Differences in percent weight growth of the Mustard Hill coral (*Porites astreoides*) from contrasting thermal environments after adaptation in a “common thermal garden”

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Acknowledgements

I would like to thank my mentor, Dr. Daniel Barshis at Old Dominion University, for all his mentorship, and for assisting me in all my scientific endeavors. I would also like to thank my teachers, Mrs. Kleinman, Ms. Foisy, and Ms. O'Hagan for their continued support for my research.

Abstract

As climate change is intensifying and anthropogenic threats, such as global warming, destructive fishing practices, pollution, excess nutrients, etc., are increasing, coral reefs are getting decimated. Reefs are dying due to coral bleaching—a break in the symbiosis between the coral host and their symbiotic algae caused by increased ocean temperatures. It has been discovered that coral preconditioned to fluctuations in temperatures just beyond their range are less vulnerable when exposed to high temperatures. The purpose of this experiment is to study whether corals from different thermal environments consistently show differential bleaching tolerances either through genetic adaptations or long-term acclimatization, after one year in a "common thermal garden." Mustard Hill coral (*Porties asteroides*) was collected from an inshore and offshore site at Carrie Bow Caye in Belize and in the Florida Keys. All fragments were kept in a "common thermal garden" of 27˚C until experimentation. The fragments were then randomly assigned to either a control tank or treatment tank with increased temperatures and were held and monitored there for six weeks. Their percent growth was calculated over the six-week treatment. Our results show that, in general, the coral samples from the more variable environments, in both locations, grew much more overall than the less variable environment. These results demonstrate that temperature variability of the local habitat can potentially indicate a coral's response to heat stress better than latitude, nationality, or other location factors.

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Introduction

The Earth's surface is made up of about 71 percent water (Universe Today, 2014). Thus, the stability of the world greatly depends on the ecological balance and health of the ocean ecosystem. As of now, more than a quarter of the world's coral reefs have been destroyed, and scientists predict that 60 percent of the world's coral reefs could be decimated by the year 2050. Coral reefs form a nursery to an estimated 25 percent of all marine animals, so it is critical that they be protected. Reefs are vital in keeping beaches and shoreline buildings intact—protecting them from intense wave action that can erode the land. Coral reefs provide an estimated $375 billion per year worldwide in goods and services such as snorkeling, scuba diving, beach protection, fishing, etc. It has also been discovered that coral reef organisms are being used in treatments for diseases like cancer, HIV, cardiovascular diseases, ulcers, and other ailments (Oceanic Research Group, 2007) (WWF, 2013).

Specifically, coral are invertebrates and Anthozoans, which are the largest class of organisms in the phylum Cnidarians. They are sessile, meaning they are immobile, so they feed by reaching out with tentacles to catch prey, such as small fish and planktonic animals. They live in colonies, and each individual coral is called a polyp. They generate a hard calcium carbonate skeleton, which serves as a uniform base or substrate for the colony. The skeleton also provides protection, as the polyps can contract into the structure if predators approach (International Coral Reef Initiative). Different species of coral build structures of various sizes and shapes ("brain corals," "fan corals," etc.). Various coral species tend to be separated into characteristic zones on a reef because of competition with other species and because of environmental conditions. Coral reefs are mostly found in tropical and semitropical waters near the equator, between the regions of 30°N and 30°S latitudes (International Coral Reef Initiative).

There are numerous and various threats to coral. Many destructive fishing practices such as cyanide fishing and bottom trawling are destroying reef habitats. Overfishing disrupts the ecological balance of coral reef communities and discarded fishing gear can entangle coral and erode polyp tissues. Careless tourism such as reckless boating, diving, snorkeling, and fishing, destroy coral reefs all over the world. Pollution, urban and industrial waste, sewage, agrochemicals, and oil pollution are harming and poisoning reefs by offsetting the chemical balance in the ocean—increasing the level of nitrogen, which causes an overgrowth of

algae, which ultimately “smothers” the reefs. Smothering of reefs can additionally be caused by excess sedimentation (NOAA, 2006).

Currently, natural disasters are occurring more frequently and hurricanes are strengthening due to climate change—exposing reefs to stronger, more frequent storms. Climate change also results in increased ocean temperature and decreasing ocean pH. This process is known as Ocean Acidification (OA). Ocean acidification reduces the availability of carbonate ions, which are needed for the formation of the coral skeleton and reef structure. Globally, ocean warming has been causing a disturbance, known as coral bleaching, in the symbiotic relationship between the coral host and its symbiotic algae, resulting in mass coral destruction (NOAA, 2013) (Coral Reef Alliance, 2013).

Coral are considered to be “plants inside animals.” They contain symbiotic, microscopic, single-celled algae called zooxanthellae within their gastrodermal cells. Most reef-building coral have a mutually beneficial symbiotic relationship with the zooxanthellae that live within the cells of the coral's gastrodermis. The coral provide these algae with a stable environment, protection, and some of the necessary organic compounds for photosynthesis. In return, the zooxanthellae supply the coral with oxygen, help them remove waste, and give them their vibrant color. They give the coral the organic products of photosynthesis to manufacture fats, as well as their calcium carbonate skeletons. Several million zooxanthellae live and produce pigments in one square inch of the coral. These pigments are visible through the clear body of the polyp and give many reef-building coral their beautiful colors. Since coral reefs are sessile organisms, they cannot migrate to new environments that are more habitable for them as ocean temperatures rise. Thus, currently, coral respond by going through coral bleaching, which is killing thousands of coral species worldwide. Bleaching is the breakdown in the symbiosis between the coral host and its’ obligate symbiotic algae. When temperatures are raised too high, the coral host expels the algae, and turns completely white. As coral can receive up to 100% of their energy requirements from their symbionts, if they do not recover quickly they may starve and die (Odyssey Expeditions: Tropical Marine Biology Voyages, 2013).

The process of and reasoning behind coral bleaching is being more deeply studied all over the world. In certain locations (i.e. American Samoa and the Florida Keys) there are two distinct environments that are both seemingly able to host sustained, living coral. These environments differ in their varying levels of temperature as well as other physical components.

While one location is more thermally variable, meaning it experiences a greater range of temperature each day, the other location experiences a more moderate range of temperatures. In a study, conducted by Oliver and Palumbi in 2011, *Acropora hyacinthus* from a thermally moderate lagoon pool (temperature range: 26.5-33.3˚C) and from a thermally variable lagoon pool (temperature range: 25.0-35˚C) were put under experimental heat stress. A comparison was conducted between mortality and photosystem II photochemical efficiency of coral fragments exposed to median or elevated temperatures. The results indicated that coral naturally growing in a more thermally variable pool resisted heat stress, while coral from a more thermally moderate pool suffered high mortalities. Additionally, results have shown that long-term increases in thermal tolerance decrease bleaching rates by the end of the century much more so than temporary increases, and higher bleaching resistance has been found at sites that have experienced more frequent or devastating bleaching events in the past (Logan, *et al* ., 2014) (Glynn, *et al.*, 2001; Maynard *et al.*, 2008; Thompson & van Woesik, 2009; Guest, *et al.*, 2012). There have been many propositions as to why coral are able to withstand consistent temperatures beyond their ranges. It has been suggested that some species are able to "shuffle" their *Symbiodinium* composition to express a clade—*Symbiodinium* subspecies—that helps the coral respond to the heat stress. Oliver and Palumbi in 2011 proposed that in high temperature environments in American Samoa, most of the surveyed coral were able to host multiple symbiont types, including *Symbiodinium* clade D. Among the coral that were able to host multiple types of symbionts, most showed higher proportions of this clade D symbiont in the hotter habitat; and even coral that hosted both members of clade C and D, showed specific preference to members of clade D. Therefore, it is feasible that the distribution of clade D correlates with the region that has experienced the greatest history of thermal stress (Stat, *et al.*, 2013). Another characteristic of the coral could be that enhanced heat stress tolerances may enable some organisms to better withstand environmental stresses in the future. Barshis, *et al.*, in 2013 found that constitutive front loading, or already expressed genes, allows an individual coral to maintain physiological resilience during frequently encountered stress. Although there are multiple experiments that investigate the variables involved in shaping holobiont (all the living organisms that make up the coral polyp) thermotolerance limits, it is still unclear whether these adaptations are temporary or long-term.

The purpose of this experiment was to expand upon Barshis' findings by looking at

existing coral colonies from thermally distinct regions. In this experiment, 22 whole coral colonies of *Porites astreoides* were collected from four different locations: ten whole colonies were collected from the inshore and offshore patch reefs in the thermally distinct reef habitats in the Florida Keys, six whole colonies were collected from the forereef site ("Karen Koltes/KK") in Belize and six additional colonies were collected from the backreef site at the Belize location. For the Florida Keys location, temperature variation differs between the inshore and offshore reefs because of physical variables. Temperature variation at the inshore reefs is greater due to the reduced heat storage capacity of the shallow waters (1-2m on average). Temperature variation at offshore corals is less extreme because the current patterns of Hawk Channel intervenes the flow from Florida Bay (See Fig.1). For Belize, the inshore site ("Backreef" or BR) was situated in 1-2 m of water while the offshore site ("Karen Koltes" or KK) was situated in 10-12 m (See Fig. 2). The purpose of this experiment was to determine whether coral from varying thermal environments still show differential bleaching tolerances after one year in a "common garden," which is a controlled tank in the lab with maintained temperatures. This essentially examined whether the differential bleaching levels were due to genetic adaptation, wherein inshore coral would be genetically adapted for higher temperature tolerance, or to long-term acclimation, wherein coral are conditioned to have higher thermal tolerance within the inshore location, but diminishes if they are removed from these conditions.

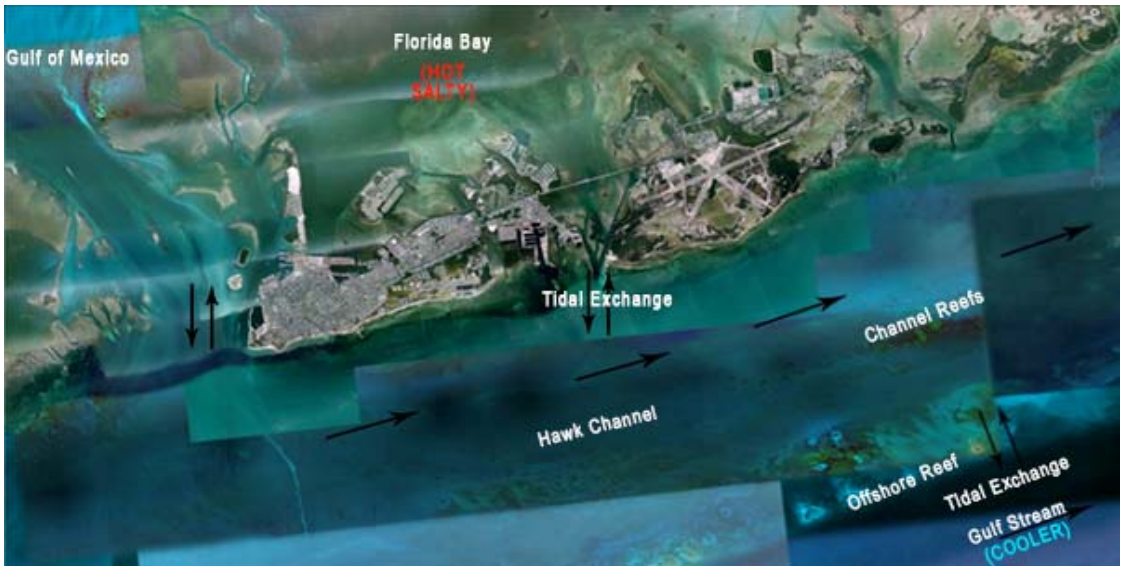

Figure 1. Florida Keys Location
http://lspolicy3.lhric.org/access/web?id=0f50f744-79ab-11e5-b327-002590c53f26

Figure 2. Belize Location
http://www.slickrock.com/Maps/detailedbelizemap.jpg

Methodology

Coral Colony Collection

Ten whole colonies of *Porites astreoides* were collected from the inshore and offshore patch reefs in Mustard Hill, Florida on August 4, 2014 and held in experimental raceways at the Keys Marine Lab. The coral were then transported to the aquatics facility at Old Dominion University and submerged in seawater on August 5th and 6th and were held at 27˚C until experimentation. Six whole colonies of *Porites astreoides* from the forereef site "Karen Koltes/KK" (average depth of 14 m) were collected on May 1st, 2015 and brought back to the dock on Carrie Bow Cay and held in the shallows underneath the dock until transport. Six additional colonies were collected from the Backreef ("BR) site (average depth of 1.5m) on May 2, 2015 and brought to the same holding area underneath the dock. Coral were packed in wet bubble wrap and moved to the dock at Pelican Beach Resort in Dangriga on May 6, 2015 and transported similarly to Old Dominion University on May 7, 2015. All coral were held in the Old Dominion University aquatics facility at 27˚C until experimentation.

Coral Fragment Collection and Stress Treatment

Using a 7/8" hole saw, cores were taken from six of the Keys' offshore coral colonies and 5 of the inshore colonies, resulting in nine pairs of offshore cores and 10 pairs of inshore cores. An air cutter was used for dividing each of the 4 Belize inshore coral colonies and 4 of the offshore colonies into 4 smaller segments. The coral were then distributed among 4 tubs labeled as Heated 1 (H1), Control 1 (C1), Heated 2 (H2), and Control 2 (C2), with 18 coral fragments in tubs H1 and C1 and 17 coral fragments in H2 and C2 (a total of 70 samples). Each tub had 4-5 offshore Keys corals, 5 inshore Keys corals, 4 offshore Belize corals, and 4 inshore Belize corals. All tubs were placed in a tank with 27˚C water, constant water flow, and artificial light. The control tubs (C1 and C2) had the surrounding water temperature of 27˚C. The heated tubs (H1 and H2) were equipped with heaters and set to 31˚C. The experiment was run for a total of 6 weeks.

Tank Quality

Figure 3.
Treatment Tank

For the six weeks that the experiment treatment was running, every day the tank sumps were cleaned 1-2 times and water was added to top off the tanks. About twice a week, a water quality check was done to monitor the levels salinity, pH, nitrate, phosphate, alkalinity and calcium carbonate. At the beginning of every week the fragments were placed in a new location in their respective treatment tanks to ensure that some fragments were not receiving more of one variable (lighting, water flow, etc.) and ultimately prevent undesired impacts/biases in the results. Additionally, once a week, observations of each individual fragment were recorded to look at changes over the experimental period.

Buoyant Weighting

Throughout the six-week treatment, the coral fragments' growth was measured 2-3 times using the buoyant weighting protocol. All the fragments were cleaned using a toothbrush to remove extraneous bacteria or algal growth then placed on a platform that was suspended in water and attached to a scale. The coral fragments were measured while suspended in water in order to

Figure 4. Buoyant Weighting Method

obtain only the skeletal growth and not the tissue density. The tissue density variable of the procedure was "eliminated" because the coral tissues in water will absorb the water, creating equilibrium with the outside water density, which leaves only the measurement of the skeletal growth. At the end of the treatment, the initial weight measurements of the fragments were subtracted from the final weight and then divided by the initial weight measure to determine the percentage of weight gained over the 5-week treatment for each fragment (Kenkel, *et al.*, 2013).

Statistics

In this experiment, an ANOVA statistical analysis was chosen to test differences in percent growth between the inshore and offshore site, between the heated and control treatment, and the interaction between the inshore and offshore and the heated and control. With an ANOVA, compared to a student's t-test, the plausibility of the null hypothesis is examined with a single statistical test run. A Two-way ANOVA with replication was used since there were two independent factors (Inshore/Offshore vs. Control/Heated).

PCR

A 108 mL solution of Guanidium was made with 50 mL H20, 50g Guanidium isothiocyanate, 5.3mL Tris (1 M, pH 7.6), and 2.125mL EDTA (0.5M). The solution was warmed for 10 minutes at 60-70˚C and dissolved by stirring. Then, 2.12g of Sarkosy and 1.05mL B-mercaptoethanol was added. The solution was adjusted to 106mL with distilled H_20 and preserved at 4˚C. The 1-3mm^3 coral fragments were obtained from the freezer and for each fragment, a small amount of ecto- and endoderm was scraped off with a sterilized razor blade. The products were placed in a tube and 400µl of Guanidium solution was added. The tubes were vortexed (until the color became brown-like because of the zooxanthellae) and heated for 10 minutes at 72˚C and vortexes 3-4 times while heated. The tubes were centrifuged for 5 minutes at maximum speed and 200µl of the supernatant was placed in a new 1.5ml collection tube. In the collection tubes, 200µl of isoproponal was added, and then the tubes were vortexed and placed at -20˚C for at least 3 hours (or typically overnight). The tubes were then centrifuged for 15 minutes at maximum speed.

With a slim (pointed) Pasteur pipette or pipette tip, all of the supernatant was removed. Once removed, 100µl of 70% EtOH was added and the tubes were vortexed and centrifuged for 10 minutes at maximum speed. All the supernatant was once again removed and the tubes were dried completely. Then 50µl of super-distilled water (or Tris 0.1M, pH 8 is even better) was added and the tubes were placed on ice for and hour while gently vortexed every 15 minutes. The DNA was stored at -20˚C until ready for PCR. A master mix was created (for 1 reaction) with 12.5µl of biomix red, 1µL of forward primer at 10µM, 1µL of reverse primer at 10 uM, 8.5 uL of water to create 23 uL, and 2µL of DNA template. In the PCR machine, the DNA was at 94˚C for 5 minutes, 10 cycles: 94˚C for 30 s, 50˚C for 30 s and 72˚C for 2 minutes, and 30 cycles: 94˚C for 45 s, 55˚C for 45 s, and 71˚C for 1 minute.

Gel Electrophoresis

A gel was created with 1% buffer: 1.7g of agarose and 170 mL of 1 x TBE. The solution was heated in a microwave in 30 sec intervals until it was transparent, then cooled until the glass could be touched for an extended period of time, then poured into the gel plate with the comb in place. A gel electrophoresis was run for all 35 coral samples and subsequent PCR and gel electrophoresis was run for the DNA samples that did not appear in the first gel viewing.

USB ExoSAP-IT PCR Product Cleanup

A mix of 5µl of post-PCR reaction product and 2µl go Exo-SAP-IT reagent was combined to create a 7µl reaction volume. The solution was incubated at 37˚C for 15 minutes to degrade remaining primers and nucleotides and incubated at 80˚C for 15 minutes to inactivate ExoSAP-IT reagent. The PCR products were treated then stored at -20˚C until sent for DNA sequencing.

The PCR results have just been received and are in the process of analysis. The results of the PCR will be present in my presentation during the competition.

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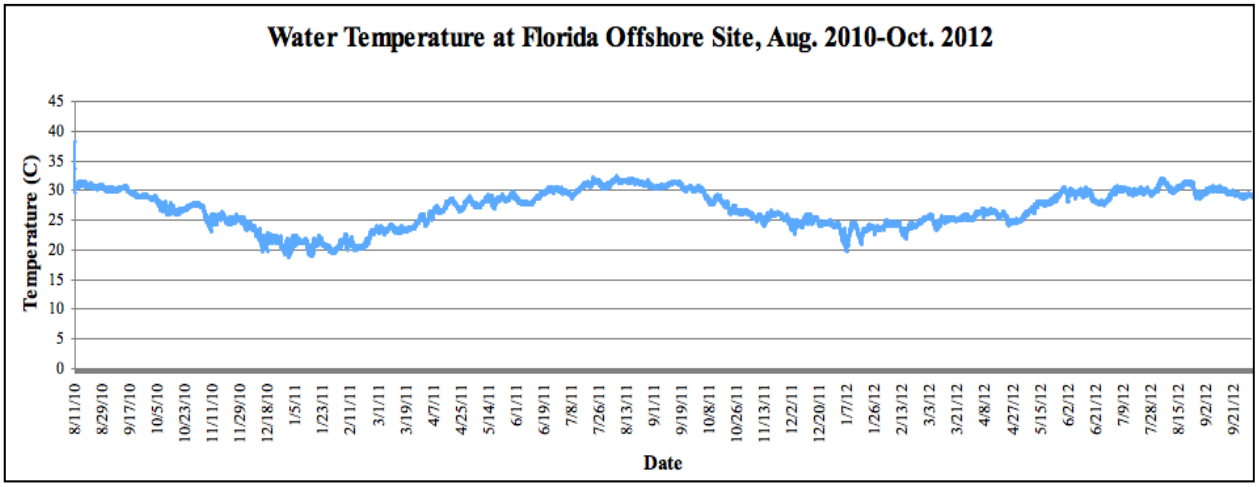
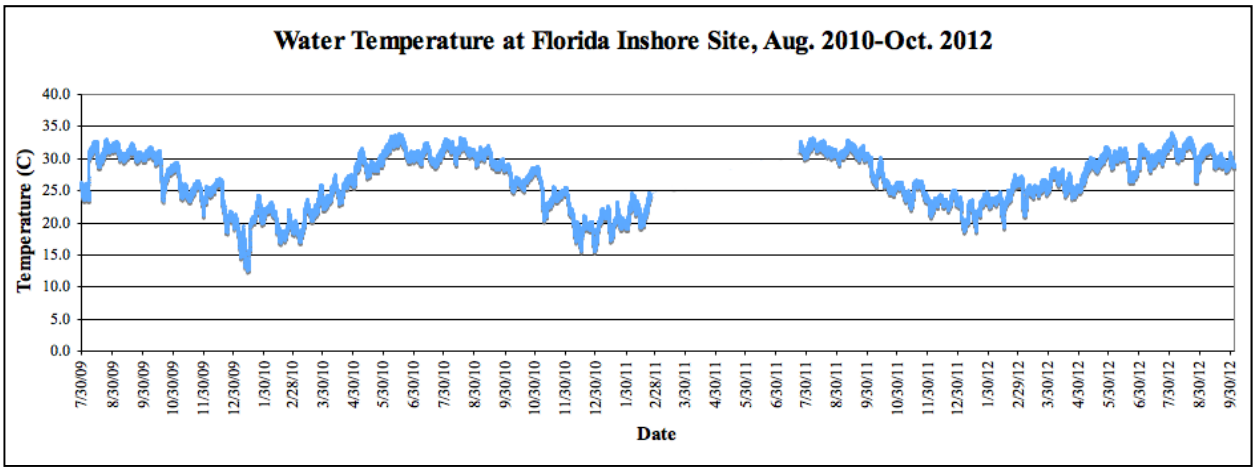
statistical test run. A Two-way ANOVA with replication was used since there were two independent factors (Inshore/Offshore vs. Control/Heated).

Data/Results

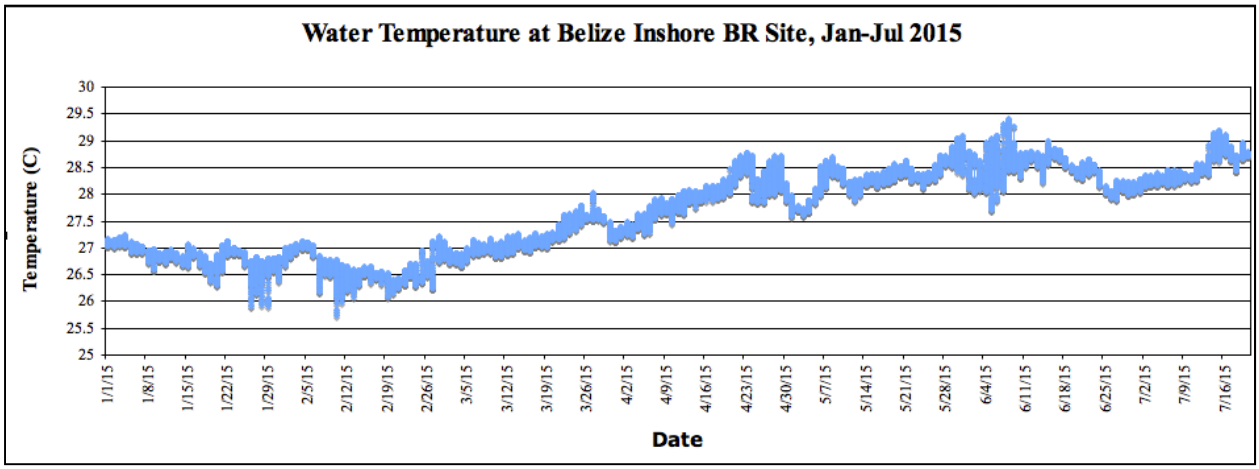
(a)



(b)



(c)



(d)

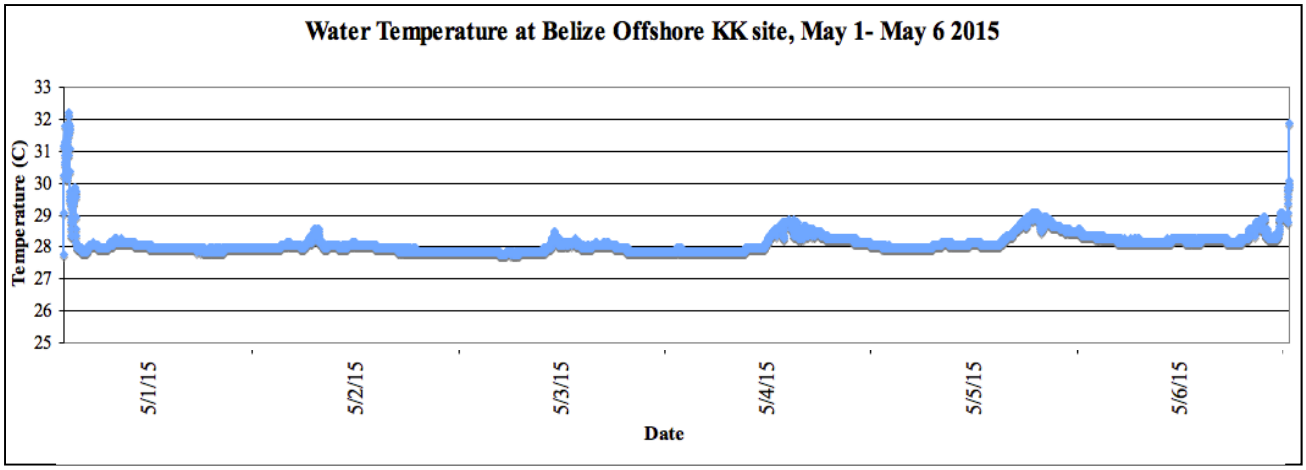


*Figure 5.*Water Temperature at Florida and Belize

(a) At the offshore location in the Florida Keys, there is low temperature variability. Over the course of two years the temperature was relatively maintained between 20-30˚C, with a few circumstances where temperatures were slightly above the 30˚C mark. The overall average temperature was 26.9˚C, the maximum temperature was 38.2˚C, the minimum was 18.7 ˚C, the range was 19.5˚C, and the rate of change was 0.0248˚C/day. (b) At the inshore location, there is high temperature variability. There was a break in temperature collection between Feb 2011 and August 11 that causes the gap in the graph. Over the course of two years the temperature was relatively maintained between 20-30˚C and also had a few circumstances where temperatures were slightly above the 30˚C mark. The overall average temperature was 26.6˚C, the maximum temperature was 34.0˚C, the minimum was 15.5 ˚C, the range was 18.5˚C, and the rate of change was 0.0235˚C/day. Although the temperature range of the inshore site was smaller than that of the offshore site long-term, there was greater fluctuation short-term. (c) At the Belize KK offshore location, over the course of seven months the temperature increased from approximately 27˚C to 29.5˚C, with a few circumstances where temperatures would decrease. The overall average temperature was 27.6454˚C, the maximum temperature was 29.414˚C, the minimum was 25.744 ˚C, the range was 3.67˚C, and the rate of change was 0.0177˚C/day. (d) At the Belize backreef offshore location, over the course of four days the temperature was relatively stable, however, there were massive spikes (May-5 7:44-17:49 and May-05 7:32-17:27) in the temperature that went beyond 31˚C, which is above the a coral's thermal threshold. The overall average temperature was 28.31388˚C, the maximum temperature was 31.472˚C, the minimum was 27.272 ˚C, the range was 4.2˚C, and the rate of change was 0.084˚C/day.

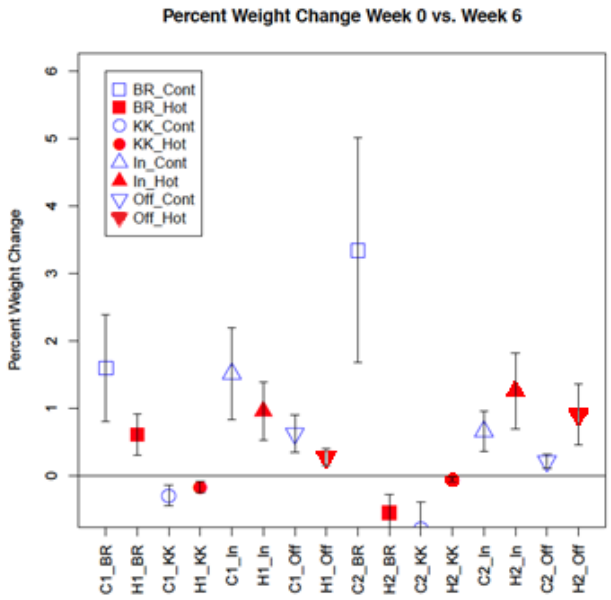


Figure 6. Percent Weight Change Week 0. Vs. Week 6

In general, the heated coral (Red; H1 and H2) had decreased growth in contrast to the control coral (Blue; C1 and C2). The Belize backreef samples, in both tank 1 and tank 2, are an example that is consistent with this trend. The coral samples from the less thermally variable habitats (KK and Off) grew less overall than the coral from the more thermally variable habitats (BR and In). The KK and Off coral in the control treatment did not grow as well and generally did not appear to be able to survive basic control treatment conditions. The In and Off coral in the tank 2 treatments showed variable responses with no observable trend to draw a conclusion. A majority of fragments showed positive percent weight change, however, four of the samples showed a negative percent weight change. The fragments that showed negative percent weight change most likely occurred due to an error in the buoyant weight measuring procedure.

Belize ANOVA: Two-Factor With Replication

SUMMARY	BR	KK	Total
Heated			
Count	8	8	16
Sum	0.1554275	-1.00541489	-0.849987
Average	0.0194284	-0.12567686	-0.053124
Variance	0.8801712	1.244110513	0.996946
Control			
Count	8	8	16
Sum	18.889782	-4.42699567	14.46279
Average	2.3612228	-0.55337446	0.903924
Variance	4.9369836	0.75118374	4.919779
Total			
Count	16	16	
Sum	19.04521	-5.43241056	
Average	1.1903256	-0.33952566	
Variance	4.1770724	0.97991738	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Inshore vs. Offshore	7.3275327	1	7.327533	3.75172122	**0.06290279***	4.19597
Heated vs. Control	18.723559	1	18.72356	9.58652481	**0.0044214****	4.19597
Interaction	15.340171	1	15.34017	7.85421889	**0.0090993****	4.19597
Within	54.687143	28	1.953112			
Total	96.078407	31				

Table 1. Belize ANOVA: Two-factor With Replication

* Approaching Significance
** Statistically Significant ($p < 0.05$)

Between the inshore (BR) and offshore (KK) site, the p-value is 0.06 which means that it is approaching statistical significance. This indicates that while although there is not a true significant difference of the corals bleaching levels between the inshore and offshore site, it suggests that there was an observable variation. For the treatment variable, there was a significant difference between the coral's response in the heated treatment compared to that of the control treatment with a p-value of 0.004. Lastly, the interaction of the two variables, inshore/offshore and heated/control, had a p-value of 0.009. This suggests that the coral

fragments from the varying thermal environments showed differential thermotolerance when in the control and heated treatment.

Florida ANOVA: Two-Factor With Replication

SUMMARY	Inshore	Offshore	Total
Heated			
Count	5	5	10
Sum	4.73252	1.35923	6.09174
Average	0.9465	0.27185	0.60917
Variance	0.27531	0.21972	0.34645
Control			
Count	5	5	10
Sum	7.33618	3.08802	10.4242
Average	1.46724	0.6176	1.04242
Variance	2.5929	0.23894	1.45911
Total			
Count	10	10	
Sum	12.0687	4.44725	
Average	1.20687	0.44473	
Variance	1.35009	0.23706	

ANOVA

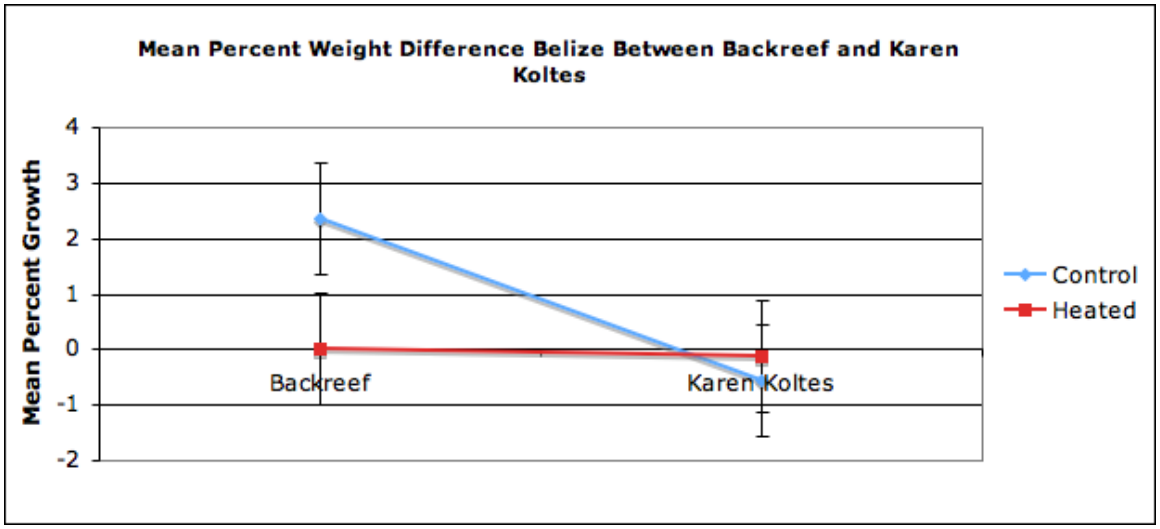
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Inshore vs. Offshore	0.93851	1	0.93851	1.1284	0.30389	4.494
Heated vs. Control	2.90432	1	2.90432	3.49196	**0.080088***	4.494
Interaction	0.03827	1	0.03827	0.04601	0.83286	4.494
Within	13.3075	16	0.83172			
Total	17.1886	19				

Table 2. Florida ANOVA: Two-Factor With Replication

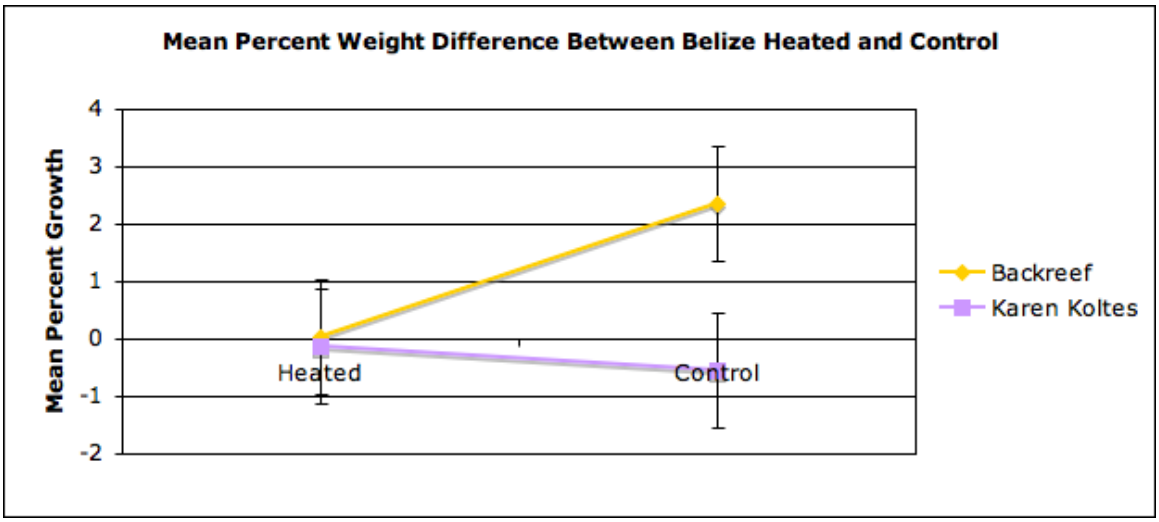
* Approaching Significance
** Statistically Significant ($p < 0.05$)

Out of the three sources of variation only one source was even approaching a significant result. The p-value for the variation between the heated and control treatment is 0.08. This indicates while although the difference was not significant, there was a reasonable difference in the coral's thermotolerance in the heated tanks in comparison to the control tanks.

(a)


Mean Percent Weight Difference Belize Between Backreef and Karen Koltes
Mean Percent Growth
4
3
2
1
0
-1
-2
Backreef
Karen Koltes
Control
Heated

(b)


Mean Percent Weight Difference Between Belize Heated and Control
Mean Percent Growth
4
3
2
1
0
-1
-2
Heated
Control
Backreef
Karen Koltes

(c)

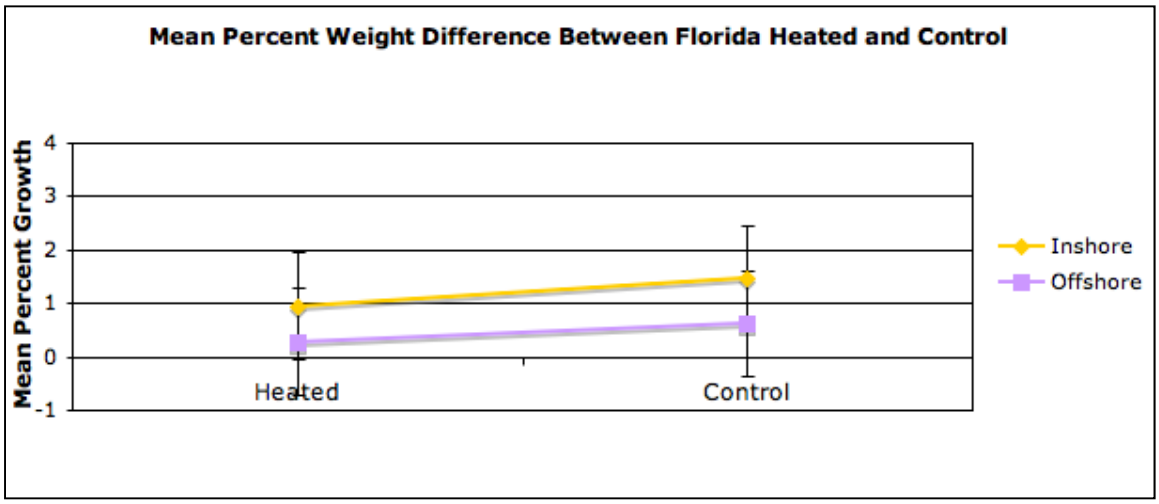

Mean Percent Weight Difference Between Florida Heated and Control
Mean Percent Growth
4
3
2
1
0
-1
Heated
Control
Inshore
Offshore

Figure 7. (a) Mean percent weight difference between Belize BR and KK sites (b) mean weight difference between Belize heated and control (c) Mean percent weight difference between Florida heated and control of just heated tank 1 and control tank 1. The Florida samples from heated and control tank 2 were not included since they were in questionable condition at the start of the experiment.

Simple comparison graphs were created with the variables that showed significant or approaching significant p-values. Figures 7a and7b indicate that KK did not grow as well as BR. The BR inshore site did significantly better than the KK offshore site, even in the case of additional heat stress. Overall, the Belize control coral tanks grew significantly more than the heated coral tanks. This is to be expected since even the slightest increase in temperature can cause stress to coral, especially after going through the sampling process. There was a significant difference, a p-value of 0.009, in the interaction between the two sets of independent variables. As indicated by the mean percent weight difference between Florida heated and control treatment approaching significance, Figure 7c supports that the inshore location had a greater mean percent growth in both the heated and the control treatments.

Conclusion/Discussion

As a whole, coral from a variable habitat in Belize and a variable habitat in Florida, separated by over 1100 km, show consistently greater tolerance of heat and treatment stress than coral from less variable habitats <5km away from each site. In other words, temperature variability of the local habitat may predict a coral's response to heat much better than even latitude, nationality, or other location factors.

The varying ranges of temperature for the inshore and offshore locations in both Florida and Belize were the projected reason for the varying levels of the coral's thermotolerance. The inshore site at both locations had more variability in temperature day-to-day while the offshore site at both locations had more moderate fluctuations. The BR and Inshore sites had greater daily variability in temperature; at the BR site there were even routine spikes of temperature of about 31˚C -32˚C which is above the coral thermal threshold. The difference in temperature range was

hypothesized to be the indicator that predicts how well a coral would respond to heat stress. In general, the data supported that the coral samples from the less variable environments from both locations grew much less overall than the more variable environments.

The two ANOVAs that were run tested the difference between the inshore and offshore sites and the heated and control treatments and their interaction in both Florida and Belize. The statistically significant and approaching significant results were put into graphs (7a-7c). For the Florida location, the fact that the inshore location had a higher mean percent growth in both the heated and control treatments indicates that the coral from the inshore site were better adapted to both regular conditions and to heat stress conditions. For the Belize site, the interactions between the inshore and offshore and heated and control coral were significant. This demonstrates that there is a variation in heat tolerance between the different locations. This is consistent with the hypothesis that coral from an inshore environment—exposed to a greater variability in temperatures each day—will grow better than coral from an offshore environment. This interaction is most pertinent to supporting the hypothesis of this experiment.

Thus, coral species from more thermally fluctuating environments may have more adaptive qualities such as possession of clade D, or constitutive frontloading, which allow them to better withstand heat stress. The samples that did not grow very well, the KK and Off corals in the tank 2 treatment, may have been because they have weaker bleaching resistance under heat stress and a stronger response to even the minimal stresses that are associated with tanks in general. Additionally, the In and Off corals that had variable responses may have been due to the fact that the nubbins themselves were in questionable condition before the experiment even began.

This experiment should be repeated with modifications that would result in more accurate and potentially more significant results. The experiment should be run for a longer period of time; a larger sample size should be used; the sampling method of the coral could be changed to impose less stress on the coral; another location to collect the coral could be used to reduce process and handling and stress during transportation; and an alternate method could be used to find the weight of the coral since the buoyant weighting method used in this experiment left a lot of room for human error. With these alterations, the hypothesis that coral from varying thermal environments will have different bleaching levels when exposed to heat stress in the experiment could be better supported. This is crucial in potentially using the information gathered to either

reproduce coral with better ability to withstand heat, or to use the heat-resilient characteristics of some coral to assist those that are vulnerable to rising temperatures and prevent bleaching.

Along with being a crucial component to the marine ecosystems, coral reefs support a variety of human needs. They are vital for fisheries, tourism, shoreline protection, and yield compounds that are important for the development of new medicines. At least 500 million people rely on coral reefs for food, coastal protection and income. Coral reefs provide goods and services such as snorkeling, scuba diving, beach protection, fishing, etc. Coral are natural barriers that protect shorelines from eroding, thus protecting coastal buildings, agricultural land and beaches. Since coral reefs are the home to millions of fish, fisheries greatly depend on reefs as their source of money. Lastly, coral reefs have been used in various treatments such as cancer, HIV, cardiovascular diseases, ulcers and other ailments. Consequently, coral reefs are integral to the health of the planet and thus require large-scale protection. This experiment marks a beginning in figuring out just how we can accomplish that.

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