OCTOCORAL PHYSIOLOGY: CALCIUM CARBONATE COMPOSITION AND THE EFFECT OF THERMAL STRESS ON ENZYME ACTIVITY

by
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ABSTRACT

HADLEY PEARSON: Octocoral Physiology: Calcium Carbonate Composition and the Effect of Thermal Stress on Enzyme Activity

(Under the direction of Dr. Tamar L. Goulet)

Octocorals are a diverse group of organisms, and within the Caribbean they contribute substantially to coral-reef cover. In this thesis, two different studies performed on these marine organisms are detailed. In Section I, the percent calcium carbonate composition of several species was analyzed. Octocorals produce sclerites: calcium carbonate crystals that serve as the skeletal elements of the coral. Determining the percent calcium carbonate composition of eight Caribbean octocoral species can give comparative insight into the form and function in these animals. This study also offers clues about potential evolutionary tradeoffs between rigidity and flexibility, as well as establishes some basic physiological information about octocoral species in the Caribbean. Since calcification in organisms such as corals can be affected by the ocean acidification seen with climate change, this baseline is an important reference for future studies. Further, the increased intensity and frequency of violent storms due to global warming might prove to be especially damaging for more rigid species.

Section II focuses on the effect of elevated temperature on one octocoral species over a period of ten days. The effect of temperature change was inferred from changes in activity of peroxidase and catalase, two enzymes that neutralize hydrogen peroxide produced in response to stress. The results show that the species did exhibit changes in enzyme activity in response to thermal stress. These enzyme activity changes will aid in understanding how this gorgonian coral species may respond to climate change.

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INTRODUCTION

Octocorals are a diverse group of cnidarians. Their forms can range from delicate, feathery fronds to almost stone-like, sparse branches. Octocorals serve as an environment for *Symbiodinium* spp., the unicellular dinoflagellate algae that engage in a mutualistic relationship with their octocoral hosts.

My thesis has two sections. The first involves the octocoral skeletal structure, the sclerites, which are formed from calcium carbonate. The sclerite percent composition may vary between species. By comparing eight species one can gain valuable insights about variability between different octocorals. This establishes a baseline for calcium carbonate composition at current ocean pH. With ocean acidification due to climate change, the percent composition may be altered. Looking at variability in this parameter also allows us to predict whether species are more flexible or rigid. With the increase in tropical storms predicted due to global warming, rigid species might be at a disadvantage.

The second section examines the relationship between host and *Symbiodinium*, and how the symbiosis might be affected by thermal stress. This is especially important in light of global warming, a phenomenon that can generate problems for corals beyond the initial stress response to elevated temperature. Increased temperature and diseases interact synergistically: thermal stress can compromise coral immunity, allowing for more rapid spread of diseases through reefs (Mydlarz 2009). In some coral species, it has

been observed that physiologically stressed individuals are also more subject to predation than unstressed corals (Slattery 2008). By measuring the concentration of certain enzymes, one can understand octocorals' reactions to thermal stress. This can allow one to make educated predictions about how climate change will affect coral reefs. Together, these two studies can allow us to better understand these valuable oceanic organisms.

SECTION I: SCLERITES

Introduction

Sclerites, also known as spicules, form the internal skeleton of octocorals, playing a pivotal role in support. They have specific shapes, sizes and patterns (Figure 1.1) in different layers of octocoral tissue: axis, axial sheath, and layers of the coenenchyme (Bayer 1961; Lewis and von Wallis 1991; Vargas et al. 2010; Tentori and van Ofwegen 2011). Sclerite characteristics vary between octocorals, and thus have been used to identify different octocoral species (Bayer 1961). Sclerites, however, can also vary within a species. For example, size and relative proportions of types of sclerites can vary between the base and the tip of a colony (van Alstyne et al. 1992; Tentori and van Ofwegen 2011). In addition, colonies within the same species that inhabit shallow water have high densities of short spindle-shaped sclerites while colonies in deeper water have fewer, but longer

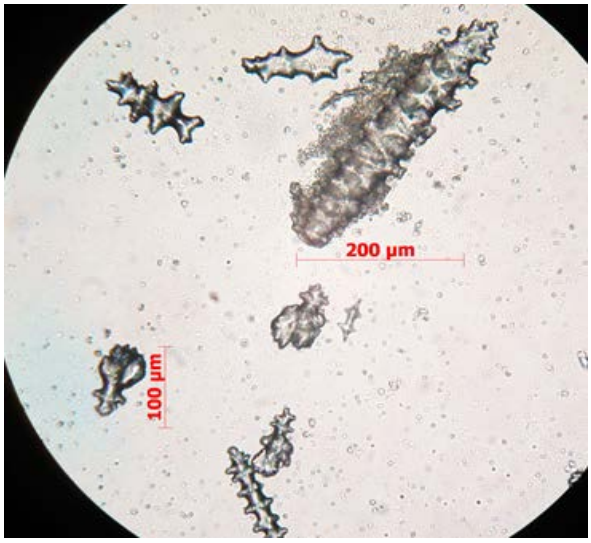

Figure 1.1: Variations in sclerite shape and size. Photo by Blake Ramsby.

spindles (Clavico et al. 2007). While alternative methods such as using DNA regions like microsatellites to differentiate between octocorals have been explored, sclerite analysis is still the most widely used method of octocoral species identification (Jamison and Lasker 2008). Recently, quantitative, computer analysis of sclerite form has been employed to identify octocorals (Vargas et al. 2010; Carlo et al. 2011).

Octocorals' calcified materials provide a skeletal structure. In addition to individual sclerites, some species have an axial skeleton formed from either fused spicular elements or amorphous carbonate (Goldberg 1987). However, most spicules are unfused and found in either the mesoglea, or tracts within cell clusters (Goldberg 1987). Octocoral sclerites are made of calcite, one of three crystalline forms of calcium carbonate ($CaCO_3$) found in nature (Velimorov and King 1979).

The formation of sclerites has been described in the gorgonian species *Pseudoplexaura flagellosa* by Goldberg in 1987. Two distinct stages of spicule formation were identified: 1) intracellular formation of crystals within the calcifying vesicles (CVs) of primary sclerocytes and 2) extracellular carbonate deposition from secondary sclerocytes that form thin cytoplasmic extensions over the immature sclerite's surface. Primary crystal formation occurs intracellularly, with sclerocytes being characterized by many mitochondria and a high density of cytoplasmic vesicles derived from the Golgi body. Larger electron lucent vesicles known as calcifying vesicles (CV) associate closely with the cytoplasmic vesicles, and serve as the site for primary crystal formation. These CV house small (0.1 μm-0.5 μm) irregular crystals. Under normal growth conditions, several CV fuse to produce 1-3 large vesicles. These vesicles become extracellular,

primordial sclerites through fusion of plasma and CV membranes, followed by plasma membrane degeneration (Goldberg 1987).

Within the mesoglea, secondary sclerocytes surround the extracellular carbonate with overlapping pseudopodia, forming a multicellular “vesicle” (MCV). Carbonate is deposited in regular, overlapping, concentric sheets. This secondary growth is followed by fusion of several large MCVs to form an immature spicule. Smaller MCVs settle onto the surface of the spicule, forming complex tubercules. Thin pseudopods envelop the maturing spicule, and secondary growth cements the tubercules onto the surface. Spicules are considered mature when the membranous pseudopods dissipate and full contact with the mesoglea is achieved. Sclerocytes that perform intracellular spicule formation are rare under normal growth conditions, but can be commonly observed in injured, recovering tissue. Since this study was species-specific, different spicular origins are possible (Goldberg 1987).

The carbon component of calcium carbonate ($CaCO_3$) can be obtained directly from carbon dioxide (CO_2) produced in glycolysis, or can be absorbed as dissolved CO_2 or bicarbonate from the water (Lucas and Knapp 1997). Dissolved CO_2 serves as the largest source of carbon in octocorals (Lucas and Knapp 1997). Most of the calcium (Ca) used in sclerite formation is taken up directly from the environment and transported by axial epithelial cells (Kingsley 1987). Kingsley and Watabe (1989) also suggested that sclerite formation may serve as a mechanism for reducing Ca^{2+} ions in cells, thus reducing Ca-toxicity in octocoral tissues.

Different sclerites have distinct physical properties, with sclerite forms having specific functions (Lewis and von Wallis 1991). For example, the shape and orientation

of club-shaped sclerites help resist deformation due to both tension and compression. Shorter, denser sclerites provide strength for shallow-dwelling octocorals to withstand the turbulent waters closer to shore (van Alstyne et al. 1992; West 1997; West 1998; Clavico et al. 2007). The selection pressures found in these environments are great enough that, even without geographic isolation, speciation can occur (Prada 2013). However, phenotypic plasticity can be seen in mature corals; transferring an adult octocoral from shallow to deep water can result in increased sclerite size, while moving from deep to shallow causes a decrease in sclerite size (Prada 2008).

Injury can also cause an alteration of sclerite characteristics. For example, in *Briareum asbestinum*, sclerites in the scar tissue are longer and less dense (West 1997). The efficacy of altered sclerite length as protection from predators is debated. Some studies suggest long sclerites are less appetizing to predators (van Alstyne et al. 1992; West 1997; West 1998; Clavico et al. 2007), while others found there is no additional protection afforded by these modified sclerites (O'Neal and Pawlik 2002; Clavico et al. 2007). Sclerite color can also change following injury. In *Gorgonia* sp., higher proportions of purple sclerites are produced after a biotic attack, resulting in 'purpling' of the surrounding tissue (Alker et al. 2004). Thus, sclerites exhibit a dynamic set of characteristics, and can vary significantly within an octocoral species.

Environmental change is another factor that may impact sclerite formation and shape. The pH of the surface ocean seawater has already dropped by 0.1 pH units, and is projected to decrease an additional 0.3-0.4 pH units by the end of the century (Bramanti 2013). Ocean acidification can affect calcifying organisms' ability to produce their skeletal structure (Cohen and Holcomb 2009, Gabay 2012, Gabay 2014). As such, it may

have significantly deleterious consequences for the more $CaCO_3$ rich stony corals (Silverman et al. 2009). Stony coral skeletons are made of aragonite, the more stable crystalline form of $CaCO_3$.

Octocoral sclerites, on the other hand, are made of calcite, which in an acidic environment is up to 5 times more soluble than aragonite (Morse et al. 2006). Surprisingly, Allemand and Grillo (1992) found that ocean water acidified by a high concentration of CO_2 increased the rate of calcite deposition in octocorals. Conversely, another study found that an increase in CO_2 reduced the rate of calcification (Gómez et al. 2010). This dichotomy could be due to the fact that different species of octocoral exhibit complex responses to lowered pH. Observed results of exposure to seawater acidification include loss of *Symbiodinium*, increased *Symbiodinium* density, atypical host sclerite formation, or no measurable effect at all (Gabay 2012, Gabay 2014). Differing capabilities in regulating internal pH, as well as the soft tissue's resistance to environmental changes, are possible sources of this variation among the taxa (Gabay 2012, Gabay 2014). If future climate change does indeed lead to the projected increase in ocean acidity, sclerite production in octocorals may be altered in a detrimental manner.

Climate change can affect these organisms in another significant way. The severity and frequency of oceanic storms has been projected to increase (Easterling 2000, Emanuel 2005). While the rigidity afforded by a high sclerite content might protect a coral from rubbing against the surrounding substrate and itself, breakage from stronger storm surge forces is more likely in rigid species. Conversely, while breakage from normal forces might be more frequent for flexible species with lower sclerite content,

storms would be less of a threat. The rigid corals might be in greater danger from the changing climate.

With the overwhelming importance of sclerites in the study of octocorals, the lack of basic physiological studies on percent sclerite composition of octocorals is surprising. The purpose of this study was to determine the average percent calcium carbonate composition of a variety of octocoral species, and thereby establish a baseline for future octocoral studies. Knowledge of octocoral species' skeletal composition also allows us to examine morphological tradeoffs of rigidity (with a high percent composition of sclerites) or flexibility (with a low percent composition sclerites), which might be especially important in light of the predicted increase in the violence and frequency of storms.

Materials and Methods

Site and Collections

This study was carried out at the field site of Universidad Nacional Autónoma de México (UNAM) at Puerto Morelos México (20°52'5.23"N, 86°51'58.92"W), during June 2011. Fifty-six octocoral colonies were sampled from a patch reef located within 50 m of the UNAM pier. There were two to eleven colonies of each species sampled in the study (number of colonies sampled follows species name in parentheses): *Eunicea flexuosa* (6), *Eunicea tourneforti* (10), *Plexaurella dichotoma* (7), *Pseudoplexaura porosa* (11), *Pseudoplexaura flagellosa* (3), *Pseudoplexaura wagenaari* (2), *Pterogorgia anceps* (9), and *Pseudopterogorgia americana* (6). The samples were stored at -80°C freezer until they were processed. Species were identified based on their colony morphology and sclerite characteristics (Bayer 1961; Sánchez and Wirshing 2005).

Tissue Digestion

Eppendorf tubes were labeled and warmed in the drying oven at 60°C for 12 hours to remove any water from humidity in the tubes. Each tube was then weighed (TW) to the closest 0.001 gm. A 0.5-cm length of frozen octocoral branch was added to each labeled tube and dried in the oven for 48 hours at 60°C, after which the tube was weighed again (DTW). The tissue was incubated in 1 ml of 5% sodium hypochlorite solution (household bleach) for 24 hours. The tube was vigorously vortex-mixed for 5 seconds, centrifuged at maximum speed for 30 seconds, and the supernatant was discarded. This process was repeated two additional times until the tissue was completely digested.

Following the last digestion, the sample was centrifuged at 13,000 rpm for 2 minutes, and then the supernatant was discarded. The sclerite-rich residue was washed three additional times with 1 ml of distilled water to remove any remaining bleach. The tube was dried in an oven at 60°C for 12 hours, and re-weighed (STW).

Calcium Carbonate Digestion

The sclerite-rich sample was incubated in 1 ml of 10% hydrochloric acid (HCl) for 24 hours. The tube was vortexed for 5 seconds and centrifuged for 1 minute at 13,000 rpm. This process was repeated three more times until the sclerites had completely dissolved. After the final treatment with HCl, the residue was washed three times with distilled water and dried for 12 hours at 60°C in the oven. The tube was again weighed (RTW), and the sclerite composition of the branches were determined using the following formulas:

TW: dry tube
DTW: dry sample + tube
STW: sclerite-rich, digested sample
RTW: residue + tube

Dry weight (DW): DTW-TW
Residue weight (RW): RTW-TW
Sclerite weight (SW): STW-TW-RW
Percent composition sclerite: (SW/DW)*100

Data Analysis

Since sclerite content represents proportional values, data were arcsine transformed. A one-way analysis of variance (ANOVA) was performed using octocoral species as the explanatory variable. Post-hoc Tukey's HSD test was conducted to explore differences in sclerite composition between octocoral species.

Results

Percent calcium carbonate content was significantly different among octocoral species (Table 1.1; P= <0.001). Post-hoc Tukey's HSD test divided the octocorals into three groups, seen in Figure 1.2. Members within each group had similar percent sclerite composition, but there were significant differences between the three groups. Group 1, with the highest $CaCO_3$ content, was comprised of *E. flexuosa*, *E. tourneforti* and *P. dichotoma*. Group 1 had twice the $CaCO_3$ content of Group 2 (composed of *P. anceps* and *Pseudoplexaura* sp.), and four times the $CaCO_3$ content of Group 3, which consisted of *P. americana*.

Table 1.1: Results of the one-way analysis of variance for $CaCO_3$ content.

Factor	*df*	Sum of Squares	Mean Square	F	P
Species	7	24470	3496	68.52	<0.001
Residuals	2347	51			

Discussion

Octocorals contain an internal skeleton made of sclerites, which are microscopic calcite structures that lend support and rigidity to the colony. Octocorals with higher sclerite density tend to be found in shallow areas, where the added rigidity may reduce chances of rubbing against the surrounding substrate due to wave action (West 1998). All of the samples, from the eight octocoral species, were sampled from the same depth.

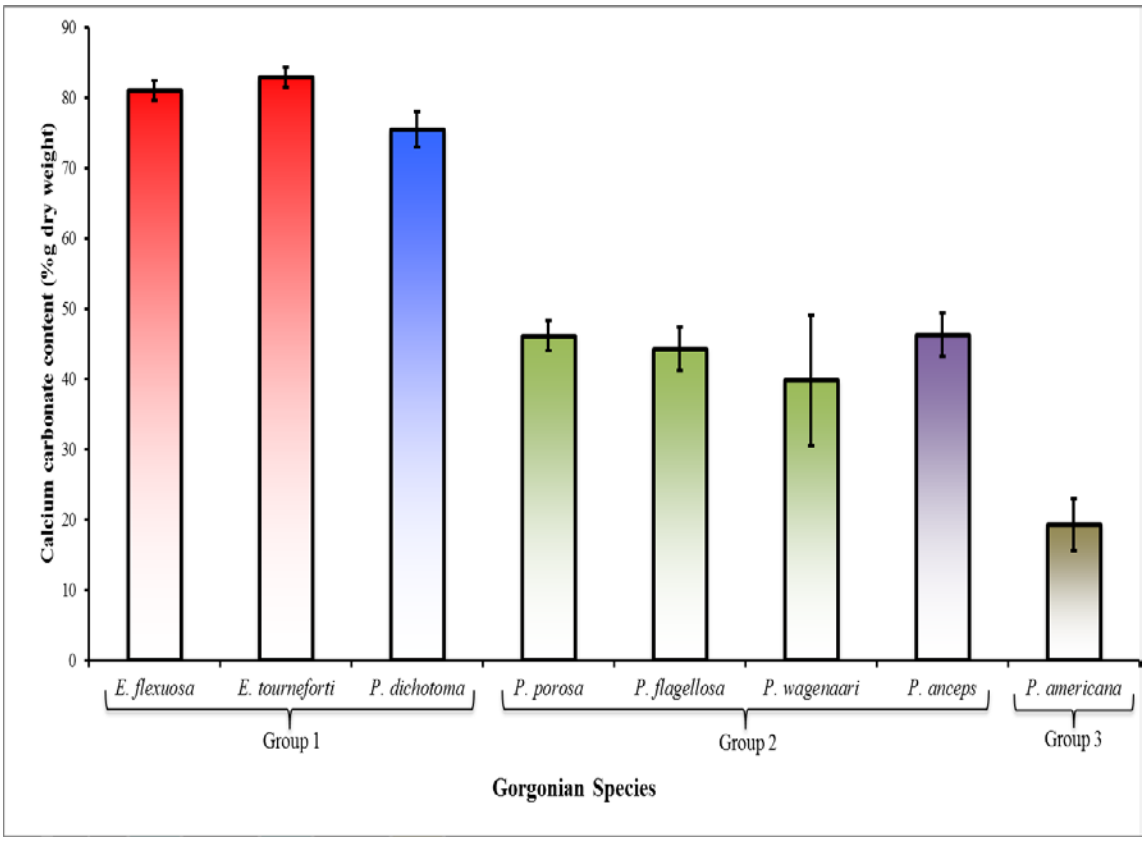

Figure 1.2: Calcium carbonate content (percent dry weight) of the eight octocoral species (*Eunicea flexuosa, Eunicea tourneforti, Plexaurella dichotoma, Pseudoplexaura porosa, Pseudoplexaura flagellosa, Pseudoplexaura wagenaari, Pterogorgia anceps,* and *Pseudopterogorgia americana*). Species of the same genera are grouped by color. Species are also divided into three groups based upon similar percent calcium carbonate composition. Data represent mean ± standard error of the mean.

Nevertheless, *E. flexuosa*, *E. tourneforti*, and *P. dichotoma*, with their high sclerite composition, would all be stronger and more durable under normal stress, while *P. americana* would be more prone to deformation and abrasion due to rubbing against the surrounding substrate. However, in the case of extreme wave stress due to storm surges, excessive rigidity could prove disadvantageous. Thus, *P. americana* may be less prone to breakage during storm surges than the more rigid species investigated in this study. With

the projected increase in storms due to climate change, this insight can be valuable in predicting which gorgonian species have the best chances of thriving.

Since sclerites provide structure, species with a higher sclerite density are more rigid than those with less. Personal observations of corals in the field, as well as additional data comparing percent sclerite composition to refractory content of tissue, support this conclusion (Shirur, pers. comm.). The refractory material consists of connective tissue and the axis of insoluble protein present in some corals (Goldberg 1974, Lawrence & Kafri 1979, Slattery & McClintock 1995). In currently unpublished data, a significant negative correlation was found between mean refractory content of tissues and percent sclerite composition of corals (Shirur, pers. comm.). *P. americana*, the most flexible species in our study (Sponaugle & LaBarbera 1991, Boller et al. 2002), had the lowest sclerite composition and highest refractory content (Shirur, pers. comm.). The most rigid species in our study (*E. flexuosa*, *E. tourneforti*, and *P. dichotoma)*, had the highest sclerite composition and lowest refractory content (Shirur, pers. comm.). These data suggest that high sclerite composition increases rigidity, while high refractory content favors flexibility (Shirur, pers. comm.). Which characteristic is most advantageous to the coral depends on its environment.

In summary, different species of octocoral can have significantly different percent calcium carbonate composition. Examining their differences can allow us insight into what tradeoffs between flexibility and rigidity were made in their evolutionary history, and how their current form affects their biological function and behavior.

SECTION II: THERMAL STRESS

Introduction

Gorgonian octocorals are important members of Caribbean reefs, where they serve as food and shelter for many fish and invertebrates (Bayer 1961, Cairns 1977, Birkeland & Neudecker 1981, Harvell & Suchanek 1987, Lasker et al. 1988). With the recent decline in scleractinian coral abundance in the Caribbean (Aronson 2006, Gardner 2003, Pandolfi et al. 2005, Ruzicka 2013), gorgonian corals may represent the future of these reefs (Ruzicka 2013). Like scleractinian corals, gorgonians host dinoflagellates from the genus *Symbiodinium*. Since these algae supply photosynthetically derived energy to corals, this symbiotic association plays an integral part of meeting the hosts' nutritional needs (Falkowski et al. 1984).

Many environmental factors can affect coral-algal symbiosis, including a key stressor, seawater temperature. Temperature higher than the mean-summer temperature can lead to a breakdown of this association (Hoegh-Guldberg 1999). This can result in increased expulsion of the algae, which turns coral tissue pale or white, and is termed as bleaching (Kleppel et al. 1989).

Only a handful of studies have reported bleaching in gorgonians, and it is generally believed that they are hardier and less prone to bleaching than scleractinian

corals (Ruzicka 2013). Most studies on bleaching in gorgonians are based on visual surveys of reefs. Since some gorgonians have pigmented tissues, loss of symbionts and discoloration or paling of tissue may be apparent in some species (Prada et al. 2010), but not in others (Lasker et al. 1984, Lasker 2003). Thus, it is important to understand if gorgonians expel their symbionts during periods of temperature stress, or if they are better suited at handling temperature changes than scleractinians.

In *Symbiodinium*, elevated temperatures affect the fluidity of thylakoid membranes within their chloroplasts (Lesser 2006). Through a set of cascade reactions, the change in fluidity can lead to photoinhibition by damage to photosystem II, causing an increase in the production of reactive oxygen species (ROS) such as hydrogen peroxide and the superoxide radicals (Lesser 2006, Flores-Ramírez & Liñán-Cabello 2007, Suggett et al. 2008). ROS can cause significant damage within cells to DNA, lipids in biological membranes, and other cellular components (Nii & Muscatine 1997, Lesser 1997, 2006). Excessive production of hydrogen peroxide is especially dangerous because the uncharged molecule can easily diffuse through membranes, causing far-reaching damage within the cell.

Different types of *Symbiodinium* exhibit different responses to thermal stress (McGinty 2012). *Symbiodinium* are classified into nine clades, lettered A-I. These clades can be further divided into numbered types. *Pterogorgia anceps*, the species under study in this experiment, has *Symbiodinium* type B1 (Goulet 2008). In a study of seven free-living *Symbiodinium* types, B1 type was found to have one of the lowest productions of ROS at basal temperature (26°C), but had one of the highest at elevated temperatures of 31°C (McGinty 2012). This result suggested that B1 type *Symbiodinium* might not be

capable of sufficiently scavenging the excess ROS they produce by themselves (McGinty 2012). Generalizations about coral-stress response based solely on symbiont type cannot be made. When two physically and ecologically similar gorgonian species (*Pterogorgia americana* and *Pterogorgia elisabathae*) with the same *Symbiodinium* clade (B1) were exposed to heat shock, they exhibited dramatically different levels of H_2O_2 release (Mydlarz 2006).

We measured the activity of two enzymes an octocoral produces to neutralize the threat of ROS: peroxidase and catalase. The enzyme peroxidase is produced to catalyze the reduction of hydrogen peroxide to water and maintain cellular equilibrium (Lesser 2006). The reduction requires an electron donor that is oxidized. The reducing agent used varies between different types of peroxidases. Catalase also aids in the reduction of hydrogen peroxide to water and oxygen (Lesser 2006). However, it differs from peroxidase in that 1) it does not require an electron source and 2) it has a high sensitivity to light, possibly due to its heme group (Lesser 2006). This second difference results in the octocoral having a high turnover of the enzyme. Factors that negatively affect the rate of protein turnover, such as thermal stress, have the potential to lower catalase activity through either rapid degradation or slower synthesis (Lesser 2006).

From a change in activity of either of these enzymes, one could infer a higher concentration in reactive oxygen species. If the enzymes are not able to quench the ROS, the corals may instead rid themselves of *Symbiodinium*, the main source of ROS (Lesser 1997, Sandeman 2006), thus leading to increased expulsion of algae and bleaching.

Since corals are indispensable to reef ecosystems, it is essential to gain a thorough understanding of how different species of coral respond to thermal stress. While there has

been a large amount of research done in relation to scleractinian corals, far less is known about non-scleractinian cnidarians. Given that gorgonians dominate Caribbean reefs, and may thus represent its future, it is imperative to understand how thermal stress might affect these pivotal organisms. Here we investigated the response of a single species of gorgonian, *Pterogorgia anceps*, to thermal stress.

Materials and Methods

Site and Collections

This study was carried out at a shallow (2–3 m) patch reef near UNAM Puerto Morelos, México (20°52'5.23"N, 86°51'58.92"W). Eight 12-cm branches were collected from ten *P. anceps* colonies. To avoid sampling clones, sampled colonies were at least 3 m apart from each other. One branch was immediately frozen in liquid nitrogen. The remaining branches were attached to cement-filled PVC stands with cable ties, which maintained the branches in a vertical orientation, and were then placed in aquaria. The branches were acclimated to tank conditions at the ambient 29°C seawater temperature for seven days. On the eighth day, one branch per colony was flash-frozen. Two branches were maintained at 29°C as controls, while two other branches were placed in an aquarium in which the seawater temperature was raised to 32°C in 1°C-per-day increments. The branches were maintained in the various treatments, and one branch was collected at both five and ten days, frozen in liquid nitrogen, and stored at -70°C.

The final two branches were placed in an aquarium where the temperature was raised to 34°C in 1°C-per-day increments, and sampling by the same method occurred at one and three days after the target temperature was reached. This early sampling was due to the visually obvious and rapid deterioration in branch viability. The data from the final branch, which was frozen on the third day of sampling at 34°C, were excluded due to discoloration and partial necrosis of branches. Samples were transported to the University of Mississippi on dry ice and stored at -80°C until processing.

Sample Extraction

Approximately 2-cm portions of frozen octocoral tissue were removed from stored samples and crushed with mortar and pestle in liquid nitrogen. A 0.1M phosphate buffer solution, pH 7.8, was added to form a thick, slushy mixture. The mixture was placed on ice for 45-60 minutes to allow complete extraction of enzymes. To remove cellular and spicular content, the extracts were spun at 2500 rpm for 10 minutes, at 4°C. The supernatant was separated and stored at -80°C until further processing. For assays, the supernatant was thawed on ice and spun at 2500 rpm for 10 minutes, at 4°C.

Protein Concentration

Protein concentration was measured using the RED 660 Protein Assay from G-Biosciences. For the assay, 10 µl of sample was added to 150 µl of RED 660, and the absorbance at 660 nm was compared to a standard curve of bovine serum albumin (0–1.5mg/ml).

Enzyme Activity

Enzyme activity for both peroxidase and catalase was measured on a BioTek microplate reader loaded with Gen5 software. For peroxidase (POX), 10 µl of the extract was diluted with 20 µl of 0.1 mM phosphate buffer (PBS, pH 6.0) and 25 mM guaiacol (made with PBS, pH 6.0). The reaction was initiated by adding 50 µl of 20 mM H_2O_2 (made with PBS, pH 6.0), and the absorbance at 470 nm was recorded every minute over a period of 30 minutes. POX activity was calculated as the change in absorbance (min^{-1}) in 10 minutes, normalized to mg protein present in the sample.

For catalase (CAT), 5µl of the extract was diluted with 120 µl of 50 mM H_2O_2 (made with PBS, pH 7.0), and the absorbance at 240 nm was recorded every minute over a period of 30 minutes. The change in absorbance (min^{-1}) over 10 minutes was converted to mM of H_2O_2 scavenged using a standard curve made with different concentrations of H_2O_2 (0–5 mM). The mM of H_2O_2 scavenged was normalized to mg protein present in the sample.

Data Analysis

A mixed model analysis of variance was utilized to assess the effects of temperature and time on the enzyme activity (acclimation at 29°C, 34°C at Day 1, 29°C at Day 5, 32°C at Day 5, 29°C at Day 10 and 32°C at Day 10) in *P. anceps* branches. Since one branch from each *P. anceps* colony was sampled under each treatment, the colony genotype was added as a random effect. Branches exposed to 34°C were sampled three days after the last day of acclimation, and four days before the next sampling of the control branch (29°C at Day 5). Therefore the aggregate enzyme activity in the two

control branches at 29°C was regarded as an appropriate control for the 34°C treatment. Five *a priori* contrasts (C_n) were used to test the effect of temperature at the different times of sampling: (C_1) 29°C branches at acclimation, at Day 5 and at Day 10; (C_2) 29°C branches at acclimation versus Day 5; (C_3) 29°C branches at acclimation and Day 5 versus 34°C; (C_4) At Day 5, 29°C versus 32°C treatments; (C_5) At Day 10, 29°C versus 32°C treatments. Since our combinations of contrasts were not orthogonal, the type I error rate was controlled by using the Bonferroni correction ($\alpha = 0.05 / 5$ tests $= 0.01$).

Results

Peroxidase (POX) activity

Log transformation was used to reduce heteroscedasticity in our data. POX activity was significantly affected by the different treatments (Table 2.1, $P < 0.001$, Figure 2.1). Peroxidase activity in control branches was not statistically different in the experiment (C_1: $P = 0.103$, C_2: $P = 0.342$). Exposure to 34°C significantly increased POX activity by 30% compared to POX activity of control branches at acclimation and Day 5 (C_3: $P < 0.001$). Conversely, after five days exposure to 32°C led to a significant decrease in POX activity by 29% compared to the POX activity in branches at the Day 5 of the ambient temperature (C_4: $P < 0.001$). But, after 10 days at 32°C, POX activity was not significantly different between branches at the ambient 29°C and the heated 32°C (C_5: $P = 0.457$).

Table 2.1: Results of the mixed-model ANOVA with REML for peroxidase and catalase activity in thermally stressed *P. anceps*.

Enzyme	Fixed Factor	*df*	F	*P*
Peroxidase	Treatment	5, 41.73	12.521	<0.001*
Catalase	Treatment	5, 33.32	3.725	0.009*

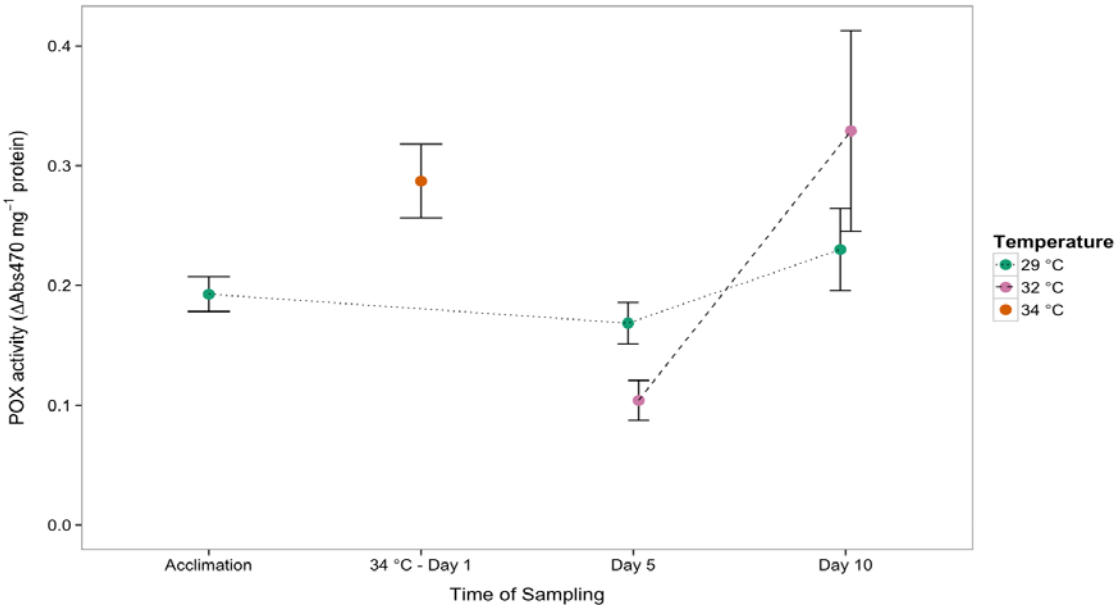

Figure 2.1: Peroxidase activity of *P. anceps* subjected to thermal stress. Data represent mean ± standard error of the mean.

Catalase (CAT) activity

CAT activity was significantly affected during the experiment (Table 2.1, P = 0.009, Figure 2.2), though this does not seem to be attributable solely to the treatments. In control branches, CAT activity was similar at acclimation and at 5 days (C_2: P = 0.112), but it significantly reduced by 30% by the tenth day (C_1: P < 0.007). CAT activity was highly variable in branches exposed to 34°C, but was statistically similar to control

branches (C_3: $P = 0.351$). CAT activity was again statistically similar between control and treated branches after exposure to 32°C for five days (C_4: $P = 0.037$). After 10 days, CAT activity in control branches was significantly lower than treated branches, by 15% (C_5: $P < 0.005$). CAT activity in the control branches sampled after 10 days was significantly lower than control branches sampled on other days. Therefore the difference in CAT activity between control and treated branches detected after 10 days may not be due to the temperature treatments, but rather some other unknown factor that lowered CAT levels for control branches on Day 10.

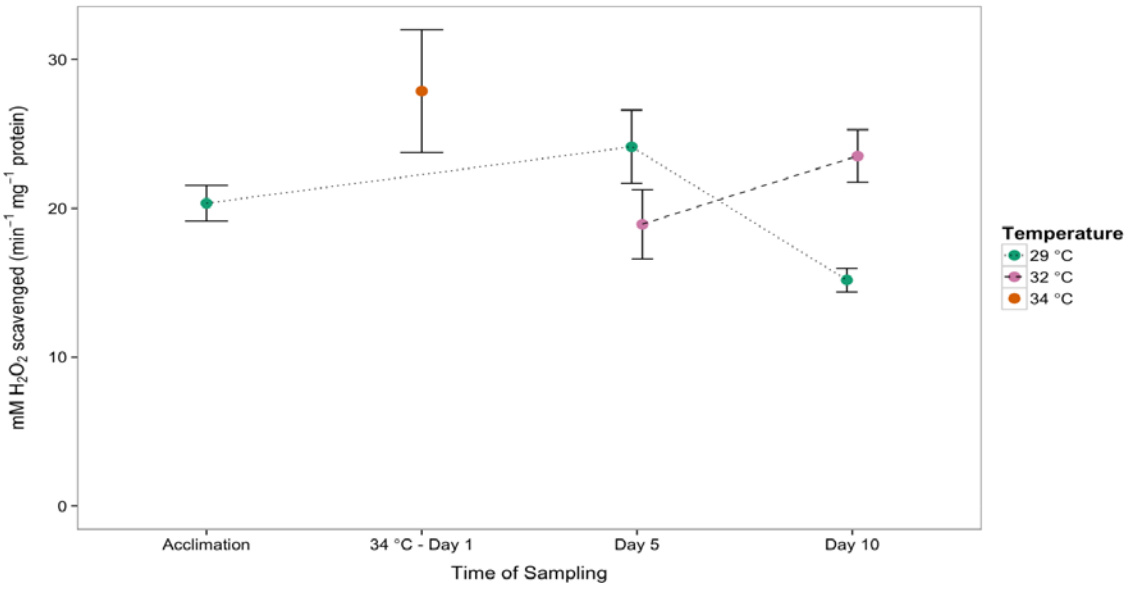

Figure 2.2: Catalase activity in *P. anceps* subjected to thermal stress. Data represent mean ± standard error of the mean.

Discussion

When stressed, organisms may produce reactive oxygen species (ROS) such as oxygen radicals and hydrogen peroxide (H_2O_2), which can be toxic to cells (Lesser 2006). To protect and maintain cellular functions, cells increase production of enzymes that neutralize ROS. Peroxidase and catalase are two enzymes that catalyze the reduction of H_2O_2 to water. We therefore used their activity to investigate the effects of thermal stress on gorgonian-algal symbiosis.

Average peroxidase activity was highest, and showed wide variability, when the branches were exposed to 34°C. This change indicated that this temperature caused significant stress for *P. anceps*, a conclusion supported by the fact that all of the branches in this treatment died before a five-day sample could be collected. At 32°C, peroxidase activity decreased to significantly lower levels than control branches after five days, and increased to above-control levels after ten days. On the tenth day at 32°C, the mean was higher than control levels, and results showed higher variation.

Catalase activity was less consistent. Branches exposed to 34°C had the highest mean concentrations of the enzyme, and the highest variability of all treatments. In the ambient (29°C) and the 32°C elevated-temperature treatments, significant differences were seen between treatments on the tenth day: the control branch showed significant variation between previous control days and 32°C Day 5 samples. Since catalase is light sensitive (Lesser 2006), variation in sunlight levels on the day of sampling might have accounted for the significant reduction seen at Day 10 in the control branches. Since the sampling for Day 10 of control and stressed branches occurred on different dates, the conditions on the sampling dates may have differed between the two treatments.

However, daily measurements of photosynthetically active radiation (PAR) do not indicate that levels differed significantly on any sampling dates except for 34°C, where PAR levels were much lower (Shirur, pers. comm.). All other sampling occurred at least three days after PAR levels returned to normal, making light levels an unlikely explanation for the discrepancy in the control.

The large standard error seen in the data at 34°C for both enzymes and for 32°C Day 10 for peroxidase indicated a variable response between *P. anceps* genotypes. Since the mean was consistently higher than controls, the variability could indicate that the degree and onset of enzymatic response varies between genotypes. Some genotypes were more thermally sensitive and reacted faster than others, which exhibited slower responses. Though the levels may vary, the trend is consistent: enzymatic activity increased in response to thermal stress.

Currently unpublished data indicates that symbiont density was significantly lower at 32°C Day 10 compared to the controls (Ramsby, pers. comm.). The lowest symbiont density was seen at 34°C Day 1 (Day 3 was also not included in this data due to coral death), though these levels were comparable to 32°C Day 10. Further, maximum photochemical efficiency of the branches significantly reduced over time for the 32°C treatment, by up to 60% on the tenth day following acclimation (Ramsby, pers. comm.). A significantly more dramatic, accelerated decrease in photochemical efficiency was seen over the four days that the branches were acclimated to 34°C, indicating production of ROS and serious damage to photosystem II (Ramsby, pers. comm.).

These data, in conjunction with the elevated concentrations of both POX and CAT on the tenth day of 32°C found in my study, is consistent with the octocoral

responding to thermal stress by attempting to 1) enzymatically quench the dangerous ROS, and 2) eliminate the main source of ROS through expulsion of the thermally stressed, ROS producing symbionts. This conclusion is further supported by the ten-day exposure to 32°C, after which the density of *Symbiodinium* cells was significantly lower compared to controls, while the thermal stress of the 34°C treatment caused faster *Symbiodinium* expulsion and more rapidly rising enzyme levels (Ramsby, pers. comm.).The net photosynthetic rate per remaining *Symbiodinium* cell, on the other hand, was significantly greater after 10 days at 32°C than control levels (Ramsby, pers. comm.). There was also a nonsignificant increase in the ratio of photosynthesis to respiration (Ramsby, pers. comm.).

Taken together, these data indicate that when *P. anceps* was exposed to thermal stress, *Symbiodinium* numbers dropped, thereby reducing the holobiont's overall potential to derive photosynthetic energy. This reduction in symbiont numbers, along with the production of ROS neutralizing enzymes, eliminated the immediate threat of the ROS. Therefore, the reduction in symbiont density, with the loss of photosynthetic input, may be offset by the reduced threat from ROS. If *P. anceps*, with the remaining *Symbiodinium* and the nutritional reserves in its tissues, can withstand the thermal event, it may be able to recover afterwards. *P. anceps* may survive a thermal stress of 32°C, but a short term thermal event of 34°C may detrimentally affect either the host or the symbiont to the point where the entire colony dies.

Gorgonians are the most abundant cnidarians on Caribbean reefs, where they support many fish and reef invertebrates. It is therefore essential to understand how climate change may affect these organisms. Since both POX and CAT are important in

eliminating ROS, understanding their use in response to thermal stress is important. This experiment demonstrated that while gorgonians may be susceptible to thermal stress, they have the potential to recover. More research is needed to understand how climate change may affect these pivotal organisms.

CONCLUSION

In the Caribbean, a shift in coral-community structure is occurring whereby octocorals are maintaining or increasing in abundance, while scleractinian coral cover is declining. During this study we examined one clear difference between scleractinian corals and octocorals, their skeletal structure, in an effort to establish baseline data about the contribution of skeletal matter to different gorgonian species. The skeletal content, which is adapted based upon environmental and biological stressors, may be affected by environmental change. In addition, we assessed the effect of thermal stress on a representative gorgonian coral species to begin to understand how, and if, gorgonian corals will survive rising seawater temperatures. Understanding why the Caribbean coral-community shift is occurring, and the physiology of gorgonian corals, will aid in predicting the future of Caribbean coral reefs.

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