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Heterotrophy and Lipids as Indicators of Resilience to Climate Change Stress in Scleractinian Corals

Erica K. Towle

University of Miami, etowle@rsmas.miami.edu

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UNIVERSITY OF MIAMI

HETEROTROPHY AND LIPIDS AS INDICATORS OF RESILIENCE TO
CLIMATE CHANGE STRESS IN SCLERACTINIAN CORALS

By

Erica K. Towle

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

August 2015

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HETEROTROPHY AND LIPIDS AS INDICATORS OF RESILIENCE TO
CLIMATE CHANGE STRESS IN SCLERACTINIAN CORALS

Erica K. Towle

Approved:

Chris Langdon, Ph.D.
Professor and Chair of
Marine Biology and Fisheries

Diego Lirman, Ph.D.
Associate Professor of
Marine Biology and Fisheries

Andrew Baker, Ph.D.
Associate Professor of
Marine Biology and Fisheries

Derek Manzello, Ph.D.
Research Oceanographer
NOAA AOML

Danielle McDonald, Ph.D.
Associate Professor of
Marine Biology and Fisheries

Dean of the Graduate School

Mark Teece, Ph.D.
Associate Professor of
Environmental Chemistry
SUNY ESF

TOWLE, ERICA K.
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Coral reefs are biodiverse ecosystems with high biological, cultural, economic, and recreational value that are facing multiple anthropogenic stressors, the greatest of which is global climate change via warming and ocean acidification (OA). Increased warming throughout the century may reach a point where frequent bleaching, the expulsion of corals symbiotic algae that can provide the coral with over 90% of its daily metabolic requirements, may cause widespread mortality. OA, a result of increased carbon dioxide dissolving into seawater, changes the chemistry of seawater such that the pH of the ocean becomes more acidic. This decrease in pH is accompanied by a decline in the saturation state of calcium carbonate, which impairs the ability of corals to build their skeletons by increasing the energetic cost of calcification. Research within the last decade has demonstrated that coral heterotrophy, the ability of the coral animal to feed on plankton in the water column, and coral energy reserves (lipid stores) may be good indicators of resilience to both warming and acidification. The gaps in our knowledge regarding heterotrophy and lipids are which coral species are capable of increasing their feeding effort, and what conditions drive this capability. To evaluate resilience capability of common corals from the Florida Reef Tract (FRT), field and lab studies were com-

pleted to address the following questions: i.) Do *in-situ* calcification rates and lipid content vary by species, season, and/or site in the FRT? ii.) Are feeding rate and lipid content plastic responses during climate change stress? iii.) Are calcification and feeding rate variable within the same species from different source locations during climate change stress? iv.) Does preconditioning to elevated CO₂ prior to thermal stress affect bleaching susceptibility? Results showed that i.) there may be metabolic tradeoffs between calcifying and storing lipids, and certain species can possibly mediate that tradeoff using heterotrophy. ii.) Past history of nutritional repletion may be an important factor in predicting resilience to climate change stress. iii.) An endangered species can increase its feeding rate and lipid stores to mitigate reductions in calcification under CO₂ stress, underscoring the importance of heterotrophy and lipids in future conservation science. iv.) Preconditioning to high CO₂ (a globally relevant scenario post 2040) will likely worsen the effects of thermal stress on calcification and feeding rate, underscoring the importance of reducing CO₂ emissions on a global scale. This dissertation demonstrates that the resilience of reef-building corals of the FRT in future warmer, more acidic oceans is affected by heterotrophy and lipids, but this response will be dependent on reefs with naturally high abundance of heterotrophic food sources. This dissertation has implications for potential feeding protocols in coral gardening and nursery programs and the planning and placement of marine protected areas.

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ERICA K. TOWLE

University of Miami

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CHAPTER 1

Introduction

1.1 Background on corals and climate change

Rapidly rising greenhouse gas concentrations are driving ocean systems toward conditions not seen for millions of years, introducing the risk of fundamental ecological transformation (Hoegh-Guldberg and Bruno, 2010). These rising atmospheric greenhouse gas emissions have increased global average temperatures by approximately 0.2 °C per decade over the last thirty years (Hansen et al. 2006), most of which has been absorbed by the oceans. Increased warming of the ocean throughout this century may reach a point where excessive coral bleaching, the expulsion of symbiotic zooxanthellae, may cause widespread coral mortality. In addition to absorbing heat, the oceans have absorbed approximately one-third of the carbon dioxide produced by anthropogenic activities resulting in a steady decrease of 0.02 pH units per decade over the last thirty years (Hoegh-Guldberg and Bruno, 2010). Ocean acidification (OA) associated with atmospheric carbon dioxide above about 450 ppm (preindustrial levels were about 280 ppm and today's levels are around 390 ppm) will push calcifying organisms into a negative carbonate balance (Hoegh-Guldberg et al. 2007), which will reduce their ability to calcify.

These changes represent a large departure from the geochemical conditions of the ocean that have been the norm for millions of years (Petit et al. 1999). How marine organisms will fare in the oceans of the future will depend on their tolerance ranges and their ability to adapt to stress.

Coral reefs are an ocean ecosystem that is especially vulnerable because in addition to coral bleaching and reduced calcification as a result of increasing atmospheric carbon dioxide, we are seeing an increased input of nutrients, pollution, and sedimentation from natural as well as anthropogenic disturbances (Bruno et al. 2007). Reefs worldwide, and especially in the Caribbean, are experiencing unprecedented declines in coral cover (Hughes 1994) for the aforementioned reasons, and coral cover is projected to decline even more in the coming decades. Resilience to stress is crucial in a changing environment, and heterotrophy in corals may contribute to resilience to climate change. Many corals rely on photosynthesis of their symbionts alone (autotrophy), but many also ingest food (heterotrophy) which may provide additional energy sources that are particularly crucial to survival in stress conditions. Heterotrophy rates of corals may vary with environmental changes such as OA and thermal stress.

The projected reductions in present-day tropical surface seawater are from a pH of 8.08 to 7.93 (with a doubling of CO₂) and aragonite saturation state from 4.0 to about 3.1 by the year 2065 and about 2.8 by 2100 (Kleypas et al. 1999). This reduction in saturation state is concerning because the deposition of calcium carbonate by corals and other reef calcifiers is partially controlled by the saturation state of calcium carbonate in seawater. Recent findings suggest that corals are approaching a critical threshold, beyond which their ability to calcify quickly enough

to effectively form reefs will be severely compromised (Langdon et al. 2000). Reduced pH and saturation state make it energetically more difficult for corals to calcify. More energy must be expended to keep the pH at the site of calcification elevated to the level needed for rapid precipitation of calcium carbonate. There may also be other consequences marine invertebrates in general may experience reduced thermal tolerance and reduced metabolic performance that could impact the ecological fitness of the organism in many ways (Portner 2008).

The intellectual merit of this dissertation is that discovering which corals will be most resilient to OA and bleaching will allow us to predict what species may become dominant on reefs of the future and what phenotypes are most resilient to stress. Moreover, knowing which corals will be most fit under climate change conditions will benefit future management decisions for reefs.

1.2 Mechanisms of coral calcification

Corals have four tissue layers: the oral ectoderm and endoderm and the aboral ectoderm and endoderm. The oral side faces seawater and the aboral side faces the coral skeleton. The oral endoderm is the layer that contains the corals symbiotic zooxanthellae. The aboral ectoderm, which faces the skeleton, is commonly referred to as the calciblastic epithelia (CE) and is the site of calcification. In order for calcium and carbonate ions, necessary for calcification, to reach the CE, they must cross four tissue layers. However, if the ions enter the coral through the coelenteron, they only have to pass two cell layers, but this can only occur if the coral has its mouth open. Calcification refers to the reaction where calcium ions

combine with carbonate ions to form calcium carbonate, the molecule that makes up the skeletons of corals. In order for this reaction to occur, not only must calcium and carbonate ions be present in the CE, but the chemistry of the site must be favorable for the reaction to occur. There has been considerable controversy regarding how calcium passes through the cell layers of a coral, specifically how it passes through the oral epithelia i.e. actively or passively. The generally accepted theory on how calcium reaches the CE is that calcium ions in bulk seawater diffuse passively across the oral epithelia (Benazet-Tambutté et al. 1996). From there, they diffuse paracellularly through the subsequent layers of tissue until they reach the border between the aboral endoderm and the CE. The exception is if bulk seawater enters the coelenteron the calcium ions diffuse paracellularly from the coelenteron through the aboral endoderm until they reach the border between aboral endoderm and CE. At this point, transport of calcium ions into the CE is active and is facilitated by a $\text{Ca}^{+2}\text{-H}^{+}\text{-ATPase}$ pump whereby calcium ions are pumped into the CE and hydrogen ions are pumped out of the CE (Tambutté et al. 1996).

More recently the field gained more clarity about the intercellular junctions through which calcium paracellularly diffuses before reaching the CE. It was demonstrated that the intercellular junctions have a resistance of about 477 Ohms cm^{-2} , a value that is intermediate between leaky and tight, but closer to the leaky end of the spectrum (Tambutté et al. 2011). These authors also demonstrated using calcein dye (which can bind to calcium) and fluorescent beads that the size of ions that can pass through the intercellular junctions must likely be larger than thirteen Angstroms and smaller than twenty nanometers (Tambutté et al. 2011). This was experimentally demonstrated because calcein could pass and has a diameter of

thirteen Angstroms, but the fluorescent beads, with a diameter of twenty nanometers, could not. While this experiment added a great deal to our knowledge of calcium transport, the authors could not generalize their results to the calcium ion specifically, even though it fits the size range they discovered, due to the charge of the ion being positive while calcein is negative. Later it was experimentally demonstrated that calcium ions can indeed pass through the intercellular junction (Gagnon et al. 2012).

The source of carbonate ions for calcification is bulk seawater which passively reaches the CE. The primary dissolved inorganic carbon (DIC) source for carbonate is bicarbonate (HCO_3^-) which is the DIC ion most abundant in seawater. Once bicarbonate reaches the CE, the following reaction, mediated by the enzyme carbonic anhydrase, occurs: $\text{HCO}_3^- \leftrightarrow \text{CO}_3^{2-} + \text{H}^+$. The carbonate ion produced by this reaction will be used in calcification, and the hydrogen ion produced will be pumped out of the CE by the $\text{Ca}^{+2}\text{-H}^+\text{-ATPase}$ pump mentioned previously. The reason that the hydrogen ion must be pumped out of the CE is so that the chemistry of the calcifying medium is favorable for calcification to occur. This can only occur when the aragonite saturation state of the medium and the pH of the medium are high. This is because other ions in bulk seawater will combine with calcium ions instead of carbonate unless the aragonite saturation state is favorable enough for calcium to react with carbonate (Allemand et al. 2011). An example of an ion that could bind to calcium over carbonate if chemistry is not favorable is phosphate (PO_4^{2-}) (Ferrier-Pagès et al. 2000). It has been experimentally demonstrated that the pH at the CE is elevated by about 0.4 pH units with respect to bulk seawater to about 8.5 (in comparison to 8.1) (Venn et al. 2011). This pH up-regulation,

(facilitated by the $\text{Ca}^{+2}\text{-H}^+\text{-ATPase}$ pump), is a necessary step for calcification to occur.

One of the mysteries of the calcification process is how the CE can be open to bulk seawater but can also have an elevated pH relative to seawater. The explanation for this is best explained by the batch seawater hypothesis put forth by Cohen and others. Cohen hypothesized that the center of calcification (COC) is a closed space with opportunities for replenishment. More specifically, a batch of bulk seawater enters the COC, the coral then closes off the COC, and via the $\text{Ca}^{+2}\text{-H}^+\text{-ATPase}$ pump, up-regulates the pH to favor calcification. When the pH and saturation state are no longer favorable for calcification, i.e. ions have been used up, the COC replenishes itself with a new batch of seawater and repeats the process of up-regulation to favor calcification. Lastly, the organic matrix, while only comprising about 0.1-1.0% of the skeleton by weight (Allemand et al. 2011), is thought to initiate nucleation and serve as the framework for newly synthesized calcium carbonate crystals, therefore control of the calcification process is thought to be both chemical and biological.

The batch seawater hypothesis makes it easy to see why OA will be a problem for coral calcification. As previously mentioned, calcification is dependent on the pH of the calcifying medium being high, so as the pH of bulk seawater gets lower, the cost of calcification will become higher because the coral will have to work harder to elevate the pH at the CE, i.e. OA will make calcification more energetically costly due to the fact that corals will have to pump more hydrogen ions out of the CE to make the pH at the site favorable. Some corals seem to be better than others at maintaining calcification rates under OA conditions, while others have

shown severe decreases or even cessation of calcification under OA conditions. Ries (2011) hypothesized that some corals have strong pumps vs. weak pumps. He believes that corals with strong pumps may be resilient to OA due to the fact that they will be able to maintain pH up-regulation at their CE, even though it will be more energetically expensive. In contrast, corals with weak pumps may not be resilient to OA due to the fact that their pumping action will not be able to keep up with the acidity of bulk seawater (Ries 2011).

1.3 The symbiont's role in coral calcification

Symbiont photosynthesis affords the coral host multiple benefits with respect to calcification. There are three predominant ways that photosynthesis stimulates calcification. 1.) Photosynthesis produces oxygen, a necessary reactant to drive coral metabolism (Allemand et al. 2011). 2.) Photosynthesis uses CO₂ as a reactant, thus drawing down pCO₂ as photosynthesis occurs, making the chemistry of the surrounding seawater more favorable for calcification (Allemand et al. 2011). 3.) Photosynthesis produces organic carbon which is translocated to the coral host to be used as an energy source to support daily life functions or to convert to ATP to drive the Ca⁺²-H⁺-ATPase pump (Allemand et al. 2011). OA decreases coral calcification rates, but it remains unclear whether OA may also increase zooxanthellae photosynthesis rates. This potential disparity in the responses of the holobiont to increasing CO₂ requires further research to determine if certain symbiont types could confer resistance and/or resilience to OA stress.

The degree to which a specific type of symbiont could confer OA resistance to a coral may depend on certain factors that can vary in Symbiodinium such as thermal tolerance (Rowan 2004) and growth rate. With respect to thermal tolerance, there is some literature showing that clade D symbionts are heat-tolerant and may confer resistance to bleaching (Baker 2003, Baker et al. 2004, Berkelmans and Van Oppen, 2006, Silverstein et al. 2014). If a coral is living in both a high temperature and high CO₂ world, based on previous literature, calcification will be more than likely to decrease. However, if a coral was hosting a heat-tolerant clade D symbiont, it might be resistant to bleaching and be able to maintain rates of photosynthesis and calcification. The trade-off for the coral host is that clade D is a slow-grower. In contrast, clade C is not thermally tolerant, but affords the coral host faster growth rates. As previously stated, clade C has been shown to grow faster than clade D (Silverstein et al. 2012). If a coral under OA stress hosts a faster-growing symbiont type like clade C, perhaps the host would be afforded maintenance of ambient calcification rates under OA. Hosting different Symbiodinium clades may influence the degree to which OA and/or bleaching will impact reef-building corals.

1.4 Calcification and resilience to climate change

Rates of coral calcification follow a typical Gaussian curve with respect to temperature (Marshall and Clode, 2004). As temperature increases, calcification rate will also increase, but only up to a certain thermal optimum after which point calcification rate will decrease with additional increases in temperature. For many

scleractinian corals, this thermal optimum is between 25 and 27 °C, but the individual maxima will vary by species and location. In a study by Reynaud et al. (2002), corals that were exposed to an increase in CO₂ level from 450 ppm to 800 ppm when held at 25 °C showed no decrease in calcification. However, corals at 28 °C that experienced the same increase in CO₂ exhibited a 50% decrease in calcification. The explanation for these results is that 25 °C is most likely the thermal optimum for the coral in this study because even a decrease in saturation state due to increased CO₂ did not affect calcification. However, at 28 °C there was a significant effect, pointing to the fact that 28 °C may be above this corals thermal optimum. This phenomenon can be explained by the fact that metabolic enzymes are temperature-dependent, and most have a relatively narrow optimal range. Outside of this range, the enzymes that control metabolism will not function optimally, contributing to decreases in metabolic responses.

With respect to CO₂ metabolic effects are less straight-forward because increases in CO₂ can positively affect the symbiont and negatively affect the coral host. Since CO₂ is a necessary reactant for zooxanthellae photosynthesis, it has been shown that moderate increases in CO₂ can increase rates of photosynthesis. In contrast, increasing CO₂ in bulk seawater decreases its pH and aragonite saturation state, making the chemistry of the water increasingly less favorable for calcium carbonate precipitation (Ries 2011, Allemand 2011). In a meta-analysis, Hendriks et al. (2010) showed that on an individual coral basis, increases in CO₂ concentration can increase coral metabolism due to the increased photosynthetic rates. However, on a community-wide scale, increases in carbon dioxide concentration contribute to decreases in metabolism and calcification (Hendriks et al. 2010). In the

last three years, other researchers have also found that increases in CO₂ cause a decrease in both coral metabolism and calcification. In a different meta-analysis, coral calcification was estimated to decrease by 22% by the end of the century due to increasing CO₂ (Chan and Connolly, 2012), and Kaniewska et al. (2012) found an overwhelming decrease in coral metabolism under increased CO₂.

Increasing the energetic status of corals by enhanced heterotrophic feeding may mitigate the negative impacts of bleaching due to increased temperature (Grottoli et al. 2006) and OA (Cohen and Holcomb, 2009) on growth and metabolism. Feeding has been shown to enhance coral growth rate and both light and dark calcification rates (Houlbrèque et al. 2003). Corals that have more energy reserves or that are able to increase their feeding rates may be better able to cope with the stress of OA and bleaching. Those that lack reserves or the ability to increase feeding may be expected to experience reduced skeletal growth. Evidence suggests that certain species may be more capable of heterotrophic feeding than others (Grottoli et al. 2006). Such species-specific differences in feeding may play a key role in determining a corals resilience to changing temperature and ocean chemistry. However, there is a paucity of information regarding the ability of various coral species to supplement autotrophy (photosynthate supplied by their symbiotic dinoflagellate algae) with heterotrophy, and the majority of OA and bleaching studies to date have placed comparatively little emphasis on the nutritional status of the organism in study.

Given the mounting concern for the potential impacts of OA and bleaching on reef-building corals, there is a need to investigate the ability of corals to mitigate these stressors and potentially acclimate to changing conditions. The ability

of corals to control their nutritional status may play a pivotal role in determining their sensitivity to near-future climate change scenarios. The successful conservation of coral populations requires an improved understanding of the major factors responsible for coral resilience to stress. In an age where 20% of the coral reefs of the world have already been destroyed, and it is predicted that another 24% are under imminent risk of collapse (Wilkinson 2004), it is increasingly important for us to understand coral nutrition patterns as well as growth and survivorship rates under acidification and thermal stress.

1.5 Coral heterotrophy

The importance of heterotrophy to the scleractinian diet has been a topic of relative controversy in the field of coral science. Coral heterotrophic capabilities were first described by Young (1930), who noted that corals were voracious carnivores of zooplankton. It was originally thought that heterotrophy was only relevant to corals with large polyps, and even then only truly relevant at depth where light for photosynthesis was limited (Porter 1976). Porter's theory implied that corals with small polyps did not use heterotrophy as a source of nutrition. Porter also believed that heterotrophy was not relevant to branching corals, only to mounding species. Porter asserted that even if corals were capable of complete reliance on heterotrophy to fulfill their daily metabolic requirements, rarely, if ever, in nature would zooplankton on a reef reach densities great enough to satiate this requirement (Muscatine and Porter, 1977). Decades later, it was found that Muscatine and Porter's belief that zooplankton densities were too low to sustain coral metabolism

were severely biased by sampling done during the day when zooplankton densities are low on the reef. However, between dusk and dawn zooplankton densities over reef systems can be very high. Muscatine and Porter also failed to realize that zooplankton are not the only food source that corals can ingest heterotrophically; additionally, they can also ingest suspended particulate matter (SPM) and detritus in the water column.

Sebens (1996) found evidence to disprove Porters (1976) theory that coral heterotrophy was only relevant in large-polyped corals. Sebens (1996) showed that size of a coral polyp did not limit its heterotrophic capability, polyp size only limited the upper size classes of material a smaller polyp could ingest, i.e. corals with small polyps might be limited to particles on the order of pico-, nano-, and micro- plankton, as opposed to meso- and macro- plankton. In a seminal study on coral heterotrophy, Palardy et al. (2005) argued that all corals are capable of heterotrophic plasticity and that different environmental conditions could allow corals to increase or decrease the contribution heterotrophy made to their diet (Palardy et al. 2005). The study argued that coral nutrition should be conceptualized as a continuum from 100% autotrophy to 100% heterotrophy with all possible combinations in-between depending on the environmental conditions being experienced (Palardy et al. 2005).

Grottoli et al. (2006) experimentally demonstrated that heterotrophy could contribute to coral resilience following a bleaching event. The authors showed that in non-bleached *M. capitata*, heterotrophy only contributed to about 40% of daily metabolic requirements (Grottoli et al. 2006). However, in bleached *M. capitata*, heterotrophy accounted for about 105% of daily metabolic requirements (Grottoli

et al. 2006). The authors also used two *Porites* species (*P. lobata* and *P. compressa*) and found they did not use heterotrophy as much as *M. capitata*. These results suggested that certain corals are more capable of heterotrophic plasticity than others, and that if corals are able to enhance heterotrophic feeding under stress, they may become more resilient to bleaching (Grottoli et al. 2006).

Other researchers wanted to test if heterotrophy could be an indicator of resilience for OA stress, too. In several studies using different species of corals and compiled by Cohen and Holcomb (2009), it was demonstrated that corals that had the opportunity to feed during exposure to acidification conditions were able to calcify at 85-100% of their ambient calcification rates. In contrast, the same corals that were not given access to food and exposed to the same acidification conditions were only able to calcify at 20-25% of their ambient rates (Cohen and Holcomb, 2009). These data suggested that heterotrophy could also be an indicator of resilience to acidification stress as well as bleaching. In a review of tropical scleractinian coral heterotrophy, Houlbrèque and Ferrier-Pagès (2009) noted that corals that use heterotrophy showed greater secretion of organic matrix, calcification rates, chlorophyll *a*, photosynthetic rates, and protein concentrations when compared to conspecifics that were not fed (Houlbrèque and Ferrier-Pagès, 2009).

There are still major gaps as to the physiological mechanisms and/or pathways that allow heterotrophy to provide some corals resilience to stress, which is a major focus of this dissertation. There have been some hypotheses put forth to address the physiological basis for resilience, which may or may not be mutually exclusive: 1.) Heterotrophy increases coral tissue biomass (Edmunds 2011) which could increase the number of pumps present to facilitate calcification. 2.) Heterotro-

phy increases amino acid content (Houlbrèque and Ferrier-Pagès 2009) that can be used in organic matrix synthesis. 3.) Heterotrophy stimulates photosynthesis rates (Houlbrèque and Ferrier-Pagès 2009) which would increase translocation of organic carbon to be used as energy for calcification. 4.) Heterotrophy increases coral metabolism which increases respiration rates which increases the internal DIC pool that can be used for calcification (Swart et al. 1983). 5.) Heterotrophy increases lipid content (Anthony et al. 2009, Tolosa et al. 2011) which can be used for ATP for ion pumping for calcification.

The relative contribution of heterotrophy to the scleractinian diet remains poorly understood. Heterotrophy can account for anywhere from 0 to 66% of the fixed carbon incorporated into a corals skeleton (Muscatine et al. 1989). Heterotrophy from zooplankton can be an important and significant source of fatty acids (Teece et al. 2001), and is thought to provide corals with essential nutrients such as phosphorus and nitrogen that are not supplied by their symbiotic photosynthetic zooxanthellae and cannot be synthesized *de novo* (Muscatine and Porter, 1977). It remains unclear which corals can use heterotrophy most efficiently. Little research has been done on the degree of coral heterotrophy with respect to OA stress. There is still a large gap in the literature regarding which corals are best at using heterotrophy and to what extent, and what mechanisms allow for increased feeding rates. In addition, much of the seminal work on coral heterotrophy has been done on Pacific species, and fewer studies have focused on Caribbean/Florida Reef Tract species. There is a great need for this research because we do not know yet how OA may affect our already dwindling populations of coral reefs. This information will expand our knowledge about how OA will affect coral reefs and will shed light on

the types of coral species that will be most resilient in the face of global climate change. In addition, these data will advance our knowledge of the potential mechanisms and roles that elevated CO₂ stress plays in the metabolism and physiology of scleractinian corals.

1.6 Coral lipids

Scleractinian corals have been described as very fatty organisms of which a third or more of their tissue by dry weight can be made up of lipids (Patton et al. 1977, Harland et al. 1993). The fatty acids that make up the lipids in corals can come directly from zooxanthellae, can be synthesized by the coral host itself from glycerol translocated from zooxanthellae, or can be obtained from a heterotrophic diet of zooplankton, suspended particulate matter, etc. (Imbs et al. 2010). Because of these different sources, lipid content in corals can vary based on zooxanthellae density, season, food availability, reproductive status, bleaching status, and rate of mucus production (Rodrigues et al. 2008). While zooxanthellae are the dominant source of lipids in corals, Teece et al. (2011) showed that heterotrophy as a lipid source is not trivial. The major function lipids serve in corals is as energy reserves (Grottoli et al. 2004, Teece et al. 2011). Patton et al. (1977) estimated that over 90% of reserves are stored in the host tissue, with less than 10% found in the zooxanthellae. Anthony et al. (2002) also demonstrated that coral tissue is mostly made up of lipids, and carbohydrates and proteins are negligible in the tissues. Grottoli et al. (2004) asserted that the excess carbon fixed by zooxanthellae photosynthesis is stored in coral tissue as lipids, representing significant energy reserves. Quan-

tifying coral energy stores (lipid reserves) is crucial to understanding resilience to stress because there may be a difference in the metabolism of lipid reserves for different species, and in their temporal responses to and subsequent recovery from that stress (Grottoli et al. 2004). Grottoli et al. (2004) found mean total lipid concentrations were about 56% lower in heavily bleached corals than in non-bleached corals, and 31% lower in moderately bleached corals than in non-bleached corals.

On an annual cycle, corals experience a natural variation in their tissue biomass, lipid content, and photosynthetic pigment concentrations. For Caribbean corals it has been observed that tissue biomass is highest during winter and spring and lowest during late summer and fall (Fitt et al. 2000). Because of this seasonal variability, it is important to examine lipid content contextually, i.e. summer vs. winter, to understand baseline values. Proteins can serve as an energy source under some circumstances, and carbohydrates can represent a small portion of a corals energy reserves, but they are generally used in the short-term (Anthony et al. 2002). However, the main role of lipids is to serve as long-term energy reserves to be used in times of stress, which is why we will quantify lipids over proteins and carbohydrates. This information may allow us to predict what corals will be most resilient under stressful conditions, and furthermore could help us target species for coral gardening and transplant experiments that we suspect will be more resilient to stress. This dissertation assesses whether the corals that exhibit plastic feeding (capability of supplementing autotrophy with heterotrophy) will also have higher levels of lipids.

1.7 Coral Metabolism - Photosynthesis, Respiration, Symbiont density, and Photosynthetic Efficiency

Animal metabolism is temperature-dependent (Hochachka and Somero, 2002) and therefore will likely be altered as warming occurs (Sanford 1999). Respiration is more sensitive than photosynthesis to changes in temperature (Urrutia et al. 2006) which may cause changes in an organisms allocation of caloric demands. Metabolic suppression has been shown in a range of marine organisms in response to CO₂ fluctuations, and while the majority of energy needs in scleractinians are supplied by zooxanthellae, host heterotrophy can sometimes meet metabolic requirements under stress (Kaniewska et al. 2012). It has been shown that Pacific coral species that maintain higher photosynthesis to respiration ratios may be more resistant to bleaching stress (Grottoli et al. 2004). Grottoli et al. (2004) hypothesized that a possible mechanism for the lack of lipid consumption in visibly bleached corals in the study could have been due to proportionally lower respiration rates as compared to other species. A lower respiration rate could allow a coral to conserve its energy reserves, and thus corals that maintain higher P/R ratios may be better adapted to survive elevated temperature stress. As such, it is important to pair growth, feeding, and lipid data with respiration data.

With respect to photosynthetic efficiency, it has been hypothesized that corals exposed to high CO₂ may increase their efficiency based on CO₂ fertilization (Bradling 2011). The rationale for this theory is that coral symbionts may be more efficient in high CO₂ conditions as CO₂ is a necessary reactant for photosynthesis. Because symbionts may be more efficient, it is possible that corals exposed to high CO₂

acclimation would be less susceptible to bleaching when exposed to temperature stress (Baker, personal communication). Since heterotrophy has been shown to increase photosynthetic efficiency (Houlbrèque and Ferrier-Pagès, 2009) part of this dissertation will assess whether corals of the Florida Reef Tract (FRT) that are feeding heterotrophically and have been acclimated to high CO₂ conditions will be less likely to bleach when exposed to heat stress.

1.8 Broader significance

Few studies to date have focused on heterotrophy and lipid content of corals in the FRT. Studies that have been done on this topic have largely focused on Pacific and Red Sea species. Teece et al. (2011) were the first to report the lipid composition of both the host and zooxanthellae in Caribbean corals. In the last five to ten years, the importance of heterotrophic capacity in corals with respect to coral resilience to climate change stressors has become a hot topic, but one that has not been looked into enough in the Atlantic/Caribbean. Earlier studies suggest that heterotrophy is correlated to lipid levels (Anthony and Fabricius, 2000), but this relationship is still unclear. Corals exhibit great plasticity in their heterotrophic behaviors and more research is needed to understand how this plasticity affects metabolism. This dissertation seeks to shed light on that relationship and how it may contribute to resilience to global climate change. Results from these experiments will enhance our understanding of coral physiology in response to climate change. This research will provide a unified approach to studying coral resiliency to climate change and will be of value to reef managers, modelers, and researchers

studying coral growth, metabolism, and physiology. Lastly, understanding how corals cope with increased CO₂ and temperature is imperative to protect corals of the FRT.

CHAPTER 2

Calcification rate and lipid content in three coral species from the Florida Reef Tract

2.1 Summary

Coral calcification rates alone may not always be the best indicator of reef health and resilience. Coral lipids have been shown to be an accurate predictor of resilience under stress, however many studies that have assessed lipid values have been laboratory-based. In this study, the goal was to gain an understanding of intra-and inter-specific variation in growth rate and lipid content, as well as symbiont density and chlorophyll *a*, of corals in the Florida Reef Tract to improve our insight of *in-situ* variation and resilience capacity in coral physiology. The Florida Keys are an excellent place to assess this question regarding resilience because the Florida Reef Tract has been decimated with respect to coral cover since the 1997-1998 El Nino event, yet is home to inshore patch reefs that remain seemingly resilient, i.e. able to recover from stress. The calcification rates of three species were monitored over a seven-month period at four sites and lipid content was quantified at two seasonal time points at each of the four sites. *Montastraea cavernosa* had the highest calcification rate ($4.3 \text{ mg cm}^{-2} \text{ day}^{-1}$) and lowest lipid content (1.6 mg

cm^{-2}). In contrast, *Orbicella faveolata* and *Porites astreoides* had lower calcification rates ($1.5 \text{ mg cm}^{-2} \text{ day}^{-1}$ and $1.7 \text{ mg cm}^{-2} \text{ day}^{-1}$, respectively) and higher lipid contents (2.8 mg cm^{-2} and 2.3 mg cm^{-2} , respectively). These data suggest that there may be a trade-off between allocating energy to calcification versus storing energy as lipid reserves. We propose that calcification measurements coupled with lipid data may provide a more complete assessment of coral health and resilience, and policy makers may want to consider using these metrics when making management decisions in the future.

2.2 Background

Corals reefs are biodiverse ecosystems with numerous cultural, economic, medical, and recreational values (Costanza et al. 1997). Today's coral reefs are facing multiple stressors including, but not limited to, anthropogenic pollution, eutrophication, overfishing, habitat destruction, and climate change. The detrimental effects of these stressors on coral reefs are no longer under debate; rather what is under debate are the metrics and baselines that should be used to improve science-based policy decisions. In order to help protect this important ecosystem and all of its resources, it is imperative to understand which coral species at which sites are going to be most resilient to stress. Resilience is the ability of an organism to return to its original state after experiencing a disturbance (Lewontin 1969), and thus resilience is about having the means to overcome stress. Identifying coral species that will be winners and losers on reefs of the future, as well as sites to focus stronger conservation efforts on, have become major foci of the field of coral eco-physiology and

conservation science. Edinger et al. (2000) asserted that coral growth rates alone may not be good enough indicators of reef health, but many studies to date only take into account growth/calcification when assessing health and resilience. Coral resilience has been shown to be largely dependent on a species lipid reserves as well as its symbiont population (Anthony et al. 2009, Pisapia et al. 2014, Grottoli et al. 2014).

Lipid content in reef-building corals represents an alternative source of fixed carbon that can be used to maintain vital processes under stress, i.e. calcification and daily metabolism (Grottoli et al. 2006, Rodrigues and Grottoli, 2007, Anthony et al. 2009). Due to the fact that lipids in coral tissue can serve as energy reserves during times of stress, measuring lipid content can be an accurate predictor of the potential resilience capacity of a certain species. The density of a corals symbiotic algal population is also a good proxy for overall coral condition and health because the symbiosis between the coral host and its symbiont is the fundamental building block of reef-building coral success (Davy et al. 2012). Under normal, unstressed conditions, a coral may receive up to 100% of its daily metabolic requirements from the transfer of photosynthetic product from the symbiont to the coral (Grottoli et al. 2006). Therefore, the amount of chlorophyll *a*, the major photosynthetic pigment of corals symbiotic algae, may also be a good indicator of the status of the symbiont. Lipids and symbiont density have been shown to vary considerably by coral species, reef site, sampling season, and sampling year (Fitt et al. 2000, Teece et al. 2011, Pisapia et al. 2014), and thus trying to compare results between studies where one or more of these factors differ can be inconclusive. Due to the variability associated with these parameters, as well as the fact that many sites

have changed since early observations were published, it is important to continue to monitor the parameters that could indicate capability for resilience or indicate a red flag regarding sites or species in distress.

Millions of people visit coral reefs in the Florida Keys every year, and these reefs alone are estimated to have an asset value of \$7.6 billion (Johns et al. 2001). Unfortunately, coral reefs in the Florida Reef Tract have experienced dramatic declines in coral cover since the 1997-1998 El Nino event, largely characterized by major losses in reef-building species like *Orbicella faveolata* (Ruzicka et al. 2013; Toth et al. 2014). An exception to this trend may be on inshore patch reefs in the Florida Reef Tract where coral cover and growth has remained relatively high (Manzello et al. 2015). Therefore, these inshore reefs of the Florida Keys present a unique opportunity to study the physiology behind this observed resilience success. These higher inshore growth rates may be due to thermal acclimatization/adaptation to higher inshore temperatures and/or relatively high inshore aragonite saturation states due to proximity to seagrass beds (Manzello et al. 2015). Previous studies exploring other mechanisms for this inshore resilience, such as lipid content, have been proposed (Teece et al. 2011), but thus far have been inconclusive because lipid content can be highly variable for the same species due to turbidity, nutrients, and plankton abundance on reefs. However, using lipids as a metric for resilience may prove to be useful when comparing between a few dominant reef-building species at specific sites and time points. Many studies to date on coral growth and lipids as indicators of resilience have been done under stress in a laboratory setting. Less is known regarding in-situ inter- and intra-specific variation with respect to calcification and lipid content. The purpose of this study was to quantify inter-and

intra-specific variation in resilience indicator parameters (namely calcification and lipid content, as well as symbiont density and chlorophyll *a*), in three common species of corals in the Florida Reef Tract at four different sites in two sampling seasons (summer vs. winter). These data may help us get a better picture of the health and resilience of corals in the Florida Keys, and may allow us to predict trends in future resilience capability. These data will also give us an important updated *in-situ* baseline with which we can compare laboratory studies done under climate change stressors.

2.3 Methods

2.3.1 Collection

Three species of scleractinian corals, *Porites astreoides*, *Montastraea cavernosa*, and *Orbicella faveolata*, were collected under Florida Keys National Marine Sanctuary Permit #FKNMS-2011-049 for the August 2013 sampling and #FKNMS-2014-002 for the March 2014 sampling from four different sites throughout the Florida Reef Tract. The four sites were Lower Keys Inshore (LKI) (24.59723N, 81.45505W), Middle Keys Inshore (MKI) (24.81216N, 80.76075W), Upper Keys Inshore (UKI) (24.939N, 80.562W) and Upper Keys Offshore (UKO) (24.946N, 80.502W). A map of the collection sites is shown in Figure 1. All sites were approximately four to six meters maximum depth. Approximately five individuals per species (x three) per site (x four) per season (x two) were collected totaling approximately 120 measurements per parameter, although there was some mortality over the seven month study. Corals were retrieved by SCUBA divers at each sampling point, cleaned

of all non-coral flora and fauna, and transported back to the University of Miami to be analyzed for calcification, total lipid content, symbiont density, and chlorophyll *a* content. HOBO loggers were deployed at all reefs sites during the seven month study to monitor sea temperature data, but unfortunately only the loggers at UKI and UKO were able to be retrieved. Molasses Reef (25.012N, 80.376W) data from the Coastal Marine Automated Network (C-MAN) was obtained from www.ndbc.noaa.gov and used as a quality control reference to compare with the HOBO logger data from the upper keys sites.

2.3.2 Calcification, total lipid content, symbiont density, and chlorophyll *a* content

Calcification was measured using the buoyant weight technique (Davies et al. 1989) in August 2013 and March 2014. Therefore, the calcification rate data shown is integrated over this seven month period. To analyze the other three parameters, coral tissue was removed from the skeleton using the air-brush technique (Szmant et al. 1990) and homogenized for 30 seconds. The aliquot for total lipids (three mL) was filtered onto a glass fiber filter (GF/A) and frozen at -80C until further analysis. Analysis followed that of Teece et al. (2011). Briefly, total lipids were extracted three times with two mL of 1:1 dichloromethane:methanol and five minutes of vortexing. The resulting organic extracts were collected, dried under a stream of nitrogen gas, weighed on an analytical balance (Mettler AE 200) and normalized to surface area.

One mL of total blastate was placed in a 1.5 mL Eppendorf tube filled with 50L of Lugols solution to be used for manual symbiont counts via microscopy. Samples

were vortexed for ten seconds, and counted twice in independent replicate counts with a haemocytometer (Hausser Scientific) using a VistaVision compound microscope at 100 magnification. Symbiont density was normalized to surface area. Lastly, one mL of total blastate was filtered onto a glass fiber filter (GF/A) for chlorophyll *a* analysis and frozen at -80 °C until further analysis following Holm-Hansen and Riemann (1978). Briefly, chlorophyll *a* samples were analyzed on a fluorometer (TD-700 Turner Designs) calibrated with purified chlorophyll *a* (Sigma-Aldrich catalog no. C6144). Pigment content was normalized to coral surface area.

2.3.3 Surface area

Scanning methodologies followed those of Enochs et al. (2014). Corals were scanned using a white light 3D scanner (HDI Advance R2, 3D3 Solutions) calibrated with a five mm glass calibration board. Each coral sample was scanned from two different angles eight times while rotated 360 degrees around a central axis. The resulting sixteen scans were aligned and compiled into a single mesh using the FlexScan3D software package. Each mesh was exported as a .stl file and imported into Leios II, where surface area was calculated.

2.3.4 Statistics

All statistical analyses were completed in the program JMP version 11.0.0. Normality and homoscedasticity were ascertained prior to testing each dependent variable using a Shapiro-Wilk test and Levenes test, respectively. A mixed model ANOVA (site, season, and species nested within site and season) was run for the dependent variables lipid content, symbiont density and chlorophyll *a*, while a full

factorial ANOVA (site x species) was run for the dependent variable calcification rate. Season was not tested as a main factor for calcification due to the fact that calcification rate was integrated over the seven month period. If significant differences were found, a post-hoc test (Tukeys HSD) was run to determine where the differences were. Correlation tests were run between each of the parameters for each species, and tested for either a quadratic or linear fit. Alpha for all tests was set at 0.05.

2.4 Results

Calcification rate was significantly affected by site as well as the interaction between species and site (Table 1, ANOVA, $p < 0.05$). Generally, UK calcification rates were higher than MKI rates (Fig. 2). Rates were variable across sites, but there were not many significant differences between species within the same site. *M. cavernosa* had the highest combined calcification rates of the three species pooled across sites and seasons ($4.3 \text{ mg cm}^{-2} \text{ day}^{-1}$, Table 2). *O. faveolata* and *P. astreoides* had similar pooled calcification rates across all sites and seasons (1.5 and $1.7 \text{ mg cm}^{-2} \text{ day}^{-1}$, respectively, Table 2).

Lipid content was significantly affected by site, the interaction between site and season, and the nested effect of species within site and season (Table 1, ANOVA, $p < 0.05$). *M. cavernosa* had the lowest mean lipid value of the three species pooled across sites and seasons (1.6 mg cm^{-2} , Table 2), whereas *O. faveolata* had the highest mean lipid value of all three species across all sites and seasons (2.8 mg cm

Symbiont density was significantly affected by season and the nested effect of species within site and season, whereas chlorophyll *a* was additionally significantly affected by the interaction between site and season (Table 1, ANOVA, $p < 0.05$). Generally symbiont density and chlorophyll *a* values were both higher in summer than in winter (Fig. 4a,b, Fig. 5a,b). However, for both parameters, differences between species within and between sites were not significant in winter.

Mean temperature data are summarized for each month of the study in Table 3 for UKI and UKO. Temperatures at the UKI and UKO sites, as well as the quality control for the Upper Keys (Molasses Reef Buoy) never exceeded local bleaching threshold (30.4°C , Manzello et al. 2007) during the seven month study. Unfortunately, temperature data from MKI and LKI were not available over this seven month period. However, historical data from the same MKI site indicated that temperatures often exceed 30.4°C in the summer, as they did in 2010 and 2011 (Manzello et al., In Review, Table 4). The same dataset reported that the LKI site also exceeded the local bleaching threshold in 2010 and 2011 (Table 4).

Correlations were run between all parameters for each species (Table 5). The only species where calcification rate was correlated to lipid content was in *M. cavernosa* with a quadratic fit (Fig. 6, $p < 0.05$). *M. cavernosa* was also the only species where zooxanthellae density was linearly correlated to lipid content (Fig. 7, $p < 0.05$).

2.5 Discussion

2.5.1 Calcification

This study quantified the calcification rates of three common coral species in the FRT over a seven month period at four sites spanning the upper to lower Florida Keys. The general trend for most of the species was that growth was higher in the upper keys than the middle keys. This finding is consistent with many previous studies on the FRT showing that coral growth in the middle keys is reduced due to the influence of water input from Florida Bay (Cook et al. 2002). Coral growth is thought to be impeded here because Florida Bay water has high turbidity levels (Roberts et al. 1982), variable temperature and salinity (Shinn 1966, Shinn et al. 1989), and elevated nutrients (Szmant and Forrester, 1996). In contrast, the upper keys is characterized by water with a low influence from Florida Bay, which could explain more favorable growth at the upper keys sites (Cook et al. 2002). The upper keys are thought to have a low influence from FL bay waters because flow out of the bay through Hawk Channel generally flows in a southwest direction (Pitts 1994). These data may explain why growth at the MKI site was very low for *M. cavernosa*, *O. faveolata*, and *P. astreoides*.

Calcification rates measured here are comparable to rates of the same three species measured on a reef in Jamaica. Mallela and Perry (2007) found the calcification rates of *M. cavernosa*, *O. faveolata*, and *P. astreoides*, to be approximately $2.4 \text{ mg cm}^{-2} \text{ day}^{-1}$, $2.3 \text{ mg cm}^{-2} \text{ day}^{-1}$, and $1.0 \text{ mg cm}^{-2} \text{ day}^{-1}$, respectively. These calcification values from the Jamaica study were lower than the values obtained in this study for *M. cavernosa* ($4.3 \text{ mg cm}^{-2} \text{ day}^{-1}$) and *P. astreoides* ($1.7 \text{ mg cm}^{-2} \text{ day}^{-1}$),

and higher than found here for *O. faveolata* ($1.5 \text{ mg cm}^{-2} \text{ day}^{-1}$). The summer of 2013 that preceded the time points taken in this study (August 2013 and March 2014) was the coolest since 1996 at Molasses Reef (Manzello et al., In Review, Sci Rpt), therefore conditions were theoretically favorable for growth (Manzello et al. 2015). These favorable conditions may partially explain why *M. cavernosa* and *P. astreoides* had higher growth rates compared to the field study from Jamaica. Lower growth rates for *O. faveolata* in this study compared to those reported from other Caribbean studies may be an indication that *O. faveolata* in the Florida Keys is less hardy in this region than previously thought. Alternatively, *O. faveolata* may not be allocating the majority of its energy to calcification, but rather to other pathways, possibly storing lipid reserves.

2.5.2 Lipid content

This study presents evidence that *O. faveolata* in the upper keys sites have elevated lipid contents compared to the two other coral species. Mean lipid content in *O. faveolata* at the UKI site in summer ($4.6 \text{ mg lipids cm}^{-2}$) was approximately two to three times the lipid content of the other species at the same site and season. Data from this study did not show a strong effect of season on lipid content. This finding is not consistent with data from Harland et al. (1992, 1993) and Oku et al. (2003) who found generally lower coral lipid values in winter months. Lower lipid content in corals in the winter is attributed to reduced temperatures and light conditions, reducing photosynthetic activity of the coral symbiont, and thereby reducing the amount of photosynthate transferred to the coral as lipids. However, in the current study, lipid content was slightly elevated in winter months compared

to summer months. While many essential fatty acids, the building blocks of lipids, can be translocated to the coral from its symbionts, heterotrophic feeding on zooplankton and/or particulate organic matter (Imbs et al. 2010) can also provide significant amounts of lipids and fatty acids for the coral host. Teece et al. (2011) also asserted that many fatty acids come from heterotrophic sources. Edmunds (1986) hypothesized that low lipid levels found in corals may be attributed to the species being largely autotrophic, as opposed to heterotrophic, suggesting that larger lipid stores may indicate greater usage of heterotrophic nutrition. Additionally, lipid content that is significantly correlated to zooxanthellae density could indicate that the bulk of the corals lipids are coming from symbiont photosynthate, as in *M. cavernosa* in this study. In contrast, lipid content that is not significantly correlated to zooxanthellae density might suggest that lipids are coming from other sources besides symbiont photosynthate, i.e. heterotrophy.

O. faveolata may be a coral species with greater plasticity in its ability to switch from autotrophic to heterotrophic nutritional inputs (Teece et al. 2011). Teece et al. (2011) asserted that heterotrophy may provide up to 40% of the fatty acids found in *O. faveolata*, which could explain its elevated lipid levels found in this study. This idea is also supported by the fact that lipid content was not significantly correlated to zooxanthellae density in *O. faveolata*. Heterotrophic feeding is contingent on the immediate area surrounding a coral i.e. turbidity, nutrient, and light levels, and possibly reproductive status (Teece et al. 2011), and is also highly species-specific (Ferrier-Pagès et al. 2010). Inshore sites tend to be more conducive to heterotrophy than offshore sites because they often have elevated nutrients due to proximity to shore (Lirman and Fong, 2007) which can indicate increased food availability.

Indeed, differences in lipid content in this study appear to be driven by site and species, especially in the upper Florida Keys.

In analyzing this dataset, it became evident that the species with higher lipid contents had lower calcification rates (i.e. *O. faveolata* and *P. astreoides*), and vice-versa, (i.e. *M. cavernosa*). This observation was initially surprising because of evidence indicating that energy reserves can protect corals from climate change stress by allowing them to maintain their calcification (Rodrigues and Grottoli, 2007, Towle et al. 2015). However, it is important to put these data in context. In this study we observed that under *in-situ* non-stress scenarios, lower lipid content appeared to favor higher calcification rates in *M. cavernosa*. Compare this finding to the idea that when a coral is undergoing a laboratory-induced stress, increasing its lipid stores can provide it with the extra energy it needs to maintain daily calcification and metabolism on the shorter-term. The two findings are not mutually exclusive, but rather, may shed light on an important and contextual balance between calcification and lipids. Under normal non-stressed conditions, a coral might benefit from a moderate balance between growing enough and storing enough lipid reserves. However, when stressed, the coral may benefit from increasing lipid reserves to use when calcification is suffering due to bleaching and/or acidification stress.

2.5.3 Symbiont density and chlorophyll *a*

Both symbiont density and chlorophyll *a* levels were highest in the summer time (Fig. 3c,d). The fact that site was not a strong cause of variability was somewhat surprising given that oftentimes inshore sites are less clear (more turbid)

with less light availability than offshore sites (Lirman and Fong 2007, Wagner et al. 2010), contributing to lower symbiont densities on inshore relative to offshore sites. However, in this study, season seemed to be a more significant source of variation on symbiont density than site (Table 1). Fitt et al. (2000) noted that symbiont parameters tend to peak in the winter because in summer corals often undergo bleaching events where symbiont populations are reduced due to exposure to higher temperatures and irradiance levels, even when those bleaching events are not fatal. Surprisingly, the symbiont and chlorophyll data from this study are not consistent with Fitt et al. (2000) because they were higher in summer rather than winter. This finding may be due to the fact previously mentioned that the summer that preceded the first time point of this study (August 2013) was the mildest summers in the last decade in the FRT (Manzello et al. 2015). Summer 2013 in the FRT was not characterized by any major bleaching events, and thus perhaps symbiont parameters were not reduced during the summer due to lack of bleaching events, but were slightly reduced in winter due to reduced light and temperature, etc. This data highlights the importance of continued monitoring, as trends from seminal Caribbean field work papers from more than a decade ago may not be broadly generalizable.

2.5.4 Calcification rate versus lipid content tradeoffs and implications for policy

Although more research is needed, the dataset suggests there could be a metabolic trade-off between calcifying and storing lipids, which may be mediated by heterotrophy in some species. Perhaps if a coral is allocating its energy toward cal-

cification and not storing enough lipid reserves, it may be worse off during stress events such as acute thermal anomalies or chronic ocean acidification conditions. Indeed, Anthony et al. (2007) found that lipid content below 1.0 mg cm^{-2} in the Pacific coral *Acropora intermedia* were a threshold level below which triggered high mortality following a bleaching event. More research is needed to determine if the physiological patterns observed in this study would hold true immediately following a stress event such as bleaching, i.e. would *O. faveolata* fare best out of the three species at the study sites due to its elevated lipid reserves? Conversely, if a coral is storing away a lot of its energy in the form of lipid reserves and not allocating enough energy to calcification, it could be less fit to compete with other corals and macroalgae for space on the reef (McCook et al. 2001), which could also lead to mortality. The potential tradeoff between calcification and lipid storage should be examined at additional sites throughout the FRT to see if the pattern holds true, as well as tested in other coral reef regions.

In conclusion, this work tends to agree with the Edinger (2000) argument that growth data alone may not be a good enough indicator of coral health. This study highlights that policy makers for coral conservation management should consider future assessments of lipid data with calcification data when predicting coral health and resilience, rather than basing decisions solely on coral cover. Measuring these parameters and identifying whether or not they are within a moderate range may help us more accurately predict stress susceptibility and/or potential for resilience on reefs of the future. To that end, these data confirm decades of previous work showing that Middle Keys Inshore sites are degraded with respect to coral health. These data also suggest that *M. cavernosa* should be monitored

closely over the coming years in the FRT based on its low lipid reserves, potentially putting it at risk following future bleaching.

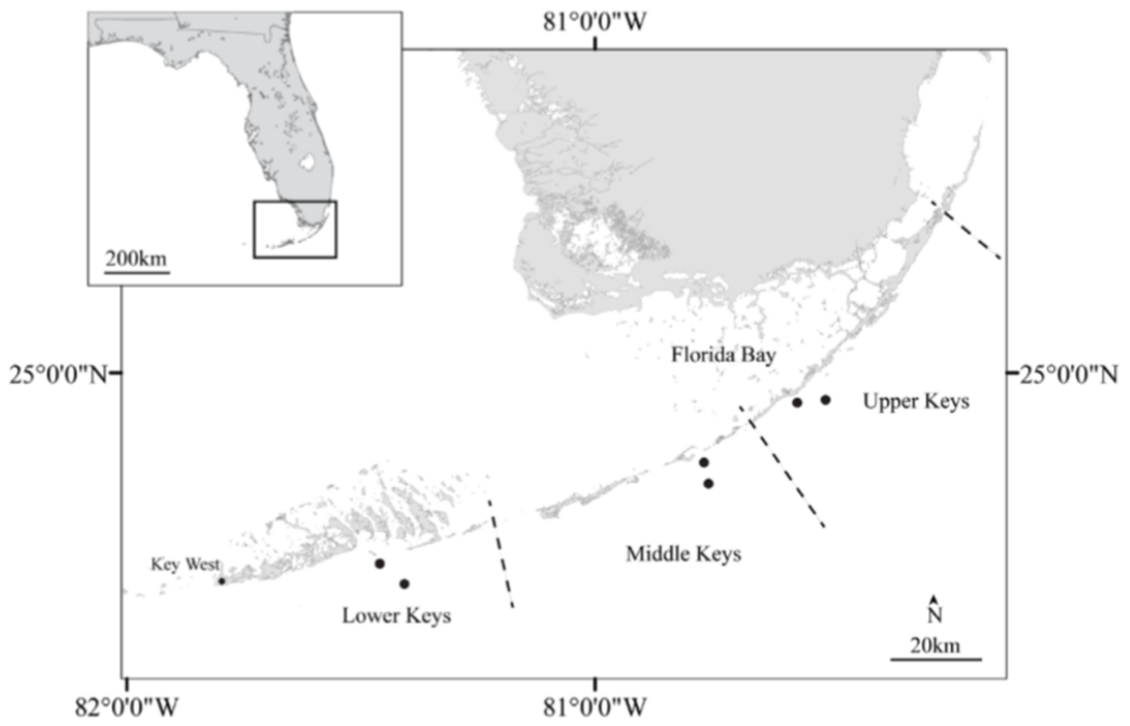


Figure 2.1: Map of the Florida Keys portion of the Florida Reef Tract courtesy of Manzello et al. (2012). Study sites are depicted by black dots.

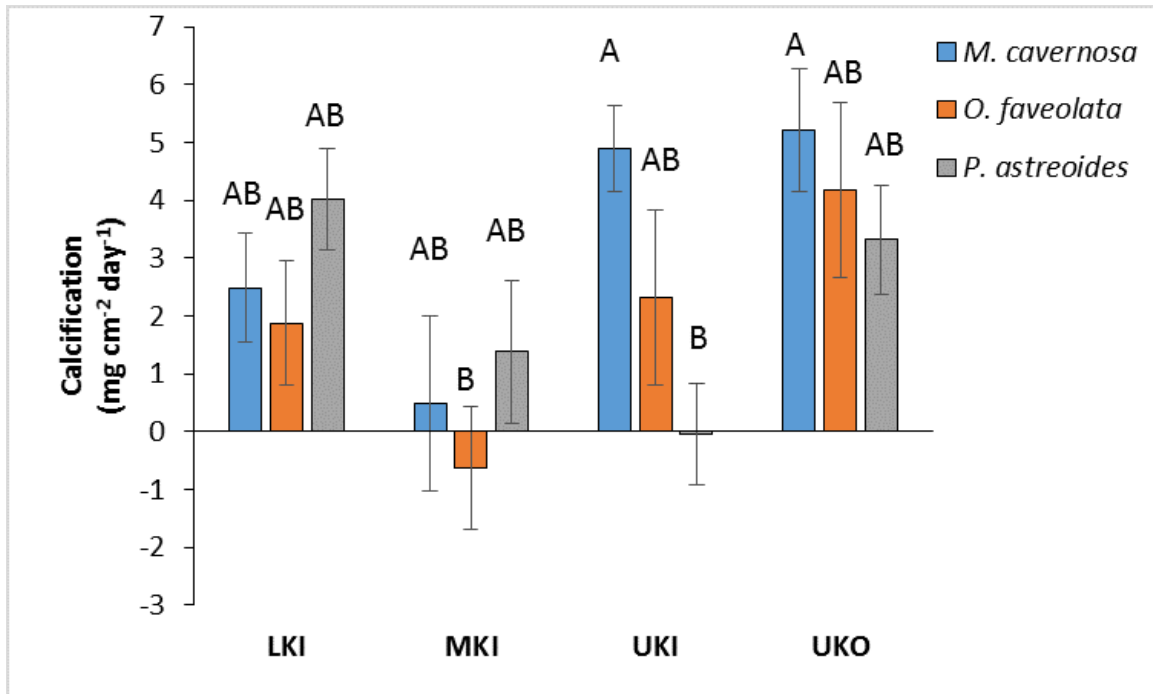
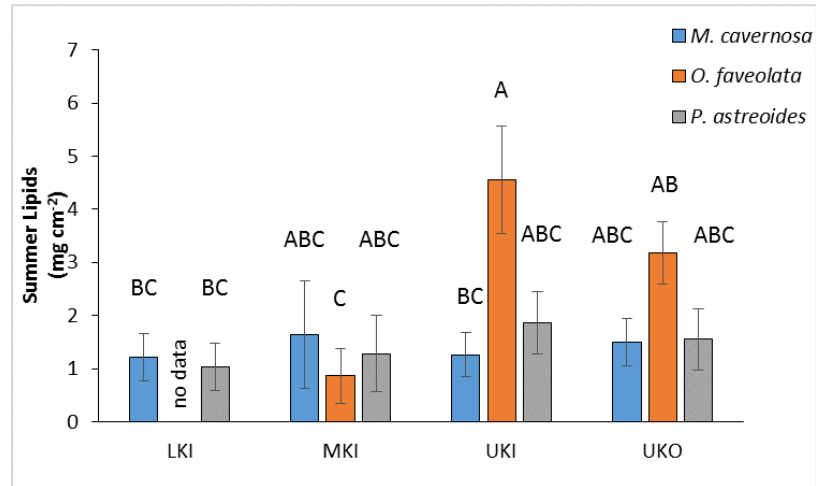
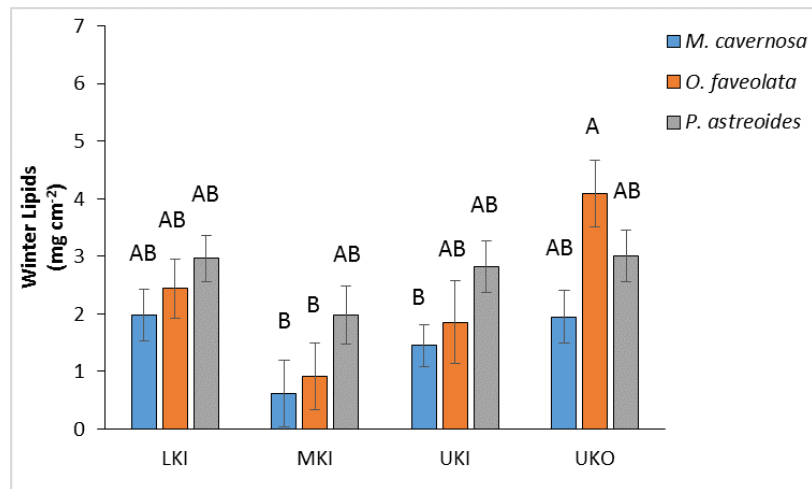


Figure 2.2: Calcification rates of the three coral species at each of the four sites over the course of the seven month study. Letters represent statistical differences based on a *post-hoc* Tukeys HSD test. Error bars represent ± 1 S.E.

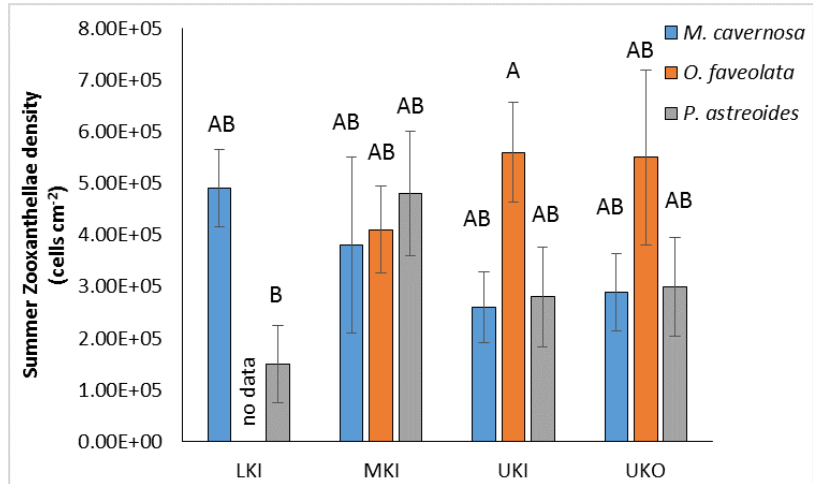


(a)

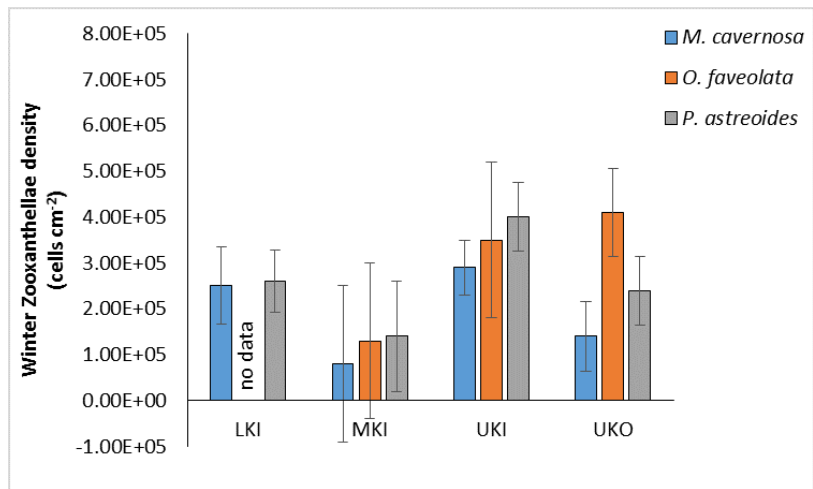


(b)

Figure 2.3: Lipid content of the three coral species at each of the four sites during (a) summer and (b) winter. Letters represent statistical differences based on a *post-hoc* Tukeys HSD test. Error bars represent ± 1 S.E.

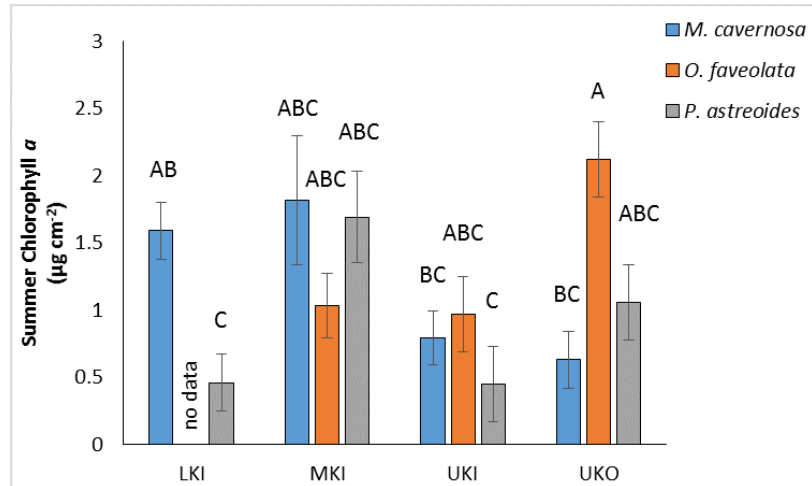


(a)

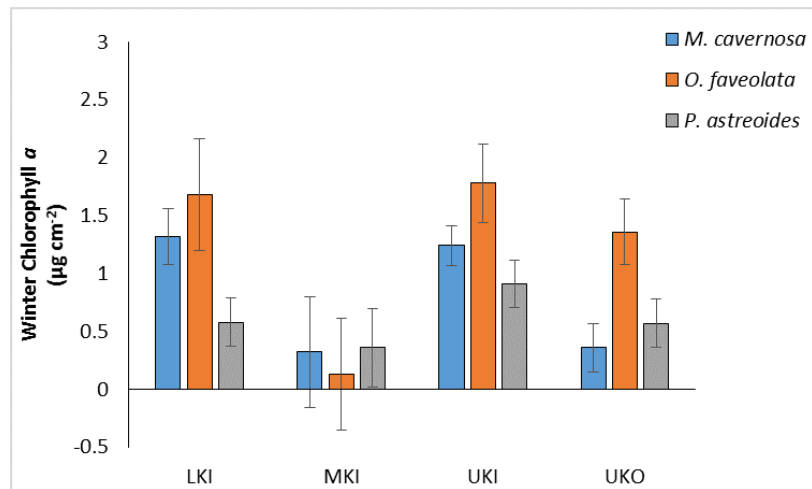


(b)

Figure 2.4: Zooxanthellae density of the three coral species at each of the four sites during (a) summer and (b) winter. Letters represent statistical differences based on a *post-hoc* Tukeys HSD test. Error bars represent ± 1 S.E.



(a)



(b)

Figure 2.5: Chlorophyll *a* content of the three coral species at each of the four sites during (a) summer and (b) winter. Letters represent statistical differences based on a *post-hoc* Tukeys HSD test. Error bars represent ±1 S.E.

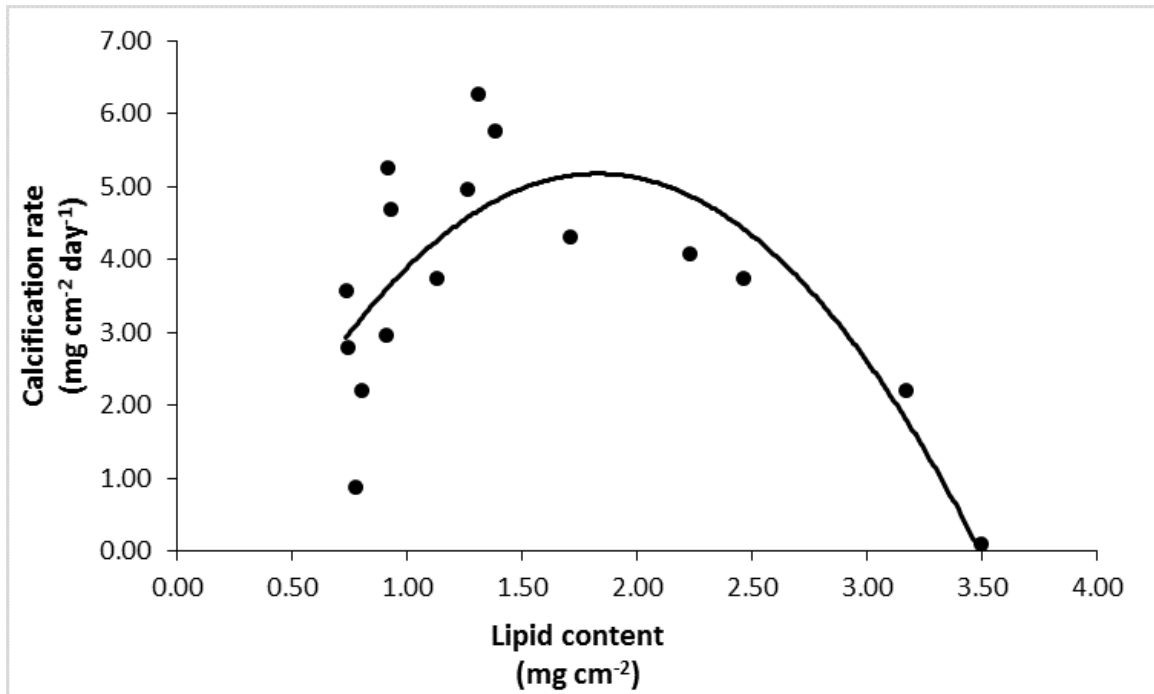


Figure 2.6: Bivariate correlation between lipid content and calcification rate in *M. cavernosa*. The equation of the quadratic fit is $y = -1.875x^2 + 6.861x - 1.097$, $R^2 = 0.62$.

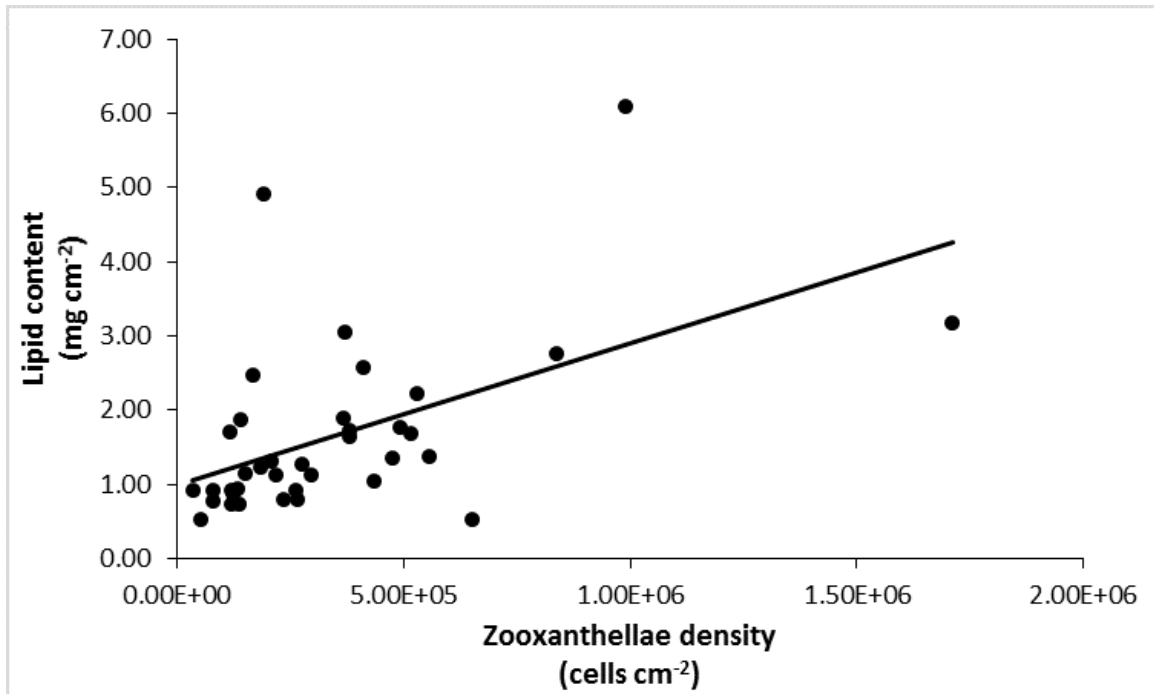


Figure 2.7: Linear correlation between zooxanthellae density and lipid content in *M. cavernosa*. The equation of the linear fit is $y = 0.988 + 1.916e^{-6}x$, $R^2 = 0.27$.

Table 1: Results of a full factorial mixed model three-way ANOVA testing the effects of site, season and their interaction with species nested within site and season on total lipid content, symbiont density, and chlorophyll *a*. Calcification rate was a two way ANOVA with site and species nested within site because growth rate values were integrated over the two seasons. Significant p values are bolded for $p < 0.05$.

Factor	Source	Degrees of Freedom	F ratio	P value
Calcification rate	Site	3	4.8343	0.0059
	Species	2	1.5609	0.2228
	Site x Species	6	2.8197	0.0224
Total lipid content	Site	3	5.6680	0.0016
	Season	1	3.0334	0.0861
	Site x Season	3	3.3705	0.0234
	Species [Site, Season]	15	2.4053	0.0074
Symbiont density	Site	3	0.7707	0.5152
	Season	1	7.9157	0.0067
	Site x Season	3	1.5432	0.2132
	Species [Site, Season]	15	1.8785	0.0487
Chlorophyll <i>a</i>	Site	3	0.3782	0.7690
	Season	1	4.0725	0.0481
	Site x Season	3	9.3702	<.0001
	Species [Site, Season]	15	3.9345	<.0001

Table 2: Summary of species means for each parameter pooled across sites and seasons \pm 1 SE.

Species	Calcification rate (mg cm ⁻² day ⁻¹)	Lipid content (mg cm ⁻²)	Symbiont density (cells cm ⁻²)	Chlorophyll <i>a</i> (μ g cm ⁻²)
<i>M. cavernosa</i>	4.34 \pm 0.73	1.59 \pm 0.24	3.43e5 \pm 5.27e4	1.23 \pm 0.17
<i>O. faveolata</i>	1.50 \pm 0.94	2.84 \pm 0.31	7.23e5 \pm 7.16e4	1.67 \pm 0.23
<i>P. astreoides</i>	1.69 \pm 0.71	2.32 \pm 0.25	2.91e5 \pm 5.66e4	0.79 \pm 0.18

Table 3: Summary of mean seawater temperature by individual month and over the total duration of the study from August 2013 – March 2014 at Molasses Reef, FL (C-MAN program) and the UKI and UKO sites from this study.

Month, Year	Molasses Reef (°C ± 1 SD)	UKI (°C ± 1 SD)	UKO (°C ± 1 SD)
August 2013	29.43 ± 0.29	29.82 ± 1.12	29.32 ± 0.85
September 2013	29.27 ± 0.25	29.68 ± 1.12	29.17 ± 0.77
October 2013	28.39 ± 0.71	28.05 ± 2.44	27.91 ± 1.85
November 2013	26.76 ± 0.65	25.10 ± 2.32	25.77 ± 2.27
December 2013	25.94 ± 0.42	24.19 ± 1.62	25.12 ± 1.21
January 2014	24.35 ± 0.86	22.03 ± 2.92	23.11 ± 2.55
February 2014	24.90 ± 0.70	24.83 ± 2.01	24.72 ± 1.46
March 2014	24.32 ± 0.54	24.74 ± 0.82	24.46 ± 0.74
August 2013 – March 2014	26.67 ± 0.55	26.03 ± 5.22	26.24 ± 4.25

Table 4: Mean summer temperatures at the MKI and LKI sites from this study in 2010, 2011, and 2012. Data from Manzello et al., In Review. Temperatures above mean local bleaching threshold (30.4°C, Manzello et al. 2007) are bolded.

Year	MKI (°C ± 1 SD)	LKI (°C ± 1 SD)
2010	30.83 ± 1.08	30.83 ± 0.91
2011	30.97 ± 0.99	30.99 ± 0.87
2012	30.19 ± 1.24	30.31 ± 1.35

Table 5: Analysis of variance for a test of bivariate fit (linear fit = degree 1, quadratic fit = degree 2) between all parameters for all species. Significant p values are bolded for $p < 0.05$.

Species	Factors	F	p	R ²	Degree of fit
<i>M. cavernosa</i>	Calcification vs. Lipids	10.5027	0.0019	0.62	2
	Calcification vs. Zooxanthellae	2.3387	0.1521	0.16	
	Calcification vs. Chlorophyll <i>a</i>	1.3308	0.2711	0.10	
	Lipids vs. Zooxanthellae	12.9200	0.0010	0.27	1
	Lipids vs. Chlorophyll <i>a</i>	8.4927	0.0062	0.20	1
	Zooxanthellae vs. Chlorophyll <i>a</i>	275.6297	<.0001	0.89	1
<i>O. faveolata</i>	Calcification vs. Lipids	0.0153	0.9048	0.00	
	Calcification vs. Zooxanthellae	0.4545	0.5253	0.07	
	Calcification vs. Chlorophyll <i>a</i>	1.1547	0.3239	0.16	
	Lipids vs. Zooxanthellae	0.0861	0.7725	0.00	
	Lipids vs. Chlorophyll <i>a</i>	1.2817	0.2724	0.07	
	Zooxanthellae vs. Chlorophyll <i>a</i>	31.8829	<.0001	0.64	1
<i>P. astreoides</i>	Calcification vs. Lipids	0.8598	0.3695	0.06	
	Calcification vs. Zooxanthellae	0.6468	0.4357	0.05	
	Calcification vs. Chlorophyll <i>a</i>	0.1682	0.6884	0.01	
	Lipids vs. Zooxanthellae	2.2043	0.1481	0.07	
	Lipids vs. Chlorophyll <i>a</i>	0.6475	0.4273	0.02	
	Zooxanthellae vs. Chlorophyll <i>a</i>	57.7911	<.0001	0.66	1

CHAPTER 3

Threatened Caribbean coral is able to mitigate the adverse effects of ocean acidification on calcification by increasing feeding rate

3.1 Summary

Global climate change threatens coral growth and reef ecosystem health via ocean warming and ocean acidification (OA). Whereas the negative impacts of these stressors are increasingly well-documented, studies identifying pathways to resilience are still poorly understood. Heterotrophy has been shown to help corals experiencing decreases in growth due to either thermal or OA stress; however, the mechanism by which it mitigates these decreases remains unclear. This study tested the ability of coral heterotrophy to mitigate reductions in growth due to climate change stress in the critically endangered Caribbean coral *Acropora cervicornis* via changes in feeding rate and lipid content. Corals were either fed or unfed and exposed to elevated temperature (30 °C), enriched pCO₂ (800 ppm), or both (30 °C/800 ppm) as compared to a control (26 °C/390 ppm) for 8 weeks. Feeding rate and lipid content both increased in corals experiencing OA vs. present-day

conditions, and were significantly correlated. Fed corals were able to maintain ambient growth rates at both elevated temperature and elevated CO₂, while unfed corals experienced significant decreases in growth with respect to fed conspecifics. Our results show for the first time that a threatened coral species can buffer OA-reduced calcification by increasing feeding rates and lipid content.

3.2 Background

Rising atmospheric carbon dioxide from anthropogenic sources is driving the oceans toward conditions not seen for millions of years and will ultimately have strong negative repercussions for corals on a global scale (Hoegh-Guldberg and Bruno, 2010). This elevated atmospheric CO₂ has increased global average temperature by 0.2 °C decade⁻¹ over the last 30 years, with much of that heat being absorbed by the ocean (Hansen et al. 2006). Increased warming throughout this century may reach a point where excessive coral bleaching, the expulsion of corals symbiotic dinoflagellates, may cause widespread mortality. In addition to absorbing heat, the ocean has absorbed one-third of the CO₂ produced by anthropogenic activities. This has resulted in a decrease of 0.02 pH units decade⁻¹ over the last 30 years, a phenomenon known as ocean acidification (OA) (Hoegh-Guldberg and Bruno, 2010). This decrease in pH is accompanied by a decline in the saturation state (Ω) of calcium carbonate which impairs the ability of corals and other calcifying organisms to form skeletons (Hoegh-Guldberg et al. 2007). Corals will need to expend more energy to achieve a constant rate of calcification as the saturation state of the ambient seawater decreases due to OA (Venn et al. 2012, McCulloch

et al. 2012). Less is known about how combined warming and OA will affect the coral holobiont, but recent work has shown that temperature can modulate the response of coral physiology to OA - in some cases mitigating OA effects, and in some cases worsening OA effects (Schoepf et al. 2013, Reyanud et al. 2003), highlighting the need for improved understanding of these interactions. Growth and survivorship of corals in the future will depend on their resilience capability in the face of climate change stress.

Identifying potential indicators of resilience to climate change stress has been a major focus of coral physiologists during the last decade. Anthony et al. (2007) showed that a corals capacity to utilize heterotrophy (feeding) and lipid content were good predictors of survivorship during a bleaching event in the Pacific coral *Acropora intermedia* because lipids stored in coral tissue represent significant energy reserves that can be used in times of stress (Grottoli et al. 2004, Teece et al. 2011). Lipids in corals can be translocated from their symbionts or obtained directly from feeding (Imbs et al. 2010). Heterotrophy may be a major source of lipids for corals because some essential fatty acids cannot be synthesized *de novo* (Teece et al. 2011), and heterotrophy may become more significant when a coral bleaches and loses its primary source of daily metabolic energy from its symbionts (Grottoli et al. 2006). Anthony et al. (2009) predicted that coral survival following a bleaching event would be strongly influenced by remaining lipid reserves and rates of heterotrophy.

Increasing the energy available to corals by enhanced heterotrophy and lipid reserves may mitigate the negative impacts of climate change stressors like OA (Cohen and Holcomb, 2009, Edmunds 2011) and warming (Grottoli et al. 2006).

Thermal bleaching results in reduced symbiont density and chlorophyll a levels (Hoegh-Guldberg 1999), and recent studies have shown that OA stress may also decrease symbiont density (Kaniewska et al. 2012, Tremblay et al. 2013). Very little is currently known about how lipids may affect a corals response to OA; however, it logically follows that corals that can increase their feeding rates and lipid reserves may be better able to cope with and recover from stressors that may reduce the amount of photosynthate they receive from their symbionts. Certain species may be more capable of heterotrophy than others (Grottoli et al. 2006, Houlbrèque and Ferrier-Pagès 2009), but there is a paucity of information regarding the ability of particular Atlantic coral species to utilize heterotrophy as a means to ameliorate the deleterious effects of warming and OA, as much of the seminal work on this topic has been done on Pacific corals. This lack of data is especially problematic for the critically threatened Staghorn coral (*Acropora cervicornis*), which was once one of the dominant reef-building coral species in the western Atlantic and Caribbean (Jackson 1994). Since the late 1970s, unprecedented declines in *A. cervicornis* populations (Wirt et al. 2013) have been documented in virtually all areas of the western Atlantic and Caribbean (Precht et al. 2002). In the Florida Reef Tract, populations have been diminished by as much as 98% (Miller et al. 2002). In order to successfully inform conservation decisions, it is imperative to understand physiological responses to stress and potential for resilience of the species chosen for restoration efforts.

The primary objectives of this study were to test if heterotrophy can mitigate reductions in growth due to climate change stress (both warming and OA), and to determine if feeding rate and total lipid content are plastic responses that change

significantly under stress in *A. cervicornis*. The effect of heterotrophy and lipids on corals is documented for a small number of species; however, most studies generally had a fed and unfed group, but did not directly quantify ingestion rates. We identify a stress buffering mechanism, i.e. the ability to plastically increase ingestion rate and total lipid content that provides the coral with extra energy. This extra energy may be used to offset reductions in growth that would otherwise result from reduced photosynthate transfer, in the case of thermal stress, or increased energy demands on calcification, in the case of OA stress.

3.3 Methods

3.3.1 Collection and Experimental Design

Due to the conservation status of *A. cervicornis*, collection of wild colonies is not permitted. Therefore, eight colonies of *A. cervicornis* were donated from three South Florida sources in May 2013: the Smithsonian Institute (Ft. Pierce, FL), the University of Miami Coral Resource Facility, and an *A. cervicornis* nursery near Broad Key, FL. The purpose of using colonies from three sources was to maximize genetic diversity across populations and individuals in this study. All colonies were taken from approximately 5m depth. Corals were fragmented into three-five cm experimental units and affixed to aluminum gutter guard using All Game epoxy. Replicates were haphazardly distributed to avoid a parental colony effect and allowed to recover from fragmentation under control conditions (26 °C/390 ppm (LT-LCO₂)) for four weeks prior to the beginning of the study. This study consisted of four treatments: 26 °C/390 ppm (LT-LCO₂), 26 °C/800 ppm (LT-HCO₂),

30 °C/390 ppm (HT-LCO₂), and 30 °C/800 ppm (HT-HCO₂) and lasted eight weeks from June to August 2013. Each treatment was replicated twice for a total of eight independent tanks, and ten corals (five fed and five unfed throughout the experiment) were in each tank. While N=2 tanks for each experimental condition may appear low, there is precedent for this design (Edmunds 2011). We believe that our design represents a reasonable tradeoff between the competing needs of replication and the space and cost limitations of adding additional experiment units. The HT level was chosen because it is immediately below mean bleaching threshold in the Florida Keys (30.4 °C, Manzello et al. 2007), and the HCO₂ level was chosen as that predicted for the year 2065 (800 ppm, IPCC, RCP 8.5). Corals were held in semi-recirculating tanks throughout the duration of the experiment. Carbonate chemistry was manipulated by direct gas injection and was monitored via direct measurement of total alkalinity (TA) and dissolved inorganic carbon (DIC).

3.3.2 Aquaria set-up

Experimental corals were maintained at the Climate Change Laboratory at the University of Miami in 45 L tanks of water replenished by a 250 L sump tank with complete water turnover every ten minutes. The high quality natural seawater supply for the tanks came from intakes in nearby Bear Cut, Key Biscayne, FL. This water was filtered to ten microns, therefore the corals in this study were not receiving significant nutrition from plankton introduced to their tanks by the seawater supply. Each sump tank contained a heating and cooling element connected to a temperature controller (OMEGA CN7533) with accuracy within 0.1 °C. CO₂ levels were achieved by bubbling the sump tanks with CO₂-enriched air produced using

mass flow controllers (Sierra Instruments model 810C). Corals experienced natural light attenuated by a neutral density shade cloth to produce light levels similar to those experienced at donor locations, but still within the range of a typical Florida patch reef environments. Daily integrated PAR averaged $5.8 \text{ mol photons m}^{-2} \text{ d}^{-1}$. The average peak midday instantaneous PAR was $353 \pm 70 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. This light level is consistent with previous work on coral physiology and heterotrophy done at $300 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Reynaud et al. 2003; Ferrier-Pagès et al. 2010).

3.3.3 Seawater chemistry

In order to monitor seawater chemistry conditions throughout the study, discrete water samples were taken from each tank weekly and poisoned with mercuric chloride to be analyzed for total alkalinity (TA) and dissolved inorganic carbon (DIC). TA was measured in duplicate on an automated Gran titrator and standardized using certified reference materials obtained from Dr. A. Dickson (Scripps IO). DIC was measured in duplicate using a DIC analyzer (Apollo SciTech Inc.) and standardized to the same certified reference seawater. Mean temperature, salinity, TA, and DIC were used to calculate pCO_2 , pH, and aragonite saturation state (Ω_a) for each treatment using the program CO2SYS using K_1 and K_2 from Mehrbach et al. (1973) refit by Dickson and Millero 1987 per Lewis and Wallace (1998).

3.3.4 Heterotrophy

In order to understand the role of heterotrophy in ameliorating thermal and OA stress effects on growth rate and lipid content, corals in the study were divided

equally into a fed and unfed group. Fed corals were placed in a plastic container and fed a diet of dried zooplankton powder (Zieglers Larval AP 100) ad libitum twice a week. Unfed corals were placed in a similar container without food for the same period of time so as to receive similar handling without the nutrition. The objective was to obtain a group of corals that had to get all their nutrition from their zooxanthellae and any plankton that might get through the ten micron water filtration system, and another group that uniformly received a supplemental source of nutrition if they were able to feed heterotrophically.

3.3.5 Feeding rate

In order to learn if *A. cervicornis* changes its feeding behavior under conditions of thermal and OA stress, we conducted feeding rate assays where the capture rate of live rotifer prey was measured on just the fed corals in each of the treatment tanks. These measurements were made four times (biweekly) on each fed coral at two, four, six and eight weeks into the experiment. Feeding rate assays were performed on days when the corals did not receive their normal twice weekly feeding with powdered zooplankton. The amount of live rotifer prey captured likely contributed an insignificant amount to the lipids of the fed corals. The purpose of the feeding rate assay was to provide information about the feeding intensity of the corals and how that feeding behavior varied with treatment. Each coral was placed in one L, well-stirred beaker of treatment water containing 10,000 live rotifers. After one hour the coral was removed and the number of rotifers remaining in the beaker was enumerated. The prey capture or feeding rate was expressed as the number of rotifers removed per cm^2 of coral surface area per hour. The ro-

tifer species, *Brachionus plicatilis*, was chosen for this study. This species is widely used as nutritious live food for the raising of larval fish, invertebrates and coral. A rotifer concentration of 10,000 L⁻¹ was selected for our experiments. This concentration is approximately five-times the natural zooplankton concentration reported for local reef waters of 1,700 zooplankters L⁻¹ (Leichter et al. 1998). Sebens et al. (1996) has recommended the use of prey concentrations up to ten-times natural prey densities in feeding rate studies so as to avoid a concentration dependence that can confound the interpretation of results. The two part feeding protocol was meant to maximize the chance of seeing treatment effects that may be small. The twice weekly *ad libitum* feeding with powdered zooplankton maximized our power to observe a heterotrophic feeding impact on long-term measures of coral condition, i.e. growth and total lipid content. The short-term live rotifer prey capture assays allowed us to probe for treatment effects on the heterotrophic feeding behavior of the corals. Doing these assays at a prey density that is likely saturating maximized our chances of seeing a treatment effect that could be masked if the capture rates were confounded by a prey concentration effect.

The details of the live prey feeding rate assays were as follows. Initial and final concentrations of live rotifers were measured and feeding rates were calculated following Coughlan (1969). Eleven one-L beakers were used, ten containing a coral and one control without coral to account for any possible changes in rotifer density not due to coral feeding. Each beaker had the same flow rate (controlled by magnetic stir bar), light conditions, and initial rotifer density from the same stock solution. Corals were allowed to feed for one hour after sunset as in Grottoli et al. (2006) and were observed to have extended tentacles in the presence of rotifers,

indicating feeding was occurring. Initial concentrations of rotifers were approximately 10,000 cells L⁻¹. After one hour, four replicate fifteen ml water samples were taken from each beaker, fixed in the preservative Lugols solution, and final rotifer concentration was quantified via microscopy. Experiments took place on days when corals were not fed the dried zooplankton diet. An advantage of this method over the gut excavation method published by Palardy et al. (2005, 2006) and Grottoli et al. (2006) is that it is non-destructive allowing us to make feeding rate, growth, chlorophyll *a*, total lipid and symbiont density measurements on all the corals in the study, rather than just a subset.

3.3.6 Coral calcification and tissue lipid content

Calcification rates were measured biweekly as changes in coral weight in air using the buoyant weight technique according to Davies et al. (1989). At the end of the study, coral tissue was removed from the skeleton using an air-brush and homogenized following Szmant et al. (1990). The aliquot for total lipids (two mL) was filtered onto a GF/A filter and frozen at -80 °C until further analysis following Teece et al. (2011). Briefly, a two ml aliquot of total coral homogenate was extracted three times (four mL 1:1 dichloromethane:methanol). The resulting organic extracts were collected, after vortexing; all three extracts were combined, dried under a stream of nitrogen gas, and weighed on an analytical balance. 3D scanning methodologies for surface area calculations followed Enochs et al. (2014).

3.3.7 Symbiont density and chlorophyll *a* concentration

One ml of total blastate was filtered onto a GF/A filter for chlorophyll *a* analysis and frozen at -80 °C until further analysis following Holm-Hansen and Riemann (1978). Chlorophyll *a* samples were analyzed on a fluorometer (TD-700 Turner Designs) calibrated with purified chlorophyll *a* (Sigma-Aldrich catalog no. C6144). Pigment content was normalized to coral surface area. Another one ml subsample of the total blastate was preserved with 50 µL of Lugols solution for endosymbiont quantification, which was calculated using two independent replicate counts on a haemocytometer (Hausser Scientific) using VistaVision compound microscope at 100 magnification. Symbiont density was normalized to surface area.

3.3.8 Statistics

All statistical analyses were completed in the program JMP version 11.0.0. Normality and homoscedasticity were ascertained prior to testing each dependent variable using a Shapiro-Wilk test and Levenes test, respectively. For the dependent variables: growth, lipids, chlorophyll *a*, and symbiont density, a three-way full factorial mixed ANOVA model was run with temperature (26 °C vs. 30 °C), CO₂ (390 ppm vs. 900 ppm), and nutrition (fed vs. unfed) as fixed factors, and replicate tanks nested within temperature and CO₂ level to account for any tank effect. For growth and lipids, there was no significant tank effect ($\alpha = 0.05$), and thus data from replicate tanks were pooled. For chlorophyll *a* and symbiont density, there were significant tank effects, thus replicate tanks were not pooled for these two dependent variables. For the dependent variable feeding rate, only fed corals were assessed, thus a two-way full-factorial mixed ANOVA model was run

with temperature and CO₂ as fixed factors and tank nested within temperature and CO₂ level to account for any tank effects. For feeding rate, there were no significant tank effects ($\alpha=0.05$), so data from replicate tanks were pooled. Where effects were found to be significant, a *post-hoc* Tukeys HSD test was run to determine which means were different.

3.3.9 Ethics statement

Eight parent colonies of *Acropora cervicornis* (Invertebrate Cnidarian) were donated from aquaria or coral nurseries in the State of Florida, USA, in May 2013, and this study was fully approved and funded by MOTE Marine Laboratories Protect Our Reefs Grant (#POR-2012-22) and the Mohamed bin Zayed Species Conservation Fund (Project #12054710). As a scientific organization, University of Miami's Rosenstiel School of Marine and Atmospheric Sciences Corals and Climate Change laboratory is empowered to conduct studies of this nature.

3.4 Results

Temperature, CO₂, and other water chemistry parameters are presented in Table 1. A three-way full-factorial ANOVA revealed that temperature, CO₂ and feeding each had significant main effects on growth rate (Table 2), e.g. HT decreased growth, HCO₂ decreased growth, and feeding increased growth. There was a significant interaction between feeding and temperature on growth (Table 2, $F(1,78)=6.64$, $p=0.012$), which is reflected in similar growth rates of fed corals under LT and HT conditions, but reduced growth rates of unfed corals under HT conditions when compared to LT conditions (Fig. 1). Growth of unfed HT-HCO₂

was significantly lower than the controls, and growth of unfed corals was also significantly lower than fed conspecifics in HT treatments at both CO₂ levels (Fig. 1). Feeding rate was significantly affected by CO₂ (Table 2, $F(1,34)=4.44$, $p=0.045$). Feeding rate was approximately 30% higher in HCO₂ treatments (1.31 rotifers hr⁻¹ cm⁻²) than in LCO₂ treatments (0.92 rotifers hr⁻¹ cm⁻²), regardless of temperature (Fig. 2). Temperature did not have a significant effect on feeding rate. Total lipid content was affected by CO₂ (Table 2, $F(1,66)=5.63$, $p=0.021$) as well as by the interaction between CO₂ and feeding (Table 2, $F(1,66)=4.14$, $p=0.047$). Fed corals in the HT-HCO₂ treatment had significantly greater lipids than unfed controls (Fig. 3). Temperature had no significant effect on lipid content. Chlorophyll *a* and symbiont density were significantly affected by replicate tank (Table 2, $F(1,74)=7.49$, $p<.0001$) (Table 2, $F(1,74)=4.52$, $p = 0.003$) respectively, and therefore replicate tanks were not pooled by treatment. Means of all eight tanks are displayed in Figure 4 for chlorophyll *a* and Figure 5 for symbiont density, and the significant main temperature effect (Table 2, $F(1,74)=55.11$, $p<.0001$) (Table 2, $F(1,74)=40.21$, $p<.0001$), respectively, can be visualized. Corals at 30 °C had less chlorophyll *a* content than corals at 26 °C (Fig. 4B). Feeding also had a significant effect on chlorophyll *a* (Table 2, $F(1,74)=8.18$, $p=.006$) whereby unfed corals had less chlorophyll *a* than fed corals (Fig. 4C). Corals at 30 °C had lower symbiont density than corals at 26 °C (Fig. 5B). Feeding also had a significant effect on symbiont density (Table 2, $F(1,74)=5.05$, $p=0.028$) whereby unfed corals had lower densities than fed corals (Fig. 5C). CO₂ did not have a statistically significant effect on chlorophyll *a* levels or symbiont density in *A. cervicornis* in this study. The reason for the significant tank effect on chlorophyll *a* and symbiont density is unknown, but this effect was

not large enough to preclude seeing significant main effects of temperature and feeding on these parameters, nor was it large enough to be significant for growth, feeding rate, or lipid content measurements. A bivariate linear fit was tested for lipid content and feeding rate, and was found to be significant, (Table 3, $R^2=0.34$, $p=0.0002$) (Fig. 6).

3.5 Discussion

3.5.1 Growth response is mediated by feeding during stress

In this study we found that the threatened coral, *Acropora cervicornis*, was able to increase its feeding rate and stored energy reserves (total lipid content) when exposed to high CO₂ conditions at 26 °C or 30 °C and mitigate reductions in calcification that caused significant decreases in growth rate in unfed corals. To our knowledge, only one previous study has reported the ability of a coral to increase its feeding rate and energy reserves under thermal (but not OA) stress and concurrently exhibit mitigation of depressed calcification. Grottoli et al. (2006) found that thermally stressed (bleached) colonies of *Montipora capitata* increased their feeding rate six-fold relative to unstressed controls while two other coral species in the study, *Porites compressa* and *Porites lobata*, did not. Growth rates were not measured as part of that lab study. However, in a related study *M. capitata* and *P. compressa* corals were bleached in the lab for one month at 30 °C and then recovered on the reef where they could feed naturally (Rodrigues and Grottoli, 2007). Growth rate data was obtained after one and a half, four, and eight months on the reef. After eight months the growth rate of *M. capitata* had recovered to the point that it was

not significantly different from the unbleached controls, while the growth rate of *P. compressa* was still significantly slower than the unbleached controls. The conclusion based on feeding data from (Grottoli et al. 2006) and growth data from (Rodrigues and Grottoli, 2007) was that the species that was able to meet its energy needs by switching from an autotrophic to a heterotrophic energy source had a faster recovery from a thermal stress event.

Edmunds (2011) performed a factorial experiment similar in design to the present study with two temperatures (26 and 29 °C), two CO₂ levels (416 and 815 ppm), and fed and unfed treatments. He reported that feeding partially ameliorated the effect of high CO₂ on biomass-normalized growth, but did not measure feeding rates so it is not possible to know if the massive *Porites* species used actively increased its usage of heterotrophy. Previous OA work with *A. cervicornis* has shown that at 25 °C (Renegar and Riegl, 2005) and 28 °C (Enochs et al. 2014), CO₂ levels between 700-900 ppm elicited decreases in growth rate, but corals in both studies did not have opportunities to feed heterotrophically. It remains unknown what minimum heterotrophy rates are necessary to elicit a stress-buffering response, and therefore corals that do obtain some food, (but perhaps below some hypothetical threshold), may still display depressed calcification. Nonetheless, this work represents the first time a study has shown increases in feeding rate and lipid content, and concurrent mitigation of OA-induced decreases in surface area-normalized calcification. Recent work suggests that food availability may also determine the response of other marine invertebrates to OA. Pansch et al. (2014) found that the barnacle *B. improvisus* from Sweden was able to withstand elevated CO₂ conditions (1,000 μ atm) over five weeks when food was plentiful, but showed reduced

growth when food was limited. This study provides further evidence that feeding and energy availability can mediate reductions in growth due to OA stress in marine invertebrates.

3.5.2 Heterotrophy and its relationship to lipids

Feeding rates reported in this study (0.9-1.3 plankters $\text{hr}^{-1} \text{cm}^{-2}$) are similar to those reported by Palardy et al. (2006) (0.70-1.0 plankters $\text{hr}^{-1} \text{cm}^{-2}$) for *Pocillopora damicornis*, a Pacific species with similar small polyps and branching morphology. Lipid content reported here ranged from 0.8 - 1.4 mg lipid cm^2 similar to what Anthony et al. (2007) reported for *Acropora intermedia* (0.5 - 2.0 mg lipid cm^2). The present data show a significant effect of CO_2 and significant interaction between CO_2 and feeding on lipid content with the highest mean lipids in fed corals at elevated CO_2 . This increase is consistent with Schoepf et al. (2013) who showed that lipid content in *Acropora millepora* also increased with elevated CO_2 , but not temperature. The current results differ, however, from those of Drenkard et al. (2013) who also tested the impact of feeding and CO_2 on larvae of the Atlantic coral *Favia fragum*. While Drenkard et al. (2013) did not directly measure feeding rate, they found that lipid content was not significantly affected by CO_2 or feeding in juvenile corals. These contrasting lipid responses highlight that juvenile and adult corals may respond differently under OA conditions. For example, juveniles may preferentially invest in tissue growth, while adults may invest in lipid storage (Drenkard et al. 2013), as the present data suggest. Heterotrophy and lipid content appear to be linked in *A. cervicornis*, as both metrics increased in response to elevated CO_2 , and there was a significant correlation between the two variables (Fig. 6). This

correlation is consistent with Teece et al. (2011), who state that heterotrophy can be a direct source of certain types of lipids for the coral host. These concurrent increases provide evidence that elevated CO₂ is a metabolic stress to *A. cervicornis*, necessitating the host to increase feeding rate and subsequently lipids.

Increased *A. cervicornis* heterotrophy at 800 ppm, but not at 30 °C, suggests that the elevated CO₂ level used was potentially stressful enough to elicit a feeding response, whereas the severity and/or duration of the elevated temperature level may not have been enough to necessitate a feeding response. Perhaps in order for temperature stress to induce a feeding/lipid response, the coral must already be at or above the bleaching threshold for a certain amount of time. We do show, however, that being fed versus unfed ameliorated the potentially stressful effect of elevated temperature on growth (Fig. 1) even though feeding rate did not increase significantly at elevated temperature. The hypothesis that these corals were close to their thermal stress threshold is supported by the observations that chlorophyll *a* content and symbiont density were both reduced in the 30 °C treatments (Fig. 4 and 5), indicating that the corals were likely experiencing the early stages of bleaching and were likely deriving less energy from their symbionts, although seemingly not dramatically enough to necessitate a significant increase in feeding rates.

3.5.3 Heterotrophic plasticity in corals - driven by metabolic stress thresholds?

Based on the reliance a coral has on its symbionts for daily metabolic energy, one might have predicted an increase in heterotrophy as a result of reductions

in symbiont density and chlorophyll *a* level in order to compensate for potential losses in photosynthate transferred to the host. We show, however, that responses are more nuanced than this. Significant decreases in symbiont density and chlorophyll *a* at high temperature were observed without seeing an increase in feeding rate, and conversely no significant decreases in symbiont density and chlorophyll *a* at high CO₂ were accompanied by increases in feeding rate. These results suggest that in *A. cervicornis*, increases in feeding rate are not related to or driven by symbiont density and chlorophyll *a* content. Instead, we propose that increased feeding is driven by exposure to the metabolic stress caused by elevated CO₂ which increases the energetic expense incurred by the Ca⁺²-H⁺¹-ATPase pump at the site of calcification (McConnaughey 1989, Ries 2011, Venn et al. 2011, McCulloch et al. 2012). Although the energetic cost of calcification is not known accurately, it has been estimated to be on the order of 30% of the total energy budget (Cohen et al. 2009, Allemand et al. 2011). Taking the view that coral calcification is an energy-demanding process and that the saturation state of the calcifying fluid starts out close to that of seawater (Tambutté et al. 2012, Gagnon et al. 2012), it stands to reason that corals will have to expend more energy to achieve a constant rate of calcification as the saturation state of the ambient seawater decreases due to OA. Following this line of logic, one might expect that energy-replete corals might be able to buffer decreases in saturation state of the ambient seawater over a certain range by devoting more energy to proton pumping. If a coral is energetically compromised due to loss of symbionts, lack of success at heterotrophy, or depleted lipid reserves, then it may be forced to devote less energy to proton pumping with the consequence that calcification declines. Our data support the idea that with-

out supplemental energy for this mechanism under stress, growth will suffer; conversely, when provided with additional energy from heterotrophy, growth will not decrease significantly from ambient rates.

In addition to feeding potentially being driven by level of coral energy reserves, the use of heterotrophy to compensate for a stressor may also be species-specific. Grottoli et al. (2006) found that 30 °C was sufficient to cause increased feeding in the Hawaiian coral *Montipora capitata* recovering from bleaching. Ferrier-Pagès et al. (2010) found that feeding rates of three species of corals varied in their responses to the same elevated temperature stress of 31 °C. These studies and the present study highlight the validity of accounting for heterotrophy when assessing coral physiological response to climate change stress. More research is needed to better understand exactly what drives increased feeding in the host. Kaniewska et al. (2012) proposed a model of cell-wide responses of corals to OA in which they describe how elevated CO₂ causes disruption to host mitochondria, decreasing cellular metabolism and increasing lipid oxidation, which could necessitate feeding to replenish cellular lipids. It is difficult to determine what rate of heterotrophy can replenish lipids to a degree whereby corals display resilience to stress. To determine this, one would have to assess the severity and duration of the stress event, the amount of lipid reserves in the coral prior to the stress event, and the degree of heterotrophy the coral is capable of (2009). Further research is necessary to determine if increased heterotrophy and lipid content under elevated CO₂ can mitigate decreases in calcification under longer-term scenarios greater than eight weeks.

This study provides a glimmer of hope that a critically endangered species with access to food sources other than photosynthate may be able to maintain growth

physiology under climate change stress. Resilience in the wild will be dependent on reefs with naturally high zooplankton abundance. Therefore, decisions concerning the placement of MPAs and/or coral nurseries may benefit from the careful consideration of natural food availability at the proposed sites. Moreover, future physiological studies on coral responses to climate change should consider assessing heterotrophy and lipid content.

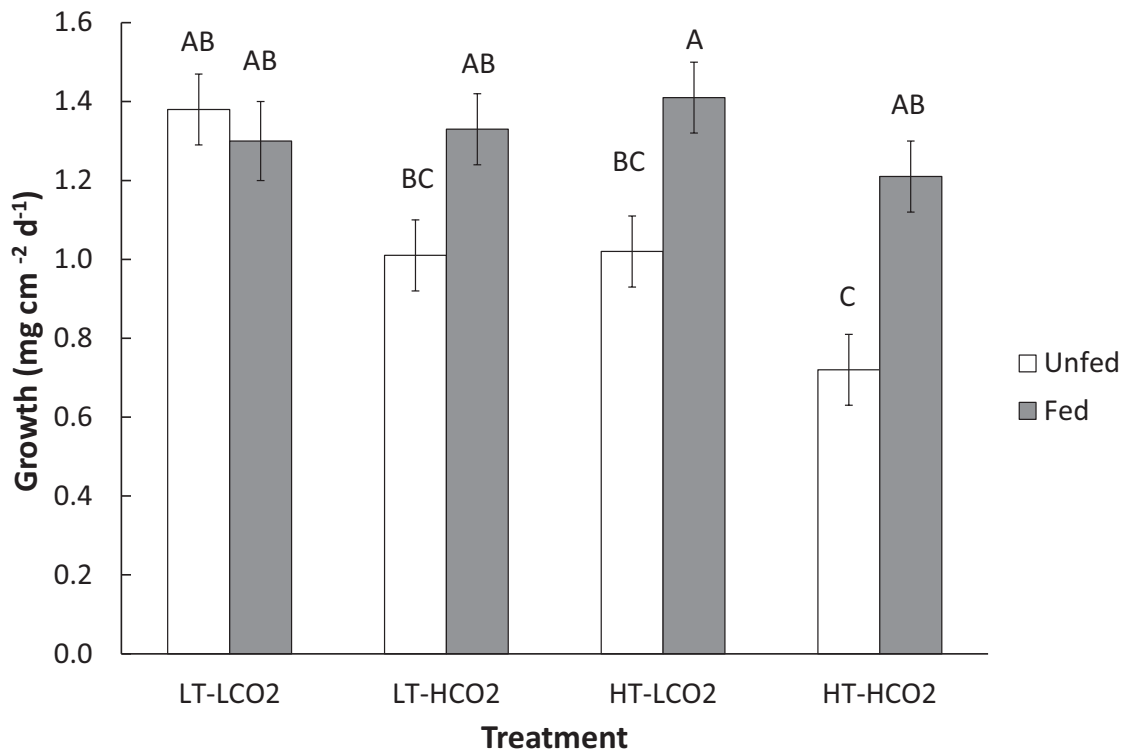


Figure 3.1: Growth of *A. cervicornis* over 8 week exposure to combinations of temperature, CO₂, and feeding. LT-LCO₂ represents control conditions, 26 °C, 390ppm, LT-HCO₂ represents 26 °C, 800ppm, HT-LCO₂ represents 30 °C, 390 ppm, and HT-HCO₂ represents 30 °C, 800ppm. Each bar represents the mean growth rate of n= 10 corals, and white bars represent unfed corals, while grey bars represent fed corals. Dissimilar letters indicate means that are significantly different following a *post-hoc* Tukeys HSD test. Error bars represent \pm one standard error.

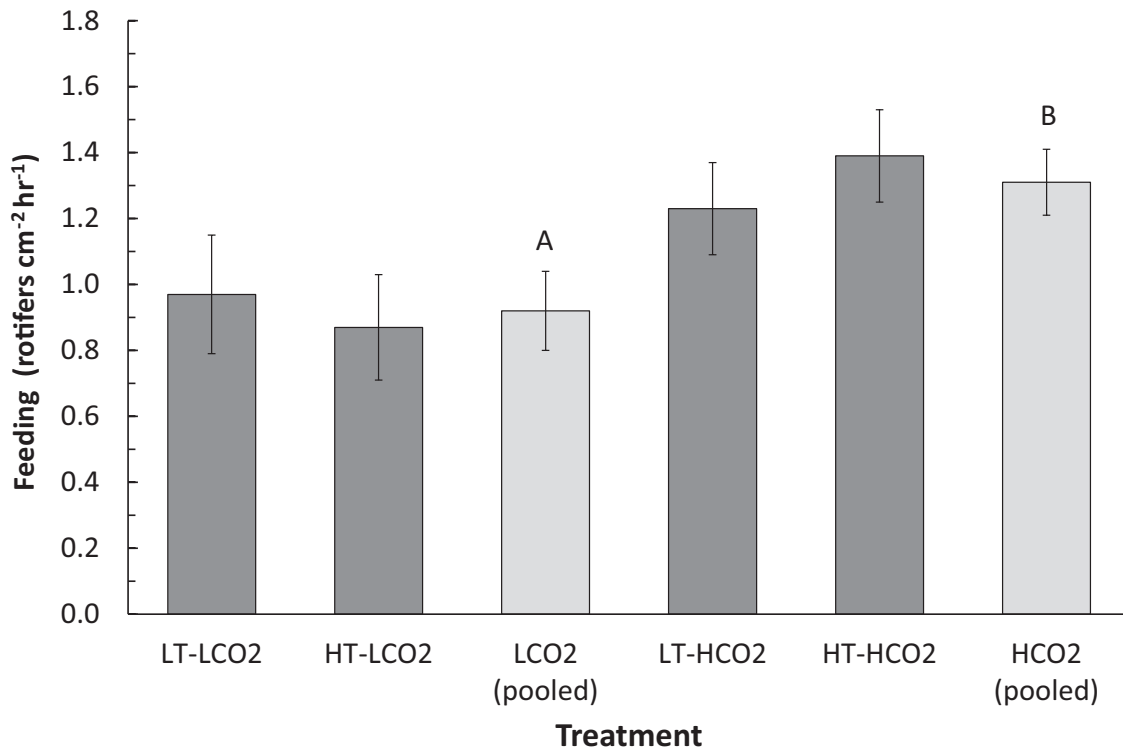


Figure 3.2: Feeding rate of *A. cervicornis* over 8 week exposure to combinations of temperature and CO₂. LT-LCO₂ represents control conditions, 26 °C, 390ppm, HT-LCO₂ represents 30 °C, 390 ppm, LT-HCO₂ represents 26 °C, 800ppm, and HT-HCO₂ represents 30 °C, 800ppm. Each dark grey treatment bar represents the mean feeding rate of n= 10 corals, while light grey LCO₂ and HCO₂ bars (pooled by temperatures) representing n=20 corals are shown to clearly depict the main effect of CO₂ on feeding rate. Dissimilar letters indicate means that are significantly different following *post-hoc* students t-test. Error bars represent ±one standard error.

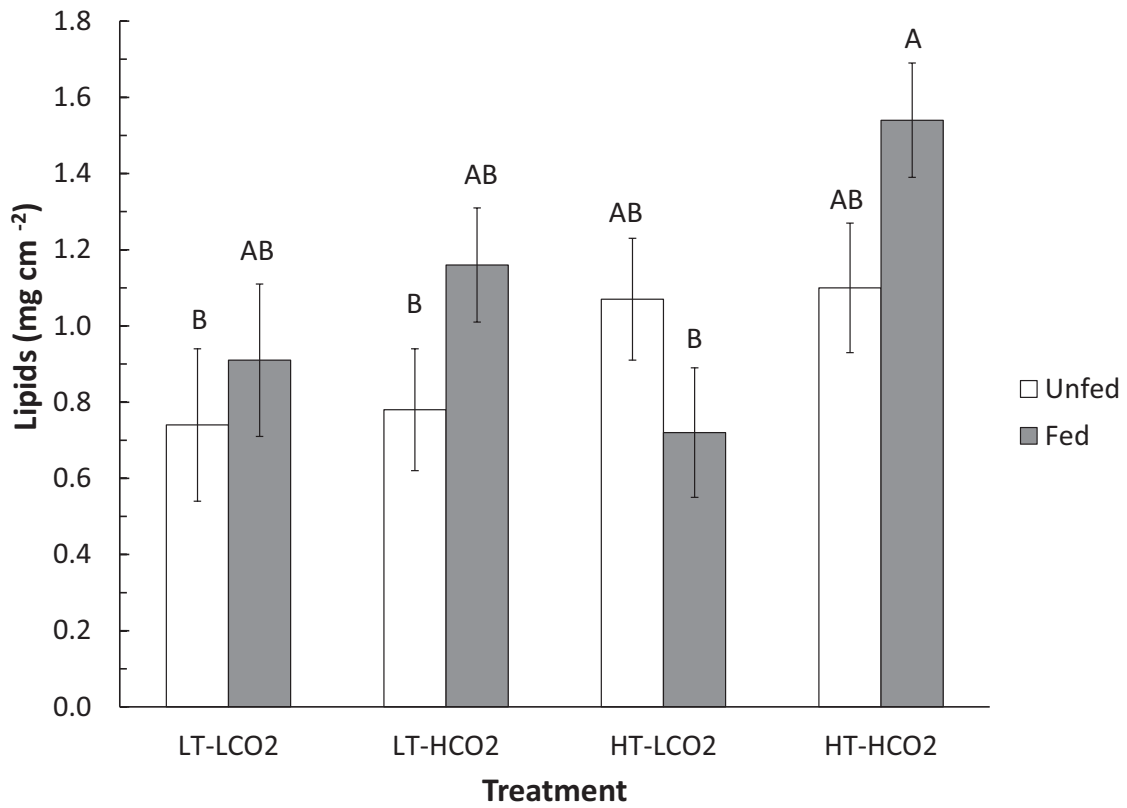


Figure 3.3: Total lipid content of *A. cervicornis* following 8 week exposure to combinations of temperature, CO₂, and feeding. LT-LCO₂ represents control conditions, 26 °C, 390ppm, LT-HCO₂ represents 26 °C, 800ppm, HT-LCO₂ represents 30 °C, 390 ppm, and HT-HCO₂ represents 30 °C, 800ppm. Each bar represents the mean lipid content of n= 10 corals, and white bars represent unfed corals, while grey bars represent fed corals. Dissimilar letters indicate means that are significantly different following a *post-hoc* Tukeys HSD test. Error bars represent ±one standard error.

Figure 3.4: Chlorophyll *a* level in *A. cervicornis* following 8 week exposure to combinations of temperature, CO₂, and feeding. A. Mean chlorophyll *a* of corals (n=10) in each individual tank, not pooled by treatment due to a significant tank effect. Therefore each treatment is shown twice, representing each replicate tank, i.e. two LT-LCO₂, two LT-HCO₂, two HT-LCO₂, and two HT-HCO₂, from left to right. Dissimilar letters indicate means that are significantly different following *post-hoc* Tukeys HSD test. Mean chlorophyll *a* of corals (each bar represents n=40) depicting the main effects of temperature (B) and feeding (C). Dissimilar letters indicate means that are significantly different following *post-hoc* students t-test. Error bars represent ±one standard error.

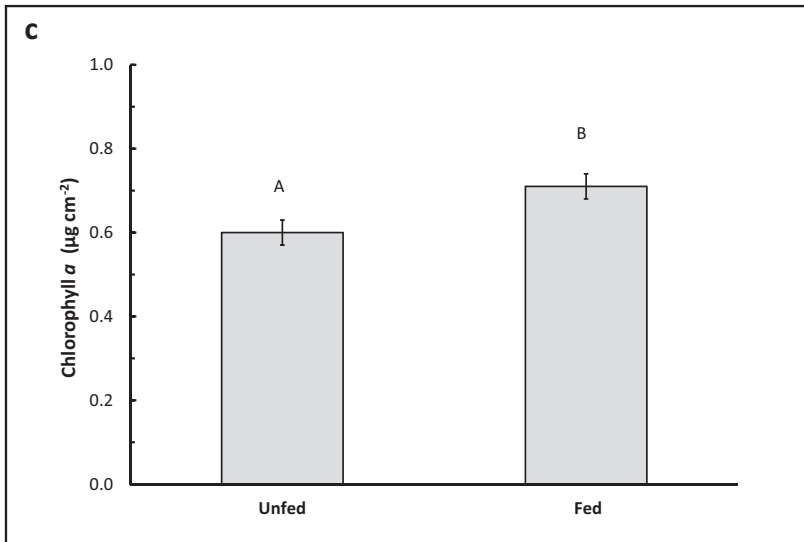
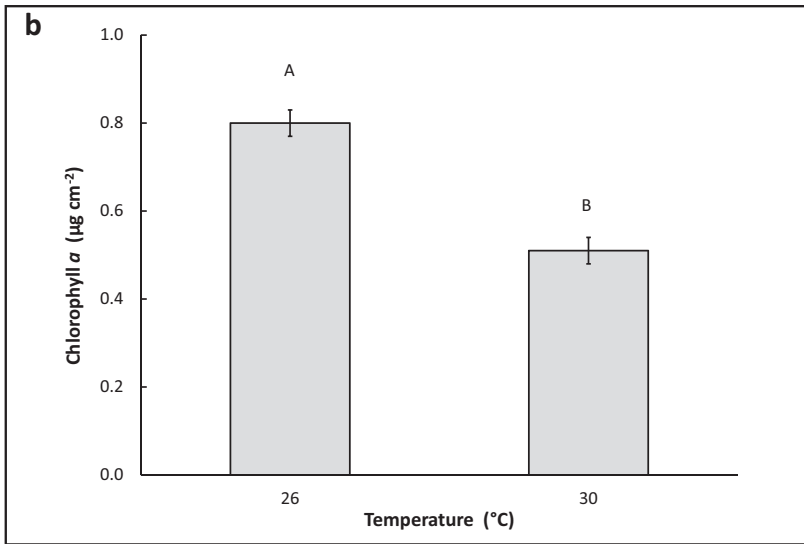
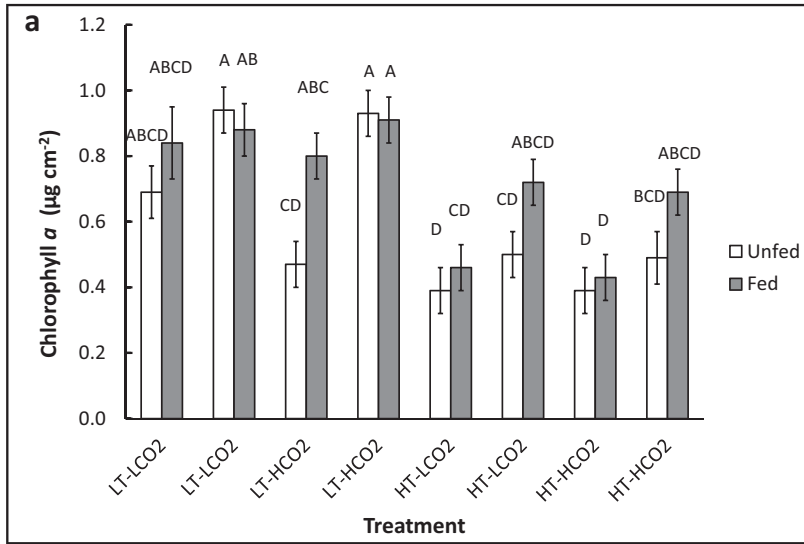
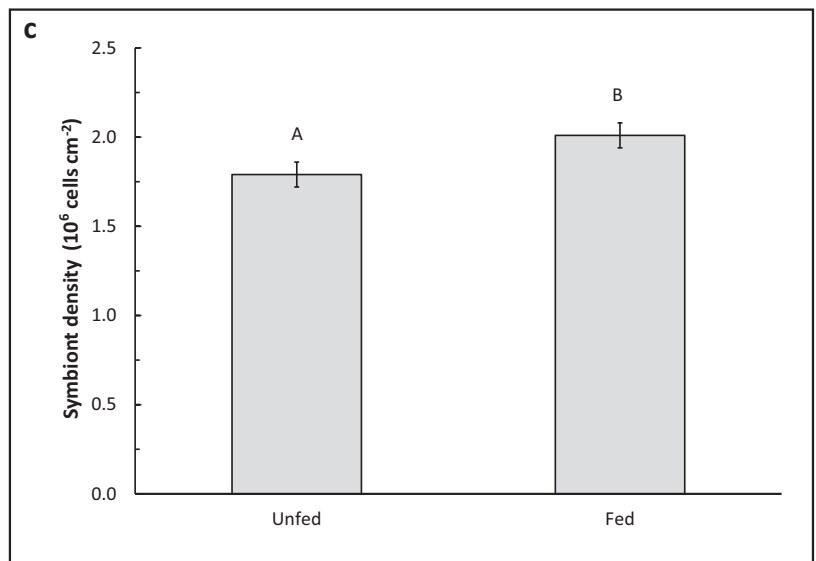
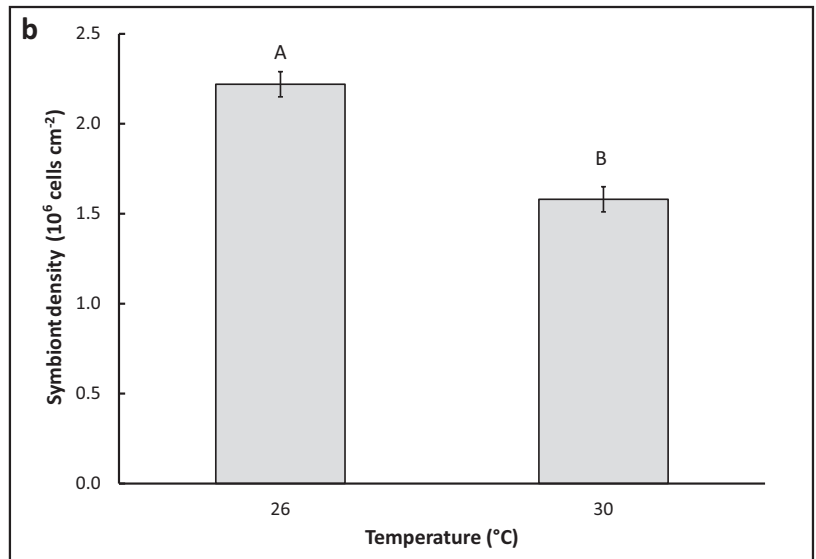
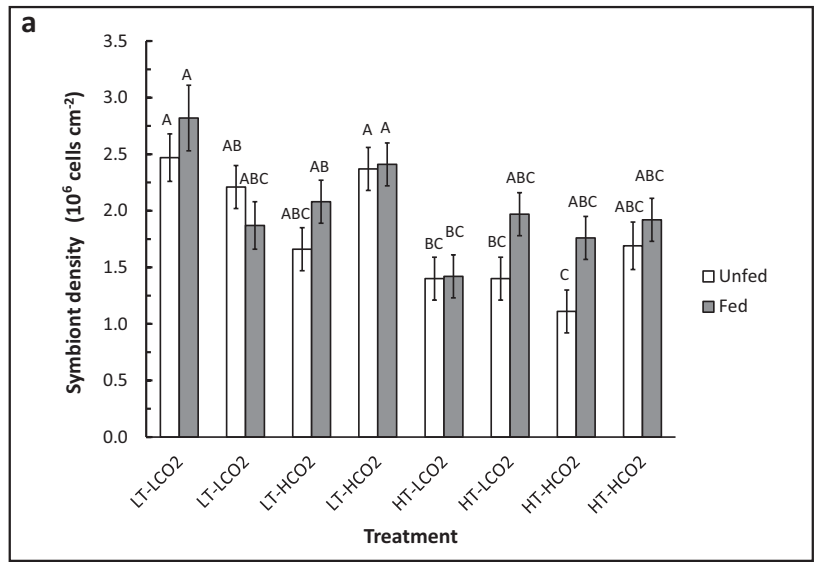


Figure 3.5: Symbiont density in *A. cervicornis* following 8 week exposure to combinations of temperature, CO₂, and feeding. Mean symbiont density of corals (n=10) in each individual tank, not pooled by treatment due to a significant tank effect. Therefore each treatment is shown twice, representing each replicate tank, i.e. two LT-LCO₂, two LT-HCO₂, two HT-LCO₂, and two HT-HCO₂, from left to right. Dissimilar letters indicate means that are significantly different following *post-hoc* Tukeys HSD test. Mean symbiont density of corals (each bar represents n=40) depicting the main effects of temperature (B) and feeding (C). Dissimilar letters indicate means that are significantly different following *post-hoc* students t-test. Error bars represent \pm one standard error.



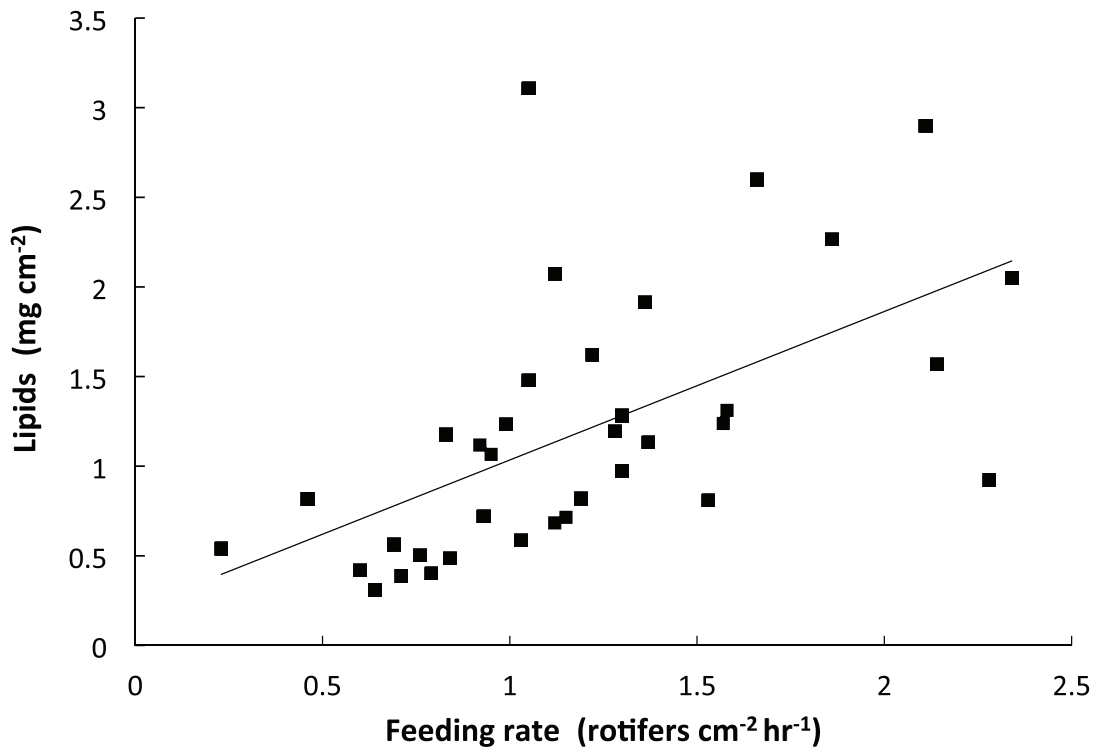


Figure 3.6: Bivariate linear fit of lipid content by feeding rate in *A. cervicornis*. Linear fit ($p=0.0002$) for $n= 36$ fed coral feeding rates and their corresponding lipid contents. The equation of the best fit line is $y = 0.82869(x) + 0.2058$, with a R^2 value of 0.3355.

Table 1: Summary of mean water chemistry parameters throughout the study period expressed as mean \pm 1 SD.

Target Treatment	Temperature (°C)	pCO ₂ (ppm)	Salinity (ppt)	pH	Ω_a	TA ($\mu\text{mol kg}^{-1}$ SW)	DIC ($\mu\text{mol kg}^{-1}$ SW)
LT-LCO ₂	26.2 \pm 0.16	380 \pm 36	32.5 \pm 1.3	8.07 \pm 0.04	3.6 \pm 0.3	2323 \pm 71	2012 \pm 63
HT-LCO ₂	30.2 \pm 0.17	393 \pm 52	32.5 \pm 1.3	8.06 \pm 0.05	4.1 \pm 0.4	2357 \pm 63	2012 \pm 56
LT-HCO ₂	26.1 \pm 0.14	789 \pm 80	32.5 \pm 1.2	7.81 \pm 0.04	2.2 \pm 0.2	2349 \pm 56	2174 \pm 54
HT-HCO ₂	30.0 \pm 0.19	823 \pm 85	32.5 \pm 1.3	7.80 \pm 0.05	2.5 \pm 0.3	2358 \pm 61	2164 \pm 50

Table 2: Results of full-factorial 3-way mixed ANOVA with replicate tank nested within temperature and CO₂ level on the dependent variables: growth, total lipid content, chlorophyll *a*, and symbiont density. Main effects are temperature (T), carbon dioxide (C) and feeding (F). Results of full-factorial 2-way mixed ANOVA with replicate tank nested within temperature and CO₂ level is also shown for the dependent variable feeding rate. Significant p values are bolded.

Factor	Source	DF	F	p value	Factor	Source	DF	F	p value
Growth	T	1	7.07	0.001	Chlorophyll <i>a</i>	T	1	55.1	<.0001
	C	1	11.3	0.001		C	1	0.83	0.366
	T*C	1	0.37	0.546		T*C	1	0.19	0.662
	F	1	19.9	<.0001		F	1	8.18	0.006
	T*F	1	6.64	0.012		T*F	1	0.27	0.603
	C*F	1	3.66	0.060		C*F	1	0.42	0.520
	T*C*F	1	1.43	0.235		T*C*F	1	1.05	0.309
	Tank [T, CO ₂]	4	1.01	0.411		Tank [T, CO ₂]	4	7.49	<.0001
Feeding rate	T	1	0.07	0.788	Symbiont density	T	1	40.2	<.0001
	C	1	4.44	0.045		C	1	0.33	0.571
	T*C	1	0.50	0.485		T*C	1	1.50	0.223
	Tank [T, CO ₂]	4	0.58	0.679					
Total lipid content	T	1	3.05	0.086		F	1	5.05	0.028
	C	1	5.63	0.021		T*F	1	2.23	0.140
	T*C	1	1.29	0.260		C*F	1	1.34	0.252
	F	1	1.64	0.206		T*C*F	1	0.15	0.701
	T*F	1	0.88	0.351		Tank [T, CO ₂]	4	4.52	0.003
	C*F	1	4.14	0.047					
	T*C*F	1	1.36	0.249					
	Tank [T, CO ₂]	4	0.92	0.46					

Table 3: Analysis of variance for a test of bivariate linear fit between feeding rate and lipid content.

Source	DF	F ratio	P value
Model	1	17.16	0.0002
Error	34		
Total	35		

CHAPTER 4

Latent effects of source location within the same coral species drive differences in calcification and feeding rate under climate change stress

4.1 Summary

Coral calcification is decreased by ocean warming, ocean acidification, and the two stressors in combination. Heterotrophy is often a pathway used by the coral host to gain additional energy while under stress to alleviate, and sometimes mitigate, reductions in calcification. While this information is a glimmer of hope for some coral species, it remains unclear which species are capable of this pathway and what *in-situ* conditions are necessary for optimal feeding, i.e. prey concentrations and host feeding effort. Additionally, the environmental conditions at a given site that a coral is acclimated to may play a role in shaping their physiological response to stress, as previous work in the Florida Reef Tract has indicated that even over small spatial scales, coral physiological response within the same species can vary considerably. In this study, we subjected fed and unfed *Orbicella faveolata* from two different locations, Emerald Reef near Key Biscayne, FL, and Truman

Harbor near Key West, FL, to the same climate change scenarios (26 °C/390 ppm, 26 °C/1000 ppm, 31 °C/390 ppm, and 31 °C/1000 ppm) to determine how corals acclimated to different environmental conditions would respond to the same treatment conditions. *O. faveolata* calcification was five times greater in corals from Truman Harbor than at Emerald Reef in the fed control group, and calcification at each stress treatment was much lower for Emerald corals than for Truman corals. Feeding rates were also variable by source location with temperature and prey concentration driving Emerald Reef feeding response, and CO₂ driving Truman Harbor response. We hypothesize that latent effects of source location within the same species of coral subjected to the same stress conditions may play a major role in understanding physiological responses to climate change.

4.2 Background

Global climate change threatens reef-building corals and overall coral reef ecosystem health via ocean warming and ocean acidification. Ocean warming contributes to coral bleaching, whereby corals expel the symbiotic algae that live within them due to their narrow thermal tolerance (Glynn 2012). These algae, under normal, unstressed, conditions can provide the coral with up to 100% of its daily metabolic requirements by transferring the products of photosynthesis to the host (Grottoli et al. 2006). When corals lose their symbiotic algae, they lose their main source of nutrition, and thus bleaching events, if sustained, often contribute to widespread mortality. Ocean acidification (OA) refers to increases in anthropogenic CO₂ that dissolve into the ocean, changing the carbonate chemistry of seawater such that

the pH of seawater decreases, or acidifies. This phenomenon impairs a corals ability to build its skeleton of calcium carbonate (Langdon and Atkinson, 2005). Coral calcification is reduced by both warming and OA, and likely to be even further reduced by the two stressors in combination (Albright and Mason, 2013, Reynaud et al. 2003).

Coral heterotrophy, or the ability of the coral host to feed on its own from the water column on zooplankton or particulate organic matter, as opposed to relying solely on photosynthetic product from its symbionts, has been shown to mitigate reductions in calcification due to thermal and/or OA stress (Grottoli et al. 2006, Cohen and Holcomb, 2009, Edmunds 2011, Towle et al. 2015). What remains unclear is which corals will be capable of using heterotrophy effectively, and what conditions will facilitate this, i.e. concentrations of zooplankton (Palardy et al. 2006) and feeding effort of the coral (Houlbrèque et al. 2015). Heterotrophy may be an indicator of resilience to climate change, but the heterotrophic capabilities of many coral species, especially the heterotrophic capabilities of Endangered Species Act (ESA)-listed species, are still unknown. One of the gaps in our knowledge about coral heterotrophy is whether intraspecific variability in feeding rate will be affected by acclimation to a given reef site that may have higher or lower densities of zooplankton. Previous work on reefs throughout the Florida Reef Tract have demonstrated that even over small spatial scales, coral physiology, i.e. calcification, lipid content, and symbiont density, can vary greatly even within the same species (Teece et al. 2011). The implications of these small spatial differences imply that creating management plans for ESA-listed species in Florida and the

Caribbean may need to be based on the synergistic impacts of both species and source location, as opposed to making decisions based on one or the other.

Therefore the aims of the study were to address how corals from different source sites of the same ESA-listed species, *Orbicella faveolata*, would respond to climate change stress, with special attention to calcification and feeding rates. Policy makers may make generalizations based on how certain species respond to climate change stress, but even within a species, physiological responses may be variable due to environmental characteristics of particular reef environments. It may not be prudent to generalize a management plan to multiple locations in the same reef tract even for the same species. In this study, we test the hypothesis that there will be no difference in the calcification and feeding rate response of *O. faveolata* colonies from an upper Florida Keys site and a lower Florida Keys site, i.e. the variation in physiology will be due to stress treatment, not source location. The results of this study have implications for coral assisted migrations and transplants to different sites for conservation efforts.

4.3 Methods

4.3.1 Collection

Orbicella faveolata colonies were collected from two sources in April 2014. Five colonies were collected by divers from Truman Harbor (TH) (24.332 N, 81.484 W), Key West, Florida under permit (FKNMS -2014-064). Three colonies were collected by divers from Emerald Reef (ER) (25.674 N, 80.099 W) Key Biscayne, Florida under Florida Fish and Wildlife Special Activities License (SAL-13-1182B-SRP). Both

collection sites were approximately five meters in depth, and the sites are approximately 112 nautical miles away from each other. 50 ER (17 per parent colony) and 50 TH (10 per parent colony) 2.5 cm diameter cores were made using a Montana Brand diamond tile drill bit (MB-65207). Cores were glued to numbered plastic tiles and allowed to recover from coring at control conditions (26 °C, 390 ppm) for one month prior to the start of the experiment.

4.3.2 Experimental design

There were four experimental treatments: 26 °C/390 ppm (LT-LCO₂), 26 °C/1000 ppm (LT-HCO₂), 31 °C/390 ppm (HT-LCO₂), and 31 °C/1000 ppm (HT-HCO₂) and the study lasted eight weeks, from mid-June to mid-August 2014. Each treatment was replicated twice for a total of eight independently controlled tanks. Twelve - fourteen corals (six-seven fed and six-seven unfed throughout the experiment) were placed in each tank for a total of 100 replicates. Replicates were haphazardly distributed between tanks accounting for source, i.e. of the six fed corals per tank, three were from ER and three were from TH, and of the six unfed corals per tank, three were from ER and three were from TH.) HT treatment water was increased from acclimation levels of 26 °C to 31 °C at a rate of 0.5 °C per day. 31 °C was chosen because it is just above mean local bleaching threshold in the Florida Keys (30.4 °C, Manzello et al. 2007). 1,000 ppm was chosen because it is the CO₂ level predicted for the end of the century (IPCC 2013).

Experimental corals were maintained in a natural light greenhouse facility at the University of Miami in 60 L tanks of water replenished by a 250 L sump tank with complete water turnover every 10 minutes. Each sump tank contained a heat-

ing and cooling element connected to a temperature controller (OMEGA CN7533) with accuracy within 0.1 °C. CO₂ levels were achieved by mixing pure CO₂ and air using mass flow controllers (Sierra Instruments model 810C) that was pumped through a Venturi injector and circulated through the sump before being pumped into experimental tanks. All tanks were connected to a HOBO U30 data logger taking measurements of CO₂, temperature, and light every five minutes. Daily PAR averaged throughout the experiment between 7:00am and 7:00pm was 350 μmoles m⁻² s⁻¹.

4.3.3 Measurements

In order to monitor seawater chemistry, discrete water samples (250 mL) were taken from each tank weekly and poisoned with 100 μL mercuric chloride to be analyzed for dissolved inorganic carbon (DIC). CO₂ was measured using an equilibrator and Licor CO₂ analyzer system calibrated against 700 ppm pure CO₂ gas. Salinity was measured using a YSI meter calibrated before each use against a 50,000 microSeimen standard solution. DIC was measured in duplicate using a DIC analyzer (Apollo SciTech Inc.) standardized using certified reference materials obtained from Dr. A. Dickson (Scripps IO). Mean temperature, salinity, pCO₂, and DIC were used to calculate pH and aragonite saturation state (Ω_a) for each treatment using the program CO2SYS using K₁ and K₂ from Mehrbach et al. (1973) refit by Dickson and Millero (1987) per Lewis and Wallace (1998).

Calcification rates were measured biweekly as changes in coral weight in water using the buoyant weight technique according to Davies et al. (1989). A skeletal density of 1.95 g cm⁻³, based on four *O. faveolata* cores sacrificed at the beginning of

the experiment, was used to calculate colony weight in air. Calcification rates were normalized to surface area to reduce variability due to differences in fragment size and to permit reporting in ecologically relevant units, i.e. $\text{mg CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$.

In order to quantify rates of heterotrophy, initial and final concentrations of live rotifers were measured and feeding rates were calculated following Coughlan (1969). An advantage of this method (see Towle et al. 2015) over those published by Palardy et al. (2005, 2006) and Grottoli et al. (2006) is that it is non-destructive. Twenty-one one-L beakers were used, twenty containing a coral and one control without coral to account for any possible changes in rotifer density not due to coral feeding. Each beaker had the same flow rate (controlled by magnetic stir bar), light conditions, and initial rotifer density from the same stock solution. Corals were allowed to feed for one hour after sunset as in Grottoli et al. (2006) and were observed to have extended tentacles in the presence of rotifers, indicating feeding was occurring. After one hour, four replicate fifteen mL water samples were taken from each beaker, fixed in the preservative Lugols solution, and final rotifer concentration was quantified via microscopy.

Two densities of rotifer concentrations were used during two separate feeding trial experiments one done at week two and one done at week six of the study. Leichter et al. (1998) found mean zooplankton density on a Florida reef was approximately $1,716 \text{ cells L}^{-1}$, but pioneering work on coral feeding rates by Sebens et al. (1996) showed that low prey densities translated into low prey capture, confounding feeding rates. Thus, Sebens et al. (2006) encouraged using as high as 10x published values. Here, in order to find a balance between not confounding feeding rates but also having more ecologically relevant concentrations, we chose

to use two concentrations: approximately 5x (8,600 rotifers L⁻¹, week two) and 6x (10,400 rotifers L⁻¹, week six) that of what Leichter et al. (1998) found. Assessing two concentrations, the higher one later in the study, allowed us to test if corals presented with more food would take the opportunity to consume more.

Fed corals were additionally offered food *ad libitum* twice weekly using a powdered zooplankton diet (Zieglers Larval AP 100) as in Towle et al. (2015). The purpose of offering food *ad libitum* separate from live rotifers offered during feeding rate experiments was so that ingestion would be more similar to *in-situ* feeding rates, as opposed to rates that could be artificially elevated if corals only had exposure to food during the feeding rate experiments. Feeding rate experiments took place on evenings when corals were not fed *ad libitum*.

In order to measure respiration of the fed corals, one hour long incubations were run using twelve 0.237 L glass chambers, ten containing a coral core and two blanks. Experiments were conducted in darkened, constant-temperature water bath maintained at either 26 or 31 °C depending on coral temperature regime. Respiration was measured as oxygen flux using the Winkler titration technique following Dickson (1994). The oxygen consumption rate was determined by regressing oxygen concentration against time. The oxygen consumption rate determined in each chamber was corrected for the background consumption rate in the control (blank) chamber multiplied by the volume of water in the chamber (0.237 L) and divided by surface area to obtain the respiration rate in $\mu\text{moles O}_2 \text{ hr}^{-1} \text{ cm}^{-2}$.

At the end of the experiment, coral tissue was removed using an air pik following Szmant et al. (1990) in order to quantify zooxanthellae density, chlorophyll *a* content, and total lipid content. Of the total blastate volume, one mL was

allocated for zooxanthellae density, one mL was allocated for chlorophyll *a*, and five mL were allocated for total lipid content. For zooxanthellae density, one mL was placed in a 1.5 mL Eppendorf tube with 50 μ L of the fixative Lugols for later quantification via microscopy using a haemocytometer (Hausser Scientific). For chlorophyll *a*, one mL was filtered onto a glass fiber filter (GF/A) and stored at -80°C until analysis. For analysis filters were thawed to room temperature, placed in centrifuge tubes with eight mL methanol, and returned to -80°C for forty-eight hours following Holm-Hansen and Riemann (1978). After forty-eight hours, samples were read on a fluorometer (TD-700 Turner Designs) calibrated with purified chlorophyll *a* (Sigma-Aldrich catalog no. C6144). For total lipid content, five mL of blastate was filtered onto a glass fiber filter (GF/A) and stored at -80°C until later analysis following Teece et al. (2011). Briefly, the five mL aliquot of total coral homogenate was extracted three times (four mL 1:1 dichloromethane:methanol). The resulting organic extracts were dried under a stream of nitrogen gas and weighed on an analytical balance. All parameters were normalized to core surface areas.

Symbiodinium types hosted by the corals were identified by denaturing gradient gel electrophoresis (DGGE) and sequencing of the second internal transcribed spacer-2 region of ribosomal DNA (ITS2 rDNA) following Cunning and Baker (2013). Briefly, this gene region was amplified using the primers ITSintfor2 and ITS2clamp and amplification products were separated by DGGE (35-75% gradient) using a CBS scientific system. Dominant bands on the gel were excised, re-amplified, and sequenced using BigDye Terminator v3.1 cycle sequencing kit and an Applied Biosystems 3130xl Genetic Analyser.

Symbiodinium in clades B, C, and D were quantified by actin-targeted quantitative PCR (qPCR) assays developed for these particular symbionts (Cunning and Baker 2013). A symbiont clade was considered present in a sample with positive amplification of two technical replicates and no amplification of no-template controls. The proportion of clade D in each colony was then calculated as D cells/(Total cells) (Cunning and Baker 2013).

4.3.4 Statistics

Statistical analyses were completed in JMP v. 11.0.0. Data were checked for normality and homoscedasticity using a Shapiro-Wilk test and Levenes test, respectively. All data were assessed for a tank effect (random factor) and source effect (fixed factor). There were no tank effects, but there was a source effect, and thus data from Emerald Reef and Truman Harbor corals were not pooled, but rather treated separately. Three-way full-factorial ANOVAs were run (temperature x CO₂ x feeding) for each site for the parameters: calcification, lipid content, symbiont density, chlorophyll *a*, and % clade D symbionts. A three-way full factorial ANOVA was run for each site for feeding rate (temperature x CO₂ x concentration of rotifers). A two-way full-factorial ANOVAs (temperature x CO₂) were run for each site for respiration. Both feeding rate and respiration were only assessed in the fed corals from each site, which is why feeding was not a factor that was tested. Alpha for all tests was set at 0.05.

4.4 Results

Mean seawater chemistry parameters for the duration of the study are summarized in Table 1. Temperatures from the collection sites are shown in Table 2. During the month corals were collected, temperatures at Truman Harbor were approximately a degree higher than at Fowey Rocks (proxy for Emerald Reef). Historical data at Truman Harbor show that in the summer of 2012, corals at this site were exposed to temperatures above bleaching threshold (Table 3). The Fowey Rocks site data suggest that during the previous two summers before collection, corals likely did not experience mean temperatures exceeding bleaching threshold (Table 2).

4.4.1 Emerald Reef corals

Calcification of ER corals was significantly affected by CO₂ (negative effect) and feeding (positive effect) (Table 3, ANOVA, $p < 0.05$). Highest calcification rate was in fed control corals ($0.30 \text{ mg cm}^{-2} \text{ d}^{-1}$) and lowest calcification rate was in the unfed HTHCO₂ corals ($-1.05 \text{ mg cm}^{-2} \text{ d}^{-1}$) (Fig 1a). Feeding rate was significantly affected by concentration of prey, temperature, and the interaction between the two (Table 4, ANOVA, $p < 0.05$). Feeding rates at HT treatments, regardless of CO₂ level, were almost 50% lower than the LT rates ($0.19 \text{ rotifers hr}^{-1} \text{ cm}^{-2}$ compared to $0.36 \text{ rotifers hr}^{-1} \text{ cm}^{-2}$, respectively) at the lower (x5) concentration (Fig 2a). However, at the higher (x6) prey concentration, HT treatment feeding rates were increased closer to LT rates (Fig 2a). Lipid content was significantly affected by the interaction between CO₂ and feeding, and the highest level was approximately

8.6 mg cm⁻² in the fed control, while the lowest level was in fed LTHCO₂ corals (4.0 mg cm⁻²) (Fig 3a). Symbiont density and chlorophyll a had the same response to treatments whereby temperature (negative effect) and feeding (positive effect) were significant (Table 3, ANOVA, p<0.05). For both parameters, maximal values were in the fed LTHCO₂ treatment (1.9e6 cells cm⁻² and 5.6 µg cm⁻²), and minimal values were in the unfed HTLCO₂ treatment (6.8e5 cells cm⁻² and 1.7 µg cm⁻²) (Fig 4a, 5a). Respiration (only assessed in fed corals) was significantly affected by temperature, whereby HT increased oxygen consumption (Table 4, ANOVA, p<0.05). Control corals respired at a rate of -1.1 µmoles O₂ hr⁻¹ cm⁻² and HT corals (regardless of CO₂ level) respired at a rate of approximately -1.6 µmoles O₂ hr⁻¹ cm⁻² (Fig 6a). Percentage of clade D symbionts was significantly affected by temperature (positive effect), whereby corals at LT and HT, regardless of CO₂ level, had 83.4% and 99.7% D, respectively (Fig 7a).

4.4.2 Truman Harbor corals

TH coral calcification was significantly affected by temperature (negative response), CO₂ (negative response) and feeding (positive response) (Table 5, ANOVA, p<0.05). However, TH calcification rate in the fed control (1.5 mg cm⁻² d⁻¹) was five times the calcification rate in the ER fed control, and the lowest calcification rate in TH corals was approximately -0.2 mg cm⁻² d⁻¹ in the unfed HTHCO₂ treatment (Fig 1b). Feeding rate was affected by the interaction between temperature and CO₂ in TH corals, but was not affected by concentration of prey (Table 6). Mean feeding rate was highest in the LTLCO₂ treatment at approximately 0.43 rotifers hr⁻¹ cm⁻² and rates were lowest in the HTHCO₂ treatment at approximately 0.23

rotifers $\text{hr}^{-1} \text{cm}^{-2}$ (Fig 2b). Lipid content was significantly affected by feeding (positive response) (Table 5, ANOVA, $p < 0.05$), and this effect was most notable in the control treatment, with fed control levels at 6.3 mg cm^{-2} , and unfed control levels at 2.9 mg cm^{-2} (Fig 3b). TH coral symbiont density and chlorophyll *a* levels were both significantly affected by temperature, feeding, and the interaction between temperature and feeding (Table 5, ANOVA, $p < 0.05$). Additionally, symbiont density was affected by the interaction between CO_2 and feeding, and chlorophyll *a* was also affected by CO_2 . For symbiont density, maximal levels at TH were 2.0×10^6 cells cm^{-2} in the fed LTL CO_2 group, and minimum symbiont density values were in the unfed HTL CO_2 group at 6.7×10^5 cells cm^{-2} (Fig 4b). Like symbiont density, maximum chlorophyll *a* levels in TH were in the fed LTL CO_2 treatment, ($5.1 \mu\text{g cm}^{-2}$), and minimum values were in the unfed HTL CO_2 and HTH CO_2 treatments, $1.6 \mu\text{g cm}^{-2}$ (Fig 5b). Respiration (assessed in fed corals only) was not affected by treatment and was approximately $-1.2 \mu\text{moles O}_2 \text{ hr}^{-1} \text{cm}^{-2}$ across treatments (Table 6, Fig 6b). The percentage of clade D symbionts was not affected by treatment, and was approximately 99.8% across treatments (Fig 7b).

4.5 Discussion

In this study, we found that for the same species of coral (*O. faveolata*), within the same region of the Western Atlantic (Florida Reef Tract), source location can have a strong impact on how a coral responds to the same climate change stressors. This finding was somewhat surprising given that all coral cores from both sites were allowed to acclimate under control conditions for a month prior to the start

of the experiment, and suggests that there may be latent effects of source location caused by fine-scale differences between sites that effect coral physiology under stress.

4.5.1 Calcification

Fed *O. faveolata* across treatments from TH did not experience significantly reduced calcification with respect to the fed control group. Calcification at all treatment levels for TH corals was positive except in the unfed group at HTHCO₂, whereas all calcification rates for ER corals were negative except for the fed control group. Though the ER fed control showed positive calcification rates, the mean was still five times lower than the TH fed control rate. All calcification rates in this study were lower than rates published in the field for *O. faveolata* (2.3 mg cm⁻² d⁻¹, Mallela and Perry, 2007). The lower calcification rates in the present study may be attributed to the fact that these were calcification rates measured in a lab setting under projected climate change scenarios, whereas the Mallela and Perry study was conducted on unstressed corals. This however, does not explain the differences observed between the two source locations in this study, which need to be addressed in the context of other physical and physiological parameters.

4.5.2 Feeding rate

Previous work has demonstrated that variability in calcification response under climate change stress can sometimes be explained by feeding rate of the coral host (Grottoli et al. 2006; Rodrigues and Grottoli, 2007; Cohen and Holcomb, 2009, Edmunds 2011; Towle et al. 2015). While coral heterotrophy may be an indicator

of resilience to climate change stress with regard to mitigating reductions in calcification, this mechanism is dependent on the heterotrophic capabilities of the coral species (Palardy et al. 2008) as well as the presence of adequate zooplankton densities. This study allowed us to test both the heterotrophic capabilities of *O. faveolata* at two densities, but also whether climate change stress treatments would affect the rate at which these corals fed. A surprising observation from the feeding data was that ER and TH coral feeding rates responded quite differently to concentration of prey and treatment. ER corals were positively affected by prey concentration, with rates greater at the higher concentration. Temperature also played a strong role in feeding rate at the lower concentration, but was much less pronounced at the higher concentration, indicating that the concentration of prey can mediate the temperature-driven feeding response. The finding that feeding was much lower at HT than LT at the x5 concentration was somewhat surprising given that other studies have shown that HT can increase feeding rate (Grottoli et al. 2006). However, this finding highlights the ideas of Palardy et al. (2008) that not all corals may be capable of increasing feeding at HT.

Contrarily, feeding rates for Truman Harbor corals were not affected by prey concentration, as rates at each density within treatments were virtually the same. However, treatment had a strong effect on TH feeding rates, with LTHCO₂ corals feeding the most, corals at both the LCO₂ treatments (regardless of temperature) feeding at an intermediate rate, and corals at the HTHCO₂ feeding the least, indicating a strong interaction effect of temperature and CO₂. The result that feeding was highest at LTHCO₂ is consistent with Towle et al. (2015) who demonstrated that another ESA-listed Caribbean coral could increase its feeding rate at HCO₂.

However, this study differs from Towle et al. (2015) in that *O. faveolata* did not sustain those elevated feeding rates at HCO₂ when experienced in conjunction with HT. Houlbrèque et al. (2015) was the first study to demonstrate that feeding rate was reduced in *S. pistillata* under OA conditions.

4.5.3 Lipids, symbiont density, and chlorophyll *a*

Lipid content at ER was significantly affected by the interaction between CO₂ and feeding, and lipid content was affected by feeding only in TH corals. This observation appears to be consistent with Towle et al. (2015) who found that feeding rate and lipid content were correlated in another endangered Western Atlantic/Caribbean coral species. Maximum and minimum symbiont density values were similar at both sites, and chlorophyll *a* followed the same trend as symbiont density in both ER and TH corals. Due to the fact that the data showed strong differences in calcification rate by source location, one might have predicted stronger differences in the lipid and symbiont density values between the two sites as well. However, that was not the case and suggests that differences in calcification must be driven by other factors besides lipid content, symbiont density and chlorophyll *a*.

4.5.4 Respiration rate and percentage of clade D symbionts

Respiration rates of ER corals were increased (more oxygen consumption) at the HT treatments, regardless of CO₂ level, indicating that ER corals may have been metabolically stressed in HT treatments. In contrast, respiration rates were not significantly different across treatments for TH corals, suggesting a greater level of

metabolic stability. DGGE and qPCR revealed that all of the corals (from both ER and TH) hosted predominantly clade D symbionts. However, ER corals at LT (regardless of CO₂ level) hosted less clade D (75-85%) than ER corals at HT (100%), indicating an increase in clade D symbionts at HT. In contrast, TH corals hosted 100% clade D symbionts across treatments, indicating no changes due to treatment groups. It has been shown that certain types of *Symbiodinium* can confer resistance to the coral host with respect to thermal tolerance. Many studies have demonstrated that corals hosting clade D symbionts are more thermally tolerant than the same species of coral hosting clade C symbionts (Glynn et al. 2001; Rowan 2004). Furthermore, corals containing thermally tolerant clade D symbionts are more abundant on reefs after episodes of severe bleaching, suggesting that adaptive shifts to hosting clade D may increase resistance to future bleaching events (Baker et al. 2004). Temperature regimes at both sites may have potentially driven the differences observed, in that TH corals appear to be exposed to slightly higher thermal regimes than ER corals (Table 2), which may have facilitated TH corals already hosting 100% D, while ER corals didn't host 100% D until exposed to 31 °C.

4.5.5 Latent effects of source location due to degree of nutritional repletion, changes in the holobiont, and fine-scale thermal history

Here, we attempt to understand why we saw such variation in the calcification response between the two source locations within the same coral species exposed to the same stressors. To summarize major trends, at ER, calcification was very low or negative. Feeding rate was sensitive to temperature, prey concentration, and

their interaction, which suggests that ER corals may have been nutritionally deplete if increases in prey availability prompted increases in consumption rate. Perhaps heterotrophy is not a substantial part of ER life history, so these corals were not satiated. The percentage of clade D symbionts increased by approximately 15% when temperature was elevated from 26 °C to 31 °C, indicating changes in the symbiosis and respiration rate was significantly increased at HT treatments, indicating changes in host metabolism. These changes potentially could have made it more difficult for the holobiont to deal with stress, and as a result, calcification suffered greatly across treatments.

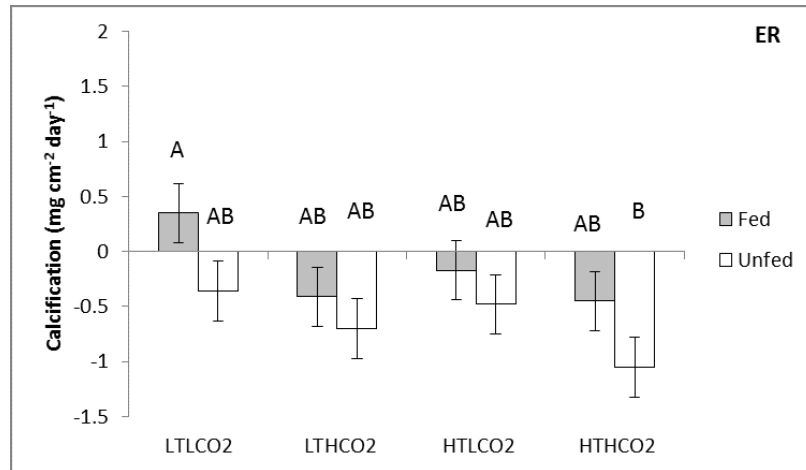
In contrast, calcification at TH was more positive under stress. Feeding was sensitive to treatment and not prey concentration. No change in consumption rates with elevated prey availability suggests that TH corals may be nutritionally replete because when offered a greater concentration of food, they did not increase their feeding rate. This finding could imply that heterotrophy is a normal part of TH coral life history. This hypothesis regarding heterotrophy is consistent with a model from Anthony et al. (2009) suggesting that corals that use heterotrophy may reduce mortality following thermal stress. At no point in the study did percentage of clade D symbionts or respiration rate in TH corals significantly change, suggesting a greater degree of stability in the holobiont compared to ER corals. This may be a healthier or a best case scenario response to stress for this species.

Another possible hypothesis regarding the differences in calcification responses between ER and TH corals is that even though the same heat stress was applied (31 °C) the degree of stress experienced may have been felt differently based on source location. For example, during the collection month of April 2014, mean

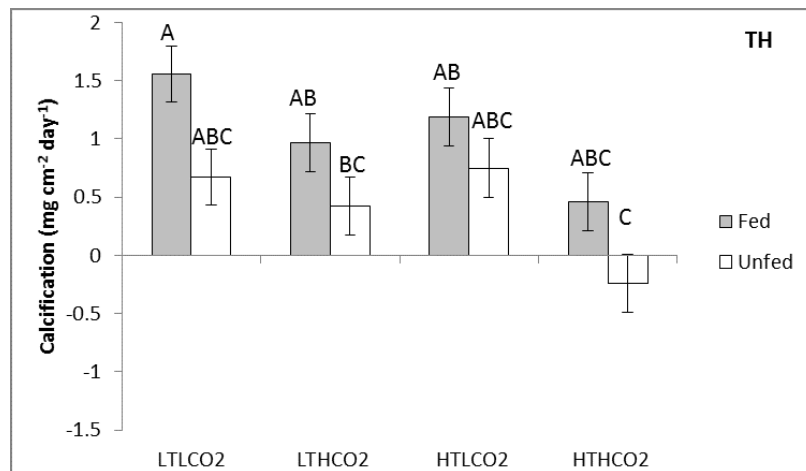
temperature was 26.5 °C at TH and 25.3 °C near ER. Though all corals were acclimated to 26 °C for four weeks before the start of the experiment, perhaps that amount of time was not long enough to reduce latent effects of source location. Corals from TH experienced $\Delta 4.5$ °C from their mean collection month temperature, while corals from ER experienced $\Delta 5.7$ °C from their collection month mean. Historical data shows that the mean temperature for the summer prior to this experiment (2013) were 30.1 °C and 29.4 °C, at TH and ER, respectively. Therefore, TH corals experienced $\Delta 0.9$ °C increase from the previous summer mean, while ER corals experienced approximately $\Delta 1.6$ °C increase from their previous summer mean. Corals from the TH site also experienced a mean summer temperature greater than bleaching threshold in the Florida Keys (30.4 °C, Manzello et al. 2007) in 2012. These historical temperature data suggest that TH corals may be acclimated to slightly higher temperatures compared to ER corals. This hypothesis is consistent with those of Oliver et al. (2009) and Oliver et al. (2011), who asserted that corals in naturally warmer environments can have higher resistance to bleaching temperatures, and can survive heat exposure that would bleach conspecifics from cooler microclimates. The results of Palumbi et al. (2014) also show that acclimatization can allow corals to acquire substantial high temperature resistance. In fact, recent work has shown that resilient corals often have higher expression or front-loading of thermal tolerance genes such as heat shock proteins, antioxidant enzymes, and genes involved in apoptosis regulation, tumor suppression, and more (Barshis et al. 2013). While genomic data was not assessed in this study, the data suggest that TH corals could be front-loading genes that confer resilience to thermal stress, while ER corals may not be. Additionally, while sea water chem-

istry data were not available from either site, it is possible that TH corals may also be more acclimated to changes in CO₂ compared to ER corals because of the nature of the harbor setting they came from, allowing them to possibly calcify better under experimental CO₂ stress. This hypothesis agrees with hypotheses from Shamberger et al. (2014) and Fabricius (2011) who suggested there may be physiological mechanisms of environmental accommodation to acidification.

In conclusion, we have reason to suspect that latent effects of source location in this species, perhaps degree of heterotrophy usage/nutritional repletion, changes in symbiont population and metabolism, and/or fine scale differences in temperature regime may have contributed to the large variation in calcification response of the same species to the same climate change stressors. Future work should examine the possibility that corals from TH are front-loading thermal tolerance genes that ER corals may not be. This study implies that latent source effects may contribute to the success or failure of assisted migrations or transplants of coral species under climate change scenarios for future conservation efforts.

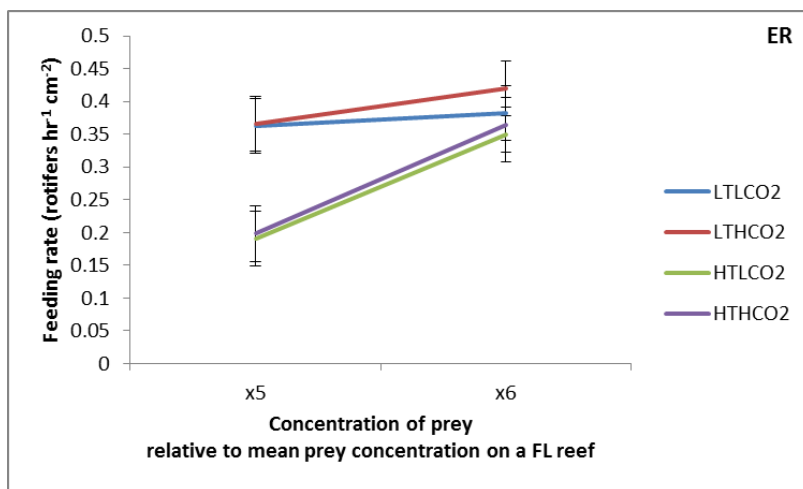


(a)

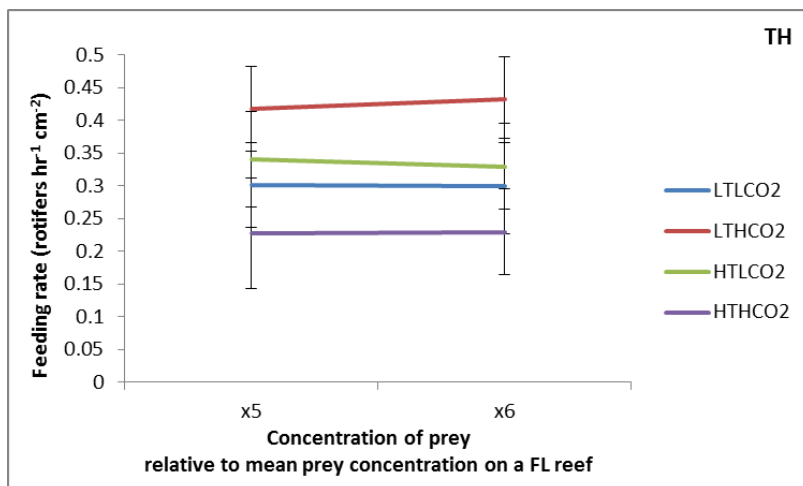


(b)

Figure 4.1: Calcification rates of corals from a.) Emerald Reef site (ER) and b.) Truman Harbor site (TH). Fed corals are represented in grey and unfed corals are represented in white for each treatment. Error bars represent ± 1 S.E. Each individual bar represents $n=6-7$ corals. Letters represent statistical differences between treatment means based on a *post-hoc* Tukeys HSD test.

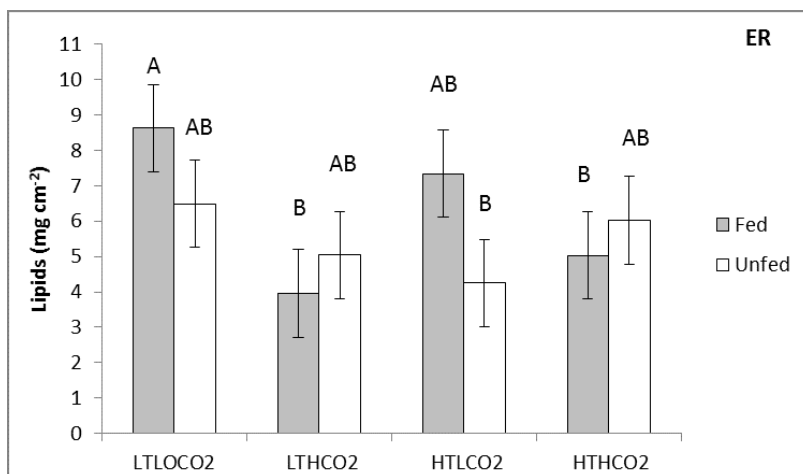


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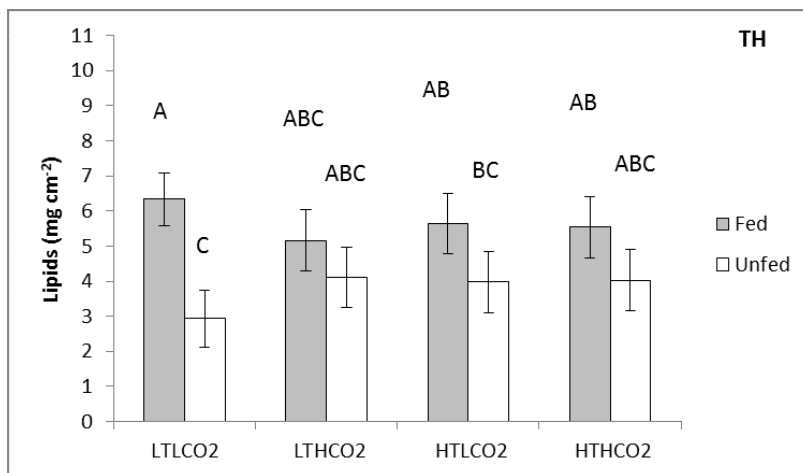


(b)

Figure 4.2: Feeding rates of corals from a.) Emerald Reef site (ER) and b.) Truman Harbor site (TH) for each treatment for each of the two concentrations of rotifers: 5x reef densities and 6x reef densities. Error bars represent ± 1 S.E. Each individual point represents $n=5$ corals.

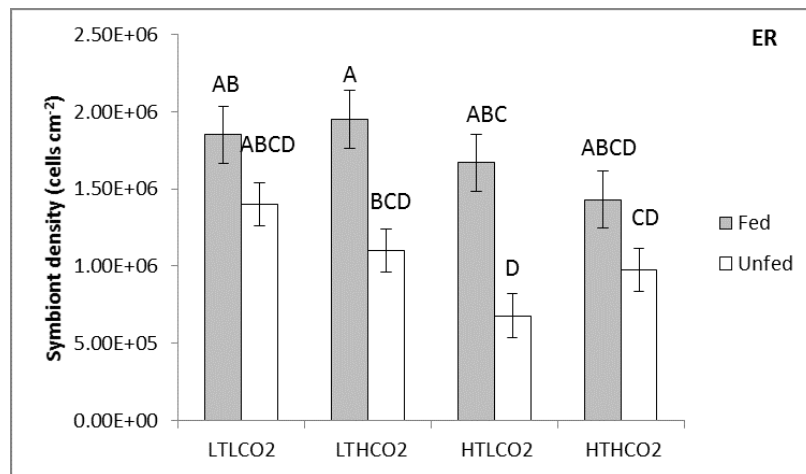


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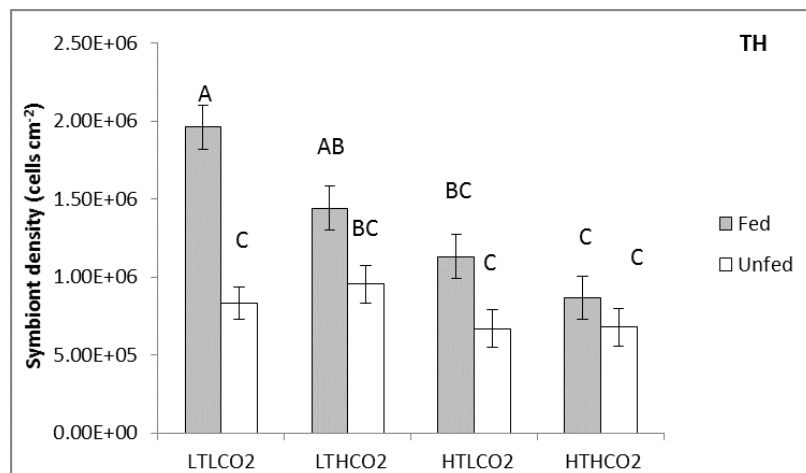


(b)

Figure 4.3: Lipid content of corals from a.) Emerald Reef site (ER) and b.) Truman Harbor site (TH). Fed corals are represented in grey and unfed corals are represented in white for each treatment. Error bars represent ± 1 S.E. Each individual bar represents $n=6-7$ corals. Letters represent statistical differences between treatment means based on a *post-hoc* Tukeys HSD test.

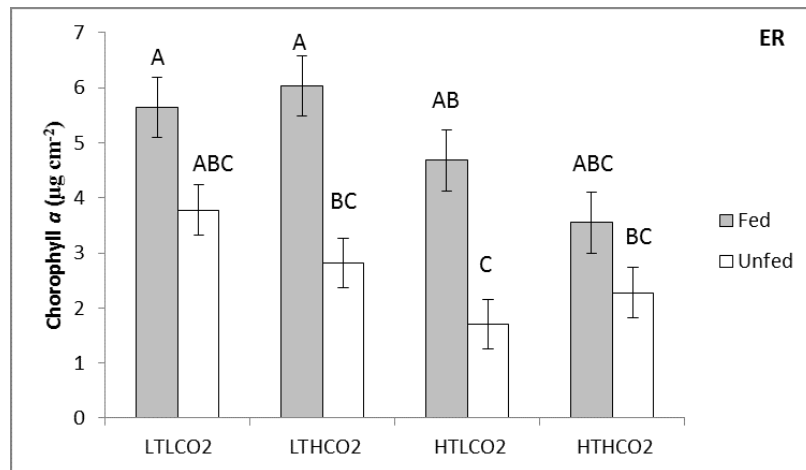


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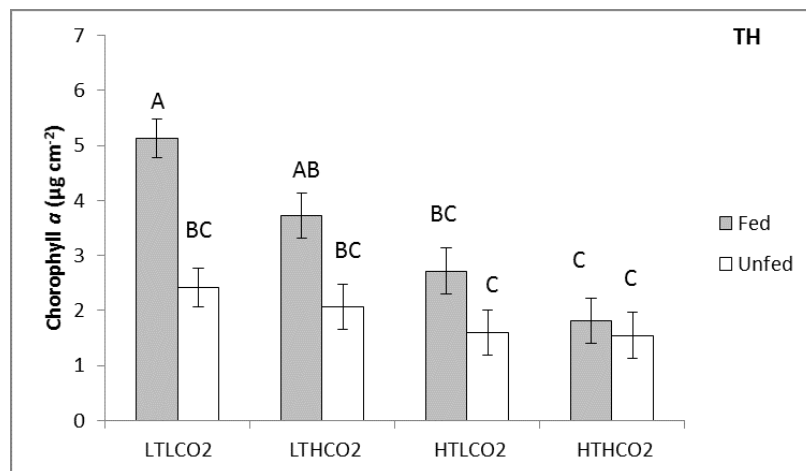


(b)

Figure 4.4: Zoonthellae density of corals from a.) Emerald Reef site (ER) and b.) Truman Harbor site (TH). Fed corals are represented in grey and unfed corals are represented in white for each treatment. Error bars represent ± 1 S.E. Each individual bar represents $n=6-7$ corals. Letters represent statistical differences between treatment means based on a *post-hoc* Tukeys HSD test.

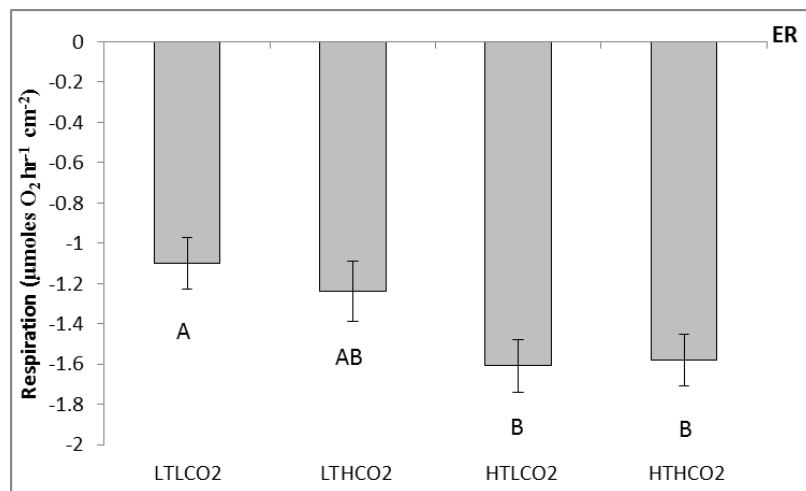


(a)

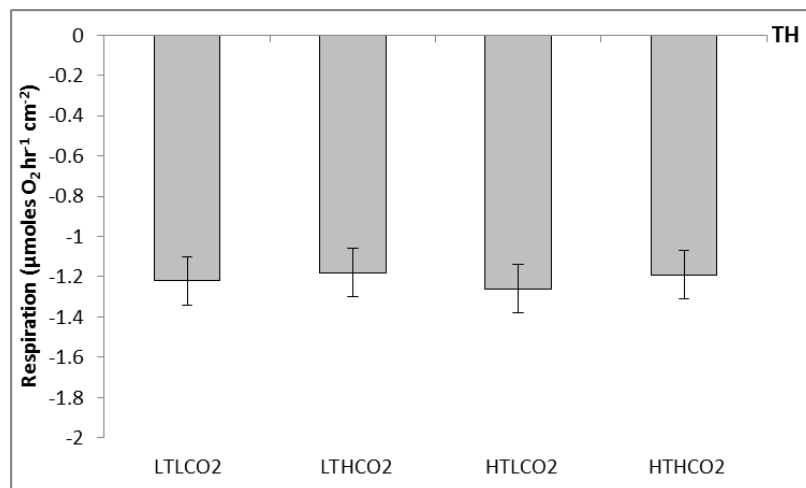


(b)

Figure 4.5: Chlorophyll *a* content of corals from a.) Emerald Reef site (ER) and b.) Truman Harbor site (TH). Fed corals are represented in grey and unfed corals are represented in white for each treatment. Error bars represent ± 1 S.E. Each individual bar represents $n=6-7$ corals. Letters represent statistical differences between treatment means based on a *post-hoc* Tukeys HSD test.

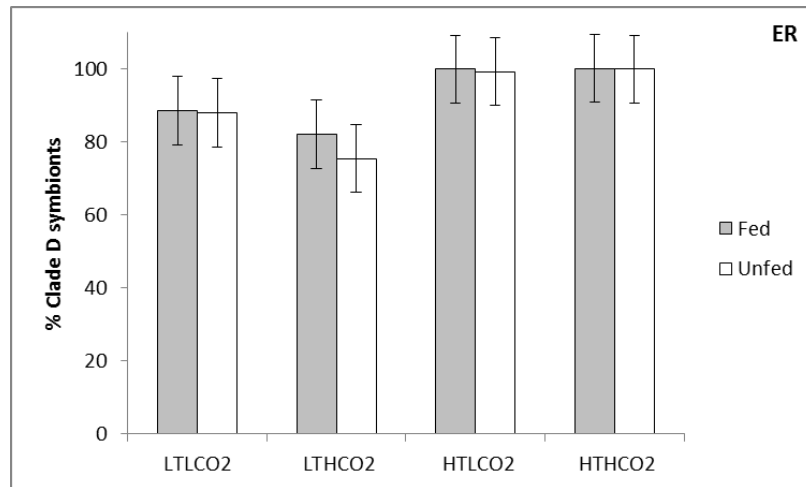


(a)

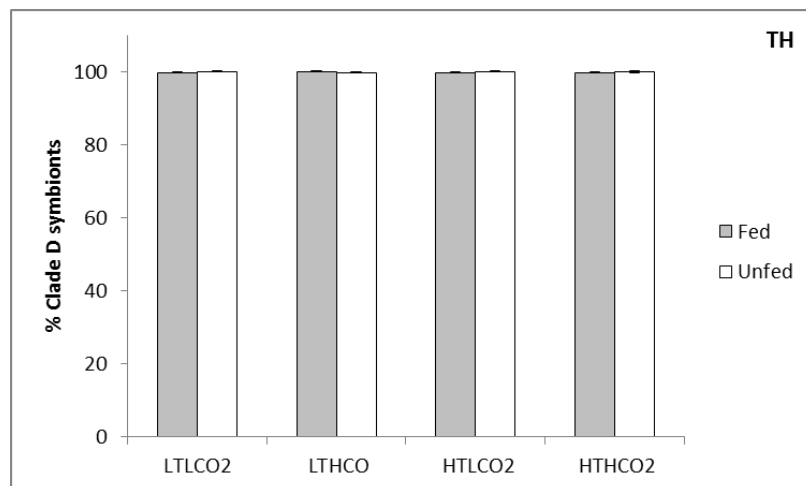


(b)

Figure 4.6: Respiration rates of corals from a.) Emerald Reef site (ER) and b.) Truman Harbor site (TH). Only fed corals were measured for respiration rates, and are represented in grey. Error bars represent ± 1 S.E. Each individual bar represents $n=5$ corals. Letters represent statistical differences between treatment means based on a *post-hoc* Tukeys HSD test.



(a)



(b)

Figure 4.7: % Clade D symbionts in corals from a.) Emerald Reef site (ER) and b.) Truman Harbor site (TH). Fed corals are represented in grey and unfed corals are represented in white for each treatment. Error bars represent ± 1 S.E. Each individual bar represents $n=6-7$ corals.

Table 1: Mean seawater chemistry parameters during the study period presented as mean \pm 1 standard deviation.

Treatment	Temperature (°C)	CO ₂ (ppm)	Salinity (ppt)	pH	Ω_a	DIC ($\mu\text{mol kg}^{-1}$ SW)
LT-LCO ₂	26.2 \pm 0.12	387 \pm 21	33.9 \pm 1.20	8.08 \pm 0.02	3.89 \pm 0.18	2060 \pm 41
HT-LCO ₂	31.2 \pm 0.15	401 \pm 28	33.9 \pm 1.10	8.07 \pm 0.02	4.50 \pm 0.22	2034 \pm 34
LT-HCO ₂	26.1 \pm 0.15	987 \pm 123	34.0 \pm 1.13	7.74 \pm 0.05	2.01 \pm 0.21	2216 \pm 38
HT-HCO ₂	31.3 \pm 0.25	984 \pm 117	33.9 \pm 1.07	7.75 \pm 0.04	2.50 \pm 0.21	2203 \pm 44

Table 2: Mean temperature ($^{\circ}\text{C} \pm 1 \text{ SD}$) at the two source sites during the collection month and the two summer means prior to coral collection. The Fowey Rocks site (25.591 N 80.097 W) was used as a proxy for the Emerald Reef site. Temperatures above mean local bleaching threshold (30.4°C , Manzello et al. 2007) are bolded.

Time frame	Fowey Rocks (proxy for Emerald Reef)	Truman Harbor
April 2014 (collection month)	25.3 ± 1.1	26.5 ± 1.4
Summer 2013	29.4 ± 1.0	30.1 ± 0.8
Summer 2012	29.3 ± 1.0	30.9 ± 1.0

Table 3: Summary of the three-way full-factorial ANOVAs for the Emerald Reef corals for calcification rate, lipid content, symbiont density, chlorophyll *a*, and % Clade D symbionts, where T represents temperature, C represents CO₂, and F represents feeding. Significant p values are bolded.

Factor	Source	Degrees of Freedom	F ratio	p value
Calcification rate	T	1	1.7840	0.1892
	C	1	6.4631	0.0150
	T*C	1	0.0992	0.7544
	F	1	6.1832	0.0172
	T*F	1	0.0118	0.9141
	C*F	1	0.0330	0.8567
	T*C*F	1	0.8815	0.3534
Lipid content	T	1	0.1760	0.6771
	C	1	3.5254	0.0679
	T*C	1	2.4820	0.1232
	F	1	0.7862	0.3807
	T*F	1	0.0874	0.7690
	C*F	1	4.2352	0.0463
	T*C*F	1	0.0572	0.8122
Symbiont density	T	1	8.6851	0.0053
	C	1	0.0679	0.7957
	T*C	1	0.2387	0.6278
	F	1	27.3386	<.0001
	T*F	1	0.0593	0.8088
	C*F	1	0.0731	0.7883
	T*C*F	1	3.1775	0.0823
Chlorophyll <i>a</i>	T	1	14.9315	0.0004
	C	1	0.5392	0.4708
	T*C	1	0.0000	0.9949
	F	1	35.5357	<.0001
	T*F	1	0.2856	0.5960
	C*F	1	0.0509	0.8226
	T*C*F	1	3.8114	0.0579
% Clade D symbionts	T	1	5.9897	0.0189
	C	1	0.4705	0.4967
	T*C	1	0.5560	0.4602
	F	1	0.0900	0.7657
	T*F	1	0.0562	0.8138
	C*F	1	0.0437	0.8355
	T*C*F	1	0.0629	0.8033

Table 4: Summary of the three-way full-factorial ANOVA for feeding rate and two-way full factorial ANOVA for respiration rate for the fed Emerald Reef corals, where T represents temperature, and C represents CO₂. Conc represents concentration of rotifers (feeding rate ANOVA only). Significant p values are bolded.

Factor	Source	Degrees of Freedom	F ratio	p value
Feeding rate	Conc	1	11.4052	0.0019
	T	1	13.0579	0.0010
	Conc * T	1	4.5267	0.0412
	C	1	0.2920	0.5927
	Conc * C	1	0.1141	0.7378
	T x C	1	0.0333	0.8564
	Conc * T * C	1	0.0497	0.8250
Respiration	T	1	9.9500	0.0065
	C	1	0.1565	0.6980
	T*C	1	0.3841	0.5447

Table 5: Summary of the three-way full-factorial ANOVAs for the Truman Harbor corals for calcification rate, lipid content, symbiont density, chlorophyll *a*, and % Clade D symbionts, where T represents temperature, C represents CO₂, and F represents feeding. Significant p values are bolded.

Factor	Source	Degrees of Freedom	F ratio	p value
Growth	T	1	4.2797	0.0448
	C	1	13.0331	0.0008
	T*C	1	1.5835	0.2152
	F	1	13.2856	0.0007
	T*F	1	0.1754	0.6775
	C*F	1	0.0131	0.9095
	T*C*F	1	0.7011	0.4072
Lipids	T	1	0.0729	0.7885
	C	1	0.0007	0.9785
	T*C	1	0.0007	0.9786
	F	1	10.0725	0.0028
	T*F	1	0.2794	0.5998
	C*F	1	1.0635	0.3082
	T*C*F	1	0.8289	0.3677
Symbiont density	T	1	30.7874	<.0001
	C	1	3.8921	0.0550
	T*C	1	0.1738	0.6788
	F	1	46.3004	<.0001
	T*F	1	8.2445	0.0063
	C*F	1	7.6503	0.0083
	T*C*F	1	1.2105	0.2774
Chlorophyll <i>a</i>	T	1	25.8621	<.0001
	C	1	5.8908	0.0194
	T*C	1	0.4815	0.4914
	F	1	26.8179	<.0001
	T*F	1	7.2600	0.0099
	C*F	1	2.9316	0.0939
	T*C*F	1	0.0273	0.8695
% Clade D symbionts	T	1	0.0010	0.9755
	C	1	0.0521	0.8205
	T*C	1	0.0732	0.7879
	F	1	0.6280	0.4323
	T*F	1	0.9148	0.3431
	C*F	1	1.3766	0.2470
	T*C*F	1	1.0655	0.3076

Table 6: Summary of the three-way full-factorial ANOVA for feeding rate and two-way full factorial ANOVA for respiration rate for the fed Truman Harbor corals, where T represents temperature, and C represents CO₂. Conc represents concentration of rotifers (feeding rate ANOVA only). Significant p values are bolded.

Factor	Source	Degrees of Freedom	F ratio	p value
Feeding rate	Conc	1	0.0002	0.9879
	T	1	2.7006	0.1115
	Conc * T	1	0.0114	0.9157
	C	1	0.0303	0.8631
	Conc * C	1	0.0230	0.8806
	T x C	1	5.4821	0.0266
	Conc * T * C	1	0.0004	0.9847
Respiration	T	1	0.0678	0.7979
	C	1	0.1857	0.6723
	T*C	1	0.0158	0.9014

CHAPTER 5

Physiological responses of *Porites porites*: can preconditioning to high CO₂ affect bleaching susceptibility?

5.1 Summary

The manifestation of thermal and acidification stress may not occur uniformly in time and space. Because acidification is a chronic stress and bleaching is an acute stress, it is likely that all bleaching events occurring post mid-century will occur at elevated CO₂ levels relative to present day levels. It remains unclear whether high CO₂ levels will benefit a corals algal symbionts due to CO₂ fertilization, or whether the negative effects of high CO₂ on host calcification will negate any potential benefit of high CO₂. This study tested whether preconditioning to elevated CO₂ prior to exposure to thermal stress could reduce bleaching susceptibility, enhance symbiont photochemical efficiency, and/or enhance coral feeding rates in the Caribbean species *Porites porites*. Coral fragments were held at 26 °C and preconditioned to either 390 ppm or 900 ppm CO₂. After three months, half of the corals from each CO₂ level were exposed to 31 °C, (i.e. 31 °C/390 ppm and 31 °C/900 ppm) for two months while the other half were maintained in their orig-

inal treatments (i.e. 26 °C/390 ppm or 26 °C/900 ppm). Calcification, feeding rate, photochemical efficiency, and bleaching percentage by treatment were measured as dependent, non-invasive response variables. Calcification was not depressed by preconditioning to 900 ppm at 26 °C relative to ambient CO₂ at 26 °C (5.18 ±0.47 vs. 5.10 ±0.62 mg cm⁻² d⁻¹, respectively). Calcification was also not significantly depressed in corals preconditioned to ambient CO₂ and then exposed to thermal stress (i.e. 31 °C/390 ppm treatment, 4.88 ±0.53 mg cm⁻² d⁻¹). However, calcification was depressed by 33%, in corals preconditioned to high CO₂ and then exposed to thermal stress (i.e. 31 °C/900 ppm treatment, 3.42 ±0.67 mg cm⁻² d⁻¹), and 85% of fragments showed visual signs of bleaching. Feeding rates were highest at 26 °C/900 ppm (0.80 ±0.11 rotifers hr⁻¹ cm⁻²) followed by 31 °C/390 ppm (0.65 ±0.15 rotifers hr⁻¹ cm⁻²), but were no different from the 26 °C/390 ppm treatment (0.29 ±0.13 rotifers hr⁻¹ cm⁻²) at 31 °C/900 ppm (0.31 ±0.16 rotifers hr⁻¹ cm⁻²). Photochemical efficiency decreased by 5% when corals preconditioned to ambient CO₂ experienced thermal stress, and by 10% when corals preconditioned to elevated CO₂ experienced thermal stress. These data indicate that preconditioning to elevated CO₂ does not reduce bleaching susceptibility in *P. porites*, and suggests that elevated CO₂ likely offers no advantage to the algal symbiont, and in fact worsens the holobiont response to thermal stress.

5.2 Background

Rising greenhouse gases are driving the ocean toward conditions that have not occurred for the last 300 million years, introducing risk of long-term ecological

transformation (Hoegh-Guldberg and Bruno, 2010; Hönisch et al. 2012). These greenhouse gases have increased global mean temperature by $0.2\text{ }^{\circ}\text{C decade}^{-1}$ over the last 30 years (Hansen et al. 2006). This increased warming is predicted to cause severe coral bleaching (loss of algal endosymbionts and/or a reduction in their photochemical efficiency), on an annual basis around the middle of the century, which will lead to widespread coral mortality (Frieler et al. 2012). In addition to absorbing heat, the ocean has absorbed one-third of the CO_2 produced by anthropogenic activities resulting in a decrease of $0.02\text{ pH units decade}^{-1}$ over the last 30 years (Sabine et al. 2004; Hoegh-Guldberg and Bruno, 2010). Ocean acidification (OA) occurring as a result of the oceanic uptake of CO_2 , is expected to reduce coral calcification and possibly push coral reefs into a negative carbonate balance (Hoegh-Guldberg et al. 2007). This decline in calcification occurs because OA lowers the oceans pH (increasing hydrogen ions that react with carbonate ions to form bicarbonate), thereby decreasing the abundance of the carbonate ion in bulk seawater that corals require to calcify (Langdon and Atkinson, 2005; Kleypas et al. 2006). The projected reduction in present-day tropical surface seawater pH is expected to decline from 8.08 to 7.93, leading to a reduction in aragonite saturation state from 4.0 to 2.8 by the year 2100 (Kleypas et al. 1999). This reduction threatens reef ecosystems because rates of coral calcification are positively related to aragonite saturation state of seawater (Ω_{arag}) (Langdon and Atkinson, 2005). Reduced pH and Ω_{arag} make it energetically more difficult for corals to calcify because more energy must be expended to elevate pH at the site of calcification to precipitate calcium carbonate (Cohen and Holcomb, 2009).

Healthy corals depend on photosynthesis of their endosymbiotic algae (autotrophy) for up to 95% of their energy demands (Muscatine and Porter, 1977), but many can also ingest food from the water column such as zooplankton (via heterotrophy). The contribution of heterotrophy to a corals diet remains poorly understood. Heterotrophy can account for anywhere from 0 to 66% of the fixed carbon incorporated into coral skeletons (Muscatine et al. 1989), and may provide corals with essential nutrients not supplied by their symbionts (Muscatine and Porter, 1977; Teece et al. 2011). Palardy et al. (2005) found that degree of coral heterotrophy is plastic and varies with changes in the physical environment. This plasticity may allow for shifts in energy input from autotrophy to heterotrophy under thermal and/or OA stress. Coral species that exhibit this plasticity may be able to offset decreased energy inputs from their algal symbionts during stress events and thereby increase resilience (Palardy et al. 2005). Coral heterotrophy has indeed been shown to account for resilience to both thermal stress (Grottoli et al. 2006, Rodrigues and Grottoli, 2007, Palardy et al. 2008, Anthony et al. 2009), and OA stress (Cohen and Holcomb 2009, Holcomb et al. 2010, Edmunds 2011, Towle et al. 2015). The gap in our knowledge is at what rate coral species can feed during thermal and OA stress, and if there is a threshold of stress exposure where feeding rate can no longer increase to compensate for the stressor.

There is plentiful evidence showing that OA negatively affects coral calcification; however, it is still unclear whether OA may benefit a corals symbiont community. CO₂ is a necessary reactant for photosynthesis, and therefore is a limiting factor in the photosynthetic process. Coral symbionts possess type II RuBisCo, a critical enzyme in carbon fixation with a low affinity for inorganic carbon, mak-

ing it under-saturated with respect to present-day oceanic CO₂ levels (Whitney et al. 1995, Rowan et al. 1996). It has been suggested that as CO₂ levels in the ocean increase, they will no longer be limiting for symbiont photosynthesis (see: CO₂ fertilization hypothesis, Brading et al. 2011). It logically follows that photochemical efficiency could possibly be enhanced by elevated CO₂. It is possible that elevated CO₂ could benefit a coral by enhancing its photochemical efficiency which could possibly enhance calcification (Allemand et al. 2011; Tambutté et al. 2011), which could confer resilience during thermal stress exposure.

The manifestation of the effects of temperature and CO₂ will not occur uniformly in space and time across the worlds reef regions (Pandolfi et al. 2011, Albright et al. 2013). Acidification is thought to be a chronic stress, whereas bleaching stress is perceived as more of an acute stress (Buddemeier et al. 2004). Recent climate models project that by mid-century, CO₂ levels will be greater than 400 ppm (IPCC 2013). Therefore, it is ecologically relevant that any coral bleaching event occurring post mid-century will involve corals living under (i.e. preconditioned to) elevated CO₂ levels (compared to the present) before they experience thermal bleaching events. The gap in our knowledge is whether pre-exposure to elevated CO₂ levels will confer potential benefits via CO₂ fertilization for symbiont photochemical efficiency, or whether the elevated levels will worsen or exacerbate the holobionts response to thermal stress.

The aims of this study were to test if preconditioning to elevated CO₂ affects bleaching susceptibility and/or enhances symbiont photochemical efficiency in the common Caribbean finger coral *Porites porites* and to test whether host calcification and feeding rate will be affected by preconditioning to CO₂ prior to thermal

stress. This study fills a gap in our knowledge of the relationship between symbiont photochemical efficiency, CO₂ level, and bleaching susceptibility, as well as coral feeding rate, which may account for resilience to climate change stress. Many studies have assessed coral physiology under climate change when the thermal and acidification stress begin at the same point in time. No studies to date have assessed how coral physiology may be affected by preconditioning to high CO₂ prior to thermal stress. This study may improve our understanding of how reefs of the future will respond to climate change stress in a more ecologically relevant way than has been commonly tested.

5.3 Methods

5.3.1 Collection

Eight colonies of the Caribbean finger coral *Porites porites* were collected at approximately four meters depth by SCUBA divers in April 2012 from Evans Reef near Broad Key in the northern Florida Keys (25.332N, 80.200W) under permit BISC-2011-SCI0022 issued by Biscayne National Park. Corals were transported to the Corals and Climate Change Facility at the Rosenstiel School of Marine and Atmospheric Science on Virginia Key, FL, where they were fragmented into 80 nubbins (each five cm in branch height) and attached to polyvinyl chloride (PVC) sleds using All Game two-part epoxy. Nubbins were allowed to recover from fragmentation under control conditions (26 °C and 390 ppm CO₂) for one month prior to the start of the experiment.

5.3.2 Experimental design

The five-month study took place between May and October 2012 and consisted of two phases, a preconditioning phase and a warming phase. On 15 May 2012, the preconditioning phase of the study began with two treatments replicated four times for a total of eight experimental tanks. Each tank had ten coral nubbins allocated haphazardly from each of the eight original colonies. Treatments were maintained at a constant temperature of 26 °C, but two different CO₂ levels: four tanks were at 390 ppm (ambient) and four tanks were at 900 ppm. They were maintained like this for three months until 15 August 2012 when the warming phase began. At this point, two tanks from each of the treatments previously described were ramped to a temperature of 31 °C, creating two new treatment conditions, 31 °C/390 ppm and 31 °C/900 ppm, each replicated twice. Tanks elevated from 26 °C to 31 °C were ramped at a rate of 0.5 °C per day over ten days. Therefore, the warming phase of the experiment consisted of four treatments (26 °C/390 ppm, 26 °C/900 ppm, 31 °C/390 ppm, and 31 °C/900 ppm, each replicated twice for a total of eight tanks. A schematic of the experimental design is shown in Figure 1. The warming phase lasted two months until 15 October 2012. The high-temperature treatment of 31 °C was chosen as the closest whole number that exceeded 1 °C above the maximum monthly mean of the local climatology (29.64 °C), which is the threshold used for NOAA's Coral Reef Watch, and is consistent with a local bleaching threshold in the Upper Florida Keys of approximately 30.4 °C (Manzello et al. 2007). The treatment carbon dioxide level (900 ppm) was chosen to represent

the value projected for the year 2065 under a business as usual trajectory (IPCC 2013).

Experimental corals were maintained in 60 L tanks replenished by a 250 L sump tank with a complete water turnover approximately every ten minutes. Each sump tank contained a heating and cooling element connected to a temperature controller (OMEGA CN5700) with a reported accuracy of 0.1 °C. Carbon dioxide levels were achieved by mixing pure carbon dioxide and air through mass flow controllers (Sierra Instruments). The gas mixture was then pumped through a Venturi injector and circulated through the sump before being pumped into the experimental tank. All tanks were connected to a HOBO U30 data logger taking measurements of carbon dioxide, temperature, and light every five minutes for the entire study. Average daily light level over the course of the five month study was 5.51 ± 1.7 mol photons $m^{-2} d^{-1}$, achieved using shade cloth and targeted to represent light levels at approximately four meters depth where experimental corals were collected.

5.3.3 Seawater chemistry

In order to monitor seawater chemistry parameters, discrete water samples (250 mL) were taken from each tank once a week for the five month study, and poisoned with 100 μ L mercuric chloride following collection. Total alkalinity (TA) was measured in duplicate on an automated Gran titrator and standardized using certified reference materials obtained from Dr. A. Dickson (Scripps IO). Dissolved inorganic carbon (DIC) was measured in duplicate using a DIC analyzer (Apollo SciTech Inc.) standardized to the same certified reference seawater. Salinity was

measured using a YSI meter calibrated against a 50,000 microSeimen standard solution. Mean temperature, salinity, TA, and DIC were then used to calculate the partial pressure of carbon dioxide ($p\text{CO}_2$), pH, and aragonite saturation state (Ω) for each treatment using the program CO2SYS using K_1 and K_2 from Mehrbach et al. (1973) refit by Dickson and Millero (1987) per Lewis and Wallace (1998).

5.3.4 Calcification

Calcification rates were measured as changes in coral buoyant weight (Davies 1989), and were measured every two weeks during the eight-week warming phase of the study.

5.3.5 Feeding rate

In order to quantify the amount of prey ingested by *P. porites*, initial and final concentrations of live rotifers were measured and feeding rates were calculated following Coughlan (1969) during the eight-week warming phase of the study following Towle et al. (2015). An advantage of this method over those published by Palardy et al. (2005) and Grottoli et al. (2006) is that it is non-destructive. Twelve one-L beakers were used, ten containing a coral and two controls (prey only, without coral) to account for any possible changes in rotifer density not due to coral feeding. Each beaker had the same flow rate (controlled by magnetic stir plates), light conditions, and initial rotifer density from the same stock solution. Corals were allowed to feed for one hour after sunset as in Grottoli et al. (2006) and were observed to have extended tentacles in the presence of rotifers, indicating feeding was occurring. Initial concentrations of rotifers were approximately 10,000 cells

L⁻¹. Leichter et al. (1998) found mean zooplankton density on a Florida reef was approximately 1,716 cells L⁻¹, but pioneering work on coral feeding rates by Sebens et al. (1996) showed that low prey densities translated into low prey capture, confounding feeding rates, thus Sebens encouraged using as high as ten times published values. After one hour, four replicate fifteen mL water samples were taken from each beaker, fixed in the preservative Lugols solution, and final rotifer concentration was quantified via microscopy. Feeding rates were normalized to coral surface area. Corals were also offered food *ad libitum* twice weekly using a commercial zooplankton diet (Zieglers Larval AP 100). The purpose of offering food *ad libitum* separate from rotifers offered during feeding rate experiments was so that ingestion would be more similar to *in-situ* feeding rates, as opposed to rates that could be artificially elevated if corals only had exposure to food during biweekly feeding rate experiments. Experiments took place on days when corals were not fed *ad libitum*.

5.3.6 PAM Fluorometry

In order to assess photochemical efficiency, the quantum yield of photosystem II (Fv/Fm), an indicator of the physiological status of symbiont, particularly the status of chlorophyll *a* fluorescence and photosystem II reaction centers, was measured. This non-invasive metric was quantified using an Imaging Pulse Amplitude Modulated (I-PAM) fluorometer (Walz, Effeltrich, Germany) approximately once weekly during the bleaching phase of the experiment according to Maxwell and Johnson (2000). Corals were dark-adapted for 45 min prior to fluorescence measurements, and analyzed in the software program ImagingWin.

5.3.7 Surface area and tissue coloration

The authors were committed to non-invasive procedures that would avoid coral mortality throughout the duration of the study, and thus surface area was calculated using the software program ImageJ (National Institute of Health) as opposed to more traditional sacrificial methods such as wax dipping. Photographs of each experimental nubbin were taken at the beginning and end of the study and analyzed for surface area using stereo-photography (3D) with two cameras mounted on a fixed frame. All photographs included a 15 cm ruler used as a reference object to normalize the surface to values in square centimeters. The same photographs were also used to qualitatively assess tissue coloration to determine bleaching susceptibility at the end of study using ImageJ. Final images of each of the corals were conservatively given categorical ranks healthy, pale, or bleached based on comparison with each corals initial photograph. If the tissue in the final picture was the same color (usually purple - dark brown) as in its initial photograph, it was classified as healthy, if the tissue was lighter in color (usually tan - light brown) than its initial photograph it was classified as pale, and if the tissue was relatively white compared to its initial photograph, it was classified as bleached.

5.4 Results

Mean seawater chemistry parameters are summarized in Table 1 for the preconditioning phase and Table 2 for the warming phase. There was no significant difference in the calcification rates of *P. porites* preconditioned to 26 °C/390 ppm treatment (5.10 mg cm⁻² d⁻¹) and then exposed to 31 °C/390 ppm (4.88 mg cm⁻²

d⁻¹), nor was there a significant difference in calcification rates of corals preconditioned to 26 °C/900 ppm (5.18 mg cm⁻² d⁻¹, Fig. 2, Table 4). However, calcification rate decreased significantly for the corals preconditioned to high CO₂ and then exposed to thermal stress (31 °C/900 ppm treatment) (Students t-test, p<0.05, Fig. 2, Table 4, 3.42 mg cm⁻² d⁻¹). The interaction between temperature and CO₂ on feeding rate was significant (ANOVA, p<0.05, Table 3). Corals preconditioned to ambient CO₂ prior to thermal stress (31 °C/390 ppm treatment) fed at approximately two times the rate of the 26 °C/390 ppm corals (Fig. 3, 0.65 vs. 0.29 rotifers cm⁻² hr⁻¹). *P. porites* exhibited the highest feeding rates in the 26 °C/900 ppm treatment, feeding at approximately three times the rate of the 26 °C/390 ppm corals (Students t-test, p<0.05, Fig. 3, Table 4, 1.01 vs. 0.29 rotifers cm⁻² hr⁻¹). However, the corals preconditioned to high CO₂ before thermal stress (31 °C/900 ppm treatment) significantly decreased their feeding rates (Fig. 3, Table 4). Temperature and CO₂ had significant effects on photochemical efficiency (ANOVA, p<0.05, Table 3). Photochemical efficiency decreased and was significantly different from the 26 °C/390 ppm treatment (0.52 ±0.01) by approximately 5% in the 31 °C/390 ppm treatment (0.44 ±0.01) and also approximately 5% in the 26 °C/900 ppm treatment (0.45 ±0.01) (Students t-test, p<0.05, Fig. 4, Table 4). Photochemical efficiency was further reduced and significantly different in the 31 °C/900 ppm treatment (0.40 ±0.01) (Students t-test, p<0.05, Fig. 4, Table 4). Corals maintained at ambient temperature and CO₂ (26 °C/390 ppm) appeared to retain healthy tissue pigmentation throughout the duration of the study, as did the corals maintained at 26 °C/900 ppm for the duration of the study, with the exception of approximately 5% which experienced tissue paling (Fig. 5). Corals in the high temperature treat-

ments that were preconditioned to ambient CO₂ experienced approximately 75% tissue bleaching, while corals preconditioned to high CO₂ prior to thermal stress experiences approximately 85% tissue bleaching (Fig. 5).

5.5 Discussion

5.5.1 Calcification and feeding rate

This study is comparable to Edmunds (2011) who showed that feeding can partially buffer the negative effects of OA on coral biomass-normalized calcification in a massive Pacific *Porites* species. This work expands on Edmunds (2011) by directly quantifying increases in feeding rates under climate change stress scenarios. Edmunds showed that feeding had a significant effect on area-normalized calcification in that fed corals had higher rates than unfed corals. While Edmunds work demonstrates that a Pacific *Porites* species has the capacity to resist the effects of one month exposure to high CO₂ through heterotrophy and changes in biomass, it did not examine the feeding rates necessary to achieve amelioration of reductions in calcification. In contrast, this study demonstrates that *P. porites* can elevate its feeding rate to almost three times its ambient rate when experiencing CO₂ stress alone (i.e. 26 °C/900 ppm), and to approximately two times its ambient rate under temperature stress alone (i.e. 31 °C/390 ppm). However, *P. porites* decreases its feeding rate when it experiences thermal stress after having been preconditioned to high CO₂ (i.e. 31 °C/900 ppm) (Fig. 3).

This research also shows that calcification is not depressed compared to ambient rates for the corals preconditioned to high CO₂ but maintained at 26 °C, nor in

corals exposed to thermal stress after preconditioning at ambient CO₂; however, calcification decreases when corals preconditioned to high CO₂ are then exposed to thermal stress (i.e. 31 °C/900 ppm) (Fig. 2). In other words, when *P. porites* experience a single stressor by itself (just CO₂ stress or just thermal stress), they can elevate feeding rate and rates of calcification are not different from ambient rates; however, *P. porites* were unable to increase feeding rate, (and also unable to mitigate reductions in calcification), when thermal stress followed preconditioning to OA stress. With both stressors, perhaps the inherent energetic costs associated with feeding such as polyp extension (Levy et al. 2006), outweigh the benefits if host metabolism is already depressed due to preconditioning to CO₂, and then further stressed by high temperature. These findings imply that *P. porites* can compensate for increased temperature or increased CO₂ with feeding when they occur separately, but not when thermal stress is compounded with OA stress, which is the more ecologically relevant scenario. A recent study found that another Caribbean species, *Siderastrea siderea*, may be tolerant to increases in CO₂ with respect to calcification when fed *Artemia* on a regular basis, but this study did not assess thermal and OA stress together (Castillo et al. 2014).

The current study highlights the idea that there may be a distinction between when two stressors begin at the same time, versus when one stress is experienced first and then another stress is compounded to the initial stress. Towle et al. (2015) found that calcification of fed *Acropora cervicornis* at 30 °C/900 ppm was not significantly different than calcification of control corals at 26 °C/390 ppm, but the temperature and CO₂ stressors were experienced for the same length of time and began at the same time. The present experiment found that calcification was re-

duced by 33% relative to the ambient treatment when *P. porites* experienced 900 ppm for three months prior to being exposed to 31 °C (while still experiencing 900 ppm) for the next two months. More research is needed to understand what the physiological basis for this distinction may be, and if coral and symbiont physiology will respond differently than *P. porites* did to CO₂ preconditioning in other coral species.

5.5.2 Photochemical efficiency

These data imply that preconditioning to high CO₂ does not stimulate or enhance the photochemical efficiency of *P. porites* symbionts. Mean photochemical efficiency decreased by 5% when corals that were not preconditioned to high CO₂ experienced thermal stress (i.e. 31 °C /390 ppm) (Fig. 5). Corals preconditioned to 26 °C/900 ppm also had similar reductions in photochemical efficiency compared to the ambient treatment, but the corals preconditioned to high CO₂ before thermal stress (31 °C/900 ppm) experienced an additional 5% reduction with respect to 26 °C/900 ppm corals and a cumulative 10% decrease with respect to the ambient treatment (Fig. 5). Reynaud et al. (2003) also showed that the photosynthetic response to elevated CO₂ can change when temperature is included as a factor. Reynaud and colleagues found that at a constant temperature of 25 °C, an increase in pCO₂ from 460 µatm to 760 µatm caused no change in photosynthesis in *Stylophora pistillata*. However, at 28 °C, the same increase in pCO₂ caused a significant reduction in photosynthesis (Reynaud et al. 2003). More recent studies have also demonstrated that photosynthetic parameters are likely reduced, not enhanced, under high CO₂. As previously discussed, studies have shown reductions in sym-

biont density under OA conditions (Kaniewska et al. 2012, Tremblay et al. 2013), as well as reductions in total chlorophyll *a* and gross photosynthesis (Tremblay et al. 2013). Still, Iguchi et al. (2012) found that chlorophyll *a* was not affected by HCO₂ when levels were elevated from 380 μ atm to 2193 μ atm, and Towle et al. (2015) also found no significant effect of 900 ppm CO₂ on symbiont density or chlorophyll *a* content in *A. cervicornis*. This inter-specific variability requires more research to understand symbiont and photosynthetic pigment responses to OA. Brading et al. (2011) showed that the response of Symbiodinium to high CO₂ is phylotype-specific, which may explain the variation between studies. Feeding has also been shown to stimulate symbiont density per unit surface area, chlorophyll *a*, and photosynthetic rates under unstressed conditions (Ferrier-Pagès et al. 2003, Houlbrèque et al. 2003, 2004a), thus heterotrophy may play a role in offsetting reductions in symbiont responses, not just host responses, under stress.

The pattern of decrease in the photochemical efficiency data are consistent with previous studies that have shown that the metabolic effects of temperature stress alone and CO₂ stress alone are, in fact, similar in the coral host. For example, Weis (2008) described increases in intracellular reactive oxygen and nitrogen species (ROS and RNS) in host cells due to thermal bleaching. Similarly under OA stress alone, Kaniewska et al. (2012) described metabolic suppression characterized by increases in oxidative stress, ROS, and RNS. Therefore, the results for the response of mean photochemical efficiency being statistically the same at 31 °C/390 ppm and 26 °C/900 ppm is consistent with previous theories on overall host response to temperature and OA stress individually. The additive effect of photoinhibition caused by both OA and heat in the 31 °C/900 ppm treatment highlights the exac-

erbation of effects when stressors occur together. This additive 10% decrease in Fv/Fm appears to be a threshold at which point *P. porites* growth will decrease, in this case, by 33% of ambient growth rates (Fig. 2). Importantly, Fv/Fm is a density-independent measure, meaning that it does not reflect the overall efficiency of the holobiont, rather only of the symbionts that remain. Therefore, a reduced rate of translocation and/or a reduced quality of the photosynthetic product being transferred to the host when photochemical efficiency is reduced by 10% could also explain the reduced calcification rate at 31 °C/900 ppm, (in addition to the lack of increased heterotrophy). However, notably, 5% reductions in Fv/Fm did not elicit significant decreases in calcification rate when coupled with host heterotrophy at either 26 °C/900 ppm or 31 °C/390 ppm.

5.5.3 Bleaching susceptibility

Data presented here show that *P. porites* that were preconditioned to 900 ppm CO₂ bleached 10% more than *P. porites* that were preconditioned to 390 ppm (ambient CO₂) prior to high temperature exposure (Fig. 5). These data imply that exposure to elevated CO₂ prior to thermal stress does not reduce bleaching susceptibility in *P. porites*. This finding may not be surprising in the context of a study by Wagner et al. (2010) that assessed the bleaching indices of 37 species of Caribbean coral following a severe bleaching event in summer 2005. Wagner et al. discovered that *P. porites* was one of species with the highest bleaching index. Perhaps in order for elevated CO₂ to afford the coral host any kind of benefit with respect to bleaching susceptibility, the coral must have a lower bleaching index, potentially determined by hosting a thermally-tolerant symbiont clade (Baker et al. 2004),

or expressing elevated levels of heat shock proteins, antioxidant enzymes and/or scattering pigments (Brown et al. 2002, Baird et al. 2008, Barshis et al. 2013). More research is necessary to examine this theory beyond the limitations of the present study. In order to more completely address the hypotheses posed in this study, future work will need to use metrics that requiring sacrificing coral tissue, i.e. symbiont density, chlorophyll *a* content, etc. However, this work provides a good baseline to address these hypotheses, and the preliminary evidence based on visual observations suggests that preconditioning to elevated CO₂ does not afford *P. porites* any benefits with respect to reducing bleaching susceptibility.

5.5.4 Implications

In summary, *P. porites* maintained ambient calcification rates when exposed to thermal stress without CO₂ preconditioning, but when thermal stress follows preconditioning to high CO₂, *P. porites* calcification rate significantly decreased. The ability to increase feeding rate may confer the energy necessary to maintain ambient calcification rates in the 26 °C/900 ppm and 31 °C/390 ppm treatments, but not in the 31 °C/900 ppm treatment, as feeding rate and calcification rate both. Additionally, *P. porites* symbionts suffered greater reductions in Fv/Fm when preconditioned to high CO₂ prior to thermal stress than with just thermal stress alone. These results suggest that *P. porites* will likely not experience any resilience benefits from natural CO₂ preconditioning once exposed to the effects of severe annual bleaching. This study should be repeated so that additional physiological parameters can be assessed such as symbiont density, chlorophyll *a*, and lipid content to have a broader understanding of the effects of CO₂ preconditioning. These kinds

of experiments will help better our understanding of coral holobiont physiology under climate change stressors that may not always be experienced in the same order or at the same time. This work highlights the overall importance of reducing CO₂ emissions on a global scale, as CO₂ will likely not afford the algal symbiont any benefit, and will likely worsen the effect of thermal stress on the holobiont.

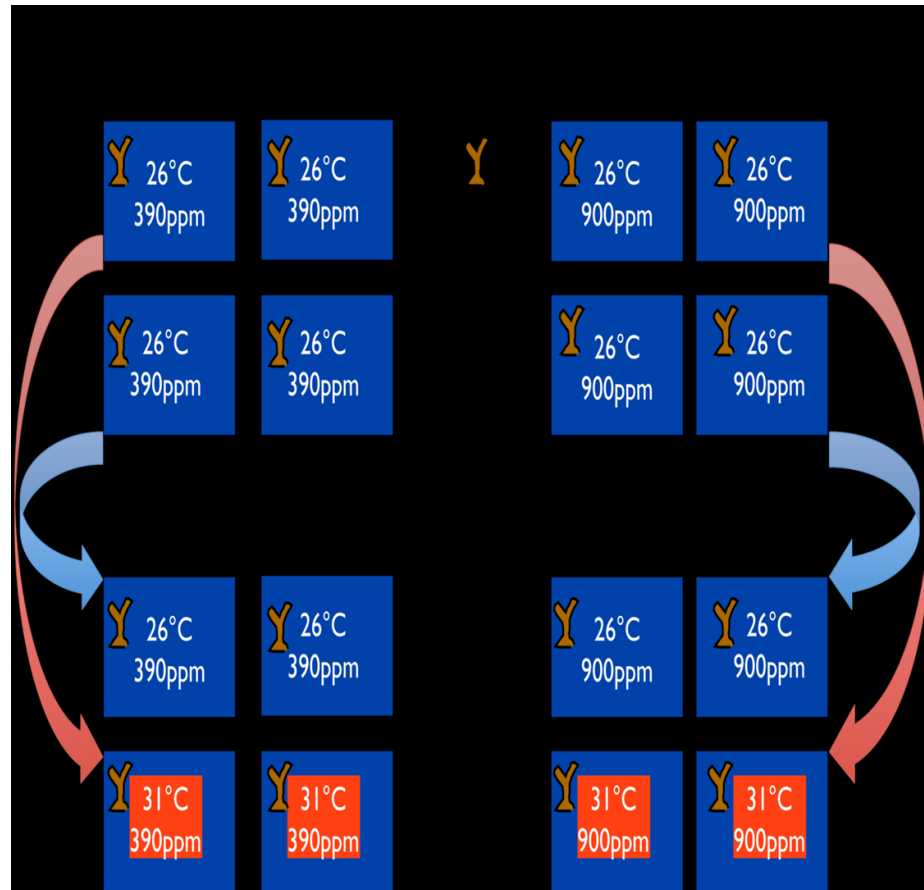


Figure 5.1: Schematic of the five-month experimental design. The top eight tanks represent the preconditioning phase of the experiment (first three months) where all corals were held at 26 °C and half were exposed to ambient CO₂ (390 ppm) while half were exposed to high CO₂ (900 ppm). The bottom eight tanks represent the warming phase of the experiment (last two months) where two of the four tanks at 26 °C/390ppm were ramped to 31 °C (represented by red arrows), while the other two remained the same (represented by blue arrows), and two of the four tanks at 26 °C/900ppm were ramped to 31 °C while the other two remained the same.

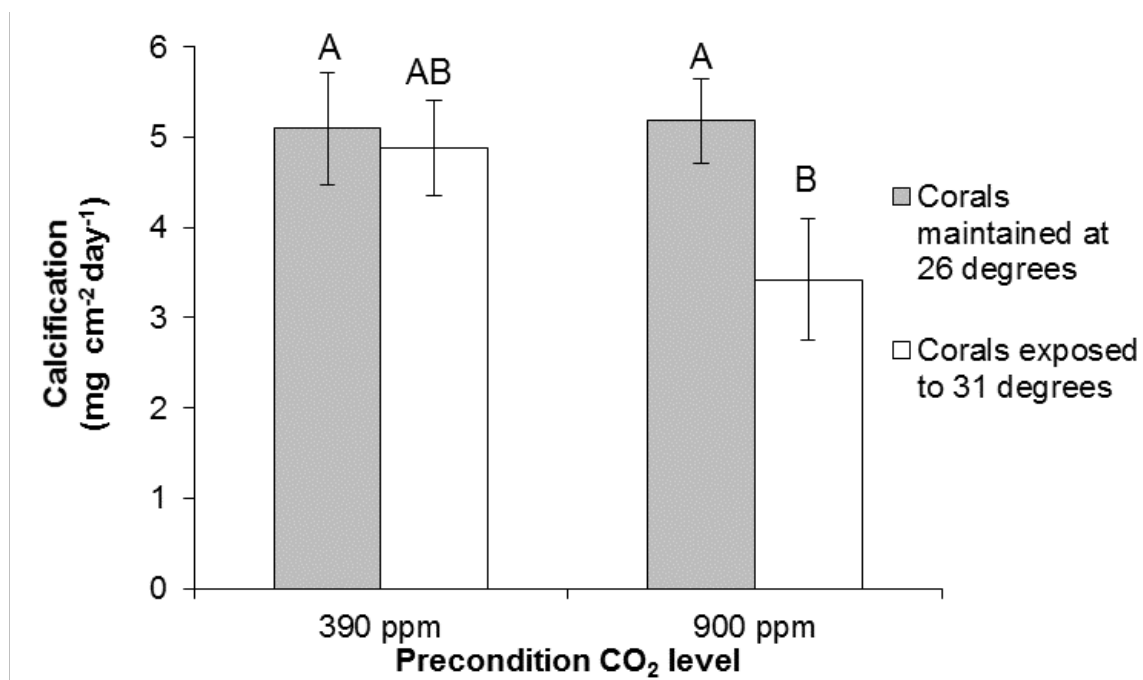


Figure 5.2: Mean calcification rates of *P. porites* in $\text{mg cm}^{-2} \text{ day}^{-1}$ at the four treatment levels during the two month warming phase of the study. Letters represent statistical differences based on *post hoc* Students t-test ($p < 0.05$). $n=20$ corals per treatment level.

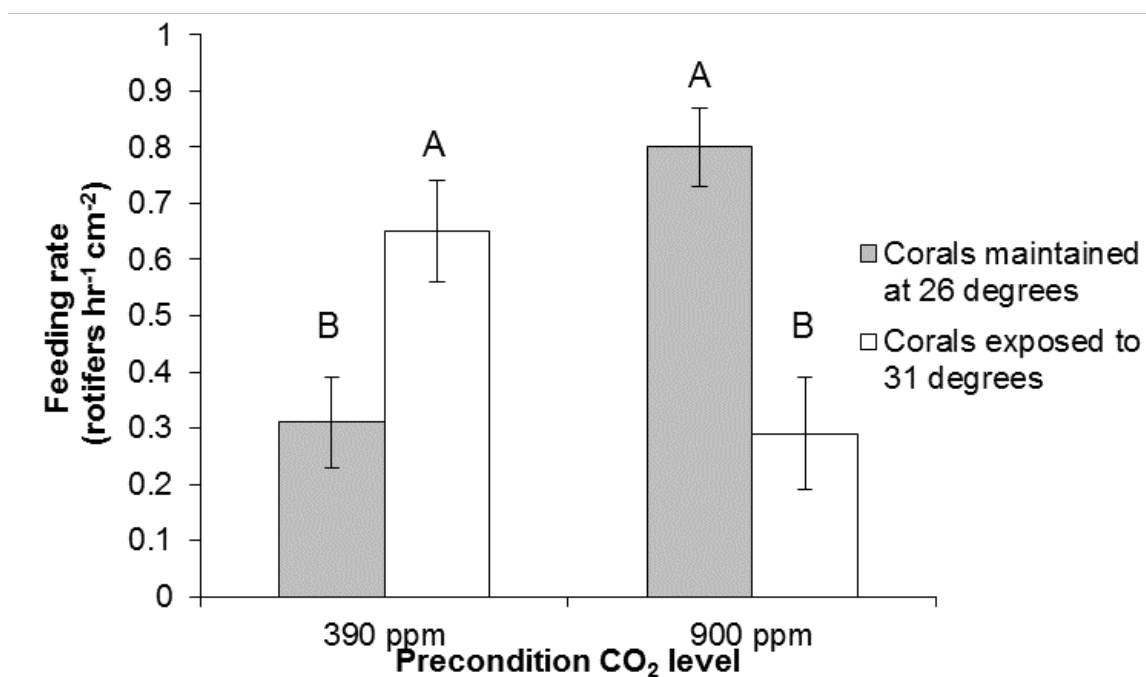


Figure 5.3: Mean feeding rates of *P. porites* in rotifers $\text{hr}^{-1} \text{cm}^{-2}$ at the four treatment levels over the two month warming phase of the study. Letters represent statistical differences based on *post hoc* Student's t-test ($p < 0.05$). $n=20$ corals per treatment level.

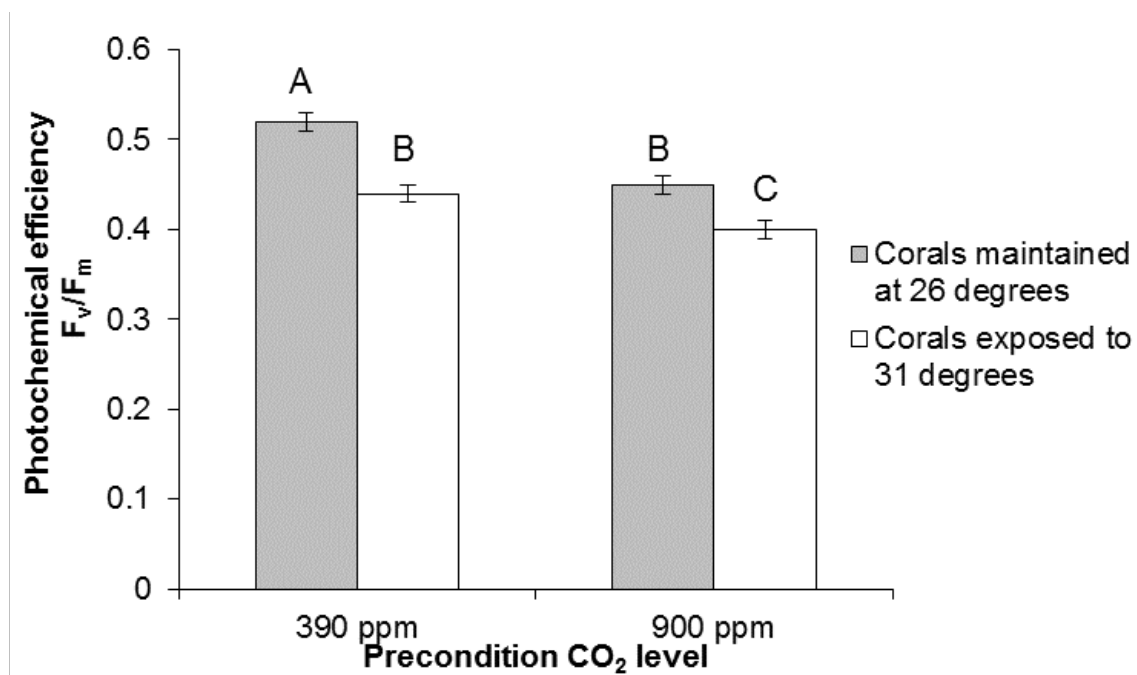


Figure 5.4: Mean photochemical efficiency (F_v/F_m) of *P. porites* symbionts pooled across all time points for the four treatment levels over the two month warming phase of the study. Letters represent statistical differences based on *post hoc* Students t-test ($p < 0.05$). $n=20$ corals per treatment level.

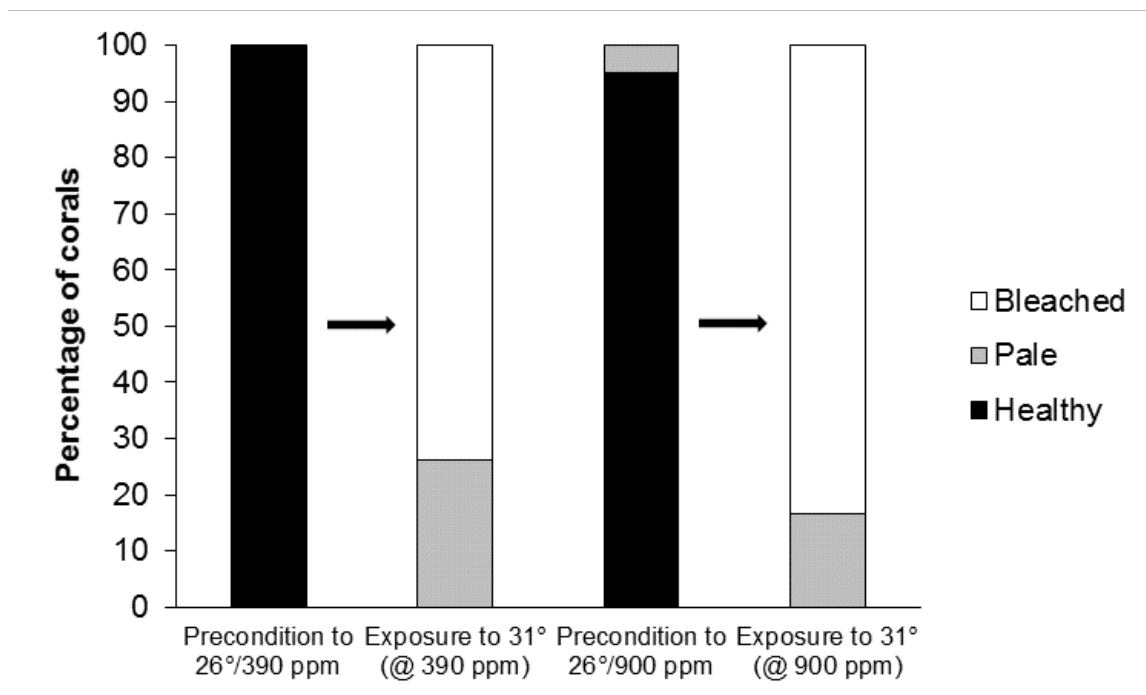


Figure 5.5: Percentage of *P. porites* with healthy, pale, or bleached tissues at the four treatment levels based on visual inspection via photographs taken at the end of the study compared to initial photographs of the same corals. n=20 corals per treatment level.

Table 1: Summary of mean seawater chemistry parameters for the preconditioning phase of the experiment: May-August 2012.

Treatment	Temperature (°C ± SD)	Salinity (ppt ± SD)	CO ₂ (ppm ± SD)	pH (unit ± SD)	Ω _{ar} (unit ± SD)
26°C, 390 ppm	26.2 ± 0.3	31.0 ± 1.0	360 ± 36	8.06 ± 0.04	3.2 ± 0.4
26°C, 900 ppm	26.2 ± 0.2	31.0 ± 1.0	907 ± 188	7.73 ± 0.09	1.8 ± 0.4

Table 2: Summary of mean seawater chemistry parameters for the warming phase of the experiment: August-October 2012.

Treatment	Temperature (°C ± SD)	Salinity (ppt ± SD)	CO ₂ (ppm ± SD)	pH (unit ± SD)	Ω _{ar} (unit ± SD)
26°C, 390 ppm	26.1 ± 0.4	32.0 ± 1.0	364 ± 39	8.04 ± 0.05	3.0 ± 0.5
26°C, 900 ppm	26.2 ± 0.9	32.0 ± 1.0	914 ± 187	7.73 ± 0.09	1.8 ± 0.4
31°C, 390 ppm	30.8 ± 0.8	32.0 ± 1.0	380 ± 34	8.06 ± 0.03	3.6 ± 0.5
31°C, 900 ppm	30.9 ± 0.2	32.0 ± 1.0	937 ± 203	7.73 ± 0.09	2.0 ± 0.5

Table 3: Effect tests (two-way ANOVA) of temperature (T), carbon dioxide (CO₂) and their interaction (T x CO₂) on calcification rate, feeding rate, and photochemical efficiency. Significant p-values are bolded for p < 0.05.

Calcification rate				
Source	DF	Sum of Squares	F ratio	p-value
T	1	15.9514	2.9402	0.0914
CO ₂	1	7.8304	1.4433	0.2342
T x CO ₂	1	9.6403	1.7769	0.1874
Feeding rate				
Source	DF	Sum of Squares	F ratio	p-value
T	1	0.0721	0.9264	0.3414
CO ₂	1	0.0456	0.5857	0.4485
T x CO ₂	1	1.8890	24.2581	<.0001
Photochemical efficiency				
Source	DF	Sum of Squares	F ratio	p-value
T	1	0.0849	18.1038	<.0001
CO ₂	1	0.0581	12.3997	0.0008
T x CO ₂	1	0.0004	0.0872	0.7688

Table 4: Results of *post hoc* ordered differences reports (Student's t-test) for calcification rate, feeding rate, and photochemical efficiency. Significant p-values are bolded for $p < 0.05$.

Calcification rate						
Level	Level	Difference	Std Err Dif	Lower CL	Upper CL	p-value
26, 900	31, 900	1.7555	0.8406	0.0751	3.4359	0.0409
26, 390	31, 900	1.6797	0.8300	0.0205	3.3398	0.0473
31, 390	31, 900	1.4598	0.8406	-0.2205	3.1402	0.0874
26, 900	31, 390	0.2956	0.7989	-1.3013	1.8927	0.7126
26, 390	31, 390	0.2198	0.7877	-1.3548	1.7945	0.7811
26, 900	26, 390	0.0758	0.7877	-1.4988	1.6505	0.9236
Feeding rate						
Level	Level	Difference	Std Err Dif	Lower CL	Upper CL	p-value
26, 900	31, 900	0.5077	0.1208	0.2636	0.7517	0.0001
26, 900	26, 390	0.4907	0.1065	0.2754	0.7059	<.0001
31, 390	31, 900	0.3587	0.1355	0.0848	0.6325	0.0115
31, 390	26, 390	0.3417	0.1230	0.0932	0.5902	0.0082
26, 900	31, 390	0.1489	0.1162	-0.0858	0.3838	0.2073
26, 390	31, 900	0.0170	0.1273	-0.2402	0.2742	0.8944
Photochemical efficiency						
Level	Level	Difference	Std Err Dif	Lower CL	Upper CL	p-value
26, 390	31, 900	0.1329	0.0262	0.0805	0.1853	<.0001
26, 390	31, 390	0.0777	0.0225	0.0327	0.1228	0.0010
26, 900	31, 900	0.0676	0.0257	0.0163	0.1190	0.0106
26, 390	26, 900	0.0652	0.0222	0.0207	0.1097	0.0047
31, 390	31, 900	0.0551	0.0259	0.0033	0.1070	0.0375
26, 900	31, 390	0.0125	0.0219	-0.0313	0.0563	0.5697

CHAPTER 6

Conclusion

Results of the studies presented here demonstrate that heterotrophic feeding in corals has the potential to mitigate reductions in calcification due to ocean acidification and thermal stress. However, the degree to which heterotrophy can act as an indicator of resilience to stress is variable by coral species, source location, and other factors in the Florida Reef Tract. In the wild, there may be a tradeoff between allocation of energy for calcification versus allocation of energy to create and maintain lipid stores. This tradeoff may be mediated by degree of coral feeding, and lipid stores may help protect corals from environmental stressors.

Under ocean acidification conditions, an endangered Caribbean coral species, *Acropora cervicornis*, can mitigate a 30% decrease in calcification rate by increasing its feeding rate and lipid content compared to unfed conspecifics. This study is the first to demonstrate the mechanism underlying the link between heterotrophy and resilience to acidification, which was only implicated in previous studies. These results underscore that food availability should be taken into account when making coral reef management decisions.

In another Caribbean endangered species, *Orbicella faveolata*, source location of coral populations can play a large role in determining the outcome of calcification and feeding rates under climate change scenarios. Physiological responses of two populations of *O. faveolata* from the Florida Reef Tract were dependent on the degree of nutritional repletion versus depletion at source site, the relative change versus stasis of the symbiont community and respiration rate, and fine-scale differences in thermal history. These results demonstrate that even within the same species in the same reef tract, corals from different sites may not respond predictably to the same climate change stressors, underscoring the importance of understanding latent effects of source location to predict resilience capability and improve conservation efforts.

Lastly, using a more ecologically relevant experimental design whereby corals are pre-acclimated to high CO₂ prior to experiencing thermal stress, this work shows that preconditioning to high CO₂ results in reduced calcification rates, feeding rates, and photochemical efficiency, potentially leading to increased bleaching susceptibility. Ultimately, preconditioning to high CO₂ will worsen the effects of thermal stress on corals, underscoring the importance of reducing CO₂ emissions on a global scale. More work is needed to understand the pathways by which heterotrophy and lipids confer resilience to corals under stress. Additionally, future research should investigate the existence of genotypes that show resilience to climate change stressors, and genes that may be frontloaded in these resilient genotypes. Focusing efforts on the protection and cultivation of coral species that are more resilient to climate change stress may improve the effectiveness of conservation efforts.

BIBLIOGRAPHY

- Albright R, Mason B (2013) Projected Near-Future Levels of Temperature and $p\text{CO}_2$ Reduce Coral Fertilization Success. *PLoS ONE* 8(2): e56468.
- Albright R, Langdon C, Anthony KRN (2013) Dynamics of seawater carbonate chemistry, production, and calcification of a coral reef flat, Central Great Barrier Reef. *Biogeosciences Discussions* 10:7641–7676.
- Allemand D, Tambutté E, Zoccola D, Tambutté S (2011) Coral Calcification, Cells to Reefs. In: Dubinsky Z, Stambler N (eds.) *Coral Reefs: An Ecosystem in Transition*. Springer.
- Anthony KRN, Connolly SR, Hoegh-Guldberg O (2007) Bleaching, energetics, and coral mortality risk: Effects of temperature, light, and sediment regime. *Limnol Oceanogr* 52:716-726.
- Anthony KRN, Hoogenboom MO, Maynard JA, Grottoli AG, Middlebrook R (2009) Energetics approach to predicting mortality risk from environmental stress: a case study of coral bleaching. *Funct Ecol* 23:539-550.
- Baird AH, Bhagooli R, Ralph PJ, Takahashi S (2008) Coral bleaching: the role of the host. *Trends in Ecology and Evolution* 24:16-20.
- Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. *Annual review of Ecology, Evolution, and Systematics* 34: 661-669.
- Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Coral reefs: corals' adaptive response to climate change. *Nature* 430: 741.
- Barshis DJ, Ladner JT, Oliver TA, et al. (2013) Genomic basis for coral resilience to climate change. *PNAS* 110: 1387-1392.
- Bénazet-Tambutté S, Allemand D, Jaubert J (1996) Inorganic carbon supply to symbiont photosynthesis of the sea anemone *Anemonia viridis*: role of the oral epithelial layers. *Symbiosis* 20:199–217.
- Berkelmans R, van Oppen MJ (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proc Biol Sci* 273: 2305-2312.

- Brading P, Warner ME, Davey P, Smith DJ, Achterberg EP, Suggett DJ (2011) Differential effects of ocean acidification on growth and photosynthesis among phylotypes of *Symbiodinium* (Dinophyceae). *Limnol Oceanogr* 56: 927-938.
- Brown BE, Dunne RP, Goodson MS, Douglas A (2002) Experience shapes the susceptibility of a reef coral to bleaching. *Coral reefs* 21:119-126.
- Bruno JF, Selig ER, Casey KS, Page CA, Willis BL, et al. (2007) Thermal stress and coral cover as drivers of coral disease outbreaks. *PLoS Biol* 5:1220-1227.
- Bruno JF, Sweatman H, Precht WF, Selig ER, Schutte VG (2009) Assessing evidence of phase shifts from coral to macroalgal dominance on coral reefs. *Ecology* 90: 1478-1484.
- Buddemeier RW, Kleypas JA, Aronson RB (2004) Potential contributions of climate change to stresses on coral reef ecosystems. *Coral reefs and global climate change*. Pew Center on Global Climate Change, Virginia, USA, 56 pp.
- Castillo KD, Ries JB, Bruno JF, Westfield IT (2014) The reef-building coral *Siderastrea siderea* exhibits parabolic responses to ocean acidification and warming. *Proc R Soc B* 281: 20141856.
- Chan NCS, Connolly SR (2013) Sensitivity of coral calcification to ocean acidification: a meta-analysis. *Global Change Biology*, 19: 282–290. doi: 10.1111/gcb.12011
- Cohen A, McCorkle DC, de Putron S, Gaetani GA, Rose KA (2009) Morphological and compositional changes in the skeletons of new coral recruits reared in acidified seawater: Insights into the biomineralization response to ocean acidification. *Geochem Geophys Geosyst* 10: Q07005.
- Cohen A, Holcomb M (2009) Why Corals Care about Ocean Acidification: Uncovering the Mechanism. In: Scott D, Fabry V, Balch B, Feely R (eds) *The Future of Ocean Biogeochemistry in a High CO₂ World*. *Oceanography* 22:118-127.
- Connolly S, Lopez-Yglesias M, Anthony K (2012) Food availability promotes rapid recovery from thermal stress in a scleractinian coral. *Coral Reefs* 31: 951-960.
- Cook CB, Mueller EM, Ferrier MD, Annis E (2002) The influence of nearshore waters on corals of the Florida Reef Tract. In: *The Everglades, Florida Bay, and Coral reefs of the Florida Keys – An ecosystem sourcebook*. Eds. Porter JW, Porter KG, 771-788. Boca Raton: CRC Press.

Costanza R, d'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, Oneill RV, Paruelo J, Raskin RG, Sutton P, van den Belt M (1997) The value of the world's ecosystem services and natural capital. *Nature* 387:253–260.

Coughlan J (1969) The estimation of filtering rate from the clearance of suspensions. *Mar Biol* 2:356-358.

Cunning R, Baker AC (2013) Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nature Climate Change* 3:259-262.

Davies PS (1989) Short-term growth measurements of corals using an accurate buoyant weighing technique. *Mar Biol* 101:389–395.

Davy SK, Allemand D, Weis VM (2012) Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiol Mol Biol Rev* 76(2):229–261.

Dickson AG (1994) Determination of dissolved oxygen in sea water by Winkler titration. WOCE Hydrographic Program, Woods Hole, MA, pp. 11.

Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research* 34:1733-1743.

Drenkard EJ, Cohen AL, McCorkle DC, de Putron SJ, Starczak VR, Zicht AE (2013) Calcification by juvenile corals under heterotrophy and elevated CO₂. *Coral Reefs* 32: 727-735.

Edinger EN, Limmon GV, Jompa J, Widjatmoko W, Heikoop JM, Risk MJ (2000) Normal coral growth rates on dying reefs: Are coral growth rates good indicators of reef health? *Marine Pollution Bulletin* 40:404-425.

Edmunds PJ (2011) Zooplanktivory ameliorates the effects of ocean acidification on the reef coral *Porites* spp. *Limnol Oceanogr* 56: 2402-2410.

Enochs IC, Manzello DP, Carlton R, Schopmeyer S, van Hooidek R, Lirman D (2014) Effects of light and elevated pCO₂ on the growth and photochemical efficiency of *Acropora cervicornis*. *Coral Reefs* 33:477-485.

Enriquez S, Mendez ER, Iglesias-Prieto R (2005) Multiple scattering on coral skeletons enhances light absorption by symbiotic algae. *Limnol Oceanogr* 50: 1025-1032.

Fabricius KE, Langdon C, Uthicke S. et al. (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nat Clim Change* 1:165-169.

- Ferrier-Pagès C, Gattuso JP, Dallot S, Jaubert J (2000) Effect of nutrient enrichment on the growth and photosynthesis of the zooxanthellate coral *Stylophora pistillata*. *Coral Reefs* 19: 103–113.
- Ferrier-Pagès C, Schoelzke V, Jaubert J, Muscatine L, Hoegh-Guldberg O (2001) Response of a scleractinian coral, *Stylophora pistillata*, to iron and nitrate enrichment. *J Exp Mar Biol Ecol* 259: 249-261.
- Ferrier-Pagès C, Witting J, Tambutté E, Sebens K (2003) Effect of natural zooplankton feeding on the tissue and skeletal growth of the scleractinian coral *Stylophora pistillata*. *Coral Reefs* 22: 229-240.
- Ferrier-Pagès C, Rottier C, Beraud E, Levy O (2010) Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress: Effect on the rates of photosynthesis. *J Exp Mar Biol Ecol* 390:118-124.
- Fitt WK, McFarland FK, Warner ME, Chilcoat GC (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnol Oceanogr* 45:677-685.
- Frieler K, Meinshausen M, Golly A, Mengel M, Lebek K, Donner SD, Hoegh-Guldberg O (2013) Limiting global warming to 2 °C is unlikely to save most coral reefs. *Nat Clim Change* 3:165-170.
- Gagnon AC, Adkins JF, Erez J (2012) Seawater transport during coral biomineralization. *Earth Planet Sci Lett* 329-330: 150-161.
- Gladfelter EH, Monahan RK, Gladfelter WB (1978) Growth rates of 5 reef-building corals in the Northeastern Caribbean. *Bulletin of Marine Science* 28:728-734.
- Glynn PW (2012) Global warming and widespread coral mortality: evidence of first coral reef extinctions, p. 103-119. *Saving a Million Species*. Springer.
- Glynn PW, Maté JL, Baker AC, Calderón MO (2001) Coral bleaching and mortality in Panama and Ecuador during the 1997-1998 El Niño-southern Oscillation event: spatial/temporal patterns and comparisons with the 1982-1983 event. *Bull. Mar. Sci.* 69:79-109.
- Graham NA, Jennings S, Macneil MA, Mouillot D, Wilson SK (2015) Predicting climate-driven regime shifts versus rebound potential in coral reefs. *Nature* 518: 94-97.
- Grottoli A, Rodrigues L, Juarez C (2004) Lipids and stable carbon isotopes in two species of Hawaiian corals, *Porites compressa* and *Montipora verrucosa*, following a bleaching event. *Mar Biol* 145:621-631.

Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. *Nature* 440: 1186-1189.

Grottoli AG, Warner ME, Levas SJ, Aschaffenburg MD, Schoepf V, McGinley M, Baumann J, Matsui Y (2014) The cumulative impact of annual coral bleaching can turn some coral species winners into losers. *Global Change Biology* 20:3823-3833.

Hansen J, Sato M, Ruedy R, Lo K, Lea DW, Medina-Elizade M (2006) Global temperature change. *Proceedings of the National Academy of Sciences* 103: 14288-14293.

Harland AD, Davies PS, Fixter LM (1992) Lipid content of some Caribbean corals in relation to depth and light. *Mar Biol* 113:357-361.

Harland AD, Navarro JC, Davies PS, Fixter LM (1993) Lipids of some Caribbean and Red Sea corals – total lipid, wax esters, triglycerides and fatty acids. *Mar Biol* 117:113-117.

Hendriks IE, Duarte CM, Alvarez M (2010) Vulnerability of marine biodiversity to ocean acidification: a meta-analysis. *Estuarine, Coastal, and Shelf Science*, doi:10.1016/j.ecss.2009.11.022

Hoegh-Guldberg O (1999) Climate change, coral bleaching, and the future of the world's reefs. *Mar Freshw Res* 50:839-866.

Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world's marine ecosystems. *Science* 328: 1523-1528.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, et al. (2007) Coral reefs under rapid climate change and ocean acidification. *Science* 318: 1737-1742.

Holcomb M, Mccorkle DC, Cohen AL (2010) Long-term effects of nutrient and CO₂ enrichment on the temperate coral *Astrangia poculata* (Ellis and Solander, 1786). *Journal of experimental marine biology and ecology* 386: 27-33.

Holmhansen O, Riemann B (1978) Chlorophyll *a* determination- improvements in methodology. *Oikos* 30:438-447.

Hönisch B, Ridgwell A, Schmidt DN, Thomas E, Gibbs SJ, Sluijs A, et al. (2012) The geological record of ocean acidification. *Science* 335:1058-1063.

Houlbrèque, F., Reynaud, S., Godinot, C., Oberhänsli, F., Rodolfo-Metalpa, R., Ferrier-Pagès, C., 2015. Ocean acidification reduces feeding rates in the scleractinian coral *Stylophora pistillata*. *Limnol Oceanogr.* 60 (1), 89-99.

Houlbrèque F, Ferrier-Pagès C (2009) Heterotrophy in tropical scleractinian corals. *Biological Reviews* 84: 1-17.

Houlbrèque F, Tambutté E, Ferrier-Pagès C (2003) Effect of zooplankton availability on the rates of photosynthesis, and tissue and skeletal growth in the scleractinian coral *Stylophora pistillata*. *J Exp Mar Biol Ecol* 296:145-166

Houlbrèque F, Tambutté E, Allemand D, Ferrier-Pagès C (2004) Interactions between zooplankton feeding, photosynthesis and skeletal growth in the scleractinian coral *Stylophora pistillata*. *Journal of Experimental Biology* 207: 1461-1469.

Hughes TP (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science* 265: 1547-1551.

Iguchi A, Ozaki S, Nakamura T, Inoue M, Tanaka Y, Suzuki A, et al. (2012) Effects of acidified seawater on coral calcification and symbiotic algae on the massive coral *Porites australiensis*. *Mar Env Res* 73:32-36.

Imbs AB, Latyshev NA, Dautova TN, Latypov YY (2010) Distribution of lipids and fatty acids in corals by their taxonomic position and presence of zooxanthellae. *Marine Ecology Progress Series* 409:65-77.

IPCC, 2013: Summary for Policymakers. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA.

IPCC, 2012: Summary for policymakers. In: Field CB, Barros V, Stocker TF, Qin D, Dokken DJ, et al. Cambridge University Press, Cambridge, UK, and New York, NY, USA.

Jackson JBC (1994) Community unity. *Science* 264:1412-1413.

Johns GM, Leeworthy VR, Bell FW, Bonn MA (2003) Socioeconomic Study of Reefs in Southeast Florida. Final Report October 19, 2001 as revised April 18, 2003 from Hazen and Sawyer to Broward County, Palm Beach County, Miami-Dade County, Monroe County, Florida Fish and Wildlife Commission and National Oceanic and Atmospheric Administration.

Kaniewska P, Campbell PR, Kline DI, Rodriguez-Lanetty M, Miller DJ, et al. (2012) Major Cellular and Physiological Impacts of Ocean Acidification on a Reef Building Coral. *PLoS ONE* 7:e34659.

Kleypas JA, Buddemeier RW, Archer D, Gattuso J-P, Langdon C, Opdyke BN (1999) Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science* 284:118-120

Kleypas JA, Feely RA, Fabry VJ, Langdon C, Sabine CL, Robbins LL (2006) Impacts of Ocean Acidification on Coral Reefs and Other Marine Calcifiers: A Guide for Future Research. St. Petersburg, FL, NSF, NOAA, and the U.S. Geological Survey, pp 88.

Langdon C, Takahashi T, Sweeney C, Chipman D, Goddard J, Marubini F, et al. (2000) Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. *Glob Biogeo Cycles* 14: 639-654.

Langdon C, Atkinson MJ (2005) Effect of elevated pCO₂ on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. *J Geophys Res-Oceans* 110:1-54.

Leichter JL, Shellenbarger G, Genovese SJ, Wing SR (1998) Breaking internal waves on a Florida (USA) coral reef: a plankton pump at work? *Mar Ecol Prog Ser* 166:83-97.

Levy O, Dubinsky Z, Achituv Y, Erez J (2006) Diurnal polyp expansion behavior in stony corals may enhance carbon availability for symbiont photosynthesis. *J Exp Mar Biol Ecol* 333:1-11.

Lewis E, Wallace DWR (1998) CO₂SYST—Program developed for the CO₂ system calculations. Carbon Dioxide Inf. Anal. Center; Report ORNL/CDIAC-105. Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.

Lirman D, Fong P (2007) Is proximity to land-based sources of coral stressors an appropriate measure of risk to coral reefs? An example from the Florida Reef Tract. *Marine Pollution Bulletin* 54:779-791.

Mallela J, Perry CT (2007) Calcium carbonate budgets for two coral reefs affected by different terrestrial runoff regimes, Rio Bueno, Jamaica. *Coral Reefs* 26:53-68.

Manzello DP, Berkelmans R, Hendee JC (2007) Coral bleaching indices and thresholds for the Florida Reef Tract, Bahamas, and St. Croix, US Virgin Islands. *Marine Pollution Bulletin* 54:1923-1931.

Manzello DP, Enochs IC, Melo N, Gledhill DK, Johns EM (2012) Ocean Acidification Refugia of the Florida Reef Tract. *PLoS ONE* 7, e41715.

Manzello DP, Enochs IC, Kolodziej G, Carlton R (2015) Recent decade of growth and calcification of *Orbicella faveolata* in the Florida Keys: an inshore-offshore comparison. *Mar Ecol Prog Ser* 521: 81-89.

Manzello DP (In Review) Rapid recent warming of coral reefs in the Florida Keys. Science Reports.

Marshall AT, Clode P (2004) Calcification rate and the effect of temperature in a zooxanthellate and an azooxanthellate scleractinian reef coral. *Coral Reefs* 23: 218–224.

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence – a practical guide. *J Exp Bot* 51: 659-668.

McConnaughey T (1989) Biomineralization mechanisms. In: Crick FC, (ed). *The Origin, Evolution, and Modern Aspects of Biomineralization in Animals and Plants*. Elsevier.

McCook LJ, Jompa J, Diaz-Pulido G (2001) Competition between corals and algae on coral reefs: a review of evidence and mechanisms. *Coral Reefs* 19:400-417.

McCulloch M, Falter JL, Trotter J, Montagna P (2012) Coral resilience to ocean acidification and global warming. *Nat Clim Chang* 2: 1-5.

Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol Oceanogr* 18:897-907.

Miller M, Bourque A, Bohnsack J (2002) An analysis of the loss of acroporid corals at Looe Key, Florida, USA: 1983-2000. *Coral Reefs* 21:179-182.

Muscatine L, Porter J (1977) Reef corals – mutualistic symbioses adapted to nutrient-poor environments. *BioScience* 27:454-460.

Muscatine L, Porter J, Kaplan I (1989) Resource partitioning by reef corals as determined from stable isotope composition. *Mar Biol* 100:185-193.

Oku H, Yamashiro H, Onaga K, Sakai K, Iwasaki H (2003) Seasonal changes in the content and composition of lipids in the coral *Goniastrea aspera*. *Coral Reefs* 22:83-85.

Oliver, T.A., Palumbi, S.R., 2011. Do fluctuating temperature environments elevate coral thermal tolerance? *Coral Reefs* 30, 429-440.

Oliver, T.A., Palumbi, S.R., 2009. Distributions of stress-resistant coral symbionts match environmental patterns at local but not regional scales. *Mar Ecol Prog Ser.* 378, 93-103.

- Palardy J, Grottoli A, Matthews K (2005) Effects of upwelling, depth, morphology, and polyp size on feeding in three species of Panamanian corals. *Mar Ecol Prog Ser* 300:79-89.
- Palardy JE, Grottoli AG, Matthews KA (2006) Effect of naturally changing zooplankton concentrations on feeding rates of two coral species in the Eastern Pacific. *J Exp Mar Biol Ecol* 331:99-107.
- Palardy JE, Rodrigues LJ, Grottoli AG (2008) The importance of zooplankton to the daily metabolic carbon requirements of healthy and bleached corals at two depths. *J Exp Mar Biol Ecol* 367:180-188.
- Pandolfi JM, Connolly SR, Marshall DJ, Cohen AL (2011) Projecting coral reef futures under global warming and ocean acidification. *Science* 333:418–422.
- Pansch C, Schaub I, Havenhand J, Wahl M (2014) Habitat traits and food availability determine the response of marine invertebrates to ocean acidification. *Glob Chang Biol* 20: 765-777.
- Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA (2014) Mechanisms of reef coral resistance to future climate change. *Science* 344: 895-898.
- Petit JR, Jouzel J, Raynaud D, Barkov NI, Barnola JM, Basile I, et al. (1999) Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* 399:429-436.
- Pisapia C, Anderson K, Pratchett MS (2014) Intraspecific Variation in Physiological Condition of Reef-Building Corals Associated with Differential Levels of Chronic Disturbance. *PLoS ONE* 9: e91529.
- Pitts PA (1994) An investigation of near-bottom flow patterns along and across Hawk Channel, Florida Keys. *Bull Mar Sci* 54:610-620.
- Portner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Mar Ecol Prog Ser* 373:203-217.
- Precht W, Bruckner A, Aronson R, Bruckner R (2002) Endangered acroporid corals of the Caribbean. *Coral Reefs* 21:41-42.
- Renegar DA, Riegl BM (2005) Effect of nutrient enrichment and elevated CO₂ partial pressure on growth rate of Atlantic scleractinian coral *Acropora cervicornis*. *Mar Ecol Prog Ser* 293:69-76.

- Reynaud S, Leclercq N, Romaine-Lioud S, Ferrier-Pagès C, Jaubert J, Gattuso JP (2003) Interacting effects of CO₂ partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Global Change Biol* 9: 1660-1668.
- Ries JB (2011) Acid ocean cover up. *Nat Clim Chang* 1: 294-295.
- Roberts HH, Rouse JLJ, Walker ND (1982) Evolution of cold-water stress conditions in high-latitude reef systems: Florida Reef Tract and the Bahama Banks. *Carib J Sci* 19:55-60.
- Rodrigues LJ, Grottoli AG (2007) Energy reserves and metabolism as indicators of coral recovery from bleaching. *Limnol Oceanogr* 52: 1874-1882.
- Rowan R, Whitney SM, Fowler A, Yellowlees D (1996) Rubisco in marine symbiotic dinoflagellates: form II enzymes in eukaryotic oxygenic phototrophs encoded by a nuclear multigene family. *Plant Cell*, 8:539-533.
- Ruzicka RR, Collella MA, Porter JW, et al. (2013) Temporal changes in benthic assemblages on Florida Keys reefs 11 years after the 1997/1998 El Nino. *Mar Ecol Prog Ser* 489:125-141.
- Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, et al. (2004) The Oceanic Sink for Anthropogenic CO₂. *Science* 305:367-371.
- Schoepf V, Grottoli AG, Warner ME, Cai W-J, Melman TF, et al. (2013) Coral Energy Reserves and Calcification in a High-CO₂ World at Two Temperatures. *PLoS ONE* 8:e75049.
- Sebens KP, Vandersall KS, Savina LA, Graham KR (1996) Zooplankton capture by two scleractinian corals, *Madracis mirabilis* and *Montastrea cavernosa*, in a field enclosure. *Mar Biol* 127:303-317.
- Shamberger KE, Cohen AL, Golbuu Y (2014) Diverse coral communities in naturally acidified waters of a Western Pacific reef. *Geophys Res Lett.* 41: 499-504.
- Shinn EA (1966) Coral growth-rate, an environmental indicator. *J Palaeont* 40:233-240.
- Shinn EA, Lidz BH, Kidinger JL, Hudson JH, Halley RB (1989) A Field Guide: Reefs of Florida and the Dry Tortugas. 28th International Geological Congress – Field Trip T176, Washington, D.C.
- Silverstein RN, Correa AMS, Baker AC (2012) Specificity is rarely absolute in coral-algal symbiosis: implications for coral response to climate change. *Proc Roy Soc B* 279: 2609-2618.

Silverstein RN, Cunning R, Baker AC (2015) Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Glob Chang Biol* 21: 236-249.

Sterner RW, Elser JJ (2002) *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press.

Szmant AM, Forrester A (1996) Water column and sediment nitrogen and phosphorus distribution patterns in the Florida Keys, USA. *Coral Reefs* 15:21-41.

Szmant AM, Gassman NJ (1990) The effects of prolonged bleaching on the tissue biomass and reproduction of the reef coral *Montastraea annularis*. *Coral Reefs* 8:217-224.

Szmant AM, Forrester A (1996) Water column and sediment nitrogen and phosphorus distribution patterns in the Florida Keys, USA. *Coral Reefs* 15:21-41

Tambutté E, Allemand D, Mueller E, Jaubert J (1996) A compartmental approach to the mechanism of calcification in hermatypic corals. *J Exp Biol* 199:1029–1041.

Tambutté E, Holcomb M, Ferrier-Pagès C, Reynaud S, Tambutté E, Zoccola D, Allemand D (2011) Coral biomineralization: From the gene to the environment. *J Exp Mar Biol Ecol* 408: 58-78.

Tambutté E, Tambutté S, Segonds N, Zoccola D, Venn A, Erez J, et al. (2012) Calcein labelling and electrophysiology: insights on coral tissue permeability on calcification. *Proc R Soc B* 279: 19-27.

Teece MA, Estes B, Gelsleichter E, Lirman D (2011) Heterotrophic and autotrophic assimilation of fatty acids by two scleractinian corals, *Montastraea faveolata* and *Porites astreoides*. *Limnol Oceanogr* 56:1285-1296.

Tolosa I, Treignier C, Grover R, Ferrier-Pagès C (2011) Impact of feeding and short-term temperature stress on the content and isotopic signature of fatty acids, sterols, and alcohols in the scleractinian coral *Turbinaria reniformis*. *Coral Reefs* 30: 763-774.

Toth LT, van Woesik R, Murdoch TJJ, Smith SR, Ogden JC, Precht WF, Aronson RB (2014) Do no-take reserves benefit Florida's corals? 14 years of change and stasis in the Florida Keys National Marine Sanctuary. *Coral Reefs* 33:565-577.

Towle EK, Enochs IC, Langdon C (2015) Threatened Caribbean Coral Is Able to Mitigate the Adverse Effects of Ocean Acidification on Calcification by Increasing Feeding Rate. *PLoS ONE* 10(4): e0123394.

- Tremblay P, Grover R, Maguer JF, Legendre L, Ferrier-Pagès C (2012) Autotrophic carbon budget in coral tissue: a new ^{13}C -based model of photosynthate translocation. *J Exp Biol* 215: 1384-1393.
- Tremblay P, Fine M, Maguer JF, Grover R, Ferrier-Pagès C (2013) Ocean acidification increases photosynthate translocation in a coral-dinoflagellate symbiosis. *Biogeosciences* 10: 83-109.
- van Hooijdonk R, Maynard JA, Manzello D, Planes S (2014) Opposite latitudinal gradients in projected ocean acidification and bleaching impacts on coral reefs. *Global Change Biology* 20:103-112.
- Venn AA, Tambutté E, Holcomb M, Allemand D, Tambutté S (2011) Live Tissue Imaging Shows Reef Corals Elevate pH under Their Calcifying Tissue Relative to Seawater. *PLoS ONE* 6: e20013.
- Venn AA, Tambutté E, Holcomb M, Laurent J, Allemand D, Tambutté S (2012) Impact of seawater acidification on pH at the tissue–skeleton interface and calcification in reef corals. *Proc Natl Acad Sci USA* 110: 1634-1639.
- Wagner DE, Kramer P, van Woesik R (2010) Species composition, habitat, and water quality influence coral bleaching in southern Florida. *Marine Ecology Progress Series* 408:65-78.
- Weis VM (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *J Exp Biol* 211:3059-3066.
- Whitney S, Shaw D, Yellowlees D. (1995) Evidence that some dinoflagellates contain a ribulose-1,5-biphosphate carboxylase/oxygenase related to that of the α -proteobacteria. *Proc R Soc Lond B Biol Sci* 259:271-275.
- Wilkinson C (2004) Status of coral reefs of the world. Vol. 2, Australian Institute of Marine Science, Townsville, Queensland, Australia.
- Wirt KE, Hallock P, Palandro D, Daly KL (2013) Potential habitat of *Acropora* spp. on Florida reefs. *Appl Geogr* 39:118-127.
- Yonge CM (1930a) Studies on the physiology of corals. I. Feeding mechanisms and food. *Sci. Rep. Great Barrier Reef Exped.* 1:13-57.