kurihara et al. 2004). However, these detrimental effects are generally only evident at pCO_2 levels not projected to occur for many decades (Caldeira and Wickett 2003). In contrast, fertilization of sub-tidal echinoids (*Heliocidaris erythrogramma, H. tuberculata, Tripuneustes gratilla, Centrostephanus rodgersii*) is not affected by acidity up to 7.6 pH units (Byrne et al 2009; 2010), which is comparable to projected pH value 300 years in the future (Bindoff et al. 2007, IPCC report). Similarly variable effects have been reported for rates of development and survivorship in a range of marine invertebrate larvae (see review by Byrne 2011).

When I commenced my PhD in 2008, there was only one published study on the effects of OA on the early life history stages of corals (Albright et al. 2008). Research in this area has since blossomed. A thorough examination of the literature on coral larvae to date (Table 1.2) suggests that effects are only evident at very high pCO_2 levels (Nakamura et al. 2011), or when gametes are past their peak (Albright et al. 2010). Nonetheless, there is critical need to explore the effects in a greater range of species from more regions of the globe.

Temperature elevation and corals

Short term rises in sea temperature may actually enhance growth and reproduction of coral species (Purcell 2005; Purcell et al. 2007), however long term exposure to elevated temperature can lead to detrimental effects on marine organisms. These effects include reduced growth (Goreau and Macfarlane 1990; Edmunds 2005; Crabbe 2007; Edmunds 2008) and fecundity (Michalek-Wagner and Willis 2001), high rates of mortality (Baird and Marshall 2002; McClanahan et al. 2004), and local extinction leading to the loss of biodiversity (Hoegh-Guldberg 2004; Kleypas and Hoegh-Guldberg 2006) Temperature also has detrimental effects on the early life history stages of corals (Table 1.3). For instance, a slight increase in temperature (1-2°C above ambient SST) enhanced the fertilization (>90%), but also resulted in higher numbers of abnormal embryos (Negri et al. 2007). Similarly, fertilization success was high for *Diploria strigosa* in elevated temperatures (1-2°C) yet throughout embryogenesis, they developed into aberrant embryos (Bassim et al. 2002). Similarly, high temperatures reduce larval survivorship (Baird et al. 2006; Table 1.3) particularly in larvae that contain symbiotic algae (Yakovleva et al. 2009; Nesa et al. 2012).

Synergistic effects

In the wild, temperature and OA clearly act on organism at the same time; however, there have been very few experiments to explore the potential synergy between these stressors. The general assumption is that the effect of OA and temperature variables will be additive. Indeed, high pCO_2 acts synergistically with high temperature to lower thermal bleaching thresholds in corals and crustose coralline algae (Anthony et al. 2008). Similarly, growth rates of *Porites panamensis* juveniles were lowest in a combined OA and high temperature

treatment (Anlauf et al. 2011, see Table 1.4). However, the interaction could be antagonistic because while temperature increases metabolism (O'Connor et al. 2009), acidosis reduces it (Pörtner et al. 2004). For example, high temperatures can compensate for dwarfism (that would otherwise eventuate due to reduced calcification) in acidified sea water in *Tripneustustes gratilla* larvae (Sheppard Brennand et al. 2010). Clearly, there is a critical need to explore the effect of this synergy in the early life history of corals.

Thesis outline

In chapter two, I test the effect of OA on the early life history of corals using gametes and larvae of four abundant scleractinian corals from the Great Barrier Reef. I used four treatment levels of pCO$_2$ corresponding to current levels of atmospheric CO$_2$ and three projected values within this century to test whether fertilization, embryonic development and larval survivorship and metamorphosis were affected by OA. Due to the nature of high mortality in invertebrate larvae (Gosselin and Qian 1997), normally within the first 24 hours the larvae population will experience up to 50% of casualties in the field. In the third chapter I test the effect of OA in combination with elevated temperature on the response variable mentioned above. I used four treatments: control, elevated temperature (+2°C), elevated acidity (600-700 ppm) and a combination of elevated temperature and acidity. In chapter 4, the final data chapter, I tested the effects of ocean acidification on its own and in combination with elevated temperature on the growth and patterns of skeleton formation in juveniles of *Acropora millepora* and *Acropora tenuis* using the treatment levels described above. Finally, I conclude with a general discussion that summarise my work and discuss areas for future work.

In addition to my data chapter I present a paper in an Appendix. The first, I co-authored with my colleague from the School of Molecular Science, JCU, Dr Aurelie Moya (Appendix I). I was involved in designing this experiment, participated in the laboratory work and provided editorial input to the manuscripts. The paper is entitled "Whole transcriptome analysis of the coral *Acropora millepora* reveals complex responses to CO_2 driven acidification during the Initiation of calcification" and it investigates the gene expression in newly settled coral juveniles under near-future acidification levels. This publication is currently in press in Molecular Ecology (Moya et al. 2012).

Figure 1.1: Coloured lines represent five projected IPCC scenario of CO_2 emissions and concentration in the atmosphere within year 2100 (from Bindoff et al. 2007, IPCC report)
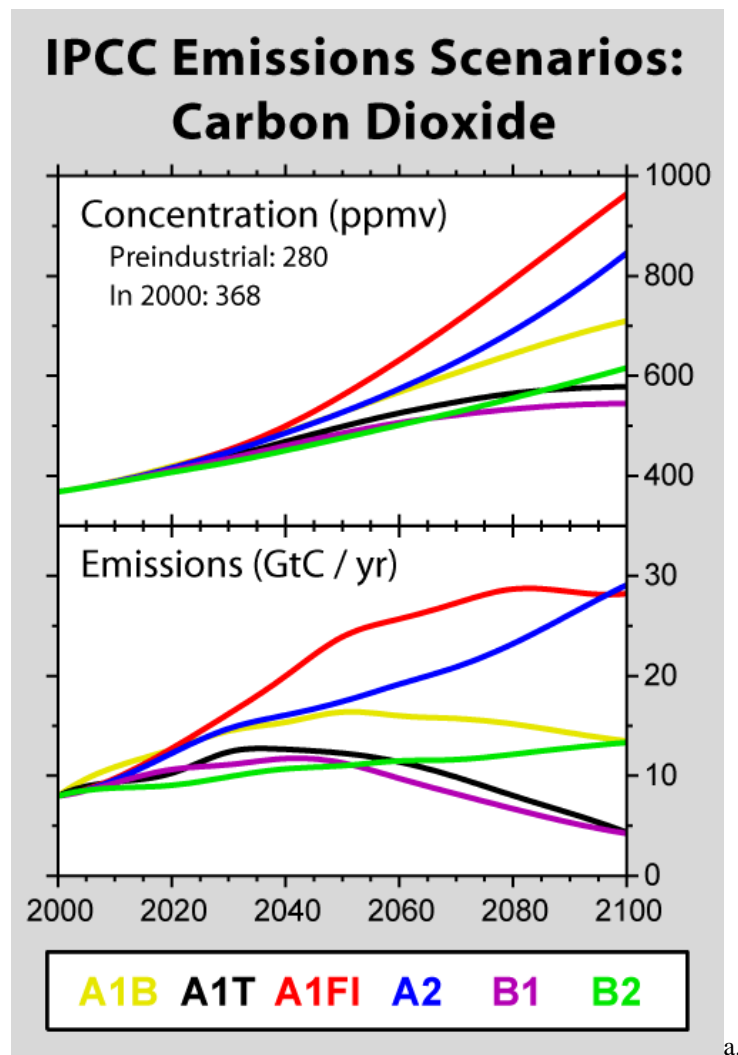
a,

Figure 1.2: Schematic diagram of the life cycle of acroporids, with emphasis on the early life history stages. (Ball 2000, unpublished diagram)
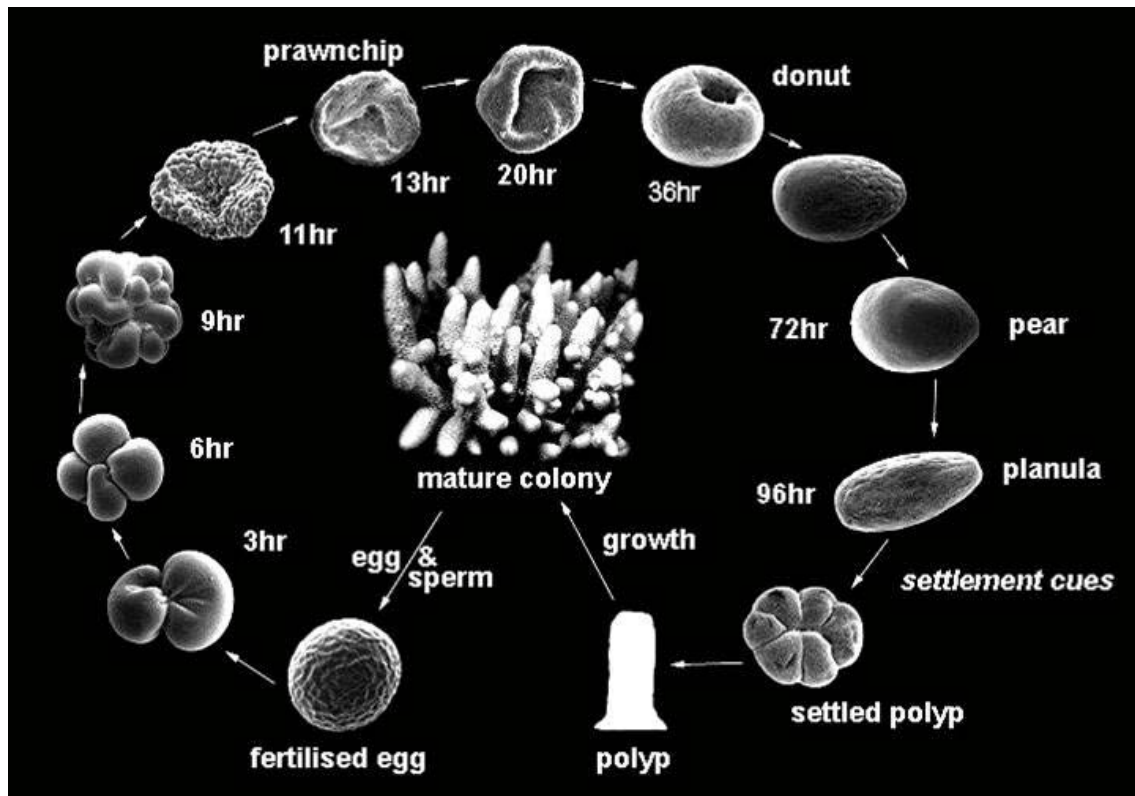
Table 1.1: Projected changes in ocean surface carbonate chemistry based on IPCC IS92a CO_2 emission scenario. pH is based on seawater scale. Percent changes from preindustrial values are in brackets. (Feely et al. 2004; Guinotte et al. 2006; Fabry et al. 2008)

Parameter	Unit	Glacial	Preindustrial	Present	2050-2100	2100 and above
Temperature (T)	°C	15.7	19	19.7	20.7	22.7
Salinity (S)		35.5	34.5	34.5	34.5	34.5
Total alkalinity (TA)	μ mol kg^{-1}	2356	2287	2287	2287	2287
pCO_2	μatm (aq)/ ppm (g)	180 (−56)	280 (0)	380 (35.7)	560 (100)	840 (200)
Carbonic acid (H_2CO_3)	μ mol kg^{-1}	7 (−29)	9 (0)	13 (44)	18 (100)	25 (178)
Bicarbonate ion (HCO_3^-)	μ mol kg^{-1}	1666 (−4)	1739 (0)	1827 (5)	1925 (11)	2004 (15)
Carbonate ion (CO_3^{2-})	μ mol kg^{-1}	279 (20)	222 (0)	186 (-16)	146 (−34)	115 (−48)
Hydrogen ion (H^+)	μ mol kg^{-1}	4.79×10^{-3} (−45)	6.92×10^{-3} (0)	8.92×10^{-3} (29)	1.23×10^{-2} (78)	1.74×10^{-2} (151)
Calcite saturation (Ω_{calc})		6.63 (20)	5.32 (0)	4.46 (−16)	3.52 (−34)	2.77 (−48)
Aragonite saturation (Ω_{arag})		4.26 (19)	3.44 (0)	2.9 (−16)	2.29 (−33)	1.81 (−47)
Dissolved inorganic carbon (DIC)	μ mol kg^{-1}	1952 (−1)	1970 (0)	2026 (2.8)	2090 (6.1)	2144 (8.8)
Total pH		8.32	8.16	8.05	7.91	7.76

Table 1.2: A summary of previous research on the effects of ocean acidification on the early life history stages of corals. Columns are categorized according to the current pCO_2, near future and past 2100 pCO_2 value. Plus signs (+) denote increase, minus signs (-) denote decrease, compared to control values.

		year	2050	2100	2100 +
		pCO_2 atmosphere	450-700	700-1000	1000 +
		pH ocean	7.9 - 8.0	7.7-7.9	<7.7
		aragonite saturation state	2.2 - 3.2	1.2-2.2	<1.5
Responses variable	**study**	**species**			
Fertilization					
optimal sperm conc.	Albright et al. 2010	*Acropora palmata*	- 7%	- 12%	
mid sperm conc.	Albright et al. 2010	*Acropora palmata*	*NS*	*NS*	
mid-low sperm conc.	Albright et al. 2010	*Acropora palmata*	- 64%	- 63%	
low sperm conc	Albright et al. 2010	*Acropora palmata*	*NS*	*NS*	
Sperm motility					
	Morita et al. 2010	*Acropora digitifera*		- 33%	- 71%
Survivorship					
	Nakamura et al. 2011	*Acropora digitifera*		*NS*	*NS*
	Suwa et al. 2010	*Acropora digitifera*		*NS*	
	Suwa et al. 2010	*Acropora tenuis*		+	
Settlement					
	Albright et al. 2008	*Porites astreoides*	*NS*	*NS*	
	Albright et al. 2010	*Acropora palmata*	- 45%	- 65%	
*non-acidified tiles	Albright & Langdon 2011	*Porites astreoides* (z)	*NS*	*NS*	
*acidified tiles	Albright & Langdon 2011	*Porites astreoides* (z)	- 45%	- 55%	
*acidified tiles	Albright & Langdon 2011	*Porites astreoides* (z)	- 42%	- 60%	
non-acidified tiles	Doropoulos et al. 2012	*Acropora millepora*		- 58%	- 75%
acidified tiles	Doropoulos et al. 2012	*Acropora millepora*		- 82%	- 45%
acidified tiles and larvae	Doropoulos et al. 2012	*Acropora millepora*		- 50%	- 60%

(for 60 days)					
2 days acidity exposure	Nakamura et al. 2011	*Acropora digitifera*		- 20 %	- 20 %
7 days acidity exposure	Nakamura et al. 2011	*Acropora digitifera*		- 80%	- 80%
Planulae Metabolism					
	Albright & Langdon 2011	*Porites astreoides* (z)	- 27%	- 63%	
	Nakamura et al. 2011	*Acropora digitifera*		*NS*	*NS*
Post-settlement Growth					
	Albright et al. 2008	*Porites astreoides*	- 45-56%	- 72-84%	
	Albright et al. 2010	*Acropora palmata*	- 39%	- 50%	
	Albright & Langdon 2011	*Porites astreoides* (z)	- 16%	- 35%	
lateral	Cohen et al. 2009	*Favia fragum* (z)		- 20%	- 75%
weight	de Putron et al. 2011	*Favia fragum*(z)	*NS*		- 37%
weight	de Putron et al. 2011	*Porites Astreoides*	*NS*		- 36%
	Inoue et al. 2011	*Acropora digitifera*		- 4%	- 25%
	Kurihara 2008	*Acropora tenuis*		- growth	
	Suwa et al. 2010	*Acropora digitifera*			- 16%
Symbiont uptake					
	Suwa et al. 2010	*Acropora digitifera*		*NS*	

*Note: The settlement substrates used in this study were: non-acidified tiles (limestone tiles conditioned with ambient seawater of 380ppm for 40 days) and acidified tiles (limestone tiles conditioned with treatment seawater of 560 or 860 ppm for 40 days). When results are insignificant, they are classified as 'no effect'. 'z' indicates coral species with vertically transferred zooxanthellae.

Table 1.3: A summary of previous research on the effects of ocean warming on the early life history stages of corals. Columns are categorized according to the current average sea temperature, near future and post-2100 sea temperature values. Plus signs (+) denote increase, minus signs (-) denote decrease, compared to control values.

Responses variable	study	species	year temperature (°C)	ambient	2012 ambient	2050 plus 2	2100 plus 4	2100 + plus 4 and above
Fertilization								
	Bassim et al. 2002	*Diploria strigosa*	30		41.3	+4.7%		
	Negri et al. 2007	*Acropora millepora*	28		>90%	NS	NS	- 87%
	Negri et al. 2007	*Acropora millepora*	26			- 30%	- 30%	- 50%
	Negri et al. 2007	*Favites abdita*	26			NS	NS	
	Negri et al. 2007	*Favites chinensis*	25.5		>80%	NS	NS	NS
	Negri et al. 2007	*Mycedium elephantotus.*	26			NS	NS	
Development								
Abnormality	Bassim et al. 2002	*Diploria strigosa*	30		22%	+18.5%		
Cell division	Negri et al. 2007	*Acropora millepora*	26			+ 10-20%	+ 10-20%	
Abnormality	Negri et al. 2007	*Acropora millepora*	26			+ 10%	+ 10%	10-50%
Cell division	Negri et al. 2007	*Favites abdita*	26			+ 10-20%	+ 10-20%	
Abnormality	Negri et al. 2007	*Favites abdita*	26			+ 10%	+ 10%	
Cell division	Negri et al. 2007	*Mycedium elephantotus.*	26			+ 10-20%	+ 10-20%	
Abnormality	Negri et al. 2007	*Mycedium elephantotus.*	26			+ 10%	+ 10%	
	Randall & Szmant 2009b	*Acropora palmata*	28			NS	+ at 31.5°C	
Survivorship								
	Baird et al. 2006	**Acropora muricata (azoox)*	28		30%		NS	
	Baird et al. 2006	**Acropora muricata (zoox)*	28		30%		NS	

	Reference	Species					
	Bassim & Sammarco 2003	*Diploria strigosa*	28	56%	- 29%	- 41%	
	Coles 1985	*Pocillopora damicornis*	25	4.3	- 28%	- 47%	- 28%
	Edmunds et al. 2001	*Porites astreoides (z)*	28	89%			- 41% at 33°C
	Randall & Szmant 2009b	*Acropora palmata*	28	34-64%	- 15%	-55% at 31.5°C	
	Randall & Szmant 2009a	*Favia fragum*	27	> 60%	*NS*	- 40%	- 50%
Post-settlement survivorship							
	Nozowa & Harrison 2007	*Acropora soliaryensis*	23	>70%	*NS* at 26°C		- 90%
	Nozowa & Harrison 2007	*Favite chinensis*	27	<20%		*NS*	- 10%
Metamorphosis							
	Bassim & Sammarco 2003	*Diploria strigosa*	28	70%	- 5%	- 64%	
	Coles 1985	*Pocillopora damicornis*	25	2.8	+ 60%	+ 30%	+ 96%
	Nozowa & Harrison 2000	*Platygyra daedalea*	27	24.6%	+27.2%		
	Nozowa & Harrison 2007	*Acropora soliaryensis*	23	2.6%	+ 2.4% at 26°C		12%
	Nozowa & Harrison 2007	*Favite chinensis*	27	25%		+ 5%	+ 15%
	Randall & Szmant 2009b	*Acropora palmata*	28	62%	- 19%	- 25% at 31.5°C	
	Randall & Szmant 2009a	*Favia fragum*	27	82%	+ 3%		- 48%

Note: *A. muricata* is a coral species with azooxanthellae larvae, zooxanthellae was introduced to *A.muricata* larvae in Baird et al (2006)

Table 1.4: A summary of results from the only publication (for the period 2008-2011) that studied the synergy effects of ocean acidification and warming on the early life history stages of a brooding coral species, Porites panamensis (Anlauf et al. 2011). Columns are categorized according to the ocean acidification and warming regimes used in this study. Plus signs (+) denote increase, minus signs (-) denote decrease, compared to control values.

Responses variable	Acidity (pH) decreased 0.2-0.25 units	Warming (°C) increased 1-1.2 °C	acidity and warming
Post –settlement survival		- 10%	- 4%
Biomass	- 20%	- 22%	- 40%
Calcification (dry weight)	- 2%	+ 9%	- 28%
Symbiont uptake	- 5%	- 50%	- 10%

Chapter 2: Near-future reductions in pH have no consistent ecological effects in the early life history stages of reef corals

This chapter was in review in PLOS One May 2012.

Introduction

Global climate change has the potential to detrimentally affect coral reefs in many ways. In particular, increasing ocean acidity is predicted to be one of the most severe threats to marine ecosystems before the end of this century (Kleypas et al. 1999; Orr et al. 2005). Ocean acidification (OA) is caused by alteration of water chemistry through CO_2 absorption from the atmosphere, in particular, a decrease in seawater pH, carbonate ion concentration (CO_3^{2-}) and the saturation state of aragonite. Recent changes in ocean chemistry, for example a decrease in pH of 0.1 units since before the industrial revolution (Caldeira and Wickett 2005), have already had pronounced effects on many calcifying marine organisms (Kroeker et al. 2012). Decreases in aragonite saturation projected to occur within this century are predicted to result in the net dissolution of calcium carbonate on coral reefs (Silverman et al. 2009).

To date, much of the research into OA has focused on its effects on adults and, in particular, the process of calcification. However, the early life history stages of marine organisms are also likely to be sensitive to changes in ocean chemistry (Portner et al. 2004; Byrne 2010; Kroeker et al. 2010). Hypercapnia, defined as an increase in the partial pressure of CO_2 in the respiratory fluids, is well known to affect acid-base regulation, oxygen transport and metabolic function (Portner et al. 2004). Similarly, high CO_2 concentration in sea water has a narcotic effect on sperm, reducing motility

(Havenhand et al. 2008, Morita et al. 2010). While hypercapnia and CO_2 narcosis may not necessarily be detrimental, responding to changes in the partial pressure of CO_2 will be energetically costly and therefore has the potential to affect growth and reproductive output following prolonged exposure (Portner et al. 2004) in the absence of acclimitization. Lowered metabolism is possibly responsible for reduced rates of early development in larval of the sea urchin *Hemicentrotus pulcherrimus* at low pH (Kurihara and Shirayama 2004). Reduced rates of larval development are likely to extend pelagic durations with possible increases in rates of mortality and reduced recruitment success (Connolly and Baird 2010). Such changes are also likely to reduce levels of connectivity among affected organisms and therefore have the potential to affect rates of recovery from disturbance (Munday et al. 2009).

The effects of decreased pH on the early life history of corals remain largely unexplored and results are variable (Table 1). For example, fertilization success in *Acropora palmata* was reduced at low sperm densities at pH of 7.85 and 7.72 (Albright et al. 2010), possibly because of reduced sperm motility (Morita et al. 2010). However, these levels of pH had no effect on fertilization success (Albright et al. 2010) when sperm concentrations were closer to those expected in the field in the hour immediately following spawning when most fertilization is likely to occur (Oliver and Babcock 1992). In addition, gametes were mixed by three hours after spawning and fertilization was scored a hour after this Albright et al. (2010) a time at which *Acropora* gametes have dramatically reduced fertility (Oliver and Babcock 1992). Similarly, larval survivorship differed among pH treatments in *A. tenuis*, but not in *A. digitifera* (Suwa et al. 2010). However, the difference in surviorship among treatments was not that expected if pH had

a dose dependent or threshold effect. Indeed, there was no difference between either pH treatment and the control in *A. tenuis*, rather the difference was between the 7.6 and 7.3 pH treatments with survivorship significantly higher in the low pH treatment (Suwa et al. 2010). The respiration of *A. digitifera* larvae was not affected by pH as low as 7.3 (Nakamura et al. 2011). In contrast, the respiration rates of *Porites asteroides* larvae declined as pH decreased (Albright and Langdon 2011). The effects of decreased pH on larval metamorphosis are also variable (Table 1). Albright et al. (2008) found no effect of pH on the metamorphosis of *Porites asteroides*. Similarly, metamorphosis of *A. digitifera* was unaffected by a 2 day exposure to pH values of 7.6 and 7.3 (Nakamura et al. 2011). However, after 7 days of exposure metamorphosis was 80% lower in the reduced pH treatments (Nakamura et al. 2011). The effects of pH on larval metamorphosis may be indirect and mediated through a change in the settlement substratum (Albright et al. 2010). Metamorphosis is often reduced when settlement substratum have been conditioned in treated seawater suggesting that low pH affects the organisms, such as crustose coralline algal, that induced larval metamorphosis, or interferes with the coral larvae's ability to sense these cues (Albright et al. 2010, Albright and Langdon 2011). However, even in this situation, the results are highly variable and do not always follow the response expected if pH was to have either a dose dependent or threshold effect. For example, metamorphosis of *A. millepora* larvae was reduced by 82% on settlement substrata that had been treated at pH 7.3 for six weeks, but there was no effect of metamorphosis on substrata treated at pH of 7.6 (Doropoulos et al. 2012). Similarly, while metamorphosis was lower when either the larvae or the substrate were treated at pH 7.6, there was no effect when both larvae and substrata were treated at this pH. Clearly,

there is an urgent need to examine the response to pH in the larvae of a greater range of coral species to see if any general patterns emerge.

Here, we manipulated pH levels to produce seawater with a range of potential pH values projected to occur at various stages later this century. The effects of these levels of pH were then tested on a number of processes crucial to successful coral recruitment: 1) larval development, 2) larval survivorship and 3) metamorphosis.

Materials and method

Collection of gravid colonies

Gametes of *Acropora tenuis* and *A. hyacinthus* were collected at Magnetic Island (19° 9'S, 146° 50'E) in October 2009 and gametes of *A. millepora* collected at Orpheus Island (18° 35'S, 146° 29'E) in November and December 2009. Adult colonies were collected a few days prior to the predicted spawning period, and maintained in outdoor aquaria. *A. tenuis* and *A. hyacinthus* spawned on the night of 9th October and 13th October respectively. *A. millepora* colonies spawned on 10th November, and the 10th December and 11th December.

Experimental manipulation of pH.

The experiments were performed in a temperature control room (26°C-27°C). A CO_2 mixing system, developed by Munday et al. (2009), was used to bubble CO_2 through seawater at concentrations chosen to match the projections of the IPCC (2007) over the next 80 years: 550, 750 and 1000 ppm. CO_2 concentrations were measured with a

Vaisala ® probe. CO_2 was bubbled through 70 l sump tanks and the enriched water flowed through replicates jars via a one-way flow-through system. pH was measured every 24 h in the sump tank of each treatment (Table 2.1, 2.2 & 2.3) using TPS WP91 meter calibrated daily using Tris and NBS buffers. Temperature and dissolved O_2 were also measured using TPS WP-91 DO, pH and temperature meter.

The effect of acidification on development

Embryos were cultured following Babcock et al. (2003). Sperm and egg bundles were collected from between four and 6 colonies of each species, each colony was presumed to be genetically distinct. Once cleavage was observed, approximately 1000 embryos were placed in each of three replicate 3.5 l plastic jars, modified to allow 0.2 μm filtered CO_2 treated sea water to flow through at the rate of 1.5 to 2 l per hour. At 12, 18, 24 and 36h after the gametes were mixed 20 embryos were removed from each jar for *A. tenuis* and *A. hyacinthus* and 10 embryos for *A. millepora* and the number that had completed gastrulation was scored. To test for differences in development time among treatments, the average time for embryos to reach gastrulation was estimated as follows

Average time to reach stage, $\overline{X}$ = Σ [time (hours) x number of propagules to reach stage]/

[number of propagules to reach stage]

The effect of pH on metamorphosis

Metamorphosis of *Acropora* larvae typically peaks between 6 and 10 days after spawning (Connolly and Baird 2010). Therefore, the age of larvae used to test for effects of pH on larval metamorphosis were: *A. hyacinthus* - 8 days old; *A. millepora Nov* - 6 days old; *A. millepora Dec* - 9 days old. Ten larvae of *A. hyacinthus* and 20 larvae of *A. millepora Nov and A. millepora Dec* were taken from each of the 3.5 l jars and placed into each well of a 6-well Iwaki cell culture plate with a modified meshed lid. A 2 mm x 2 mm crustose coralline algae (CCA) chip (*Neogolithion* sp. for the *A. hyacinthus* assay and *Titanoderma* sp. for *A. millepora*) was also placed in each well to induce metamorphosis (following Heyward and Negri 1999) and the wells, covered by plankton mesh to retain the larvae, were completely immersed in the CO_2 treated seawater and the number of larvae that completed metamorphosis was assessed 24 h later. Larvae were defined as metamorphosed once a basal disc had been deposited (Baird and Babcock 2000).

The effect of acidification on larval survivorship

To test whether reduced pH would have a direct effect on larval survivorship, 50 motile larvae (4 days old) were introduced into three replicate 200 ml plastic jars with mesh lids which were then immersed in the CO_2 enriched seawater. The number of surviving larvae was counted every 24 h at which point the seawater in the jars was competently replaced. This period is sufficient for dead larvae to lyse and disappear (Baird et al. 2006). Larvae were followed for between five and 7 days depending on the experiment.

Data analysis

Mean differences in the time to gastrulation were tested with 1-way ANOVA: the treatment factor was pH with four levels that differed slightly among assays (Tables 2.1, 2.2 and 2.3). Mean differences in the number of larva completing metamorphosis were tested with 2-way ANOVA: the first factor was pH (fixed) with four levels; the second factor, plate (random, n=3) was nested within treatment. There were 6 replicate wells in each plate. Each species on each occasion was analyzed independently (i.e. three to five separate assays depending on the response variable) because the pH values were slightly different in each assay (Tables 2.1, 2.2 and 2.3). Bonferroni correction was used to adjust the probability of type I error (i.e. probability was considered significant when $p<$ 0.05/number of assays). Tukey's HSD multiple comparison tests were conducted when ANOVAs detected significant differences among the main factors. Any bias in these data was explored by residual analysis. Only the *A. millepora Nov* data for metamorphosis required a $\log_{10} (x + 1)$ transformation. Differences in the median survival time in days among treatments were tested using Kaplan-Meier survival analysis. All analyses were performed in SPSS version 20.

Results

The effect of pH on larval development

The mean time to gastrulation did not vary consistently among as a function of pH (Fig. 2.1). *A. tenuis* larvae developed more slowly at the lowest pH (Fig. 2.1A; $F_{3, 225} =$ 4.27; p = 0.006). In contrast, *A. hyacinthus* larvae developed more slowly in the control and low pH (Fig. 2.1B; $F_{3, 225} = 4.31$; p = 0.006). In the *A. millepora Nov* assay there was

no significant difference in development times among the pH treatments (Fig. 2.1C; $F_{3, 116}$ = 2.5; p = 0.06). Finally, in *A. millepora Dec,* development was slower at the lower and lowest pH (Fig 2.1D; $F_{3, 116}$ = 3.13; p = 0.029).

The effect of pH on larval survivorship

Median survivorship did not vary among pH treatments in either of the two species tested (Fig 2.2). In *A. tenuis*, median survivorship was highest in the lowest pH treatment; however, the 95% confidence intervals indicate that this was not significantly different to the control pH (Fig 2.2A). In *A. millepora Nov* median survivorship was lower in the two lowest pH treatments; however, there was no significant difference between these values and the control (Fig 2.2B).

The effect of pH on metamorphosis

The mean number of larvae completing metamorphosis was only affected by pH in the *A. millepora Dec* assay (Fig. 2.3). The number of larval completing metamorphosis did not differ among the pH treatments in *A. hyacinthus* (Fig. 2.3A $F_{3, 2}$ = 1.54; p < 0.211) or *A. millepora Nov* (Fig. 2.3B; $F_{3, 2}$ = 0.71; p = 0.551). In contrast, the number of *A. millepora Dec* that completed metamorphosed was higher in the lower and lowest pH treatments (Fig. 2.3C; $F_{3, 2}$ = 7.77; p < 0.001).

Discussion

Simulated levels of pH projected to occur at various stages later this century did not have a consistent effect on the pre-settlement stages of these three *Acropora* species. In most

assays, no significant differences were apparent between the three pH treatments and controls. In the few assays where an effect was detected, this was often opposite to the expected trend. For example, the metamorphosis of *A. millepora* was higher at the two lowest pH levels (Fig. 2.2C) and rates of development were slower in *A. hyacinthus* at the two highest levels of pH. This suggests that projected levels of pH will not threaten the early life history stages of these corals species until at the at least the end of the century.

Development rates of coral embryos were not consistently affected by pH. In two assays the prediction of a slower rate of development at reduced pH did occur (Fig 2.1A, C), however, effect was small, with a increase in the mean time to complete gastrulation of approximately of between 2 to 4 h (approximately 15%). This difference is much lower than the typical differences among individuals within a cohort in the time taken to reach other important dispersal related stages, such as the time to become competent (Connolly and Baird 2010). These results contrast with the effects of pH on development rates in other organisms. For example, *Littoria obtusata* embryos in a pH 7.6 treatment took 1.5 days longer to hatch when compared to controls (Ellis et al. 2009). Similarly, larval development in sea urchins (Kurihara and Shirayama 2004) and oysters (Kurihara et al. 2007) is slower at low pH. However, both these species have a larval test and it is perhaps the absence of calcareous skeleton that makes coral larvae less likely to be affected by low pH.

Rates of larval survivorship were not affected by reduced pH. Similarly, larval survivorship did not differ between controls and reduced pH treatments for *A. digitifera* or *A. tenuis* larvae (Suwa et al. 2010). In contrast, in sea urchins (Kurihara 2008) and brittle stars (Dupont et al. 2010) survival was lower at reduced pH. However, once again,

both these species have a larval test which may predispose them to being affected by low pH.

Metamorphosis was not consistently affected by pH. Indeed, metamorphosis in *A. millepora_Dec* was higher under the two lowest pH treatments (Fig. 2.3C). Results from the literature are similarly inconsistent. Albright et al. (2008) found no effect of pH on metamorphosis in *Porites asteroides* larvae. Similarly, rates of metamorphosis in *Porites panamensis* were not affected by a 0.2-0.25 unit decrease in pH (Anlauf et al. 2011). In contrast, Nakamura et al. (2011) did find an effect but only in *A. digitifera* larvae exposed for 7 days to pH levels not expected until well into the next century. While Albright et al. (2010) did record reduced metamorphosis under reduced pH treatments, they suggested the effect was indirect, i.e. through changes in the nature of the settlement substratum rather than a direct effect on the larvae; an hypothesis supported to some extent by more recent work (Albright and Langdon 2011; Doropoulos et al. 2012). Nonetheless, no mechanism has been proposed to explain this phenomenon. Furthermore, the effects presented by Doropoulos et al. (2012) are not consistent with either a dose dependent or threshold effect in all their experiments, as discussed above.

The gametes and larvae of many marine invertebrates are exposed to large daily fluctuations in environmental variables, including pH. For example, the pH in many shallow coastal areas, including coral reefs, varies markedly over very short periods. For example, in sub-surface waters directly above the reef crest, pH varied by 0.2 units in 4 h (Gagliano et al. 2010). Similarly, diurnal oscillations in pH above coral colonies varied by 0.5 units (Odhe and van Woesik 1999). Indeed, naturally oscillating pH actually stimulates the growth of coral recruits (Dufault et al. 2012). The gametes of many species

are equipped with constitutive defenses against low pH in order to deal with these environmental fluctuations (Hamdoun and Epel 2007; Byrne et al. 2009 & 2010). Given these fluctuations in pH in the reef environment it is perhaps no surprise that effects of pH are generally inconsistent and often only evident at very low levels (Table 1.2).

In conclusion, projected ocean acidification levels in the near future appear unlikely to have major direct ecological effects on the non-calcifying early life history stages of corals. Our results, and a careful reading of the literature on the effects of reduced pH on coral larvae (Table 1.2), suggests that direct effects are only evident at very low pH levels (Nakamura et al. 2011), or when gametes have lost vitality (Albright et al. 2010). One possible source of the inconsistency in our results is the small number of adults, typically between four and six individuals, used to produce the larvae for experiments. Sampling effects from the use of low numbers of brood stock may result in high variability in the genetic composition among different larval cultures. Parental effects on coral larval traits, such as metamorphosis and metabolism, can be large (Meyer et al 2009). Consequently, future work should aim to incorporate parental effects into experimental designs. Alternatively, organism may be able to acclimatize relatively quickly to changes in pH, particularly as large diurnal fluctuations appear common in the environment.

The lack of major affects on larval ecology does not mean that ocean acidification is not a threat to other stages in a corals life history. There are strong theoretic and empirical reasons for expecting an effect on physiology (Portner et al. 2004) and patterns of gene expression vary dramatically as levels of pH decrease (Moya et al. 2012). The consequences of energy expenditure on cellular acid-base regulation and lowered

metabolism are perhaps unlikely to be apparent in short term ecological experiments. In particular, growth, reproduction and competitive ability are all likely to be affected by increases in ocean acidity over a longer time frame (Anthony et al. 2008; Fabricius et al. 2011). Finally, increased concentrations of atmospheric CO_2 are also resulting in the warming of the ocean via the green house effect (Hendriks et al. 2010). Consequently, marine organisms must deal with both low pH and high temperatures and future work should explore the possible synergistic effects of these stressors on coral larval ecology.

Table 2.1: Water parameters measurements Oct 2009 with 1 standard error value for experiments conducted

Desired-pCO_2 in ppm	pH	Dissolved O_2 (% saturated)	Temperature (°C)
Ambient	8.18 ± 0.01	89.7 ± 2.0	25.4 ± 0.3
550	8.07 ± 0.01	88.3 ± 1.9	25.2 ± 0.2
750	7.97 ± 0.02	85.3 ± 2.1	25.5 ± 0.2
1000	7.87 ± 0.02	85.9 ± 2.3	25.7 ± 0.3

Table 2.2: Water parameters measurements Nov 2009 with 1 standard error value for experiments conducted

Desired-pCO_2 in ppm	pH	Dissolved O_2 (% saturated)	Temperature (°C)
Ambient	8.17 ± 0.03	87.3 ± 2.5	27.0 ± 0.4
550	8.14 ± 0.03	85.3 ± 1.6	26.8 ± 0.2
750	8.02 ± 0.02	84.5 ± 2.1	26.9 ± 0.4
1000	7.87 ± 0.02	84.4 ± 2.2	26.8 ± 0.4

Table 2.3: Water parameters measurements Dec 2009 with 1 standard error value for experiments conducted

Desired-pCO_2 in ppm	pH	Dissolved O_2 (% saturated)	Temperature (°C)
Ambient	8.13 ± 0.04	82.1 ± 0.8	27.5 ± 0.2
550	8.05 ± 0.01	80.5 ± 0.9	27.6 ± 0.2
750	7.96 ± 0.03	80.4 ± 1.4	28.0 ± 0.1
1000	7.84 ± 0.02	78.5 ± 2.7	27.8 ± 0.1

Figure 2.1: Time in hours to reach gastrula (mean + SE) in four separate experiments (A = *Acropora tenuis*, B = *A. hyacinthus*, C = *A. millepora Nov* and D = *A. millepora Dec*) under four acidity treatments (1 = pCO$_2$ of 380ppm, 2 = 550 ppm, 3 = 750 ppm and 4 = 1000 ppm).
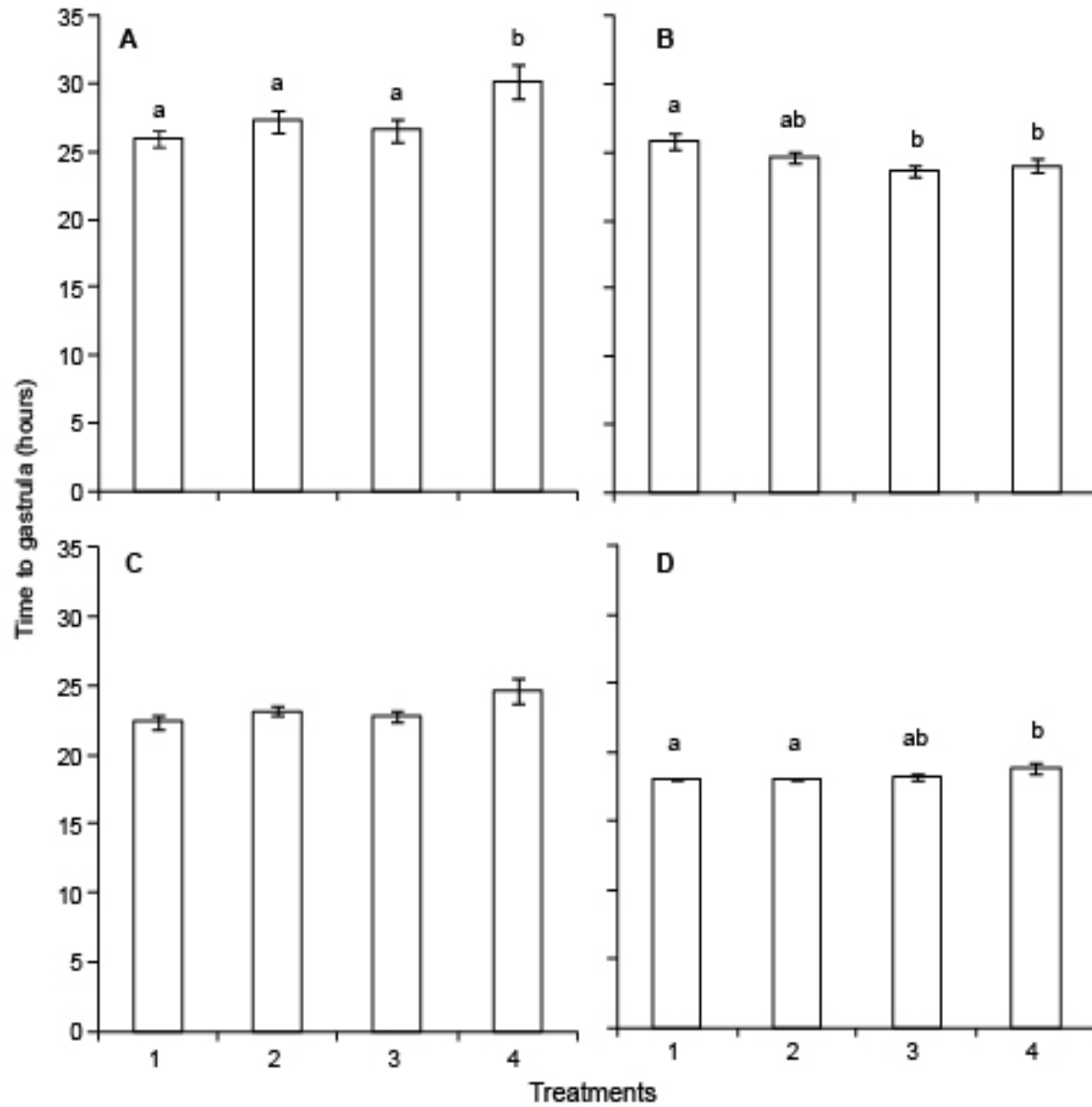
Figure 2.2: Survivorship (mean number of larvae alive ±SE) in three separate experiments (A = *A. tenuis*, B = *A. millepora Nov*) under four acidity treatments (Key: 1 = Ambient pCO_2, 2 = 550 ppm, 3 = 750ppm and 4 = 1000 ppm).
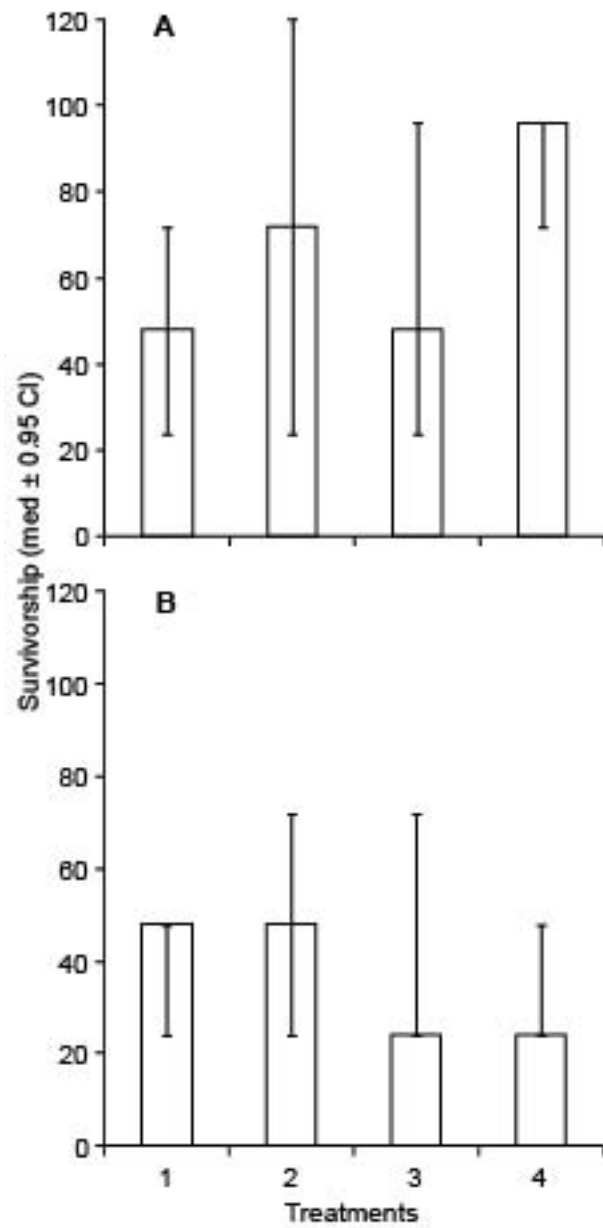
Figure 2.3: Metamorphosis (mean % ±SE) in three separate experiments (A = *Acropora hyacinthus*, B = *A. millepora Nov* and C = *A. millepora Dec*) under four acidity treatments (1 = pCO$_2$ of 380ppm, 2 = 550 ppm, 3 = 750 ppm and 4 = 1000 ppm).
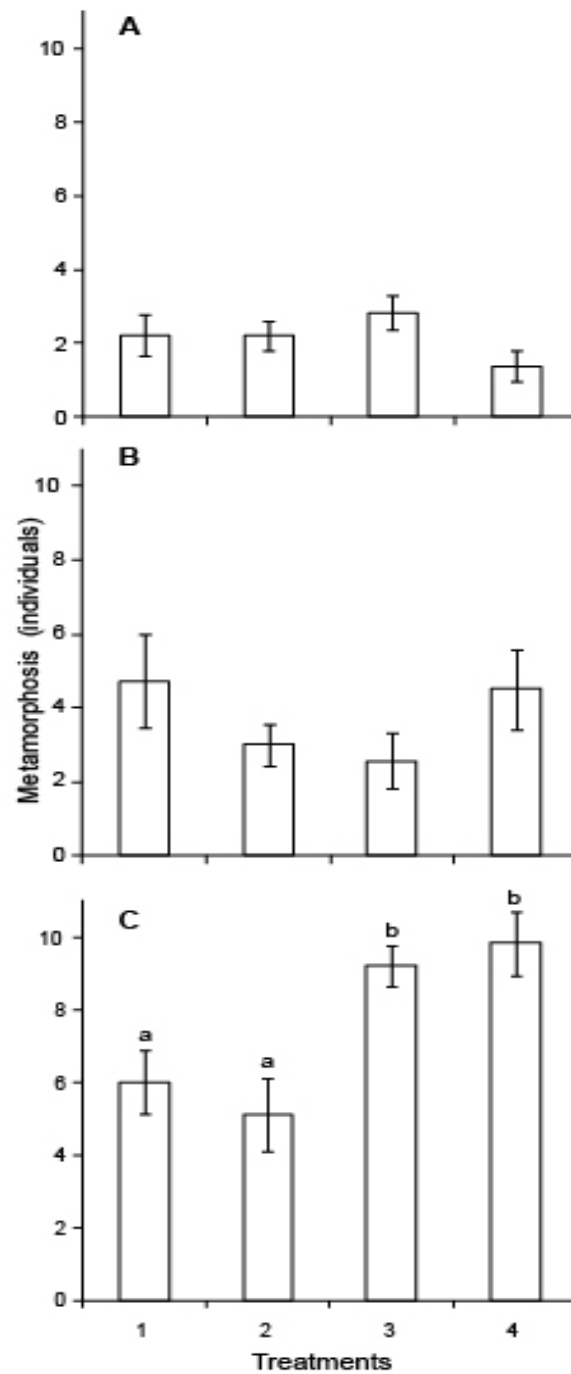
Chapter 3: Effects of ocean acidification on ocean warming on the early life history stages of corals.

This chapter was in review in Marine Ecology Progress Series in April 2012.

Introduction:

Increasing carbon dioxide (CO_2) concentrations in the atmosphere are causing the oceans to warm. Sea surface temperatures have risen by a global average of 0.7 °C since the industrial revolution (Feely et al. 2009) and are predicted to rise by a further 2 to 3 °C by the end of the century under the IPCC A2 scenario (Bindoff et al. 2007, IPCC report). In addition, the absorption of atmospheric CO_2 is changing ocean chemistry; decreasing seawater pH and reducing the availability of carbonate ions (CO_3^{2-}). Ocean pH has decreased by 0.1 units since the 1950s and is projected to decrease by a further 0.2 to 0.3 units by the end of the century (Feely et al. 2009).

The effects rising sea surface temperature on marine organisms are well documented. Symbiotic organisms, such as corals, seem particularly sensitive to positive thermal anomalies (Baird et al. 2009), and the resulting coral bleaching has a range of ecological effects from reductions in growth, reproduction and competitive ability to high mortality of individuals over large spatial scales (see review in McClanahan et al. 2004). In contrast, the effects of ocean acidification are less obvious, in part because the effects are often sub-lethal (Fabricius et al. 2011). Early experiments suggested a linear decline in calcification in response to a declining aragonite saturation state (Langdon and Atkinson 2005), however, more recent work indicates that the response is highly variable among species and dependent upon experimental technique (see review in Pandolfi et al. 2011).

The effects of temperature on the early life history stages of coral are also well documented. For example, increases in temperature of 1-2 °C above ambient levels result in high numbers of abnormally developed larvae (Negri et al. 2007; Bassim et al. 2002). Similarly, high temperatures reduce larval survivorship (Baird et al. 2006) particularly in larvae that contain symbiotic algae (Yakovleva et al. 2009; Nesa et al. 2012). In contrast, the effects of OA on coral larval ecology are much more variable and direct effects are only evident at very high pCO$_2$ or when gametes and larvae are past their peak (see discussion in Chapter 2).

In the wild these two stressors clearly act on organism at the same time, however, there have been very few experiments to explore the potential synergy between OA and temperature. The general assumption is that the effect of OA and temperature variables will be additive. High pCO$_2$ acts synergistically with high temperature and significantly lowered thermal bleaching thresholds in corals and crustose coralline algae (Anthony et al. 2008). Similarly, growth rates of *Porites panamensis* juveniles were lowest in a combined OA and high temperature treatment (Anlauf et al 2011). However, the interaction could be antagonistic because while temperature increases metabolism (O'Connor et al. 2009) acidosis reduces it (Pörtner 2004). For example, high temperatures can compensate for dwarfism that would otherwise eventuate due to reduced calcification in acidified sea water in *Tripneustustes gratilla* larvae (Sheppard Brennand et al. 2010).

In this chapter, I tested the synergistic effects of elevated temperature and CO$_2$ on the early life history stages of two common spawning acroporids of the Northern Great Barrier Reef region. **Hypothesis: elevated temperature and CO$_2$ will decrease the success of fertilization, development, survivorship and settlement of *A. tenuis* and *A. millepora* propagules.**

Materials and method

Collection of gravid colonies

Gametes of *Acropora tenuis*, and *A. millepora* were collected at Magnetic Island (19° 9'S, 146° 50'E) and Orpheus Island (18° 35'S, 146° 29'E) in 2010. Adult colonies were collected a few days prior to the predicted spawning period, and maintained in outdoor aquaria. *A. tenuis* and *A. millepora* from Magnetic Island spawned on the nights of the 28th of October, the 1st of November and the 2nd of November 2010, respectively. *A. millepora* colonies from Orpheus Island spawned on the 22nd of November and the 30th of November 2010. In each experiment sperm and eggs from 4-6 colonies were used to represent a population of spawners in the field.

Experimental manipulation of ocean acidification and warming.

The CO_2 tanks were prepared in a temperature control room (26°C-27°C). Two levels of CO_2 concentration (ambient and elevated pCO_2) and two levels of temperature exposure (ambient 27°C and +2°C) were selected as recommended by the EPOCA protocol (Barry et al. 2010). The treatments levels were: ambient (normal temperature and CO_2 concentration), 650-750 ppm CO_2 (High CO_2), 29°C (High Temp) and a combination of elevated pCO_2 and 29°C (T x CO_2).

A CO_2 mixing system developed by Munday et al. (2009) with modifications to the gas mixing chamber and gas mixing outlet was used to suit the coral larval culture. Experimental temperatures were maintained by using water baths via heating using aquarium heaters (Eheim Jager 300W aquarium heaters) and tank temperatures were measured twice daily throughout the experimentation period. The desired gas CO_2

concentration was produced using a CO_2 mixing system and the CO_2 concentration was measured in ppm units using a CO_2 gas probe (Vaisala ®). The mixed gases were bubbled through 250 L sump tanks to enrich the water (refer to methods in Chapter 2) and water chemistry parameters (pH, DO and temperature) and corresponding total alkalinity (TA) were measured for each treatment throughout the experiment (Table 3.1) by using fixed end point titration methods.

The effect of elevated pCO2 and warming on fertilization

For this experiment I used similar method of fertilization experiment in Chapter 2, however the fertilization was induced to elevated CO_2 and temperature regimes.

The effect of elevated pCO2 and warming on development

For this experiment I used similar method of time to stage developmenyt experiment in Chapter 2, however the fertilization was induced to elevated CO_2 and temperature regimes.

The effect of elevated pCO2 and warming on metamorphosis

Metamorphosis of *Acropora* larvae typically peaks between 6 to10 days after spawning (Connolly and Baird 2010). The age of larvae used to test for effects of pCO2 on larval metamorphosis were: *A. hyacinthus* - 7 days old; *A. millepora* - 7 days old. Ten or 20 larvae from each of the 3.5 L jars were placed into each well of a 6-well Iwaki cell culture plate with a modified meshed lid. A 2 mm x 2 mm crustose coralline algae (CCA) chip (*Titanoderma sp.*) was also placed in each well to induce metamorphosis following Heyward and Negri (1999) and the wells were then immersed in treated seawater and the

number of larvae that completed metamorphosis was assessed 24 h later. Larvae were defined as metamorphosed once a basal disc had been deposited (Baird and Babcock 2000).

The effect of elevated pCO_2 on survivorship

The survivorship was conducted with fertilized larvae (2 cells stage) and was unsuccessful due to the high mortality and subsequent contamination in survivorship replicate jars (due to the high buoyancy of the new born larvae). The motile larvae are chosen as it will represent the time in plankton prior to settlement under stressed conditions minus the issue of larvae density. Fifty motile larvae (4 days old) were introduced into 3 replicate jars with a mesh lid, and immersed in the CO_2 enriched tanks. Numbers of surviving larvae were scored every 24 hours. This period is sufficient for dead larvae to lyse and disappear from the cultures (Baird et al. 2006). Larvae survival rates were followed up to 14 days in each treatment.

Data analysis

Mean differences in the proportion of eggs fertilized, time to complete gastrulation, time to become motile and the number of larvae completing metamorphosis among the four treatments were tested with a fully factorial two-way ANOVA. The factors were temperature (fixed, two levels: 27 and 29 C) and pCO_2 (fixed, 380 and 700). Each experiment was analyzed independently (i.e. 2 to 4 experiments depending on the response variable). Bonferroni correction was used to adjust the probability of a type I error occurring (i.e. probability was considered significant when *beta* < alpha/n where beta equals the Bonferoni corrected probability, alpha = 0.05 and n = the number of experiment run for the specific response variable). Any bias in these data was explored by residual

analysis and if detected the data were transformed as noted in the ANOVA tables. Differences in the median survival time among treatments for each experiment were tested using Kaplan-Meier survival analysis. All statistical analyses were performed using SPSS 16.

Results:

The mean proportion of eggs completing fertilization was not affected by either temperature or pCO_2 in three of the four experiments (Fig 3.1 A, B & C; Table 3.2). In all of these, experiment fertilization success was uniformly high (Fig 3.1 A, B & C). In one experiment, *A. millepora* Nov22, the mean proportion of eggs fertilized was lower at high temperatures: 100% of eggs were fertilized at 27°C vs. 89.5 ± 3.8 eggs (mean ± SE) fertilized at 29°C (Fig. 3.1D; Table 3.2).

The effect of temperature and pCO_2 on time to complete gastrulation was variable. In all but one experiment, *A. millepora* Nov22, embryos completed gastrulation more quickly at high temperatures (Fig. 3.2; Table 2.3). For example, the average time to complete gastrulation in *A. millepora* Nov01 was 22.0 ± 0.49 h at 27°C vs. 18.6 ± 0.16 h at 29°C. In one experiment, *A. tenuis*, pCO_2 also had an effect, with embryos in high pCO_2 completing gastrulation more quickly (17.8 ± 0.18 h) than those at ambient pCO_2 (18.7 ± 0.28 h) (Fig. 3.2A; Table 3.3). In no experiment was there any interaction between temperature and pCO_2 (Table 3. 3). Further, the effects of temperature and pCO_2 on time to motility were highly variable. In all but one experiment, *A. millepora* Nov22, motility was achieved more quickly at higher temperatures (Fig.3.3; Table 3.4); however, in two experiments, temperature interacted with pCO_2. In *A. tenuis*, there was no difference between the pCO_2 treatments at 27°C; however, at 29°C motility was reached more rapidly

in the high pCO_2 treatment (Fig.3.3a). In *A. millepora*_Nov01, there was no difference between the pCO_2 treatments at 29°C, however, at 27°C, embryos in the high pCO_2 treatment became motile more quickly (Fig. 3.3B). In *A. millepora* Nov22 motility was reached more rapidly in high pCO_2 (Fig.3.3C; Table 3.4) than at ambient pCO_2 (59.2 ± 1.7 h vs. 66.8 ± 2.84 h) (Fig. 3.3C; Table 3.4).

The effects of temperature and pCO_2 on larval survivorship were also variable. In *A. tenuis*, median survivorship was significantly lower in the high temperature treatment, but did not vary among the other three treatments (Fig. 3.4; Table 3.5). In *A. millepora*_Nov22, median survivorship was lower in the high temp and high temp & elevated pCO_2 treatments when compared to the ambient temperature treatments (ambient and high pCO_2), however, there was no significant difference in median survivorship between the elevated pCO_2 treatment and any other treatment (Table 3.6).

The effects of temperature on larval metamorphosis were also variable. In *A. tenuis*, metamorphosis was higher at 29°C (55.1% ± 0.45 vs. 70.3% ± 0.25) (Fig. 3.5; Table 3.7). In contrast, metamorphosis was lower at 29°C in *A. millepora*_Nov22 (54.9% ±0.30 vs. 40.2% ± 0.45) (Fig. 3.5; Table 3.7). pCO_2 did not have any effect on metamorphosis, either on its own, or in synergy with high temperature (Fig. 3.5; Table 3.7).

Discussion:

The effects of temperature on the early life history stages of corals were more prevalent than those of OA. In general, rates of development were faster at high temperatures, as predicted by metabolic theory (Gillooly et al. 2001). In contrast, OA rarely affected development and, with one exception, did so only in combination with temperature. However, the effect was contrary to predictions. In both experiments where an interaction

was detected (Fig. 3A & B; Table 4) elevated pCO$_2$ decreased the time to motility whereas hypercapnia or CO$_2$ narcosis would be expected to slow rates of development (Pörtner et al. 2004). Similarly, in the only experiment where OA had a significant effect on development, time to motility was faster in high pCO$_2$ treatments. Neither temperature, OA nor the combination of these variables affected fertilization, larval survivorship or metamorphosis in any consistent or predictable direction. We conclude that projected changes in temperature and OA this century are unlikely to have major ecological effects on the early life history of corals, either alone, or in synergy, with the possible exception of temperature speeding up rates of larval development.

Fertilization was robust to both elevated OA and temperature within the range of the variables used in the experiments. This is a result that is in good agreement with all work done previously with corals (see Table 1.2 and 1.3). Albright et al. (2010) reported a 64% decrease in fertilization rates at similar levels of OA when sperm concentrations were low, however, the age of the gametes used in this experiment (gametes were mixed after 3 h) suggests they were past their prime (Oliver and Babcock 1992). In general, fertilization rates are only reduced at + 4 °C (e.g. Negri et al. 2007 and see Table 1.3). Similarly, sea urchin fertilization is robust to these levels of OA and temperature (Byrne 2011, Sheppard Brennand et al. 2010). Marine invertebrate fertilization is generally robust to acidity and minor increases in temperature because the propagules are often equipped with cellular defences, present in the egg before fertilization (Hamduon and Epel 2007; Portune 2010), probably because the gametes are regularly exposed to fluctuating temperature and OA in the plankton (Gagliano et al. 2010).

Rates of development were predictably increased by temperature, a result consistent with most of the literature (see Table 1.2 & 1.3), and one to be expected on the basis of

metabolic theory. However, it was surprising that OA also on occasion increased rates of development, both on its own, and in synergy with high temperature, in contrast to a prediction of reduced metabolism from hypercapnia.

Metamorphosis was affected by temperature but not OA, however, the temperature effect was inconsistent. Again, these results are consistent with the literature (see Table 1.2 & 1.3). Temperature of + 2 °C above ambient average sea temperature have little effect on metamorphosis, and both positive (Coles 1985) and negative effects (Randall and Szmant 2009) have been reported. Metamorphosis is rarely directly affected by OA (Nakamura et al 2011, Albright et al 2010; 2011), indeed, only in larva that have passed peak metamorphosis (Nakamura et al. 2011) has a direct effect been reported. The effect of OA on metamorphosis, if any, is indirect, and mediated through OA-induced changes in the composition of the substratum, in particular, the health of crustose coralline algae that is often required to induce metamorphosis in coral (Albright et al 2010; Doropoulos et al. 2012). In the only other test of a synergistic effect between these stressors, metamorphosis was not affected (Anlauf et al. 2011).

Survivorship was not consistently affected by OA or temperature. The temperature results is perhaps surprising because most previous experiments report an effect at temperature levels above ambient (e.g. Coles 1985; Bassim and Sammarco 2003 but see Yakovleva et al. 2009). In contrast, larval survivorship is rarely affected by OA (Chapter 2; Table 2.2). This suggests that some species of corals are more susceptible to temperature and acidity stress than others (Fabricius et al. 2011).

In conclusion, the effects of a +2 °C increase in temperature were relatively minor and had no effect on fertilization, and mixed effects on larval survivorship and metamorphosis. The only consistent effect was on rates of development which were

typically more rapid at high temperatures. The effects of increased rates of development on coral demography are difficult to predict. If larvae develop competence more quickly, average dispersal distance should decrease which is therefore likely to reduce levels of reef connectivity. However, greater levels of retention are also likely to increase the rates of recruitment. Consequently, the effect of more rapid rates of development in response to increasing temperatures is likely to be highly dependent on local conditions such as reef density and hydrodynamics. In contrast to temperature, OA had no predictable or consistent effect, either alone, or in synergy with temperature. It is concluded that temperature increases associated with global warming are more likely to have ecological consequences than OA (see also Cooper et al. 2012).

Table 3.1: Water chemistry parameters (±SE) value throughout the experiment with saturation values of Aragonite and Calcite and actual saturated values of pCO$_2$ in treatments tanks calculated using CO2SYS. (Parameters: K1, K2 from Mehrbach et al. 1973 refit by Dickson and Millero 1987; KHSO$_4$ from Dickson 1990; pH: NBS scale/kg-H$_2$O (Pierrot et al. 2006)

Treatment	pH	TA (μmol/kg as CaCO3)	Temperature (°C)	Ω calc	Ω arag	pCO$_2$ calculated
Ambient	8.12 ± 0.02	2076 ± 40	26.5 ± 0.08	4.2 ± 0.2	2.8 ± 0.1	446 ± 18
High Temp	8.11 ± 0.02	2094 ± 43	28.9 ± 0.04	4.2 ± 0.2	2.8 ± 0.1	509 ± 21
High CO$_2$	7.96 ± 0 .02	2015 ± 37	26.5 ± 0.05	3.0 ± 0.1	2.0 ± 0.1	675 ± 26
T x CO$_2$	7.98 ± 0.01	2036 ± 47	29.3 ± 0.11	3.3 ± 0.2	2.2 ± 0.1	710 ± 37

Table 3.2: Two-way ANOVA results for every fertilization experiment (single species tested against elevated pCO_2 (abbreviated pCO_2) and temperature treatment) with Bonferroni correction (probability was considered significant when $p < 0.013$)

Cohorts	n	df	F value	*p* value
A. tenuis	20			
Temperature		1	0.078	0.783
pCO_2		1	0.000	1.000
Temp vs. pCO_2		1	1.255	0.279
A. millepora Nov 01	20			
Temperature		1	3.189	0.093
pCO_2		1	0.925	0.351
Temp vs. pCO_2		1	5.453	0.033
A. millepora Nov 02	20			
Temperature		1	3.723	0.072
pCO_2		1	2.492	0.134
Temp vs. pCO_2		1	5.200	0.037
A. millepora Nov 22	20			
Temperature		1	10.376	0.005
pCO_2		1	3.976	0.063
Temp vs. pCO_2		1	3.976	0.063

Table 3.3: Two-way ANOVA results for every 'average time to gastrula stage' experiment (single species tested against elevated pCO$_2$ (abbreviated pCO$_2$) and temperature treatment) with Bonferroni correction (probability was considered significant when $p < 0.013$).

Cohorts	n	df	F value	p value
A. tenuis	238			
Temperature		1	14.475	0.000
pCO$_2$		1	10.067	0.002
Temp vs. pCO$_2$		1	0.696	0.405
A. millepora Nov 01	232			
Temperature		1	50.353	0.000
pCO$_2$		1	2.525	0.113
Temp vs. pCO$_2$		1	0.157	0.692
A. millepora Nov 22	240			
Temperature		1	0.090	0.765
pCO$_2$		1	0.050	0.823
Temp vs. pCO$_2$		1	0.090	0.765
A. millepora Nov 30	237			
Temperature		1	18.846	0.000
pCO$_2$		1	2.143	0.145
Temp vs. pCO$_2$		1	3.993	0.047

Table 3.4: Two-way ANOVA results for every 'average time to motile stage' experiment (single species tested against elevated pCO_2 (abbreviated pCO_2) and temperature treatment) with Bonferroni correction (probability was considered significant when $p < 0.013$).

Cohorts	n	df	F value	*p* value
A. tenuis	142			
Temperature		1	323.125	0.000
pCO_2		1	4.935	0.028
Temp vs. pCO_2		1	44.796	0.000
A. millepora Nov 01	218			
Temperature		1	53.519	0.000
pCO_2		1	10.597	0.001
Temp vs. pCO_2		1	17.654	0.000
A. millepora Nov 22	142			
Temperature		1	3.015	0.085
pCO_2		1	10.101	0.002
Temp vs. pCO_2		1	3.622	0.059
A. millepora Nov 30	210			
Temperature		1	13.202	0.000
pCO_2		1	0.070	0.792
Temp vs. pCO_2		1	0.587	0.444

Table 3.5: Median survivorship results for *A. tenuis*, Kaplan-meier analysis. df = 3, χ^2= 16.4, p< 0.001. Abbreviation: LCL = Lower confidence level, UCL = upper confidence level.

Treatments	n	Median	0.95 LCL	0.95 UCL
Ambient	150	168	158.33	177.68
High Temp	150	72	45.90	98.10
High CO_2	150	168	125.70	210.30
T x CO_2	150	168	115.74	220.26

Table 3.6: Survivorship results for *A. millepora*, Kaplan-Meier analysis. df = 3, χ^2= 4.814, p = 0.028. Abbreviation: LCL = Lower confidence level, UCL = upper confidence level

Treatments	n	Median	0.95 LCL	0.95 UCL
Ambient	150	168	104.13	231.87
High Temp	150	72	47.18	96.83
High CO_2	150	96	0.12	191.88
T x CO_2	150	72	57.70	86.30

Table 3.7: Two-way ANOVA results for every 'metamorphosis' experiment (single species tested against elevated pCO_2 (abbreviated pCO_2) and temperature treatment) with Bonferroni correction (probability was considered significant when $p < 0.025$)

Cohorts	n	df	F value	*p* value
A. tenuis	72			
Temperature		1	7.30	0.009
pCO_2		1	0.04	0.845
Temp vs. pCO_2		1	0.04	0.850
A. millepora	72			
Temperature		1	7.24	0.009
pCO_2		1	1.47	0.229
Temp vs. pCO_2		1	1.82	0.182

Figure 3.1: Average percentile of fertilization under both elevated temperature and acidity exposure (±SE) for 4 experiments across 2 acroporid species (n = 20 for both species). Key: white bars = Ambient temperature 27°C and ambient pCO_2 (446ppm and 509ppm) and black bars = High CO_2 (675 ppm and 710 ppm)

A= *A. tenuis*, B= *A. millepora* Nov 01, C = *A. millepora* Nov 02, D= *A. millepora* Nov 22.
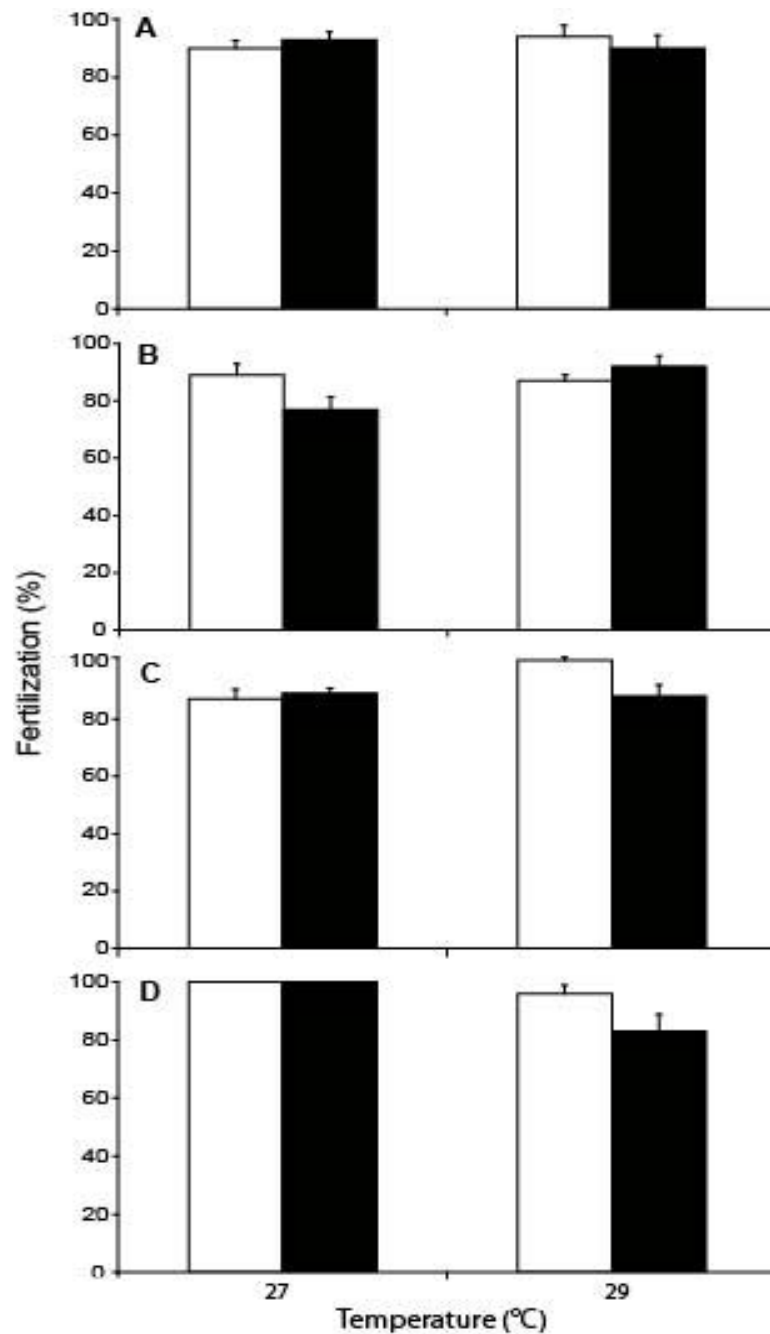
Figure 3.2: Average time to gastrula under both elevated temperature and acidity exposure (±SE) for 4 experiments across 2 acroporid species (*A. tenuis* n= 238; *A millepora* n= 232 – 240).

Key: white bars = Ambient temperature 27°C and ambient pCO_2 and black bars = High CO_2; A= *A. tenuis*, B= *A. millepora* Nov 01, C = *A. millepora* Nov 22, D= *A. millepora* Nov 30.
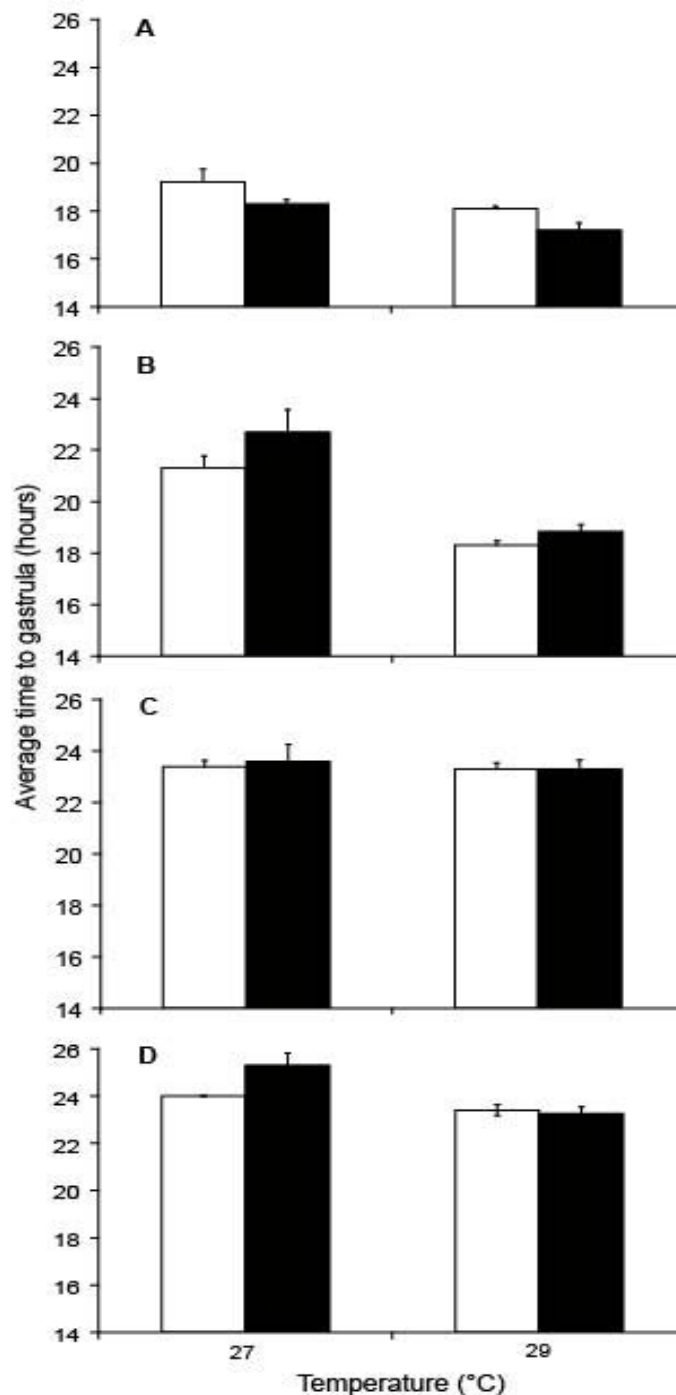
Figure 3.3: Average time to motile under both elevated temperature and acidity exposure (±SE) for 4 experiments across 2 acroporid species (*A. tenuis* n= 142; *A millepora* n= 142–218).

Key: white bars = Ambient temperature 27°C and ambient pCO_2 and black bars = High CO_2

A= *A. tenuis*, B= *A. millepora* Nov 01, C = *A. millepora* Nov 22, D= *A. millepora* Nov 30.
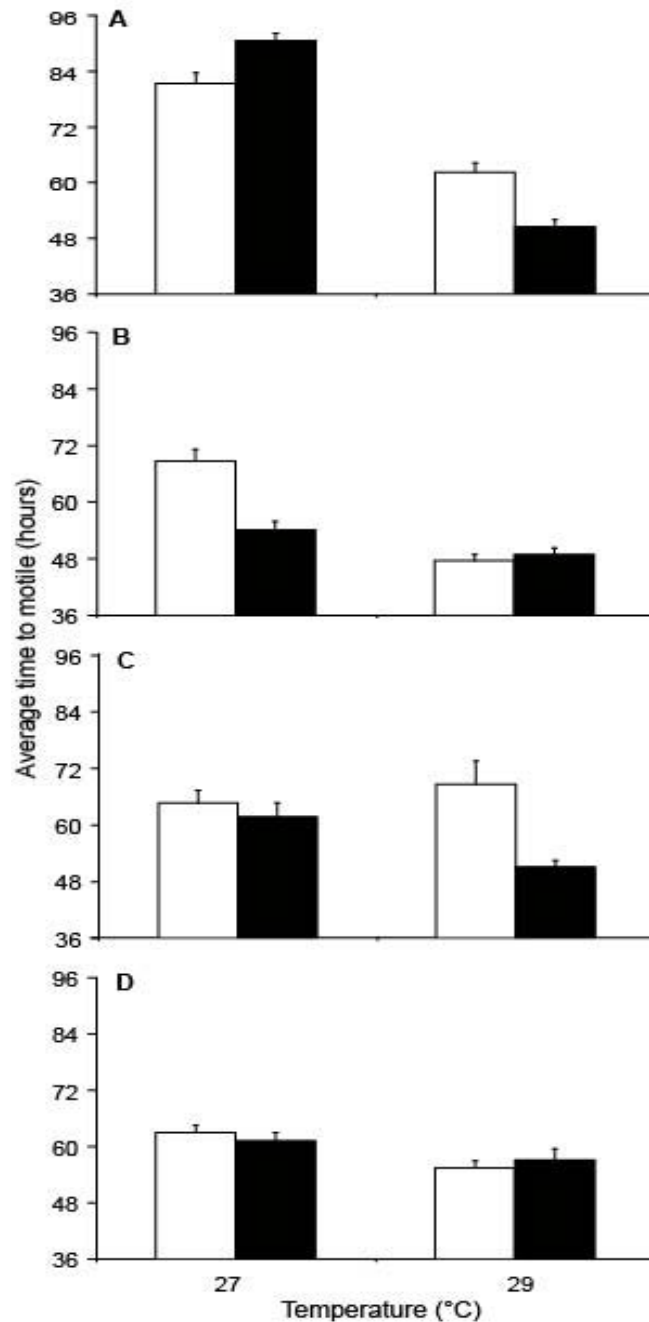
Figure 3.4: Number of surviving propagules under both elevated temperature and acidity exposure (±SE) for 2 experiments across 2 acroporid species (n= 150 for borth species). Key: closed squares = Ambient temperature 27°C and ambient pCO2 (446ppm), open squares = high temp (29°C, 446ppm), closed circle = high acid (27°C and 675 ppm) and open circles = acid x temp (29°C and 710 ppm). A= *A. tenuis*, B= *A. millepora*.
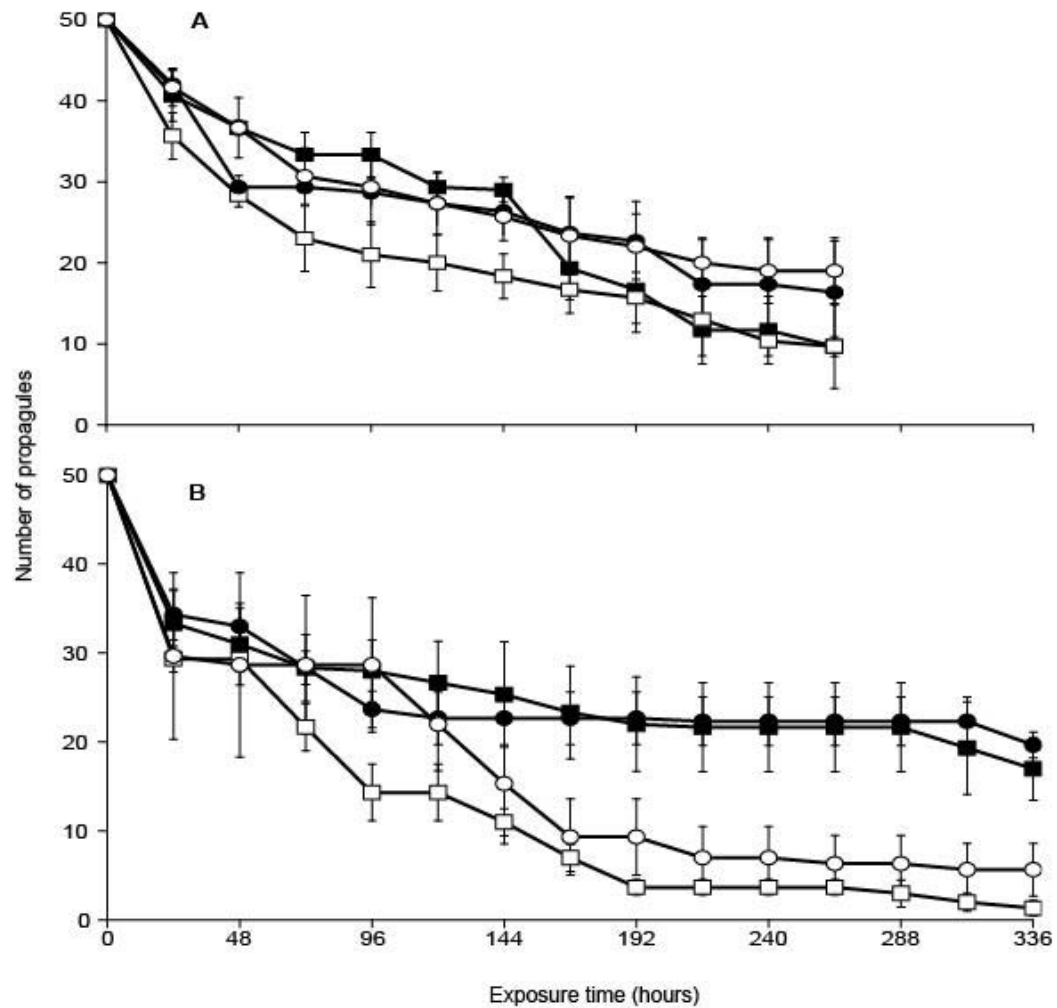
Figure 3.5: Average percentile of metamorphosis under both elevated temperature and acidity exposure (±SE) for 2 experiments across 2 acroporid species (n= 72 for both species).

Key: white bars = Ambient temperature 27°C and ambient pCO_2 and black bars = High CO_2; A= *A. tenuis*, B= *A. millepora*
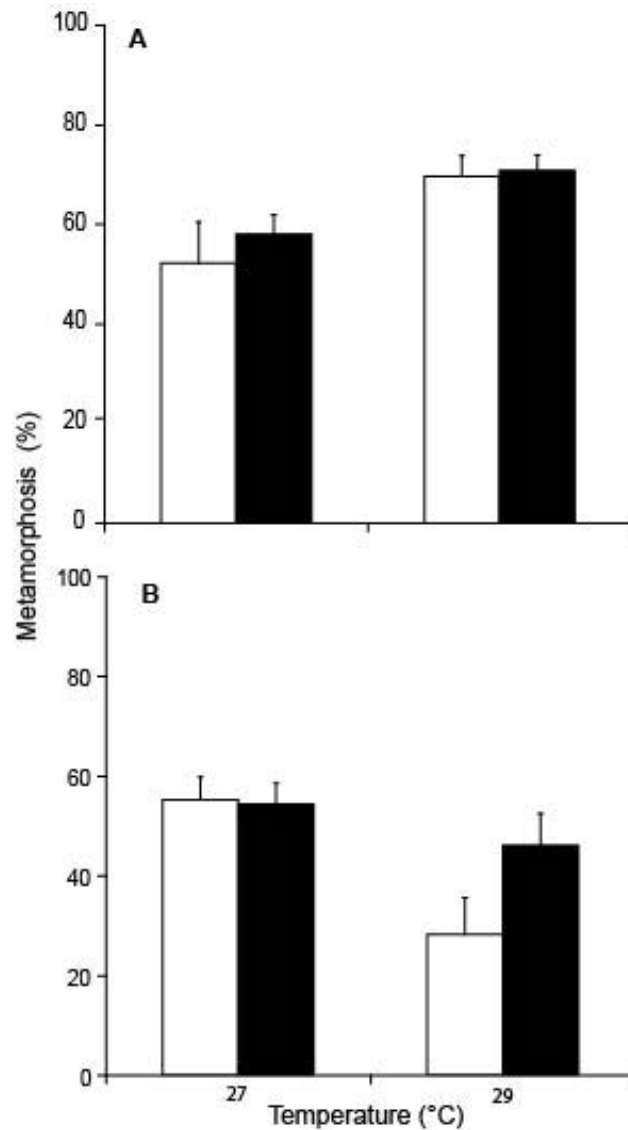
Chapter 4: Effects of ocean acidification on metamorphosis of larvae of a brooding and spawning coral

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Introduction

Climate change has the potential to detrimentally affect coral reefs. In particular, increasing ocean acidity as a result of increased atmospheric concentrations of CO_2 is expected to present a severe threat to marine ecosystems before the end of this century (Kleypas et al. 1999; Orr et al. 2005). Ocean acidification is caused by alteration of sea water chemistry through CO_2 absorption from the atmosphere leading to a decrease in seawater pH and carbonate ion concentrations (CO_3^{2-}). Recent changes in ocean chemistry, including a decrease in pH of 0.1 units since the 1950s, have reduced the rates of calcification in many marine organisms (Langdon 2000; Cooper et al. 2008; Pörtner 2009). By 2050, ocean pH is expected to be lower than at any point in the last 20 million years (Caldeira and Wickett 2003; Guinotte et al. 2006; Turley et al. 2007).

To date, much of the research on the effects of ocean acidification on corals has focused on adults, in particular the process of calcification. However, the early life history stages of marine organisms are also likely to be sensitive to changes in ocean chemistry (Pörtner et al. 2004; Byrne 2010). Hypercapnia, defined as an increase in the partial pressure of CO_2 in the respiratory fluids, affects acid-base regulation, oxygen transport and metabolic function (see review by Pörtner et al. 2004) and reduced sperm motility in seawater artificially enriched with CO_2 (Havenhand et al. 2008, Morita et al. 2010).

Many ecological processes are affected by differences in the life history traits of organisms. For example, the mode of larval development in marine invertebrates (often called the reproductive mode) affects patterns of dispersal (Baird et al. 2009b), rates of recruitment (Hughes et al 2002) and post-settlement mortality (Marshall et al. 2010). Corals have two contrasting modes of reproduction: brooding and broadcast spawning. These modes of reproduction are distinctive in many ways (Baird et al 2009b), however, potentially the most important difference in terms of the larva's response to stress, such as increased acidity, is whether or not they contain photosynthetic symbionts, *Symbiodinium*. Interestingly, all brooded larvae (except those of the *Isopora sp*.) contain *Symbiodinium* on release from the mother, whereas only four of 85 genera of broadcast spawning species have *Symbiodinium* in the eggs (Baird et al. 2009b). Symbiotic organisms are generally more susceptible to stress: for example, few other organisms respond to the relatively small changes in sea surface temperature associated with mass coral bleaching events (Baird et al. 2009a; McClanahan et al 2009). Similarly, *Acropora* larva experimental infected with *Symbiodinium* have higher rates of mortality when exposed to temperatures 2°C higher than ambient (Yakovleva et al 2009), and naturally occurring levels of ultra-violet radiation

(Baird et al unpublished data) when compared to larvae of the same species that lack *Symbiodinium*.

Here, we test whether ocean acidification affects metamorphosis in the larval of a brooding coral that contains *Symbiodinium*, *Leptastrea cf transversa* and a spawning coral that does not, *Goniastrea retiformis*. **Hypothesis: elevated CO_2 will decrease the success of settlement of *L. transversa* and *G. retiformis* propagules.**

Materials and method

Goniastrea retiformis is a hermaphroditic broadcast spawner. The eggs of *G. retiformis* lack *Symbiodinium* which are typically not take up by larvae until settlement (Fig. 1a; Babcock and Heyward 1986). *Leptastrea cf transversa* is a gonochoric brooder. Larvae are released containing *Symbiodinium* that have been transmitted from the mother to the developing oocytes (Fig. 4b).

Eight mature colonies of *G. retiformis* were collected from the fringing reef in Pago Bay, Guam (13° 25'N, 144° 48'E) and maintained in outdoor aquaria until they spawned on the night of 15th June 2009. Following spawning, larvae were cultured as outlined in Babcock et al. (2003) and maintained in 0.2μm filtered sea water (FSW) until competent to settle (Connolly and Baird 2010). Approximately 20 colonies of *L. cf transversa* were collected from the lagoon behind the fringing reef fronting the Guam Port Authority (13°27'54.98"N, 144°40'16.10"E) on the 15th June and maintained in flow through aquaria at the University of Guam Marine Laboratory. At night, the water flow was stopped, and in the morning larvae were collected from the aquaria with pipettes. A total of 75 larvae were collected on

the 17th June 2009 and the larvae were maintained in 0.2μm filtered sea water for three days prior to the settlement experiment that commenced on 20th June 2009.

Experimental manipulation of ocean acidification.

The CO_2 tanks were prepared in a temperature control room (25°C-26°C). A CO_2 mixing system developed by Munday et al. (2009) was used to manipulate pCO_2 in seawater. Four levels of pCO_2 (ambient 380 ppm, 550 ppm, 750 ppm and 1000 ppm) were used, as recommended by Barry et al. (2010). CO_2 concentration was measured in ppm units using a CO_2 gas probe (Vaisala ®). The mixed gases were bubbled through 70 l sump tanks to enrich the water. The enriched water was flowed through replicates via a one-way flow-through system. pH conditions and corresponding total alkalinity (TA) were measured for each treatment by using auto Gran titration (APHA 2320). Experimental calcite (Ω_{calc}) and aragonite saturation (Ω_{arag}), carbonate (CO_3^{2-}) and bicarbonate ion concentration (HCO_3^-) were calculated from TA, pH, salinity and CO_2 concentration (pCO_2) using CO2SYS (Pierrot et al. 2006; Table 4.1).

The effect of pCO₂ on metamorphosis

Ten *G. retiformis* larvae from each of the 3.5 l jars were placed into each well of a 6-well Iwaki cell culture plate with a modified meshed lid. Similarly, one *L. cf transversa* larva was placed in each well with a total of 18 larvae per pCO_2 treatment. A 5 mm x 5 mm crustose coralline algae (CCA), *Hydrolithion sp.* was placed in each well to induce metamorphosis. Three plates (with 18 wells as replicates) were then immersed in the four pCO_2 treatments and the number of larvae completing metamorphosis was assessed after 24

h. Larvae were defined as metamorphosed if a basal disc had been deposited (Baird and Babcock 2000).

Data analysis

Differences in the mean number of *G. retiformis* larvae completing metamorphosis among the four pCO_2 treatments were tested with 1-way ANOVA. Tukey's HSD multiple comparison test was used to determine which pCO_2 levels differed. Any bias in these data was explored by residual analysis and if detected the data were transformed as noted in the ANOVA tables. A contingency table was used to test whether the number of *L. cf transversa* larvae settling was independent of the level of pCO_2.

Results

Ocean acidification did not affect metamorphosis as predicted in either species. The mean number of *G. retiformis* larvae completing metamorphosis did not differ among the different pCO_2 levels ($F_{1,46}$ = 2.386, p = 0.1292; Fig. 4.2). While the number of larvae completing metamorphosis in *L. cf transversa* was dependent on the level of pCO_2 ($\chi^2_{1, 3}$ = 9.0625, p = 0.02847), the pattern was not that expected if the response was does dependent. The highest number of *L. cf transversa* larvae metamorphosed in the 1000 ppm the lowest number in the 750 ppm treatment and there was no difference between ambient and 500 ppm (Table 4.2).

Discussion

Metamorphosis was not consistently affected by pCO_2 in either species. These results suggest that the mode of reproduction does not affect the larval response to pCO_2 and

furthermore, there will be no direct effects of ocean acidification on settlement rates of reef corals, at least in the near future.

Our results are consistent with other recent research on the effect of OA on coral larval metamorphosis. A reduction in metamorphosis is typically only apparent at very high levels of pCO2 or when mediated by OA induced changes in the quality of the substratum (see review by Albright 2012; Chua 2012). There are a number of reasons why corals may be robust to OA. Adult corals thrive in an environment with high natural fluctuations in pH (Gagliano et al. 2010) which may pre-adapt the offspring to withstand high level of OA. For example, sea urchins that live in tidal pools with high natural fluctuations in pH produce offspring that are robust to OA (Byrne 2010). Similarly, larvae of the Sydney Rock Oyster (*Saccostrea glomerata*) produced by adults subjected to elevated pCO$_2$ during gametogenesis had faster development, greater shell length and metabolic activity in elevated CO$_2$ conditions, compared to juveniles from wild caught adults (Parker et al 2012). Similarly, brooded larvae produced by adult byrozoans (*Bugula neritina)* grown in elevated copper concentration were larger, dispersed farther and were tolerant of copper toxicity (Marshall 2008). Such maternal effects are likely to be greater in species with larvae that rarely leave the maternal habitat, such as those species that brood larvae. However, we found no difference between these two coral species with different modes of larval development. Alternatively, the process of calcification may pre-adapt corals to fluctuation in pH. During calcification, corals elevate the extracellular pH under the calicoblastic epithelium by 0.2-0.5 units (Venn et a. 2011) and therefore, corals may have evolved mechanism for the tissue to adjust to high pH.

In conclusion, projected ocean acidification levels in the near future appear unlikely to have major ecological effects on coral settlement. The lack of major affects on larval ecology does not mean that ocean acidification is not a threat to other stages in a coral's life history. There are strong theoretic and empirical reasons for expecting an effect on physiology (Pörtner et al. 2004) and patterns of gene expression vary dramatically in coral larvae as levels of pCO$_2$ increase (Moya et al. 2012). The consequences of energy expenditure on cellular acid-base regulation and lowered metabolism are perhaps unlikely to be apparent in short term ecological experiments. In particular, growth, reproduction and competitive ability are all likely to be affected by increases in ocean acidity over a longer time frame (Anthony et al. 2008; Fabricius et al. 2011). In addition, increased concentrations of atmospheric CO$_2$ are also resulting in the warming of the ocean via the green house effect (Hendriks et al. 2010). Consequently, marine organisms must deal with both high pCO$_2$ and high temperatures and future work should explore the possible synergistic effects of these stressors on coral larval ecology.

Acknowledgement

Table 4.1: Water chemistry parameters (±SE) value throughout the experiment with saturation values of Aragonite and Calcite and actual saturated values of pCO$_2$ in treatments tanks calculated using CO2SYS using parameters: K1, K2 from Mehrbach et al 1973 refit by Dickson & Millero 1987; KHSO4 from Dickson 1990; pH: NBS scale/kg-H$_2$O (Pierrot et al 2006)

Treatment	pH	TA (μmol/kg as CaCO$_3$)	Temperature (°C)	Ω calc	Ω arag	pCO$_2$ calculated
Ambient	8.12 ± 0.02	2076 ± 40	26.5 ± 0.08	4.2 ± 0.2	2.8 ± 0.1	446 ± 18
600	8.11 ± 0.02	2094 ± 43	28.9 ± 0.04	4.2 ± 0.2	2.8 ± 0.1	509 ± 21
750	7.96 ± 0.02	2015 ± 37	26.5 ± 0.05	3.0 ± 0.1	2.0 ± 0.1	675 ± 26
1000	7.98 ± 0.01	2036 ± 47	29.3 ± 0.11	3.3 ± 0.2	2.2 ± 0.1	710 ± 37

Table 4.2: The number of *Leptastrea cf transversa* larvae completing metamorphosis under four level of pCO_2.

pCO2	Metamorphosed	Not metamorphosed
Control	9	9
500	9	9
750	4	14
1000	13	5

Figure 4.1: (a) A broadcast spawned larva (*Leptasrea cf. transversa*) with no symbionts (b) a brooded larvae (*Stylophora pistillata*) with symbionts evident as brown splotches in the gastroderm
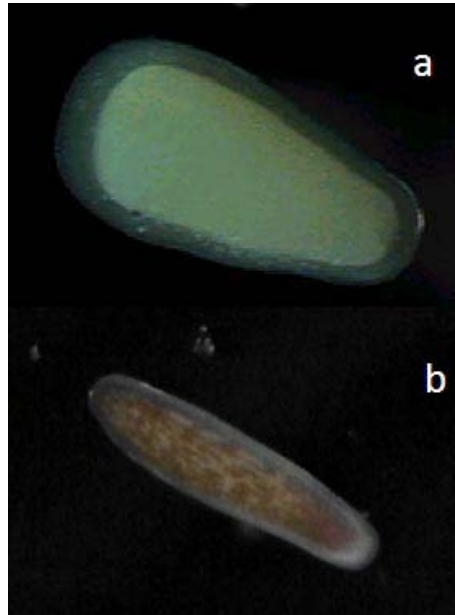
Figure 4.2: The proportion of *Goniastrea retiformis* larval complete metamorphosis under four acidification regimes (mean + SE). Letters denote groups that are not significantly different as indicated by Tukey's HSD test.
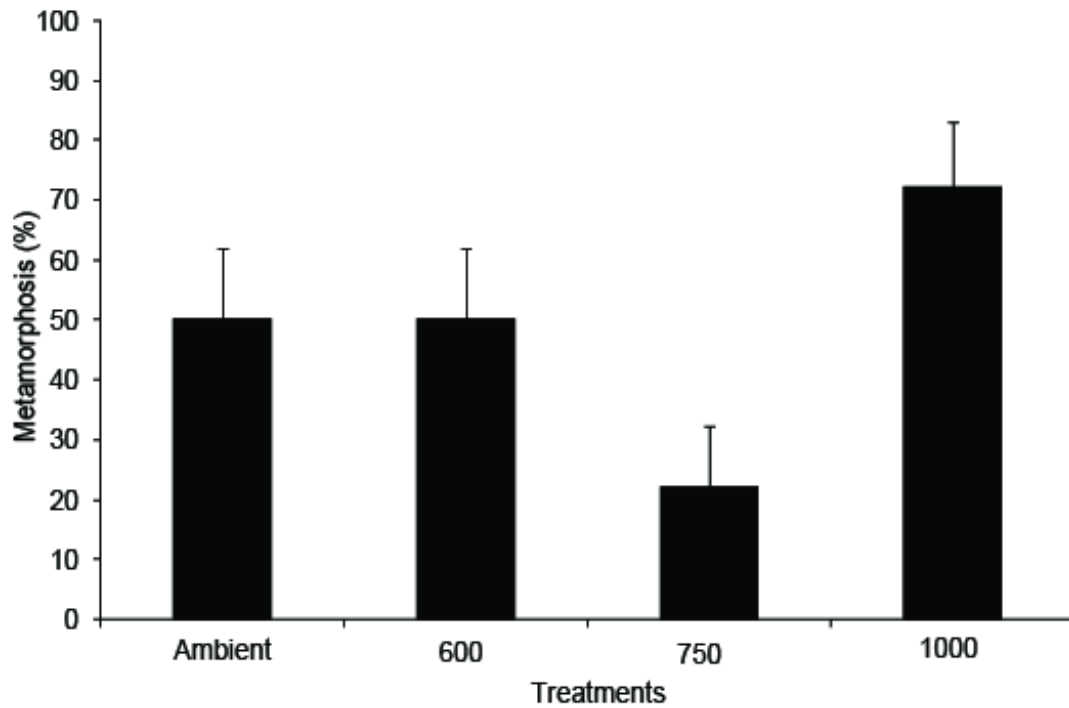
Chapter 5: Effects of ocean acidification and ocean warming on the growth and patterns of skeletogenesis in juvenile corals.

Introduction

Up to 70% of the world's corals may be lost within the next 40 years if climate related stressors and human influences continue to intensify (Wilkinson 1993). Recovery of damaged coral populations generally depends on successful sexual recruitment to reseed the damaged reefs (Fan et al. 2002; Arthur et al. 2005; Hughes & Tanner 2000). Therefore, it is essential to study how the recruitment of stony corals will be affected by climate change, especially the combination of ocean acidification and warming at levels projected to occur in the near future.

Coral recruitment plays a primary role in: maintaining genetic diversity; populating denuded areas; determining the community structure of coral reefs; and replenishing reefs post-disturbance (Morgan 2001). Environmental factors, such as temperature, can reduce recruitment with profound effects for marine population dynamics (Gaines and Roughgarden 1985; Harrison and Wallace 1990; Doherty and Fowler 1994; Riegl and Purkis 2009; Hughes et al 2000). Following settlement, coral recruits secrete a calcium carbonate skeleton (Vandermeulen and Watabe, 1973; Le Tissier, 1988) without which growth and further development cannot proceed (Chambelain 1978; Vandemulen and Watabe 1973).

Ocean acidification decreases the availability of carbonate ions for calcification and is therefore likely reduce growth rates (Kleypas et al. 1999; Kleypas and Langdon 2000) making juveniles more vulnerable to predation which is an important source of mortality

early in life (Penin et al. 2011). In addition to ocean acidification, ocean warming is also likely to affect coral growth. Small temperature rises are likely to increase growth rates (Coles 1985; Jokiel and Coles 1990), however, once temperature exceeds critical thresholds growth rates are likely to decline (De'ath et al. 2009). The effects of these stressors in synergy are, however, largely unexplored. Calcification of *Stylophora pistillata* colonies maintained at an elevated temperature declined by 50% in response to increased acidity (Reynaud et al. 2003), suggesting temperature has an additive effect on decalcification. Similarly, Anlauf et al. (2011) found temperature exacerbated the effects of ocean acidity on the early calcification of a brooding coral species *Porites panamensis*. However, the effects of these stressors both independently and in synergy have been largely unexplored in coral juveniles.

The objective of this study was to test whether growth and the patterns of skeletogenesis were affected by near future levels of ocean warming and acidification. **Hypothesis: elevated temperature and CO_2 will decrease the skeletal growth and morphology development of *A. tenuis* and *A. millepora* propagules.**

Materials and method

Larval culture and settlement

Adult colonies of *Acropora tenuis*, and *A. millepora* were collected a few days before they were expected to spawn from Magnetic Island (19° 9'S, 146° 50'E) and Orpheus Island (18° 35'S, 146° 29'E) in 2009 and 2010. Adult colonies were maintained in JCU outdoor aquaria until spawning. In 2009, colonies from Orpheus Island spawned on the 11th of December. In 2010, *A. tenuis* from Magnetic Island spawned on the night of 28th October,

and *A. millepora* colonies from Orpheus Island spawned on 30th November. In each experiment sperm and eggs from 4-6 colonies were cross-fertilized and cultured in the treatments (Oliver and Babcock 1992), with three replicate 3.5L jars in each treatment, modified to allow 0.2 μm filtered treated seawater to flow through at the rate of 1.5 – 2L per hour. Once the larvae were motile (6-7 days after fertilization) they were infected by zooxanthellae according to Baird et al. (2006). Ten larvae were removed haphazardly, mounted on a slide and viewed under blue-violet light using fluorescent microscope (Leica MZ FLIII). The average number of symbiont cells per larva was 8.7±5.

Larvae were settled onto sterile petri dishes when 6 days old. Two to three larvae were contained in a water droplet (4ml) together with a CCA chip (*Titanoderma* sp.) on the surface of the Petri-dishes. Planulae were allowed 24 hours to metamorphose after which remaining swimming planulae were removed. Planulae settled individually and in aggregations of numerous fused primary polyps. Individual settled and aggregations of spats were used for different analyses in this experiment. One to two Petri-dishes were collected every sampling period to ensure that 5- 10 juveniles were sampled on each collection.

Experimental manipulation of pCO2 and temperature.

Refer to methods section in Chapter 2 (for 2009 acidification experiments) and 3 (for 2010 acidification and warming experiments) for full description of methods

Effects of acidification on juvenile growth

The CO_2 tanks were prepared in a temperature control room (26°C-27°C). In 2009, four levels of ocean acidity treatments were used: ambient, near future, mid range and high range pCO_2, refer to Chapter 2 for actual estimated values of pH. In 2009, the experiment was conducted for 28 days. Five to ten samples were collected on day 1, 2, 3 and 7, and subsequent weekly collection was conducted up to day 28.

Tissue was removed by submerging samples in 10% NaCl solution for 24 hours after which spats were washed with tap water (a gentle stream for 5 seconds), distilled water (a gentle stream for 5 seconds), and then soaked in distilled water for 1 hour. The skeletons were then air-dried before microscopy. The maximum diameter of each spat was measured to the nearest unit with a graticule eyepiece. Representative specimens were photographed under a Nikon SMZ800 stereo-dissecting microscope with a Nikon Coolpix 4500 camera.

Effects of acidification and warming on juvenile growth

In 2010, two levels of CO_2 concentration (ambient 380 ppm and 750 ppm) and two levels of temperature exposure (ambient 27°C and +2°C) were selected as recommended by the EPOCA protocol (Barry 2010). The treatments were ambient (27° plus 380 ppm CO_2); high temp (29°C plus 380 ppm); high CO_2 (27°plus 750 ppm CO_2) and combination of high temperature and acidity (29°C plus 750ppm CO_2). The actual pCO_2 was recalculated using CO2SYS (Chapter 3, Table 3.1). Larvae were settled onto Petri dishes as described above. Five to ten samples were collected daily for 7 days. Spats were bleached, measured and photographed as described above.

Data analysis

A one-way analysis of covariance was used to test for mean differences in the growth (maximum diameter) of juveniles among the four levels of acidity with age in days used as the covariate. A two-way analysis of covariance was used to test for mean differences in the growth (maximum diameter) of juveniles among the four treatment combinations of heat and acidity with age in days used as the covariate. Each species was tested independently. Statistical analysis of variance was conducted using SPSS.

Results

Growth of recruits

Growth rates did not differ among juveniles exposed to the four levels of pCO_2 ($F_{3,243}$ = 1.792, p = 0.162, Fig. 5.1). Similarly, neither elevated CO_2 nor temperature affected the growth rate of either species, nor was there any interaction between these factors (Fig. 5.2A & B).

Patterns of skeletogenesis

Levels of pCO_2 equivalent to those projected to occur by the end of this century had no obvious effect on the patterns of skeletogenesis. Skeletogenesis began with the deposition of a basal plate with 12 basal ridges in a single cycle. On day 2-3, lateral processes were evident on the inner end of basal ridges in a single cycle. These processes developed into rods (or synapticulae) that grew perpendicular to the basal ridges and fused with adjacent synapticulae to form the corallite wall. The first fused rings of the corallite wall were evident after 3-4 days (Fig 5.3). After 7 days, the samples exhibited prominent laminar

septa, and a porous coenosteum. In contrast, there were some minor malformations of the first fused ring of corallite wall in both *A. millepora* and *A. tenuis* after 7 days in the high pCO_2 treatment, compared to the ambient, high temp and high T x CO_2 treatments (Fig 5.4; Fig. 5.5); however, these are unlikely to be ecologically important.

Discussion

Neither juvenile growth nor the patterns of skeletogenesis of two common species of coral were affected by a range of pCO_2 levels corresponding to the A2 scenario up to the end of the century (IPCC 2007). Similarly, neither growth nor patterns of skeletogenesis were affected by 2°C above ambient or pCO_2 levels of 650-750 ppm nor where these variables affected by these stressors in combination. We conclude that the juvenile growth and patterns of skeletogenesis are likely to be affected by near future levels of climate change.

The results are in contrast to many other studies (Table 1.1 and Table 1.2). This is perhaps because I used near future levels of pCO_2 and temperature (IPCC 2007). Most effects were found in experiments using the more extreme values (reviewed in Chapter 1).

Another possible reason is that we used juveniles with low densities of zooxanthellae. Anlauf et al. (2011) and Albright and Langdon (2011) both used species that have high densities of zooxanthellae at settlement (e.g. *P. cylindrical* with 3000 zooxanthellae Hirose and Hidaka 2006). Symbiotic organisms appear particularly vulnerable to stress (Baird et al. 2009; Yakovleva et al. 2009) and the low densities of zooxanthellae in the larvae may have lessened the chance of oxidative stress, particularly under increased temperature. Photosynthetic electron transport occurring within the zooxanthellae produces reactive oxygen species (ROS) when exposed to high temperature,

causing the photosystem II to overload even under normal light (Takahashi et al. 2004). Possible tissue damage beyond the zooxanthellae can occur as ROS were permeable though cell walls (Nakamura and van Woesik 2001), which may have explained the drastic responses from zooxanthellae larvae species (Chapter 1, Table 1.1).

In general, newly settled juveniles developing skeletons are particularly vulnerable to physical disturbance and acidosis, as seen in bivalves and crustacean juveniles with external skeleton structure (Arnold et al. 2009; Cigliano et al. 2010; Byrne et al.2011). Unlike bivalves or crustaceans, corals are protected by a tissue layer, which serves as a buffer. Rodolfo-Metalpa et al. (2010) demonstrated that exposed skeleton in *Cladocora cespitosa* and *Balanophyllia europaea* dissolved more than tissue covered areas on the same coral colony. Therefore, the anatomy of corals may pre-adapt them to cope with OA more effectively than some other organism.

In conclusion, these results suggest that growth rates and patterns of skeletogensis in juvenile corals are unlikely to be affected by near future levels of elevated pCO$_2$ or temperature, at least in species which do not possess symbionts in high densities at settlement.

Figure 5.1: Mean maximum diameter of *A. millepora* juvenile in four pCO_2 treatments ($\pm$SE) with n = 248. (Closed squares = ambient CO_2 concentration, open squares = near-future, closed circle = mid-range and open circles = high-range CO_2). See Table 2.1 for actual values of pH.
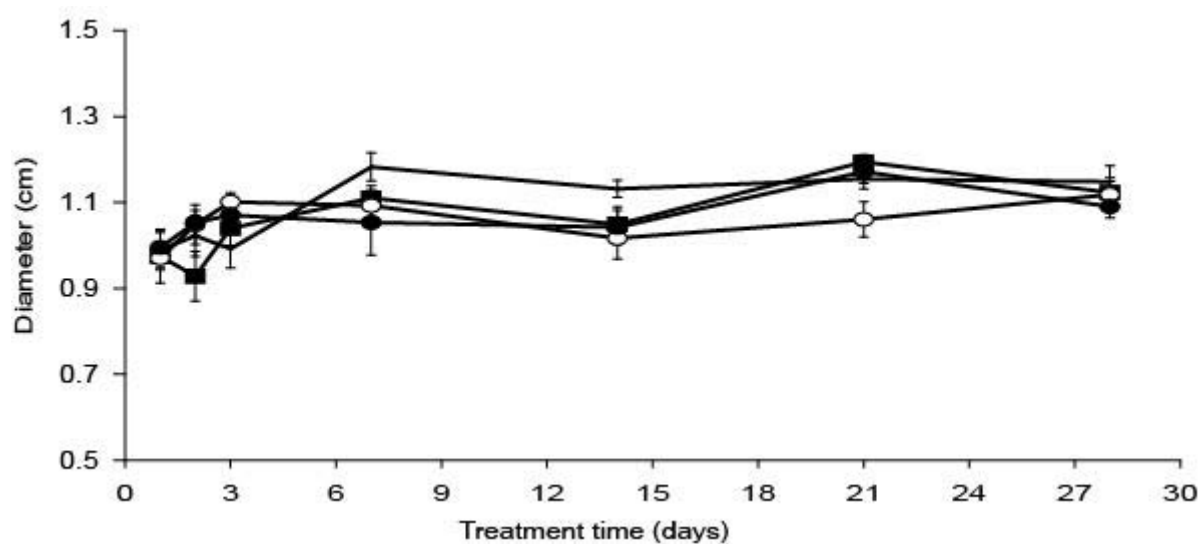
Figure 5.2: Average maximum diameter of *A. tenuis* (A; n= 153) and *A. millepora* (B; n= 184) juveniles in four combinations of pCO_2 and temperature through time in 2010. (Closed squares = Ambient temperature 27°C and ambient pCO_2 (446ppm), open squares = high temp (29°C, 446ppm), closed circles = high CO_2 (27°C and 675 ppm) and open circles = T x CO_2 (29°C and 710 ppm). See Table 3.1 for actual temperature and pCO_2 values.
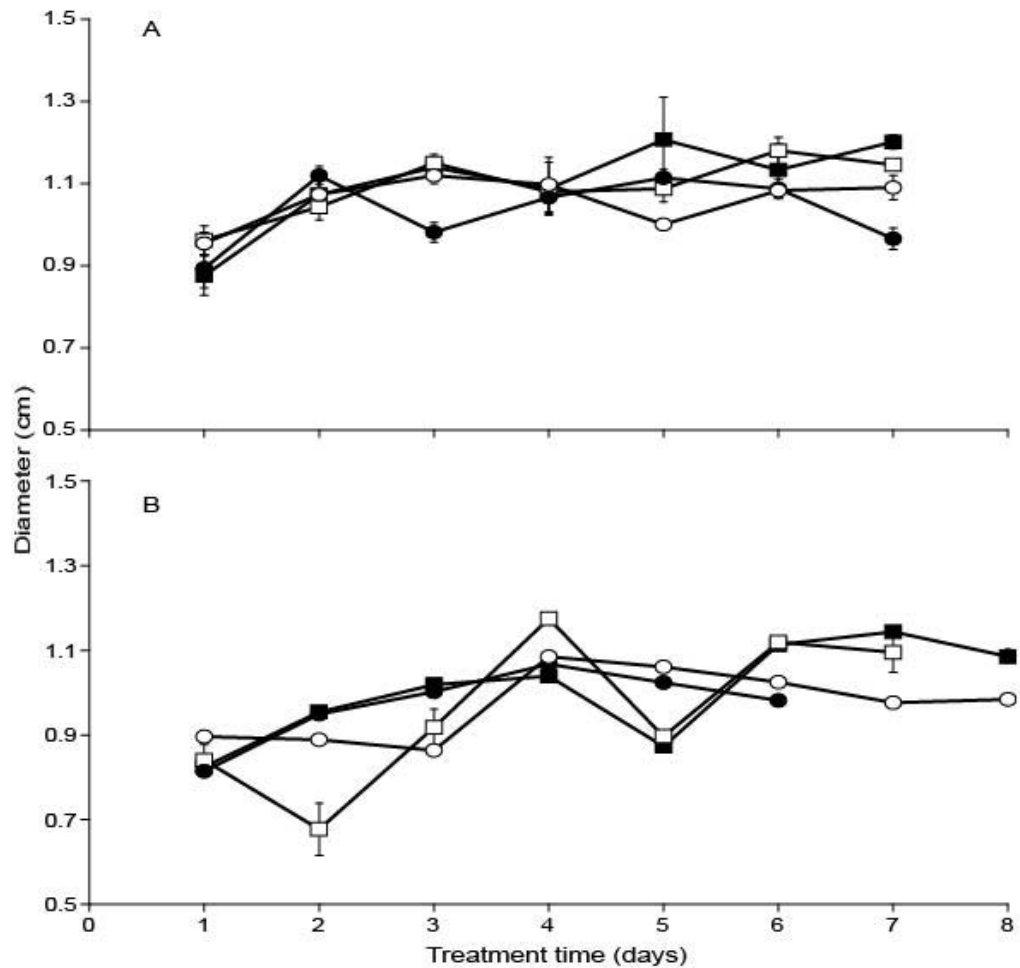
Figure 5.3: Stereo-micrographs of representative coral spats of *A. millepora* in four different pH treatments. Refer to Chapter 2, Table 2.1 for actual pH values.
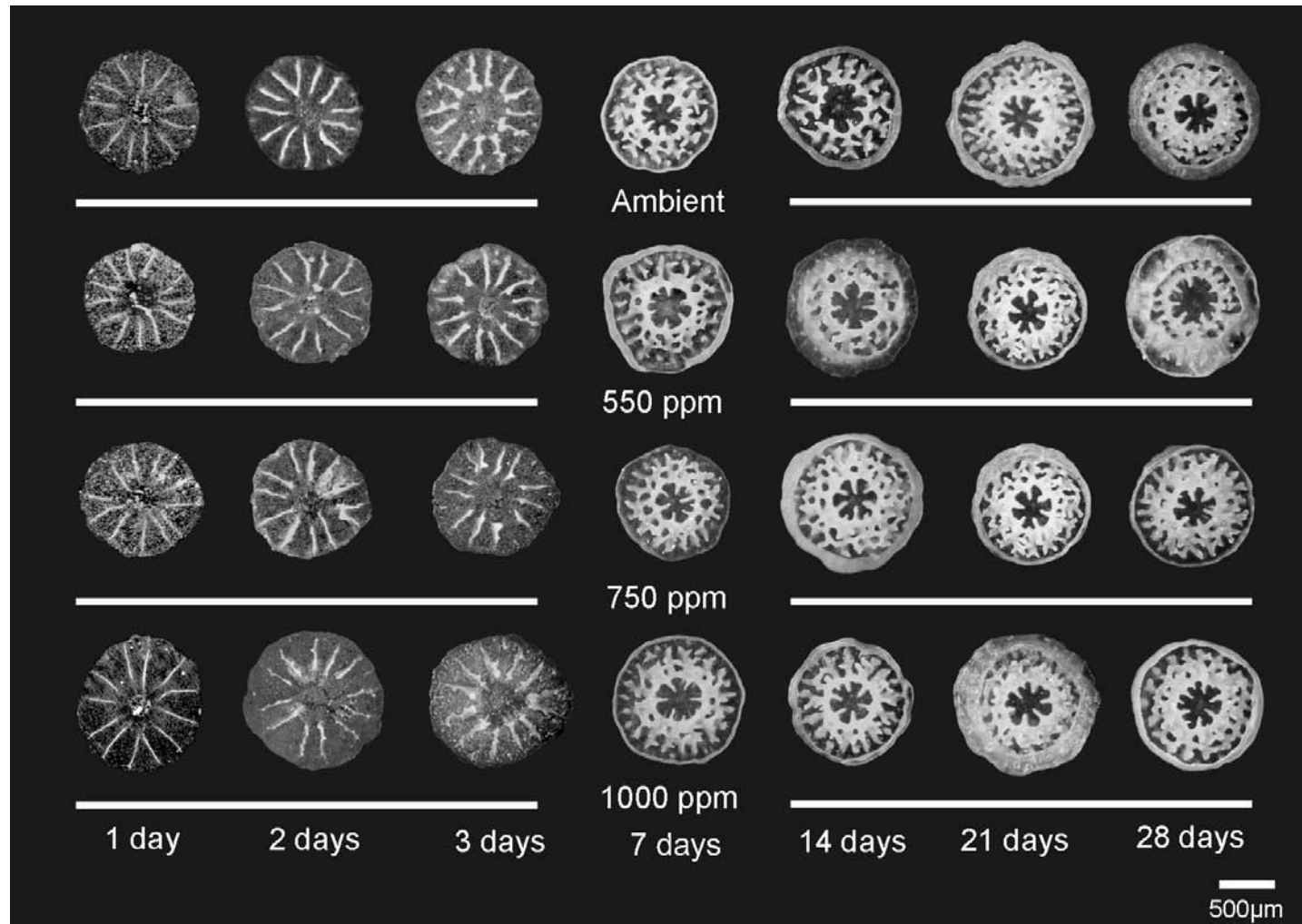
Figure 5.4: Stereo-micrographs of representative coral spats of *A. tenuis* in four different CO_2 and temperature treatments (ambient, High Temp, High CO_2 and T x CO_2). Refer to Chapter 3, Table 3.1 for actual temperature and pCO_2 values.
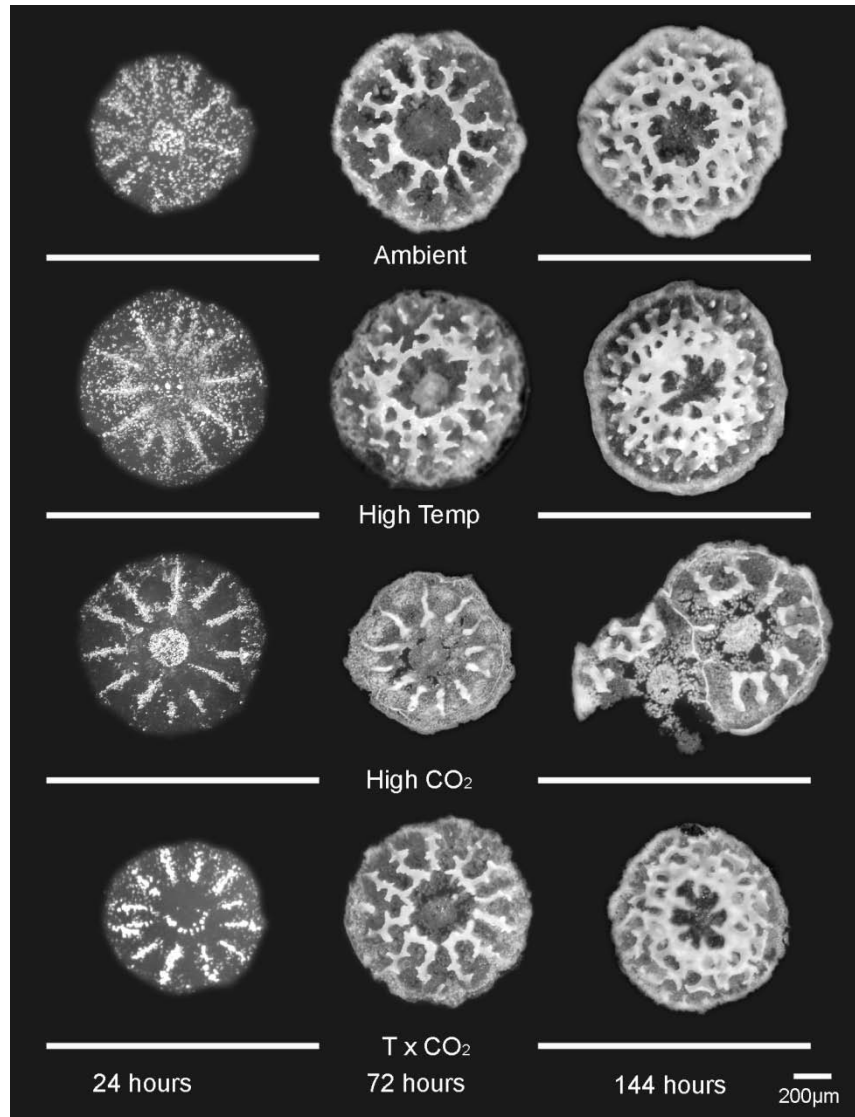
Figure 5.5: Stereo-micrographs of representative coral spats of *A. millepora* in four different elevated CO_2 and temperature treatments (ambient, High Temp, High CO_2 and T x CO_2). Refer to Chapter 3, Table 3.1 for actual temperature and $p$$CO_2$ values.
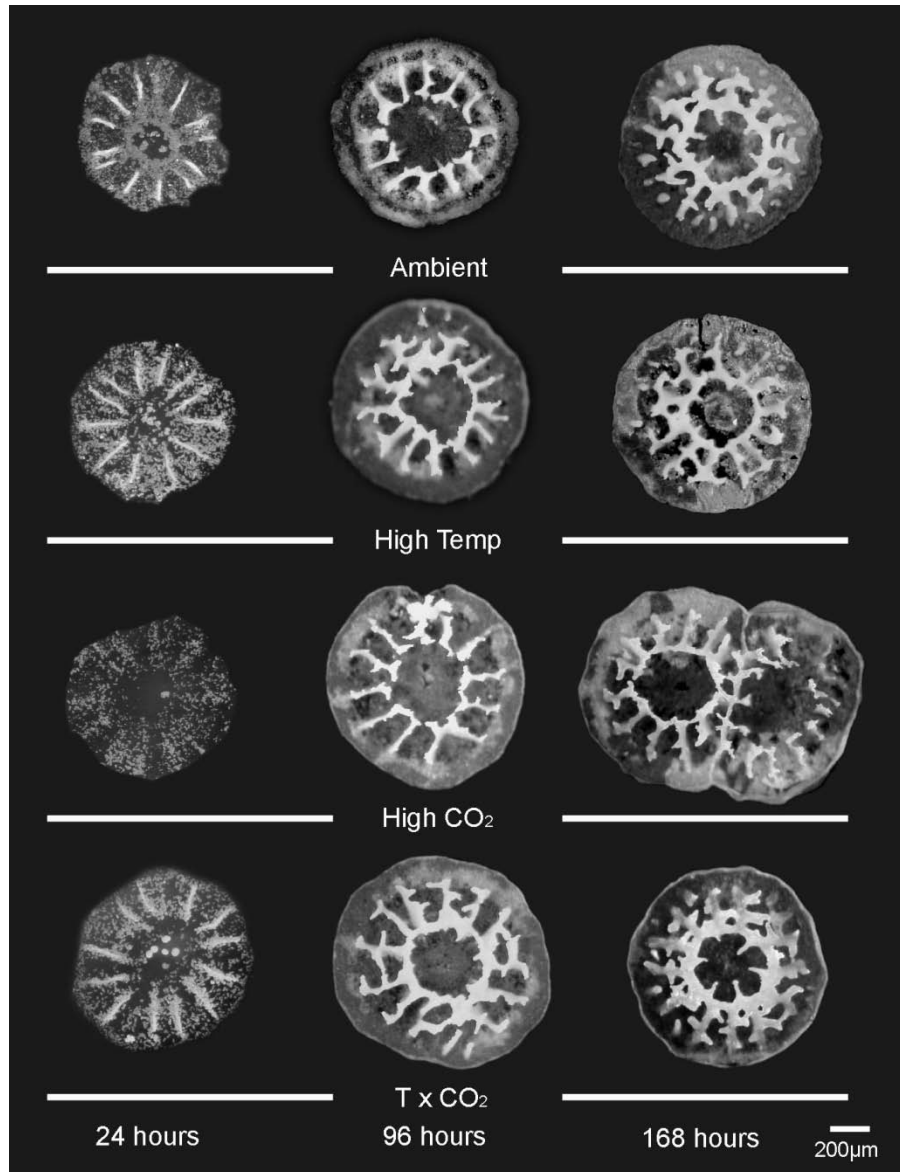
Chapter 6: General Discussion

The results of my thesis suggest that near-future levels of ocean acidification are unlikely to have major significant ecological effects on the early life history stages of corals. I detected no consistent effects of elevated CO_2 on fertilization success, embryonic development, larval survival, larval settlement or growth of juveniles (Chapter 2, 4 and 5). In contrast, ocean warming enhanced larval development (Chapter 3) and therefore may have an effect on patterns of dispersal by reducing the time for larvae to become competent. Here, I elaborate on these findings and suggest some ideas for future research.

This thesis has advanced our understanding of coral recruitment ecology, specifically on the effects of climate change related stressors. The results presented in my thesis were the first studies in Australia to look at the synergistic effects of ocean acidification and warming on the early life history stages of common and ecologically important acroporids. This study builds substantially on previous work (Table 1.2) with an intensive compilation of data *on A. tenuis, A. hyacinthus* and A. *millepora* (a summary of my results is presented in Table 5.1). When these studies are considered in total, they support my contention that ocean acidity does not represent a direct ecological threat to the early life history stages of corals. Effects of OA on early life history of corals are only apparent at unrealistically high levels of pCO$_2$ or when gametes or larvae are weak. The only consistent result in the literature is a reduction of coral larval metamorphosis on settlement substratum pre-conditioned in an acidified treatment. However, even here no plausible mechanism has been advanced.

I found no significant effects of OA and warming on the early life history stages of two spawning acroporids, prior to settlement (as outlined in Chapters 2 and 3). This chapter

highlights the possibility of maternal influence on the quality of offspring; in particular, withstanding climate related stressors early in their life cycle. The robustness of acroporids' fertilization was further affirmed in Chapters 2 and 3. Maternal factors fulfil an important role in the survival and success of offspring (Marshall 2008, Marshall et al 2008). For instance, the environment where the adult was cultured induced persistent carry-on maternal effects on the offspring (Sanford and Kelly 2011). Sydney Rock Oyster (*Saccostrea glomerata*) adults subjected to elevated pCO$_2$ during gametogenesis have produced positive carry-over effects on larvae, with larvae from pre-exposed adults being less affected in elevated CO$_2$ conditions (Parker et al. 2012). Similarly, byrozoan (*Bugula neritina)* adults were pre-conditioned in multiple elevated copper concentrations prior to brooding, and the larvae produced were larger, more dispersive and more stress tolerant (Marshall 2008). However, maternal exposure history may also have pervasive, negative effects on the post-metamorphic performance (particularly survival) of offspring: offspring from toxicant-exposed mothers had poorer performance after six weeks in the field, especially when faced with high levels of intraspecific competition (Marshall 2008). Hence, maternal experience can have complex effects on offspring phenotype, enhancing performance in one life-history stage while decreasing performance in another (Marshall and Morgan 2011). Therefore, I suggest that future studies explore the effect of OA on gametogenesis of corals, to examine the effects of ocean acidification throughout early life history.

Another alternative for contrasting results between my own and other's work, particularly with respect to juvenile growth, was that previous experiments have examined species that differ in terms of physiology and biology. The major difference being that I

used the larvae of broadcast spawners that lack maternally provided zooxanthellae, whereas most other experiments have used larvae with maternally provided zooxanthellae (Table 1.2 and Table 1.3). While I did infect my larvae with symbionts prior to settlement, the densities were low (Chapter 5). The lack of zooxanthellae in the coral larvae is a plausible beneficial factor, at least early in life. The presence of zooxanthellae significantly affects survival of *A. intermedia* and *A. muricata* larvae exposed to thermal stress, suggesting that symbionts are a severe burden to symbiotic coral larvae under heat stress (Baird et al 2006; Yakovleva et al 2009). In future, it would be substantial to assess more directly whether symbionts may also predispose coral larvae to OA.

In conclusion, understanding the vulnerability and robustness of earlier developmental stages is fundamental to the persistence of tropical coral populations and crucial in relation to the varied responses of species in the suite of future acidity and temperature elevation scenarios. The varied response indicates a possible phase shift of community with the change of species composition under predicted climate change scenarios. For example, from hard coral dominant reef, to algae or sea grass dominated (Fabricius et al. 2011, in high CO_2 volcanic vents) or sponges dominated habitat (Rutzler 2002, following a decrease of live coral in Belize). Repeating this experiment, combined with more potential stress factors (e.g. anthropogenic activities), is required to make reliable predictions about the future of entire coral communities and reefs in the Great Barrier Reef. Rapid increases in the atmospheric $p\mathrm{CO_2}$ level in conjunction with human exploitation have led the reef to a state of constant stress. It is crucial to incorporate anthropogenic activities in future research, as it will give a more realistic representation of

the A2 scenario in conjunction with capital growth and it will provide a more strategic

management approach, particularly in countries that rely heavily on marine resources.

Table 6.1: A summary of the results compiled in this thesis. The effects of ocean acidification and warming on 3 species of acroporids were investigated. Plus signs (+) denote increase, minus signs (-) denote decrease, compared to control values.

		Elevated CO$_2$ (pH)			Acidity (pH)	Warming (°C)	acidity and warming
	year	2009			2010	2010	2010
	temperature					+ 2 °C	+ 2 °C
	pCO$_2$ (ppm)	550	750	1000	750		750
Responses variable	**species**						
Fertilization							
	Acropora tenuis	NS	NS	NS	NS	NS	NS
	Acropora hyacinthus	NS	NS	NS			
	Acropora millepora	NS	NS	NS	NS	NS	NS
Development (hours)							
	Acropora tenuis	NS	NS	- 4h		- 1h	- 2h
	Acropora hyacinthus	NS	NS	+ 2h			
	Acropora millepora	NS	NS	NS	NS	- 2h	- 2h
Motility (hours)							
	Acropora tenuis				+ 9h	-19h	- 29h
	Acropora millepora				no consistent effect	no consistent effect	no consistent effect
Planulae survival							
	Acropora tenuis	NS	NS	NS		NS	NS
	Acropora hyacinthus	NS	NS	NS			
	Acropora millepora	NS	NS	NS		- 80%	- 80%
Metamorphosis							
	Acropora tenuis	NS	NS	NS	no consistent effect	no consistent effect	no consistent effect
	Acropora millepora	NS	+ 17%	+ 19%	no consistent effect	no consistent effect	no consistent effect
Post-settlement growth							
	Acropora tenuis	NS	NS	NS	NS	NS	NS
	Acropora millepora	NS	NS	NS	NS	NS	NS

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Appendix

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