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**A correlation between larval size and spat growth and development in coral species *Porites astreoides*: An approach to understanding the effects of Ocean Acidification on calcifying organisms**

By
Kascia Q. White

A Thesis Submitted to
Saint Mary's University, Halifax, Nova Scotia
in Partial Fulfillment of the Requirements for
a Bachelor of Science, Honors Biology

March, 2014, Halifax, Nova Scotia



Approved: Dr. Ron Russell
Internal Supervisor

Approved: Dr. Colleen Barber
Reader

Date: April 7, 2014

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**A correlation between larval size and spat growth and development in coral species *Porites astreoides*: An approach to understanding the effects of Ocean Acidification on calcifying organisms**

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## Abstract

Ocean acidification poses an extreme threat to coral colony survival. The rise of carbon dioxide in atmospheric pressure decreases the seawater carbonate ion concentration resulting in lower rates of calcium carbonate deposition in coral skeletons. If this rise continues, rates of coral erosion will exceed coral accretion, causing coral reef systems to significantly decline. Coral calcification rates could decline by 20% to 80% of modern values by the end of this century. *Porites astreoides* from Bermuda's Rim Reef release larger planulae than *P. astreoides* from Bermuda's Patch reefs, so adult P. *astreoides* colonies were collected from two Patch Reef sites and two Rim Reef sites in Bermuda. Planulae were settled in three target $pCO_2$ levels (420, 1200, and 1670 ppmv) and reared in experimental conditions for two weeks. My objective was to determine whether there is a species-specific differential response to $CO_2$. I am focusing on answering whether 1) larval sizes across the north lagoon differ, and 2) larvae differ in their response to $CO_2$ variations. I predict that if Rim Reef larvae are larger than Patch reef larvae of the same species, they will be more resistant to ocean acidification effects. Rim planulae were significantly larger. Rim and Patch corals differed significantly for both total calcification and settlement proportions, with higher rates of calcification under ambient $CO_2$ conditions, but no significant difference among $CO_2$ conditions in settlement proportions. This study provides suggestive evidence to support the hypothesis that increased larval size leads to increased resistance to enriched $CO_2$ in *P. astreoides* recruits.

April 7, 2014

# CHAPTER 1 **INTRODUCTION**

## 1.1 Introduction

Since life began on earth, changes in the global climate have affected the interactions between various organisms as well as their distribution. However, anthropogenic increases in atmospheric concentrations of greenhouse gases have caused much more rapid changes in the earth's climate than have been experienced for millennia. It is predicted that these elevated rates of change will result in local if not total extinction of some species, changes in species interactions with one another due to alterations of species distribution, and variations in the flow of energy and cycling of materials within ecosystems (Kennedy et al. 2002).

These changes in global climate are causing a dramatic decline among one of the most dynamic, diverse, and productive ecosystems on earth: coral reefs (Sorokin 1993, Birkeland 1997). Though this ecosystem makes up less than 0.2 % of the oceans, coral reefs are home to almost one-third of all described marine fish species (Bryant *et al*. 1998; Moberg and Folke 1999), as well as four thousand species of reef fish and 800 species of hermatypic (reef-building) corals (Bryant *et al*. 1998; Moberg and Folke 1999). There are up to 60,000 described reef animals and plants and it is estimated that one- to nine-million reef species exist (Bryant *et al*. 1998; Moberg and Folke 1999). Coral reefs provide habitat, food and breeding grounds for countless marine species (Bryant *et al*. 1998; Cohen and Holcomb 2009).

This ecosystem is integral to the life of so many marine species; however, coral reefs are in global decline (Pandolfi, *et al.* 2003). While reefs are resilient when faced

with periodic disturbances such as hurricanes or predator outbreaks, they seem to exhibit less of this resilience with persistent anthropogenic disturbances such as overfishing, eutrophication and climate change (Bellwood *et al.* 2004; Moberg and Folke 1999). No unaffected reefs remain; 30% of reefs are already severely degraded and 60% may be lost by 2030 (Hughes *et al.* 2003, Pandolfi *et al*., 2003). As a form of adaptation to climate change and other human induced changes, many reefs, especially in the Caribbean, have shifted from coral-dominated to macroalgal-dominated systems, reducing their structural complexity and diversity (Aronson and Precht 2000; Bellwood *et al.* 2004; Hughes 1994; Lapointe *et al.* 2004; McCook 1999; Rasher and Hay 2010). Overfishing and eutrophication caused most reef systems to degrade before 1900, while the past three decades have seen extensive bleaching, disease and coral mortality partially due to the effects of climate change (Pandolfi, *et al*., 2003, 2005). Many factors contributing to coral decline are local, but threats to coral reef systems such as increasing $CO_2$ concentrations are progressively becoming a global issue. Consequently, sea surface temperatures are elevated while oceanic pH is decreased. (Cohen and Holcomb 2009). Thus, it is imperative that we better understand coral responses to climate change if we are to conserve reef ecosystems.

## 1.2 Coral Biology and Physiology

Though often referred to as a single coral, hermatypic corals are made up of hundreds of individual coral polyps. These tiny polyps are connected by a shared underlying gastrovascular cavity (Figure 1). The tissue of each coral polyp contains two dermal cell layers, the external epidermis and the internal gastrodermis, these two layers

are separated by the mesoglea, a thin layer of connective tissue. Like other cnidarians, coral polyps also contain a mouth surrounded by a ring of tentacles. Beneath the polyp tissue, the calicoblastic epidermis lies against the accreted calcium carbonate skeleton that provides a structural foundation for the polyps (Muller-Parker and D'Elia 1997). Endosymbiotic algae in the family *Symbiodinium*, often referred to as zooxanthellae, live within the gastrodermal tissue of hermatypic corals. Mixotrophic and hermatypic corals rely on zooxanthellae photosynthate, ingestion of organisms ranging from bacteria to mesozooplankton, and absorption of inorganic nutrients such as nitrogen and phosphorous for survival (Houlbreque and Ferrier-Pages, 2009).

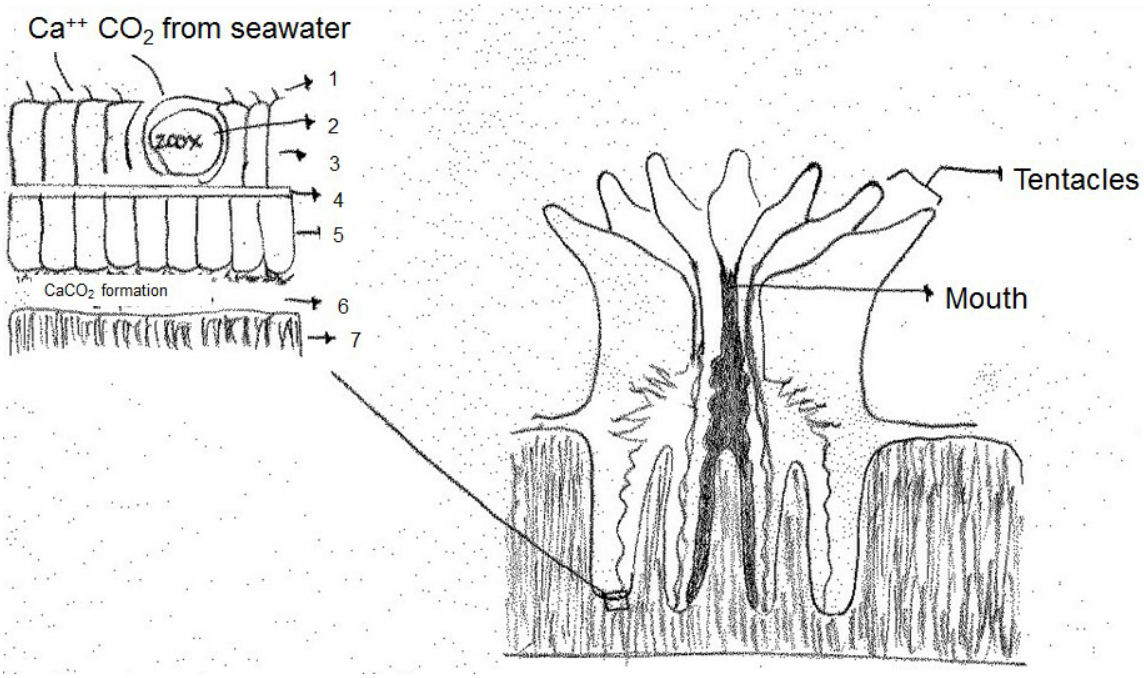


**Figure 1: The anatomy of a coral polyp, including the seawater in the gastrovascular cavity (1), the gastrodermis (2), symbiotic zooxanthellae in the gastrodermis (3), the mesoglea (4), the epidermis (5), the sub-epithelial space (6), and the aragonite skeleton (7). Figure from Baker 2003.**

Corals can reproduce both asexually by budding or fragmentation and sexually through the fertilization of eggs and sperm (Richmond, 1997). There are two kinds of sexual reproduction and spawning in hermatypic coral species: broadcasting, in which corals synchronously release eggs and sperm with fertilization occurring in the water column, and brooding reproduction where fertilization occurs inside the coral polyp. After fertilization, the coral larvae, called planulae, swim freely in the water column. The period of which the planulae remain in the water column ranges among species, with pelagic swimming lasting from hours to weeks. Once ready to begin a benthic lifestyle, the planulae then settle onto the reef substrate and undergo metamorphosis, at which time the larvae change from the initial pelagic planktonic phase to the final benthic polyp stage. Once this process has occurred, the polyp begins calcification (Richmond 1997, Harrison et al. 2011).

## 1.3 Calcification in Coral Recruits

As with many ecosystems, reproduction is the backbone of the coral reef ecosystem. Studying the calcification in young coral recruits provides an opportunity to examine how the mechanism of calcification works and how different environmental conditions may affect it. Semi-metamorphosed or newly settled corals have no calcium carbonate skeleton. Therefore, any skeletal growth that occurs throughout this study will occur within an experimental framework and does so only under varying $pCO_2$ conditions. In addition, early calcification of coral recruits is extremely fast, which allows for a shorter experimental period for studying calcification of these recruits (Maida et al. 1994). Limitations in our understanding of recruitment become especially detrimental as

we seek to understand long-term reef population dynamics and community structure. This knowledge is vital if we are to recognize the response of coral reef recruitment to predicted changes in climate variability (Richmond 1997, Albright et al. 2008).

## 1.4 Carbonate Chemistry

Over the past 250 years atmospheric $CO_2$ concentration has increased from 280 parts per million by volume (ppmv) to greater than 385 ppmv due to anthropogenic modifications (IPCC WG 1. 2007). Ice core data indicate that this level exceeds the natural range of atmospheric $CO_2$ (180-300 ppmv) from the past 650,000 years (IPCC WG 1. 2007). In addition, atmospheric $CO_2$ is predicted to double pre-industrial levels by the year 2065 (Kleypas *et al.* 1999). The ocean is the largest $CO_2$ sink, absorbing approximately 30% of all $CO_2$ emitted from anthropogenic sources. Therefore, as atmospheric $CO_2$ has continuously risen, so has oceanic $CO_2$. This rise causes the proportion of $CO_2$ absorbed by the ocean to simultaneously rise as emissions rise (Sabine *et al*. 2004, 2011). Because of this phenomenon, corals are adversely affected as the absorbed $CO_2$ reacts chemically with seawater and reduces the oceanic $CO_3^{2-}$ concentration. Though the precise mechanism of coral calcification is unknown, studies have demonstrated that corals depend on high concentrations of $CO_3^{2-}$ for the formation of their aragonite skeleton (Cohen and Holcomb, 2009). Increases in $CO_2$ reduce the pH of seawater in a process known as ocean acidification. Anthropogenic ocean acidification is being exacerbated by the process of hydrolysis of $CO_2$ that occurs in the seawater when hydrogen ion concentrations $[H^+]$ increase. Over the past

century, oceanic pH has already decreased 0.1 units (IPCC WG 1. 2007). As atmospheric $CO_2$ concentrations increase, seawater pH is expected to decrease 0.3 units by 2100 (Kleypas *et al.* 1999; Blackford and Gilbert 2007**)**. A decrease in the concentration of $CO_3^{2-}$ results in a decrease in the saturation state of seawater with respect to $CaCO_3$.

## 1.5 Ocean Acidification

Most studies on coral and ocean acidification have focused on the response of adult corals, but there are a few studies on the effects of ocean acidification on recruitment and early growth. De Putron *et al*. (2010) investigated the calcification of new recruits of the Golf-ball coral, *Favia fragum* and Mustard-Hill coral, *Porites astreoides*. Two experiments were conducted, one in which they manipulated aragonite saturation state by addition of HCl at constant $pCO_2$ (this method decreases both $[HCO_3^-]$ and $[CO_3^{2-}]$ and one in which they manipulated aragonite saturation state by $pCO_2$ enrichment at constant alkalinity (this method increases $[HCO_3^-]$ and decreases $[CO_3^{2-}]$. They found that both species showed a negative response to decreased $CO_3^{2-}$, regardless of method, and concluded that $[HCO_3^-]$ does not impact skeletal growth *Porites astreoides*.

## 1.6 Experimental Background and Objectives

Conducting an experiment using planulae and new recruits of a brooding species provides the opportunity to examine environmentally-based population differences in a

controlled lab experiment. Working with planulae and new recruits is also advantageous in that all skeletal components are deposited within the experimental framework and there is no need to control for pre-experimental calcification. Lastly, the use of planulae and new recruits allows for the examination of the effect of ocean acidification on recruitment and an early life stage, a topic rarely addressed in the literature. However, it is essential for an understanding of reef resilience, which can provide a greater understanding of the differential parental investment on coral resilience.

The island of Bermuda is comprised of three distinct reef zones, two of which are the focus of this study. The first is the off-shore Rim reef zone, a shallow, 2 to 6 m depth coral reef region that encircles the 775 $km^2$ Bermuda platform (Figure 2). The second zone is the enclosed shallow Patch reef lagoon found just inshore of the Rim reef, which harbors numerous isolated, roughly circular, patch reefs, commonly at a depth of 1 to 4 m. The other reef zone is the terrace reef that extends toward the sea from the rim reef, gradually sloping away from the platform with an average depth of 18 m. These reef systems undergo various levels of sediment loading, light regime, and wave energy. The sediment load is highest at the inshore Patch reefs that are within the lagoon and decreases offshore. Conversely, wave energy is significantly decreased within the sheltered lagoon of Patch reefs while peak intensity is reached at the Rim reef. The high sediment loads and low wave energy found at the Patch reef favors the development of increased coverage of soft corals and other reef invertebrates, and inorganic material. In contrast, the Rim reef favors higher coverage of hard coral with a decline in coverage of soft coral, reef invertebrates and inorganic material (Wood and Jackson, 2005).

Dr. Samantha de Putron Bermuda Institute of Ocean Science (BIOS) observed

that *P. astreoides* planulae released from Rim Reef parental colonies are larger than those released from Patch Reef parental colonies (unpublished data). Larval size increases the ability to withstand increases in $CO_2$ (Kurihara, 2008). By understanding the variability in which corals respond to ocean acidification, we can better predict the biological consequences of anthropogenic induced $CO_2$ over the next few decades. The objectives of this study are to determine whether larval size and lipid content can be used as a measure of energetic status and coral resilience. By examining two reef platforms with different larvae sizes within the same species (*Porites astreoides*), I predict that larger larval size confers greater resilience; measured by total calcification, zooxanthellae density and lipid content, to ocean acidification in the Atlantic coral *Porites astreoides*.

The questions, then, are whether there will be a differential response to $CO_2$ and whether this is species specific. This experiment seeks to determine the inter-site variation of *Porites astreoides* measuring coral response and fitness (ability to settle and grow) to various $CO_2$ conditions. Focusing on answering two questions: to confirm whether larval sizes across the north lagoon differ? And, to confirm if these larvae differ in their response to $CO_2$ variations? It can therefore be hypothesized that planulae collected from Rim reef parental colonies will be larger than planulae collected from Patch reef parental colonies. It is then expected that Rim reef planulae will exhibit elevated lipid content and higher zooxanthellae counts. It can also be hypothesized that settlement will decrease under enriched $CO_2$ conditions. Due to their larger size, it can be predicted that the effects of enriched $CO_2$ will be less severe for Rim planulae. Lastly, it is hypothesized that total calcification (measured by corallite weight/area) of both Rim and Patch polyps will decrease under enriched $CO_2$ conditions. Because calcification is

an energetically expensive process, the increased area of Rim planulae will cause the effects of enriched $CO_2$ to be less severe for Rim polyps.

# CHAPTER 2 **MATERIALS AND METHODS**

To determine the independent and interactive effects that ocean acidification has on larval size and spat growth, a two-week experiment was conducted using juvenile *Porites astreoides* coral recruits that were collected from both Patch and Rim reef parental colonies in an experiment using manipulations of three $pCO_2$ variations. Before the planulae were put into experimental conditions the planulae area was measured using ImageJ v.1.45 (Rasband, 2014) software in order to verify size differences between the planulae from the two sites. Settlement was determined after a 48 hour (2 day) period after the planulae were introduced into the experimental tanks. After two weeks, the number of surviving polyps was determined and measured for coralite (skeleton) weight, lipid content and zooxanthellae density in order to determine the effects ocean acidification has on the larval size and spat (a settled planulae beginning to form its calcium carbonate skeleton) growth of juvenile *Porites astreoides*.

## 2.1 Experimental Design

Triplicate sets of three glass 20L aquaria were set up and filled with filtered seawater (filtered with 50µM, then 5µM filters in succession) in two fresh water baths. Each water bath had six aquaria consisting of two replicates of each $pCO_2$ condition (380-420 ambient, 1200 medium, 1670 high) positioned randomly (Figure 2). The polyps were fed every five days with approximately 5mL brine shrimp per aquaria and the aquaria were maintained on a 12- hour light/dark cycle with three fluorescent aquarium lights: mean ± SD 34.5 ±12.5 lmol m-2 s-1. (AquaSun 10,000K T5 Fluorescent

Aquarium Lamps by UV Lighting Co.) above each water bath. The water bath temperatures were maintained through a combination of a chiller-pump system and aquarium heaters and were monitored with Hobo submersible temperature loggers (Hoboware Pro V2, Onset Corp., Bourne, USA) that measured temperature every 30 minutes. The average seawater temperatures for the 2-week period were: 27.3 °C ± 0.02 (mean ± SD).

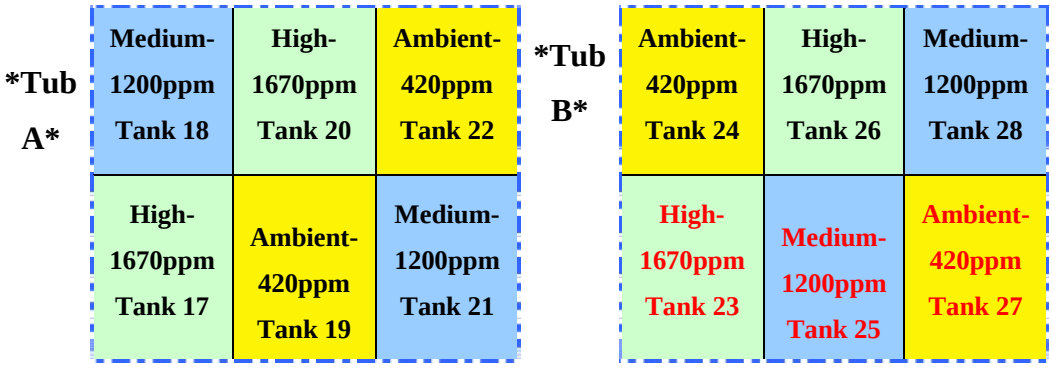


**Figure 2: The experimental set-up consisted of two water baths each with six aquaria ranging from $pCO_2$ values of approximately 490ppmv – 1670ppmv. Both Patch and Rim planulae were settled in each aquarium. There are nine experimental tanks and three holding tanks. *Tub A contains 6 experimental tanks *Tub B contains 3 experimental and 3 holding tanks**

To influence the amount of calcium carbonate skeleton secreted, $pCO_2$ was manipulated. Dr. Dan McCorkle (Woods Hole Oceanography Institute- WHOI) set up a system through which an air compressor pressurized ambient air and then mass flow controllers mixed this air with compressed $CO_2$ in order to achieve the three target $pCO_2$ levels (420, 1200, and 1670 ppmv). These $pCO_2$ levels were chosen to span a range of aragonite saturation states, including one undersaturated state (high $pCO_2$). Air at these three $pCO_2$ levels was then bubbled into each aquarium via a micropore wand for the duration of the experiment. Water changes were conducted weekly to prevent the buildup

of dissolved inorganic nitrogen and other wastes. During water changes, corals were placed into their replicate aquaria until the new seawater equilibrated to the appropriate temperature and $CO_2$ levels. Before each water change, discrete water samples from each aquaria were taken to measure salinity, dissolved inorganic carbon (DIC) and alkalinity (Alk). Salinity samples were analyzed at Bermuda Institute of Ocean Science (BIOS) using an Autosal salinometer; they had a mean of 35.63 ± 0.22 psu (mean ± SD) (n = 115). Alk/DIC samples were poisoned with mercuric chloride and subsequently analyzed by Dr. McCorkle using a Marianda VINDTA-3C analysis system at WHOI. Table 1 lists the average temperature, salinity, pH and $pCO_2$ for the three treatments.

**Table 1**: **Seawater chemistry and temperature for the three $pCO_2$ treatments. Values are recorded as mean ± SD**

| Treatment | Temperature (°C) | Salinity (psu) | pH | $pCO_2$ (ppmv) |
|---|---|---|---|---|
| **Ambient** | 27.3 ± 0.30 | 35.56 ± 1.68 | 8.18 ± 0.05 | 424.6 ± 9.66 |
| **Mid** | 27.3 ± 0.28 | 35.73 ± 1.34 | 7.80 ± 0.06 | 1252.5 ± 11.54 |
| **High** | 27.3 ± 0.33 | 35.59 ± 1.26 | 7.65 ± 0.05 | 1782.7 ± 14.24 |

## 2.2 Laraval Collection and Settlement

A total of 30 (15 from two sites at each of the two reef systems) mature colonies of the brooding scleractinian (stony) coral *P. astreoides* were collected in July 2012 from offshore rim reefs 32°27’453” N, 64°50’098” W and inshore patch reefs 32°22’323” N, 64°44’518” W sites in Bermuda (Figure 3) a few days prior to their predicted time of larval release (according to the lunar cycle, de Putron 2003). PVC

pipe rings with a 15cm diameter were used to locate mature coral colonies that would likely spawn. The specimens were transferred to BIOS in coolers filled with seawater and then transferred to an aquaria on the BIOS wet bench. The aquaria were maintained at ambient conditions (27.5 °C ± 0.4 (mean ± SD)), light pollution was reduced at night via the use of heavy blinds) and were randomly assorted by site to control for any temperature or light variation along the wet bench. Corals were kept at the BIOS in outdoor flow-through seawater aquaria under ambient temperature and light conditions. During nights of predicted planulae release, the corals were kept in mesh bags that allowed for seawater flow-through (Figure 4). The bags allowed for the planulae to be collected while still completely submergered.

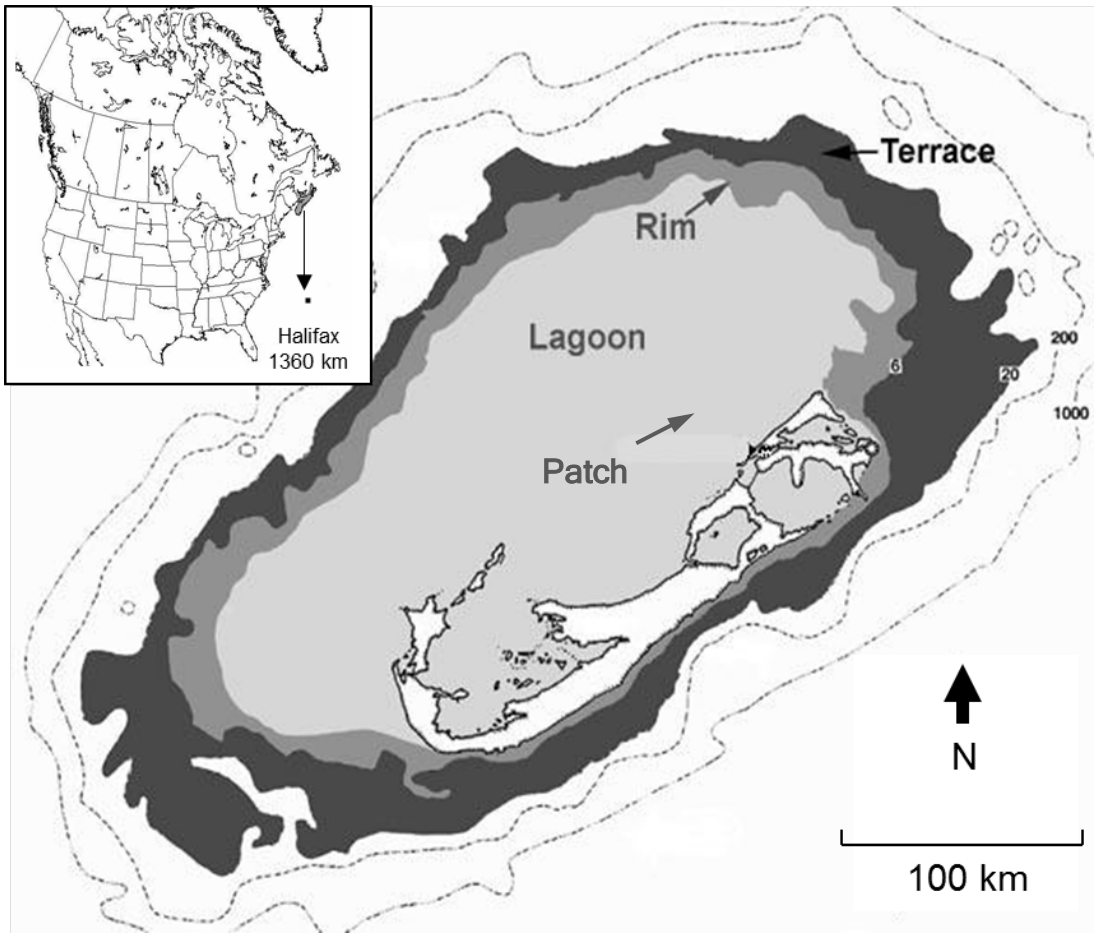


**Figure 3: The Bermuda Platform, including the Bermuda Islands and reefs. *P. astreoides* colonies were collected from Patch Reef and Rim Reef. Planulae were settled on tiles preconditioned in Whalebone Bay. The experiments were conducted at BIOS.**

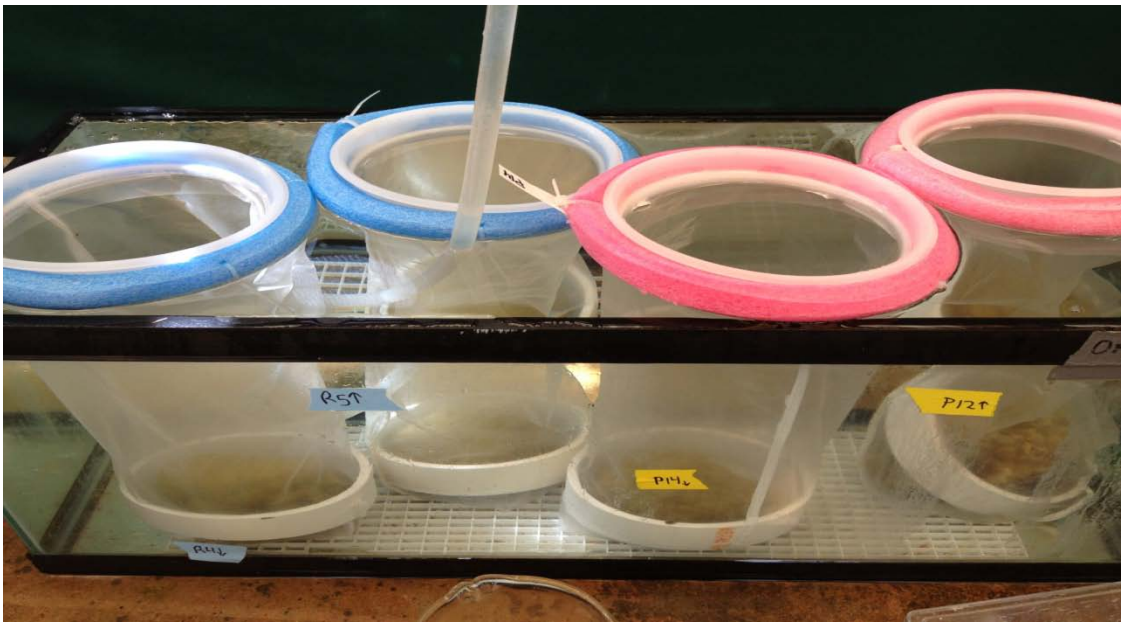

**Figure 4: *P. astreoides* colonies kept in fine mesh bags on the BIOS wet bench. The mesh bags had floatation devices around the rim to assure that planulae released during each night of spawning could be collected efficiently.**

Two to four weeks prior to the start of the experiment, tiles were preconditioned in racks on a nearby reef to develop an algal biofilm suitable to induce larval settlement (de Putron et al. 2010). Approximately 50-70 planulae were settled on two preconditioned terracotta tiles (~ 4cm x 4cm x 1cm) in small plastic containers (~0.5L) of seawater at the $pCO_2$ condition of each experimental aquarium. Each container was covered with 100 μM mesh lid so that seawater could pass through the containers freely without allowing planulae to escape. Corals were kept without lights for the 48-hour settlement period. Additionally, crushed crustose coralline algae were placed on the tiles, which acts as a larval settlement cue (Negri et al. 2001, Harrington et al. 2004). After 48 hours, each settlement tile was checked individually for settlement of metamorphosed primary polyps under a microscope. The number of metamorphosed polyps on each tile (Figure 5) was recorded as the settlement number for each tile. Tiles were then

transferred to the experimental aquaria were they remained until the experiment concluded. After spawning was completed, parental colonies were replaced on their respective reefs using underwater cement (de Putron 2003).

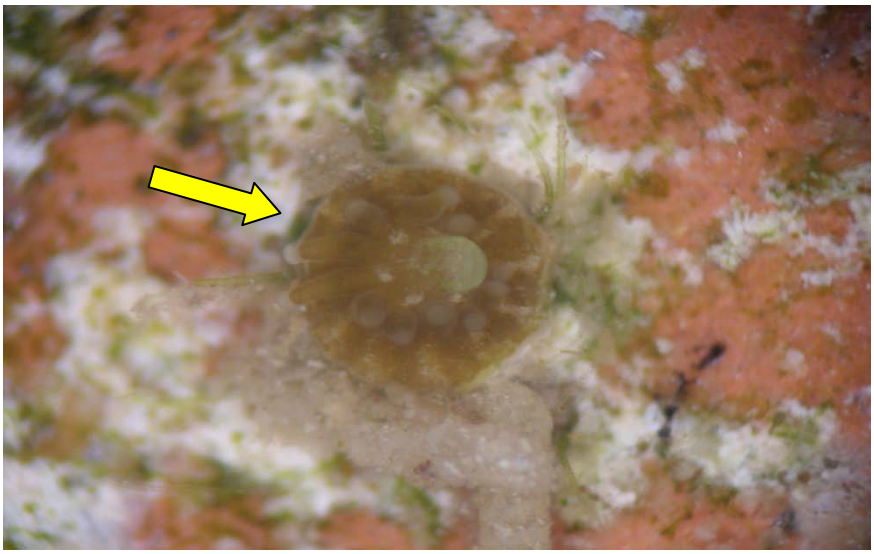

**Figure 5**: ***P. astreoides*** **metamorphosed coral polyp after 48hr settlement on terracotta tile.**

## 2.3 Planulae Area

Ten planulae from both Rim and Patch reef sites were photographed each day using and Olympus digital camera with a scopetronix microscope adapter. To create a reference for measurement a standard 12 inch ruler was also photographed under the microscope. Using ImageJ v.1.45, the scale was set using the photographed ruler. Each planula was digitally measured by obtaining length and width and multiplying the length by width by 0.8 to obtain the estimated area of each planula.

## 2.4 Skeletal Analysis

Skeletal weight provides a quantitative measure of the amount of calcium carbonate accreted under various experimental conditions. At the end of the two-week experiment, approximately 50 two-week old polyps in each of the three $CO_2$ conditions for each reef system (Rim and Patch reef) were sacrificed by placing the tiles in a 10% bleach solution for one hour. This process removes any living tissue leaving only the calcium carbonate skeleton. The tiles were then rinsed with distilled water and allowed to air dry. At Woods Hole Oceanographic Institute (WHOI) each skeleton was carefully removed from the tile using forceps and weighed using a Mettler MT5 microbalance (Cohen *et al.* 2009).

## 2.5 Lipid Analysis

Samples of 3 vials (10 planulae per vial) per day were collected for lipid analysis from both Rim and Patch reefs during each day of spawning. Theses samples were kept frozen at -80° C at BIOS and shipped frozen to WHOI. During a four day period a modified Folch extraction method was used to extract of lipids from frozen planulae samples (Folch *et al.* 1957, Harland *et al.* 1992, 1993) at WHOI. For more precise lipid analysis, samples of ten planulae were used because individual planulae are too small. Day one of analysis involved adding 1mL of dichloromethane (DCM) and methanol (MeOH) solution (2 DCM: 1 MeOH) to each glass vial of ten planulae. In order to break the cell walls each vial was placed in the sonicator for five minutes and the samples were kept at 4° C for 24 hours. A blank sample containing only DCM: MeOH solution was also ran as a control. On day two, the extractions were again sonicated and the organic

extract was separated into another vial with an addition of 0.5mL of 0.88% KCl in $dH_2O$ to the extract. Samples were then left overnight at 4° C. On day three, the clear aqueous phase was removed with a pipette; this process was completed once the separation between the aqueous phase and organic phase was obvious (the upper layer was clear). The organic phase was then washed with 1mL of methanol and water (1 MeOH: 1 $H_2O$) and let stand for 30 minutes at 4° C. Once the aqueous layer had clearly separated, the vials were allowed to thermo-equilibrate to room temperature and the aqueous top layer which consisted of water and salts was removed.The remaining solvent was dried using nitrogen and the organic phase was transferred into pre-weighed acetone washed aluminum trays. The aluminum trays were then placed in a drying oven at 60° C overnight. The final day, the aluminum trays were thermo-equilibrated to room temperature to obtain a stable mass then weighed using a Mettler MT5 microbalance. The final weight of each sample was determined by subtracting the weight of the aluminum tray and the weight of the blank from the total weight of each sample.

## 2.6 Zooxanthellae Analysis

At the end of the two-week experiment, approximately 30-40 spat per $CO_2$ condition were sacrificed for zooxanthellae density analysis. Each polyp was carefully removed from the tiles using a razorblade, individually placed into microcentrifuge tubes, and frozen for subsequent analysis. At WHOI, remaining water was removed from each microcentrifuge tube and 300µL of filtered seawater was added. The tissue sample was homogenized with a microcentrifuge pestle, the pestle was rinsed with an additional 300µL of seawater to prevent sample loss, and then the samples were spun in a

microcentrifuge for ten minutes at 10,000 rpm. After centrifugation, the supernatant of each sample was removed and 50µL of filtered seawater was added to resuspend the pellet (Barkley, 2011). To analyze the zooxanthellae density, this solution was briefly vortexed, then pipetted into a hemacytometer (Bright Line Counting Chamber, Hausser Scientific, Horsham, PA, USA; dimension of a single square = 1mm x 1mm x 0.1mm), and counted the number of algae per square for 18 squares was counted. The density of zooxanthellae was calculated using the following equation:

$$\frac{\#\ zooxanthellae\ x\ dilution\ factor}{\#\ squares\ counted\ x\ vol.per\ square}$$

The dilution factor was 0.050 and the volume per square was 0.0001 $cm^3$.

## 2.7 Statistical Analysis

All statistical analyses were conducted using SYSTAT 13® (Wilkinson 2009) statistical software. To determine the normalized lipid content (µg/$mm^2$), a calculation of mean lipid: area for planulae from each site per day of the spawning event was calculated and then used for statistical analysis. To normalize for total calcification (mg/$\mu m^2$) a calculation of weight: area ratio for each coral polyp that was sampled; this data was then used data for subsequent statistical analysis. A Shapiro-Wilk test of normality was conducted for all analysis, revealing that sample distributions were not normal. Due to non-normal distributions, non- parametric Kruskal-Wallis and Man-Whitney tests were used for overall statistical analysis of planulae area, settlement proportions, skeletal areal density, lipid content and zooxanthellae counts. This was followed by a Dwass-Steel-

Critchlow-Fligner pairwise comparison. Results were considered significant when $p \leq 0.05$.

# CHAPTER 3 **RESULTS**

## 3.1 Planulae

Analysis of planulae area data reveled that planulae released from Rim reef parental colonies were 1.67 times larger than planulae released from Patch reef parental colonies. The difference mean area between the Rim and Patch reef was found to be statistically significant (Mann-Whitney, U = 4,100, F = 0.038, $n_1$= 110, $n_2$ = 90, Figure 6).

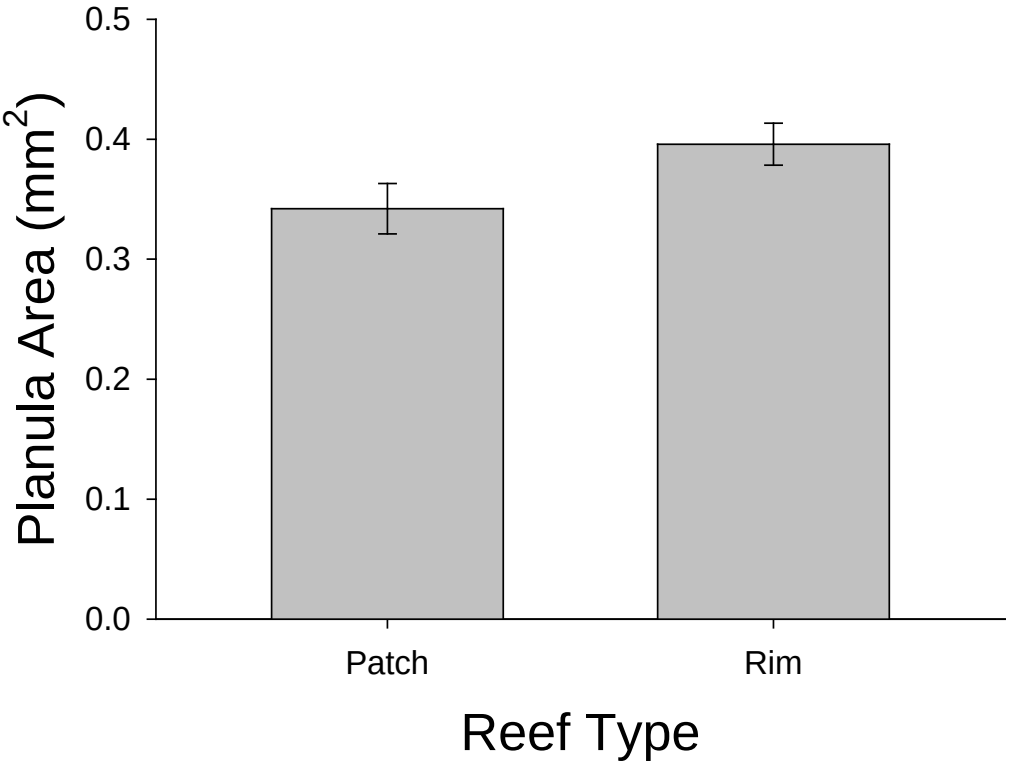


**Figure 6: Planulae area *Mean ± SE area (mm$^2$).* Planulae released from Rim reef parental colonies are significantly larger than planulae from Patch reef parental colonies (n=200).**

## 3.2 Settlement

Analysis of settlement data reveled that on average 10.9 Rim planulae settled per tub as compared to 6.1 Patch planulae. The proportion of planulae settled per tub was measured by dividing the sum of planulae settled on each of the two tiles by the number of planulae deposited into each tub. A total of 482 of approximately 2,781 Rim reef planulae settled (17.3 %) and 624 of approximately 6,910 Patch reef planulae settled (9.03 %). The settlement proportions per settlement tub of Rim reef planulae were significantly higher than that of Patch reef planulae (Mann-Whitney test statistic, $U = 1.967$, $p = 0.0001$, Figure 7). $CO_2$ enrichment did not have a significant effect on the settlement proportions of Rim or Patch reef (Kruskal-Wallis test statistic, $H = 1.546$, $p = 0.462$).

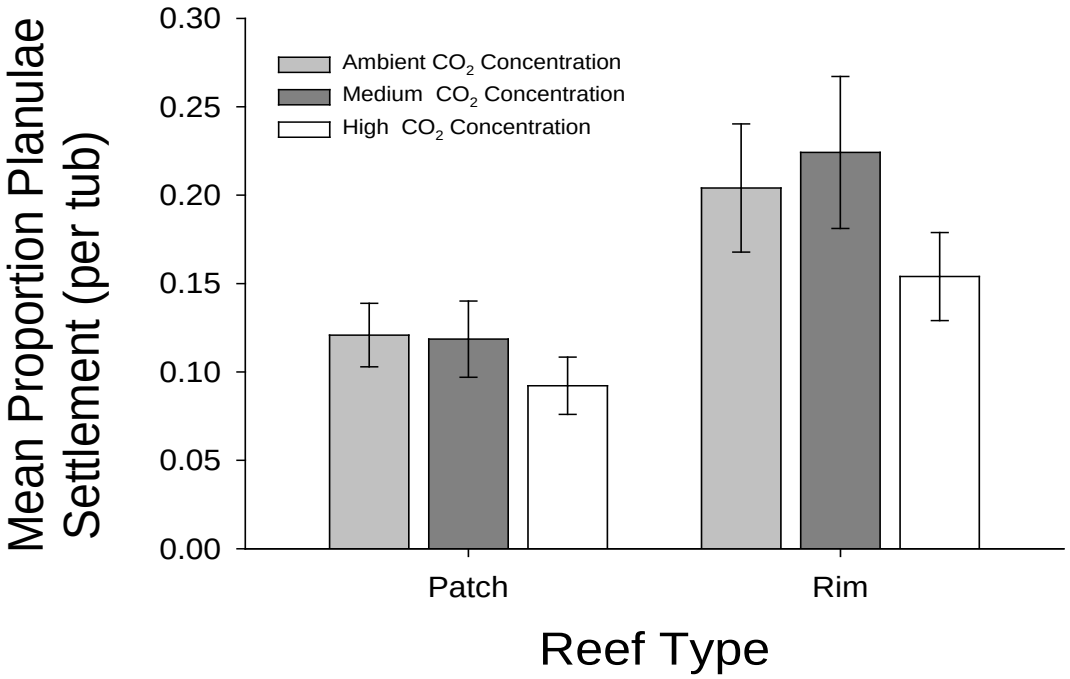


**Figure 7: The mean (± SE) number of planulae from each site and $CO_2$ condition that settled pertub (n = 175). A statistically significantly greater number of Rim planulae settled per settlement tub than Patch planulae. There was no statistically significant difference in settlement between treatments of $CO_2$.**

### 3.3 Skeletal Areal Density

To obtain an overall interpretation of the total calcification, each coral skeletal weight (mg) was divided by its area ($\mu m^2$); a total of 319 coral skeletons were measured: 147 Rim reef coral skeletons and 172 Patch reef coral skeletons. Analysis revealed that Rim reef skeletons had a mean skeletal areal density of 8.65E-08 and Patch reef skeletons had a mean of 6.73E-08. Rim reef corals accreted significantly more skeleton than Patch reef corals (Mann-Whitney, U = 6,298, $p < 0.0001$, Figure 8). To determine if enriched $CO_2$ affected the amount of skeleton the corals accrete, a Kruskal-Wallis test was completed for 118 ambient, 99 medium and 102 high coral skeletons indicating significant differences between the $CO_2$ conditions (Kruskal-Wallis test statistic, H = 6.565, df = 318, P = 0.0375). Significant differences were found between the ambient and high $CO_2$ conditions with higher total calcification in skeletons reared in ambient $CO_2$ conditions (Dwass-Steel-Chritchlow-Fligner, pairwise comparisons = -8.91, p = 0.000002, Figures 9). A significant difference was also found between the ambient and medium $CO_2$ conditions with ambient skeletons again having a higher rate of calcification (Dwass-Steel-Chritchlow-Fligner, pairwise comparisons = -6.58, p = 0.000011, Figure 9). There was no significant difference found between the high and medium $CO_2$ conditions, indicating the total calcification of skeletons reared in high and medium $CO_2$ conditions was relatively equivalent (Dwass-Steel-Chritchlow-Fligner, pairwise comparisons = 2.36, p = 0.218, Figure 9).

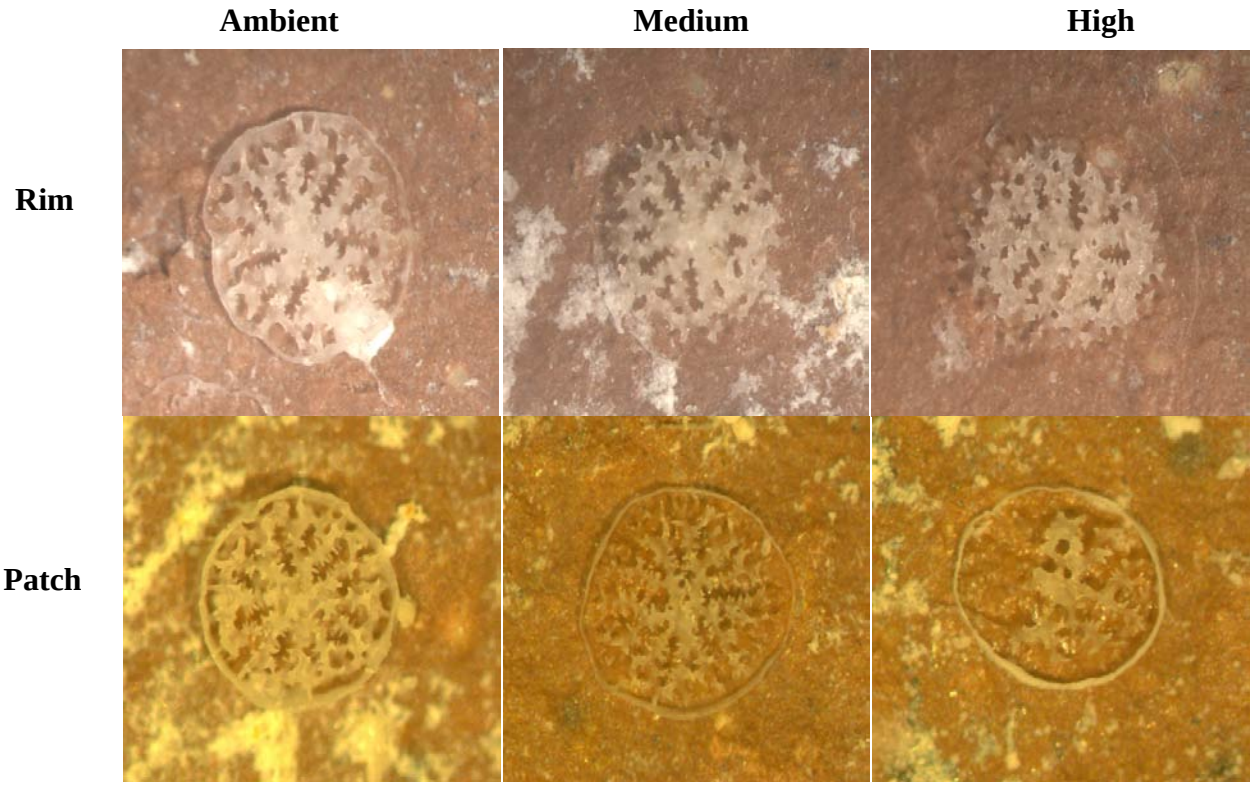


**Figure 8**: **Two-week old *P. astreoides* skeletons from both Rim Reef parental colonies and Patch Reef parental colonies at ambient $CO_2$ (420 ppmv), medium $CO_2$ (1200 ppmv) and high $CO_2$ (1670 ppmv). $CO_2$ enrichment had more of a statistically significant effect on the calcification of Patch reef polyps than of Rim reef polyps. Both reef types showed negative effects to increased $CO_2$.**

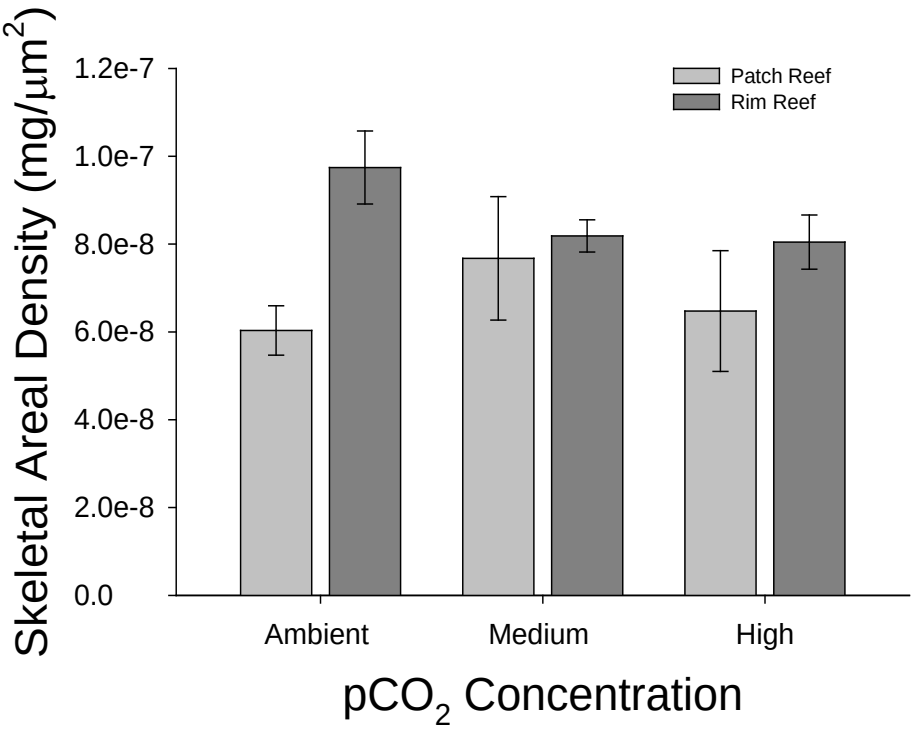


**Figure 9: Mean Skeletal Areal Density (± SE) of 2-week old *P. astreoides* coral skeletons. A significant difference was found between Rim and Patch reef skeletal areal densities. Coral skeletons reared in ambient $CO_2$ conditions had significantly higher rates of calcification than that of coral skeletons in high $CO_2$ conditions (n=320).**

### 3.4 Lipids

Total lipid analysis was conducted on aggregated samples of 10 coral polyps and normalized to area ($mm^2$) to obtain lipid content per polyp. There were no significant differences in total lipid content found between Rim and Patch reef polyps (Mann-Whitney U = 194, P = 0.311, $n_1$ = 18, $n_2$ = 18 Figure 10). $CO_2$ enrichment had no effect on lipid content when normalized to area (Kruskal-Wallis H = 2.896, df = 35, p = 0.235, Figure 10). This lack of statistical significance can be attributed to small sample size only having analyzed 36 aggregated samples of 10 polyps, 18 from both Rim and Patch reef.

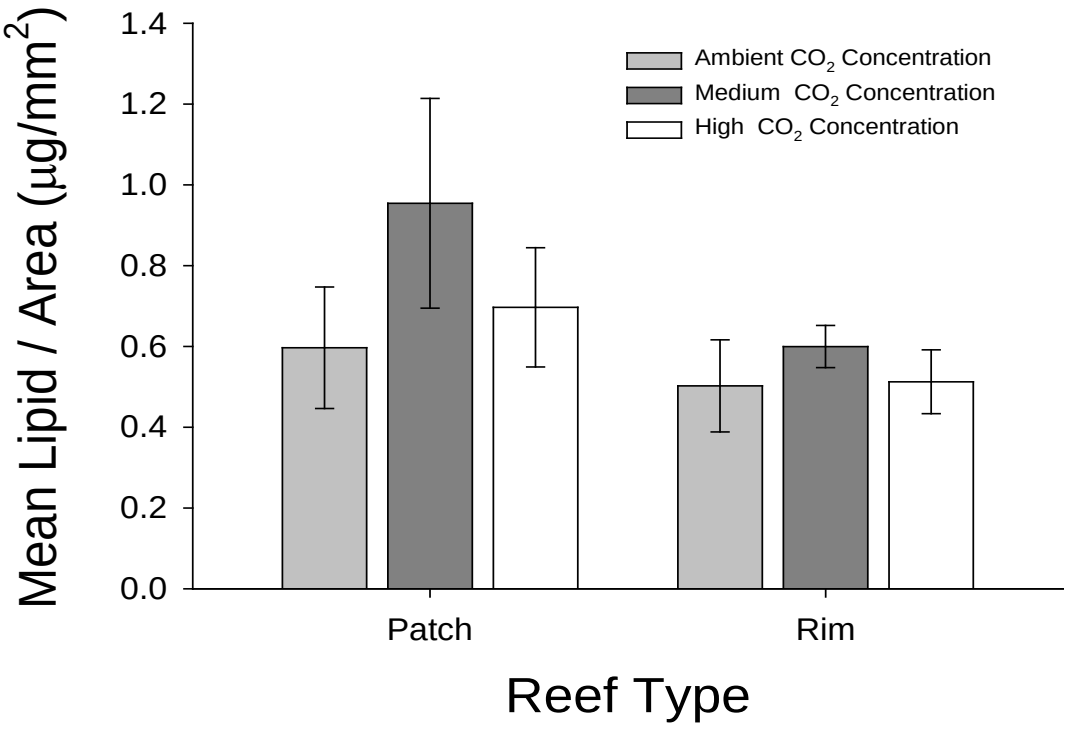


**Figure 10: Mean Lipid Content (µg/mm$^2$). No statistically significant difference existed between Rim and Patch lipid content across the $CO_2$ conditions when normalized to area (n=36).**

### 3.5 Zooxanthellae

The density of symbiotic zooxanthellae present in the host tissue of *P. astreoides* coral polyps did not vary between the two reef systems. However, a trend towards a higher zooxanthellae density in Rim polyps than Patch polyps was observed (Mann-Whitney U = 1,138, n1 = 47, n2 = 59, P = 0.114, Figure 11). A significant difference was found in the zooxanthellae counts among the three $CO_2$ conditions (Kruskal-Wallis, H = 7.583, P = 0.023, Figure 12). Zooxanthellae counts were higher in coral polyps reared in the high $CO_2$ conditions than that of coral polyps reared in ambient $CO_2$ conditions (Dwass-Steel-Chritchlow-Fligner, pairwise comparisons = 4.153, P = 0.009, Figure 12). A significant difference was also found between polyps in ambient and medium $CO_2$ conditions with ambient coral polyps having higher zooxanthellae counts (Dwass-Steel-Chritchlow-Fligner, pairwise comparisons = -8.173, p = 0.000002, Figure 12). Zooxanthellae counts were significantly higher in high $CO_2$ conditions than medium $CO_2$ conditions (Dwass-Steel-Chritchlow-Fligner, pairwise comparisons = -12.289, p = 0.000002, Figure 12).

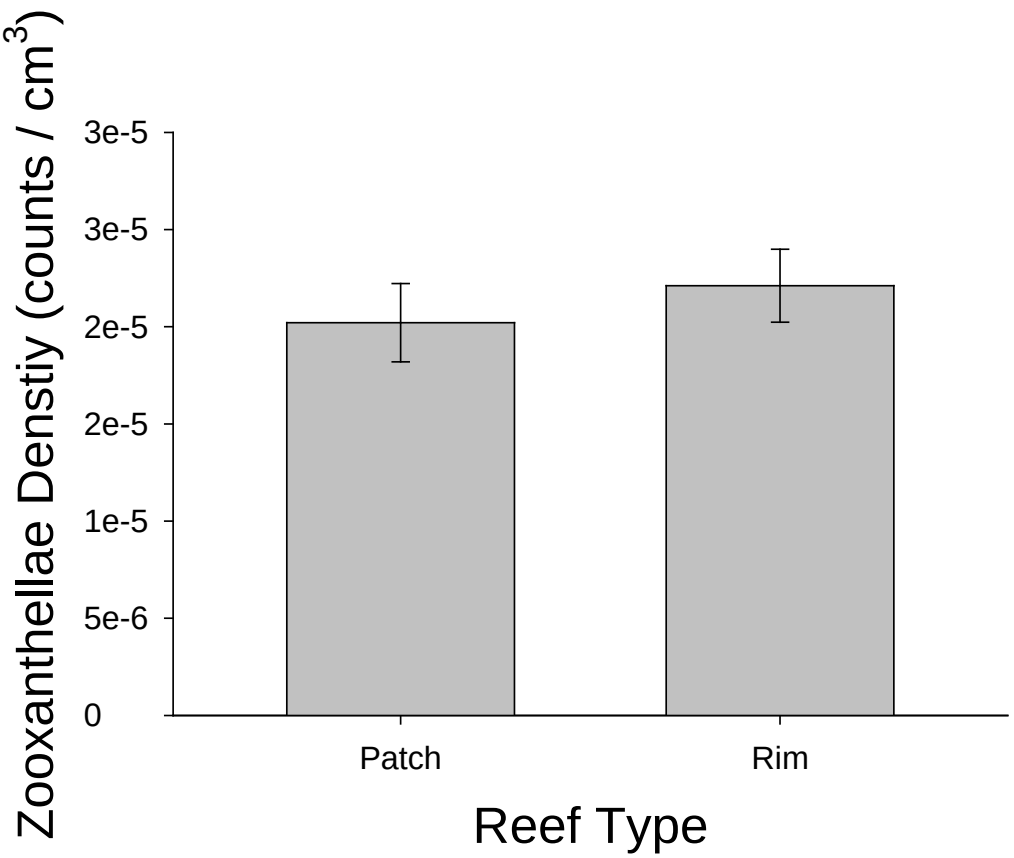


**Figure 11: Zooxanthellae density (counts/cm$^3$) of Patch vs Rim planulae. No statistical significance was found between reefs however a trend existed towards higher counts in Rim polyps (n=106).**

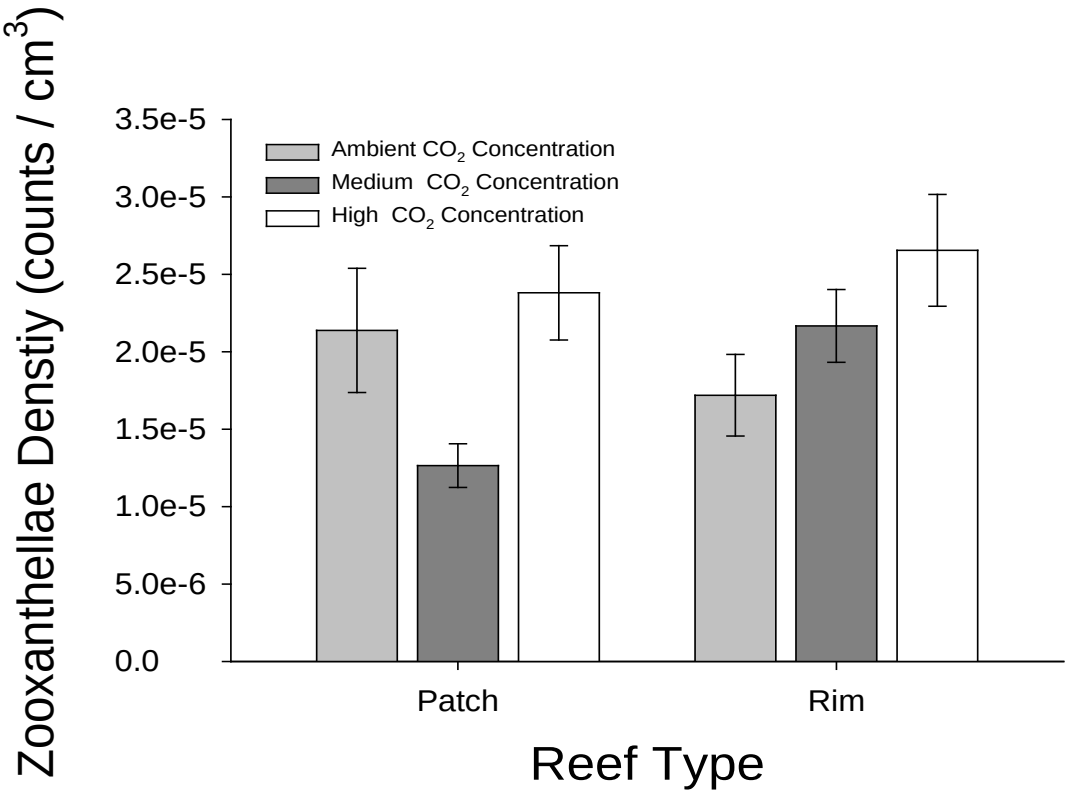


**Figure 12: Zooxanthellae density (counts/cm$^3$) of 2-week old *P. astreoides* of Rim and Patch reef in enriched $CO_2$. A statistically significant difference was found between Rim and Patch polyps as well as across the $CO_2$ conditions (n=106).**

# CHAPTER 4 **DISCUSSION**

## 4.1 Planulae

Larval sizes across the Bermuda reef platform differ; *P. astreoides* planulae released from Rim reef parental colonies have a larger mean area than planulae released from Patch reef parental colonies. Various environmental parameters across the Bermuda platform favor stony coral such as *P. astreoides*; the high wave energy paired with low sediment loads at the Rim reef favors these hard coral while low wave energy and high sediment loads at the Patch reef favor varying soft coral and invertebrates (Wood and Jackson, 2005). *P. astreoides* is a brooding species of coral in which development of the planulae takes place within the parental colony and is subsequently released into the water column (Richmond 1997). Thus, differences in planulae size can be attributed to the environment of the parental colony. Rim reef and Patch reef corals are exposed to environmental differences in nutrient, zooplankton, light, sedimentation and saturation state(Wood and Jackson, 2005), all of which can affect the energetic status of parents, consequently affecting the amount of energy allocated for reproduction (Wood and Jackson, 2005). Bermuda does not have a national sewage system; the sewage released pollutes the inshore reef system degrading the Patch reef that is closer to shore more than the Rim reef, which has the open ocean behind it (Locke et al., 2013). A eutrophication gradient with increased nitrogen, and greater sedimentation and turbidity has been observed closer to land having a greater effect on the Patch reef than the Rim reef (McGlathery, 1992). The negative effect of increases in sediment and turbidity to corals is the inhibition of coral growth due to suspended particles scraping the coral tissue. In addition, suspended particles absorb light and high sediment and turbidity decreases

phototrophy (Anthony and Fabricius, 2000). It is likely that Patch reef corals are subject to more environmental stressors which limit the amount of energy available for reproduction thus, smaller planulae are produced.

The differences in planulae size may also be attributed to genetic or epigenetic differences between the *P.astreoides* populations at the Rim and Patch reef sites. However, the distance between the two sites is not large enough that gene flow would be inhibited. In addition, because Bermuda is isolated from other reef ecosystems, there is likely to be limited variation among the coral populations of a given species (Locke et al., 2013). Further research on possible genetic and epigenetic differences should be conducted to determine whether or not inter-site variation among *P. astreoides* planulae is due to genetic diversity.

## 4.2 Settlement

When examining settlement proportions it was observed that planulae released from Rim reef parental colonies had much higher rates of settlement than those from Patch reef. However, $CO_2$ enrichment did not have a significant effect on settlement proportions of Rim or Patch reef planulae. This observation that increases in $CO_2$ does not affect settlement proportions is supported in previous studies (e.g. Albright *et al*., 2008). When planulae are in their planktonic stage they do not calcify and so it is unlikely that increased $CO_2$ would affect the planulae before settlement occurs (Albright *et al*., 2008). Coral metamorphosis is stimulated by chemical cues from microbial biofilms and crustose coralline algae (Negri *et al*., 2001; Albright *et al*., 2008, 2010). Crustose coralline algae are beneficial to the settlement process because they precipitate

high amounts of magnesium calcite, a form of $CaCO_3$ that is more soluble than aragonite (crystallized form of $CaCO_3$). $CO_2$ enrichment reduces the recruitment of crustose coralline algae by 78% at $pCO_2$ = 560ppmv (Albright *et al*., 2008, 2010; Hofmann *et al*., 2010). The trend observed in this study that calcification decreases as $pCO_2$ increases is supported by the reduction in crustose coralline algae. This idea is further supported by Abright et al's (2010) and Hofmann et al.'s (2010) findings that the abundance of filamentous algae increases with $CO_2$ enrichment, thus inhibiting coral settlement. This experiment does not address the possibility of algal interference however it controls for differences in relative algal abundance as all tiles used for settlement were preconditioned at the same location and time period. Albright et al. (2010) completed a similar study however tiles used for settlement were preconditioned under experimental $pCO_2$ conditions for 40 days prior to the experiment. Tiles preconditioned under high $CO_2$ were observed to have reduced crustose coralline algae and increases in filamentous algae abundance (Albright *et al*., 2010). Supporting the hypothesis that settlement proportions will decrease under enriched $CO_2$ conditions.

### 4.3 Skeletons

The results of this study indicated that total calcification is negatively affected by $CO_2$ enrichment. Both Rim and Patch reef coral skeletons showed decreases in calcification as $pCO_2$ increased. Coral skeletons from Rim reef parental colonies had larger skeletal areal densities than Patch reef skeletons across each $CO_2$ condition. These results coincide with the growing body of literature that proposes that ocean acidification will have a negative effect on coral calcification that will vary between and within species and reefs. Slower rates

of calcification can be an effect of a longer period of time spent in juvenile, non-reproductive life stages which ultimately reduces the effective population size and fecundity (Albright *et al*., 2008). Future studies could vary the settlement period allowing for both early and late metamorphosed planulae to be studied .The results of this study are far from conclusive and subsequent experiments should be conducted with larger sample sizes and an extended experimental period.

Cohen and McConnaughey's (2003) model of coral calcification further supports the results of this study. It indicates that corals raise the pH (it is important to note that pH is directly correlated with $CO_2$; $CO_2$ increases as pH decreases) in isolated extracellular compartments to transform $HCO_{3^-}$ into $CO_3^{2^-}$ and finally $CaCO_3$. This model indicates that calcification is an energetically expensive process that decreases with ocean acidification. Nutrient enrichment is thought to compensate for increases in $CO_2$ which can either lessen or normalize the effect of ocean acidification on calcification. In this experiment, nutrient levels were increased with heterotrophy which is thought to give the corals excess nutrients that it cannot obtain from its algal symbiant zooxanthellae (Houlbreque and Ferrier-Pages, 2009). Heterotrophy is considered to increase skeletal growth, however this study did not test the effect of heterotrophy on coral growth but instead controlled for it by feeding all corals. Additional studies should be conducted analyzing heterotrophy and its correlation to ocean acidification and coral growth.

## 4.4 Lipids

Due to the necessity of pooling groups of ten coral to compose a sample in order to analyze the data, the outcome of the lipid analysis was limited by a very small sample size. An increased number of replicates would have provided more conclusive results. Results for lipid analysis were statistically non-significant however, interesting observations can be made. Lipid content seemed to peak in the medium $CO_2$ condition for both Rim and Patch reef. Subsequent studies should look at the relationship between lipid content and zooxanthellae counts and determine whether a relationship exist between the two.

## 4.5 Zooxanthellae

Zooxanthellae is a symbiotic algae found in tropical reef building corals(Goreau and Goreau, 1959). While the zooxanthellae provide the coral with various nutrients crucial to survival, the coral provides the zooxanthellae with a safe habitat. It has been observed that corals with symbiotic algae calcify at much faster rates than corals that have lost their zooxanthellae (Goreau and Goreau, 1959). Zooxanthellae influence coral calcification by removing $CO_2$ from the environment which increases the amount of carbonate ions available thus, stimulating coral calcification (Pearse and Muscatine, 1971). In addition, zooxanthellae remove algal phosphates that act as crystal poisons which in turn induces crystallization of calcium carbonate. Zooxanthellae also provide the coral with organic products of photosynthesis required for skeletogenesis aiding in faster rates of calcification (Goreau and Goreau, 1959).

This study indicated that zooxanthellae density increased with enriched $CO_2$ while coral calcification decreased with $CO_2$ enrichment. Suggestive evidence supports the idea that this symbiotic alga can boost coral resistance to increases in $CO_2$ up to a certain threshold. However, as $CO_2$ continues to rise over time both species in this symbiotic relationship risk extinction. The corals limited \inability to secrete a calcium carbonate skeleton in enriched $CO_2$ will leave zooxanthellae without an adequate habitat to survive.

An overall trend was seen towards Rim reef having higher zooxanthellae counts than Patch reef corals. Environmental differences at the two sites can be the basis of the differences observed. Continuing research should include determining whether there are correlations between environmental differences and larval size. In addition to determine whether correlations exist between larval size and zooxanthellae counts as well as an analysis of coral and zooxanthellae genes and protein expression.

Like a canary in a coal mine, coral reefs serve as an indicator of the overall environmental change. As a productive ecosystem providing a home, food and nutrients to over a third of all marine species; coral reefs are seeing a major decline (Bryant *et al*. 1998; Moberg and Folke 1999). As global anthropogenic $CO_2$ emissions continue to rise coral reefs and its habitants face an eminent threat.

## 4.6 Challenges and Limitations

This study represents a limited analysis on the effects of ocean acidification to coral recruits. When experimenting with live organisms, many parameters play a pivotal role.

This study was limited by the peak spawning events of *P.astreoides* and the amount of planulae released from each adult coral colony. The amount of larvae released directly affected sample size throughout the experiment. Small sample size likely contributed to statistical non-significance in some analysis. Mortality played an effect as fewer coral polyps were available for analysis of skeletal density, lipid content and zooxanthellae counts. In addition it was challenging to keep with time constraints as it pertained to settlement and settlement checks, water changes, feeding schedules, water quality tests and light/dark cycles. Ethics and fishery policy limited the number of adult coral that were collected for the experiment. Lastly, as with many studies, experimental design can be a limitation to what the study will reveal. Lab experiments allow control of factors that cannot be controlled for in the environment and allow us to closely examine various responses of a particular species independent of its natural environment. However, species may respond differently to a given stimulus in its natural habitat, and it is important to acknowledge this possibility.

# CHAPTER 5 **CONCLUSIONS**

This study sought to determine the extent to which ocean acidification affects coral recruits. As atmospheric $CO_2$ enters the ocean through diffusion; the process of ocean acidification limits the available carbonate ions corals need to accrete their calcium carbonate skeleton. Bermuda has different varying reef systems: Rim reef and Patch reef. This study sought to answer whether the planulae sizes at these two reefs differed and if the planulae had differential responses to increases in $CO_2$. By manipulating $pCO_2$ concentration in various experimental tanks these questions were answered. It was determined that planulae released from Rim reef parental colonies were significantly larger than planulae released from Patch reef parental colonies. In addition, the planulae differed in response to increases in $CO_2$ with total calcification decreasing as $CO_2$ increased. Throughout the analysis it seemed that Rim reef corals had better adaptations to the effects of ocean acidification. Perhaps they could have pre-existing traits that make them more fit for dealing with environmental stressors. For future studies it will be interesting to determine if genetic differences between *P.astreoides* at Rim and Patch reef exist. If genetic differences do exist, this species faces an even bigger threat of extinction caused by ocean acidification due to the isolation of Bermuda's reef platform. With isolated populations, chances of recolonization significantly decrease. Ocean acidification poses an extreme threat to the existence and productivity of Bermuda's reef system. It is important that global $CO_2$ emissions decrease and that more is learned about coral reef ecology and indicators of coral reef resilience. Future research should address how coral reefs can be maintained and advocate the importance of this ecosystem to human life. As anthropogenic $CO_2$ in the atmosphere increases and climate change continues to

accelerate it is imperative that we understand the impact of large-scale climate variability and construct conservation and management policies to conserve and protect this beautiful, delicate ecosystem.

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