EFFECTS OF HEAT STRESS ON SURVIVAL AND PRODUCTIVITY OF POCILLOPORA ACUTA CORAL

(Climate change: It's not so "acuta" when corals bleach)

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I. Abstract

Due to global climate change, atmospheric and oceanic temperatures have risen dramatically, and are expected to continue increasing. Ocean warming disproportionately impacts species with a narrow range of thermotolerance, such as corals. Tropical corals are especially vulnerable to increasing ocean temperatures due to their unique symbiotic relationship with the photosynthetic algae, *Symbiodiniaceae,* which is disrupted under extreme heat events. The species *Pocillopora acuta*, a thin branching species of coral, is known to exhibit a wide range of thermotolerance. However, it is unknown whether *P. acuta*'s thermotolerance is dictated by genomic differences, the species of symbiont hosted, or a combination of these factors. To investigate this, we conducted a lab-based heat challenge experiment over ten days. Genetically identical *P. acuta* individuals were either held constant at 26° C in a control treatment or temperature increased by 1° C each day from 26° C to 34° C in a heat challenge treatment. We observed that only certain *P. acuta* individuals completely bleached under heat stress while others remained resistant. In our heat challenge treatment, we observed decreased levels of photosynthetic efficiency via pulse amplitude modulation (PAM) and decreased color intensity, signifying bleaching. However, some individuals were more negatively affected than others. These findings suggest that genetic advantages or differences in symbiont communities could allow some *P. acuta* corals to be more resistant to extreme temperatures. Future work investigating symbiont genotypes could provide more insight into what makes some corals more resistant to heat stress. These findings could aid in informing coral conservation decisions in the face of global climate change.

II. Introduction

Tropical coral reefs are complex ecosystems that host a significant amount of the ocean's biodiversity. While coral reefs cover less than 1% of the ocean floor, they are estimated to support 25% of all known marine life (Knowlton *et al.* 2010). Coral reefs are also extremely economically valuable–the value of the world's coral reefs have been estimated at bringing coastal communities $375 billion dollars each year in tourism, goods, and services (Spalding *et al.* 2017). It is estimated that there are over 800 unique species of reef-building corals, each filling an important niche in the ecosystem (Knowlton *et al.* 2010). However, reef-building corals are under increasing threat due to climate change. Today CO_2 levels are 414 ppm (NOAA 2022), which is about 50% higher than pre-industrial levels, which were 278 ppm (Keeling *et al.* 2005). These high CO_2 levels in the atmosphere create a stronger greenhouse effect, causing air and water temperatures to increase. In addition, around 30% of global CO_2 is absorbed by the ocean, causing ocean water to acidify and creating suboptimal conditions for corals (Hönisch *et al.* 2012). As ocean acidification and temperatures continue to increase, they can cause harmful effects on tropical coral species and predicting how corals will respond remains a top research priority.

Coral symbiosis is a relationship between a coral host and a microbial algae from the genus *Symbiodinium.* The coral host provides shelter and micronutrients for its symbionts and the endosymbiotic dinoflagellate algae supplies the host with carbon sugars through photosynthesis (McIlroy *et al.* 2022). This relationship is essential to the health

and survival of most corals, but can break down under external conditions of stress such as extreme heat and excessive light, which leads to algal cell death or expulsion (Douglas, 2003). While most corals host symbionts, not all symbionts affect the host equally. There are four major clades of symbionts, and each clade is thought to facilitate certain advantages or disadvantages in its host coral. *Durusdinium sp.*, for example, is commonly considered to be the most thermotolerant symbiont strain, but in turn, is thought to provide fewer nutrients to its coral host (Ros *et al.* 2021). Understanding what corals host what symbiont type, and why, is a key component in understanding coral resilience.

Pocillopora acuta is a species of branching, reef-building coral found throughout the Central Pacific and Indian Ocean which relies on its symbiotic relationship with algal species in the genus Symbiodiniaceae to gain nutrients (Poquita-Du *et al.* 2020). *Pocillopora acuta* is able to host multiple Symbiodiniaceae strains, but most commonly hosts *Durusdinium* (Ros *et al.* 2021). It is also possible for *P. acuta* to shift, or "shuffle" the type of Symbiodiniaceae it hosts, presumably in response to environmental changes, such as heat stress (Ros *et al.* 2021). *Pocillopora acuta* has an optimal temperature range of approximately 25-26°C; however, previous studies have found that their thermotolerance can vary. For example, in Southern Taiwan, *P. acuta* specimens survived a nine month experimental exposure of 30°C and bleaching was observed (Mayfield *et al.* 2018). Likewise, another study found that during the 2016 mass-bleaching event in the Great Barrier Reef, ten *P. acuta* colonies displayed no signs of bleaching when other coral species on the same reef experienced widespread mortality (Epstein *et al.* 2019), highlighting the variation in bleaching tolerance within this species. While we know that symbiont type and host genetic differences play a role in determining *P. acuta* resilience, the factors that determine this thermotolerance remain largely unknown.

Here, we compared the physiology of individual *P. acuta* colonies that host putatively different algal symbionts under control temperatures (26 °C) relative to the same individuals under simulated heat challenge. Corals were monitored for survival, photosynthetic efficiency via pulse amplitude modulation (PAM), and coral color through photo analysis as a proxy for bleaching intensity. We expect that individuals in our heat stress treatment will experience higher levels of bleaching and mortality, and lower photosynthetic efficiency by the end of the experiment. We also expect to see certain individuals show more resilience under extreme temperatures, indicating that their genes, symbionts, or a combination of the two, are allowing *P. acuta* to better withstand heat stress. *P. acuta* is ecologically important because it builds structure and adds diversity to coral reefs, and it is frequently used as a model organism to better understand the impacts of climate change on reef systems. Understanding *P. acuta's* thermotolerance range is vital to predict how the coral will react to rapid temperature change and high temperatures in the future. In addition, learning more about *P. acuta's* sensitivity to heat stress could help determine whether to focus conservation efforts on this species or to conduct further study on its resilience.

III. Methods

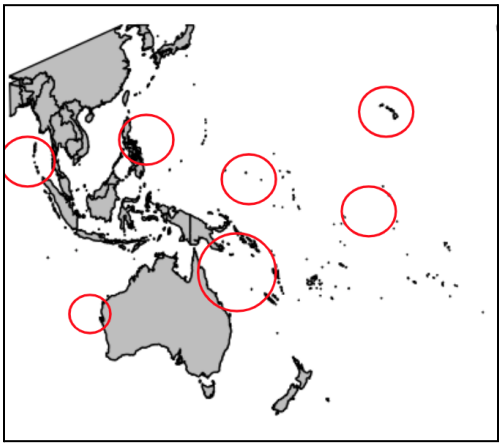


Figure 1. Global geographic distribution of *Pocillopora acuta* throughout the Indo-Pacific Ocean. Red circles indicate known areas of *P. acuta* locality (Schmidt-Roach *et al.* 2014).

Experimental design

Two experimental systems were each maintained at one of two temperature treatments: 1) Control conditions maintained a temperature of 26° C, and 2) a heat challenge treatment in which the temperature was increased by 1° C every day over 9 days from 26-34° C and then held constant at 34 °C on days 9 and 10. Each system contained three separate tanks. Temperature and salinity for each tank were recorded three times daily for the duration of the experiment. Salinity was measured using a refractometer with a target range of 34-35 ppt, and temperature was measured using a mercury thermometer. All tanks experienced a 12:12-hour light-dark cycle with a light intensity of 120-130 photosynthetically active radiation units (PAR). Each tank contained a powerhead to facilitate water flow. Flow conditions were identical across tanks.

Three coral fragments from each of six genotypes of *Pocillopora acuta* were acquired through the aquarium trade and were acclimated long-term in Boston University's aquarium facility. One fragment of each genotype was assigned to each treatment for a total of N=6/treatment (N=12 fragments total). In addition, a third fragment from each genotype was flash frozen in liquid Nitrogen prior to the experiment for downstream genomic and protein analyses. Fragments were color-coded via zip ties and glued to stands. A total of two fragments were placed in each tank. Fragments were rotated daily around 10:00 am to ensure that each fragment was experiencing identical conditions within each tank. Survival of all fragments was monitored and recorded in the morning during temperature ramping. Corals were not fed for the duration of the experiment.

Pulse Amplitude Modulation (PAM)

To measure algal photosynthetic efficiency of photosystem II (Fv/Fm), pulse amplitude modulation (PAM) was measured using a Walz Junior PAM. These measurements were taken for each experimental fragment six times during the experiment (days 2, 3, 6, 8, 9, and 10). Corals were dark acclimated for one hour prior to PAM measurement being taken. Measurements were taken from random locations on each fragment until three measurements were obtained within 0.05 Fv/Fm of each other.

Photo Analysis

Photos of each coral fragment were taken via an iPhone model 13 on days 1, 4, 7, and 10. The heat challenge temperature for these days was 26° C, 29° C, 32° C, and 34° C, respectively. Each fragment was placed on a CORAL WATCH Coral Health Chart card inside a black lightbox. Photos were white-balanced in Adobe Photoshop to control for lighting differences across photos. Photos were then analyzed in Matlab (version R2022b) to extract Red, Blue, and Green (RGB) values. The program for value extraction, "Analyze Intensity Macro", was provided by Winters *et al.* (2009). Ten random points were taken from the coral in each photo to calculate RGB. In Rstudio, RGB values were inverted, meaning a lower red channel value (R) indicates lower color intensity, suggestive of coral bleaching.

Statistical Analysis

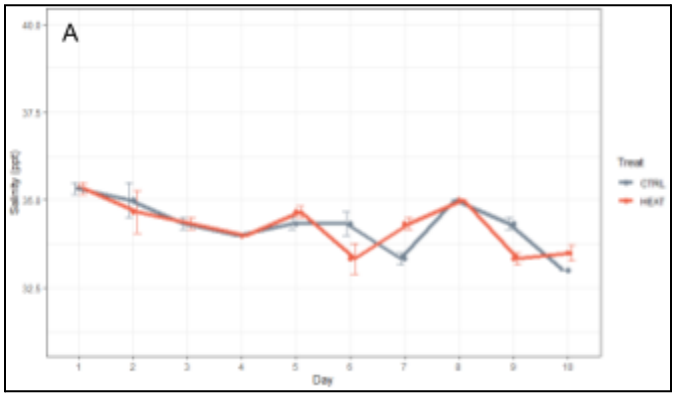
PAM data was analyzed using ANOVA and Tukey Post-HOC test. Changes in coral color intensity were analyzed using a paired T-test. Percent change in R channel intensity for each heat stressed fragment was calculated. Statistical analyses were performed in RStudio (version 2022.07.01+554) or VassarStats.com.

IV. Results

Water Quality

Temperature of the control tank ranged from 24° C to 26° C, with an average temperature of 25.67° C across all 10 days (Figure 2b). The heat treatment tank temperature ranged from 24° C to 34° C, with temperature increasing by approximately 1° C each day (Figure 2b). Salinity in the control tank ranged from 33 ppt to 37 ppt with an average salinity of

34.30 ppt, and the heat treatment salinity ranged from 32 to 37 ppt with an average salinity of 34.25 ppt (Figure 2a). Salinity did not differ between control and heat treatments (p=0.639).



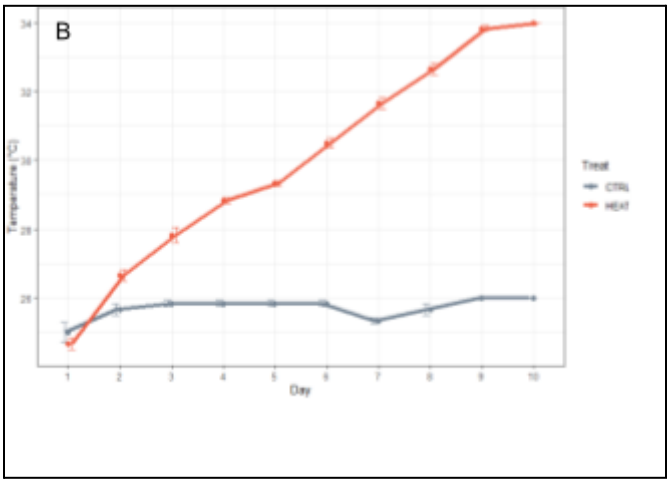


Figure 2. (A) Average salinity in control (26° C) and heat (26-34° C) treatment tanks over the 10-day experiment. Salinity was measured via refractometer three times per day and averaged. Error bars represent the standard error of the mean. **(B) Average temperature in control (26° C) and heat (26-34° C) treatment tanks over the 10-day experiment.** Temperature was taken via mercury thermometer three times per day and averaged. The heat control tank was increased 1 °C each day from days 2-9 and held constant at 34° C on day 10. Error bars represent the standard error of the mean (± SE).

<u>Photosynthetic efficiency</u>

Photosynthetic efficiency (Fv/Fm) was measured 6 times throughout the experiment. Fv/Fm values of the control corals increased slightly throughout the experiment (Figure 3). Fv/Fm values of the heat challenge corals decreased dramatically after day 5, corresponding with the temperature increasing to 29° C (Figure 3). This data, represented by genet in Figure 4, shows the same trend. From days 1-5, Fv/Fm values were similar regardless of treatment. After day 5, when the temperature increased to 29°C, Fv/Fm values started to decrease for the corals in the heat tanks (Figure 4). Specifically, genotypes ORA-PG and P have the lowest values on day 7. On day 9, where the temperature was held at 34°C, genotype ECA-H yielded very low Fv/Fm values, corresponding to Table 1, where it showed symptoms of bleaching. On day 10, there was an overall decrease in Fv/Fm values in the corals but the ones that displayed the most variance were genotypes P, ECA-H, ECG, and ORA-PG (Figure 4). This trend can also be seen in Figure 5, which displays the difference in the average Fv/Fm values for each individual between the control and heat tanks. Figure 6 looks specifically at the average Fv/Fm values of the genotypes on day 10. The gray lines pair the same

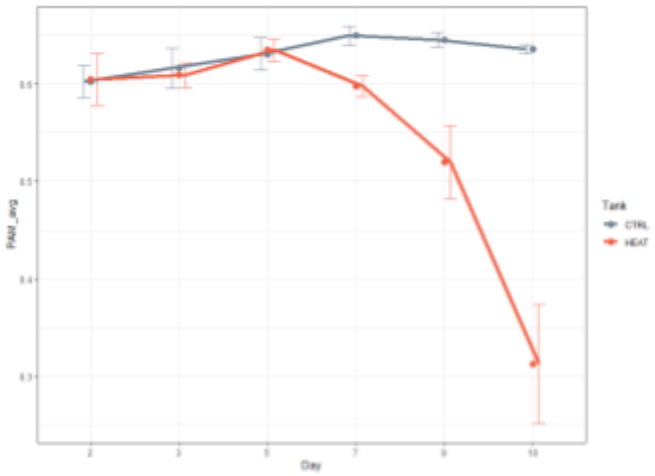


Figure 3. PAM (Photosynthetic yield) of *Pocillopora acuta* decreased in the heat challenge over 10 days. The average of heat challenged *P. acuta's* photosynthetic activity decreased after day 5. Control tank is the gray line and the heat tank is the red line. PAM was performed 6 times. Error bars of Fv/Fm values indicate standard errors of the mean (± SE).

genotypes from the control treatment to the heat challenge to visualize the difference. An ANOVA test revealed a statistically significant difference between the Fv/Fm values of each genotype between control and heat challenge treatments on day 10 of the experiment (p=0.003) (Figure 6). A Tukey post-HOC test yielded the same value (p=0.003) between the two treatments (Figure 6).

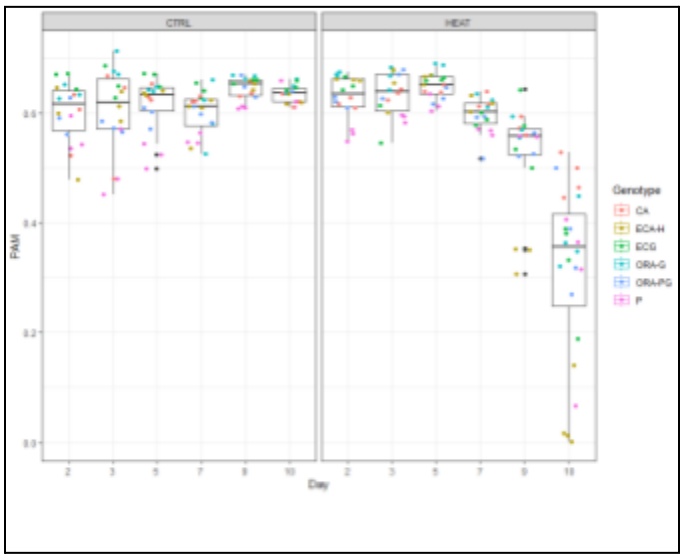


Figure 4. Total PAM (Fv/Fm) values of *Pocillopora acuta* genotype across 10 days. 3 replicate Fv/Fm (Photosynthetic efficiency) values were taken per fragment on days 2, 3, 5, 7, 9, and 10. Colored points represent different genotypes. CA is red, ECA-H is yellow, ECG is green, ORA-G is light blue, ORA-PG is blue, and P is pink. Black points represent outliers. Over 10 days, Fv/Fm values decrease in the heat challenge while the control values remain consistent. Boxplots of Fv/Fm values show the distribution of the data with error bars indicating standard errors of the mean (± SE).

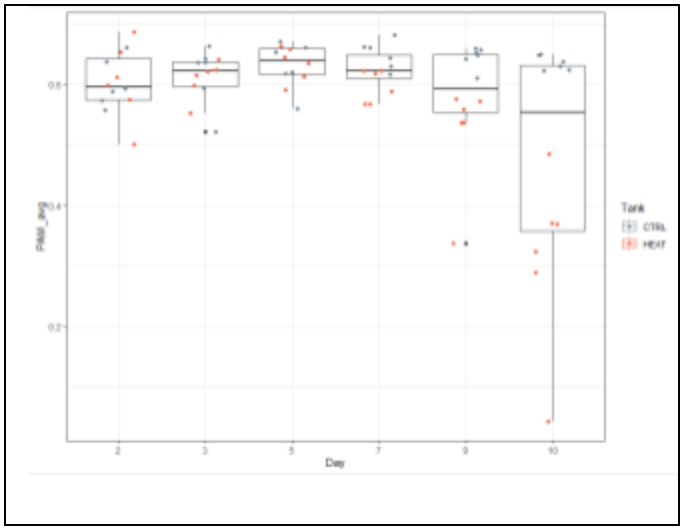


Figure 5. PAM (Fv/Fm) average of *Pocillopora acuta* in control and heat challenge over the course of 10 days. Fv/Fm (Photosynthetic efficiency) values were averaged for each genotype in the control (gray points) and heat challenge (red points) over 10 days. Black points represent outliers. Boxplots of Fv/Fm values show the distribution of the data with error bars indicating standard errors of the mean (± SE).

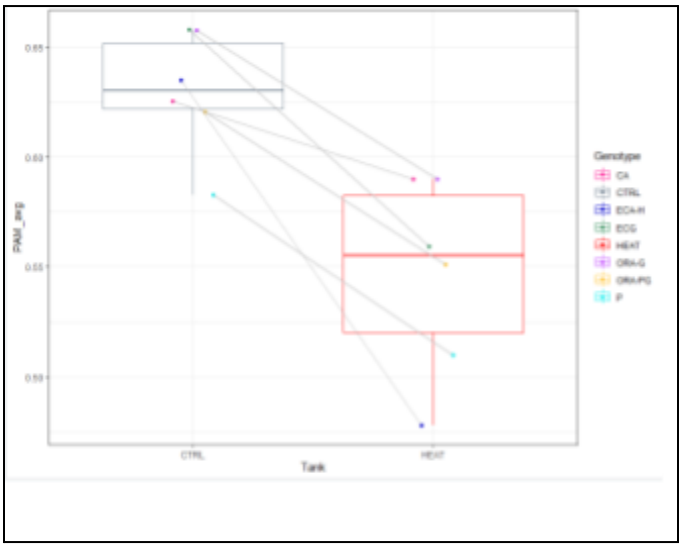


Figure 6. PAM (Fv/Fm) average differences between *Pocillopora acuta* genotypes in control and heat challenge treatments on day 10. Three replicate Fv/Fm values for each fragment were averaged. Light gray lines pair genetically identical fragments from the control treatment on day 10 to the heat challenge on day 10. Each colored point represents a specific genotype. Pink is CA, royal blue is ECA-H, green is ECG, purple is ORA-G, and yellow is P. Boxplots show the distribution of the data with error bars indicating standard errors of the mean (± SE).

Color Intensity

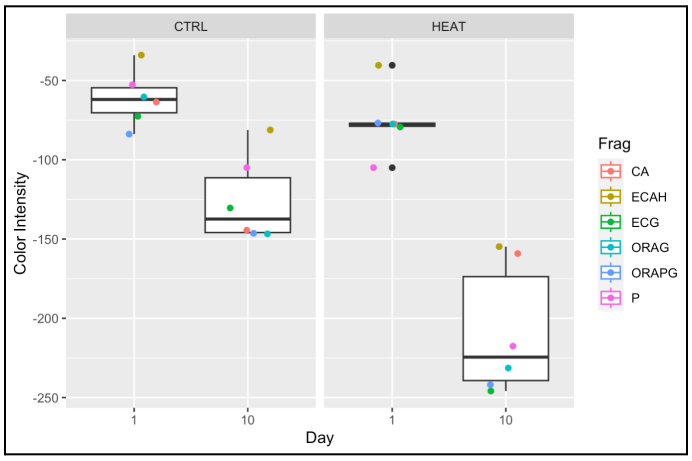


Figure 7. Change in red channel intensity (R value) of *Pocillopora acuta* control and heat stressed fragments on the first and final days of the experiment. Photographs of each *P. acuta* fragment were taken on days 1 (first experimental day) and 10 (final experimental day). A lower color intensity indicates fewer symbionts present in the fragment's tissue. Photographs were analyzed for red channel intensity using Adobe Photoshop and Matlab. Colored points correspond to the coral genotype. Black points represent outliers in the data. Error bars represent the standard error of the mean (± SE).

Color intensity (R values) for both control and heat challenge treatments on day 1 of the experiment did not differ ($p=0.059$). On day 10 of the experiment, there was a significant difference between the R values between the control and heat challenge ($p<.0001$). Corals in the control treatment moved from an overall R value of -61.71 (±3.33, N=60) on day 1 to -125.68 (±5.62, N=60) on day 10, whereas heat treatment corals moved from an overall R value of -70.45 (±3.55, N=60) to -208.45 (±6.12, N=60) (Figure 7).

All heat challenged corals experienced an extent of color change recorded on day 10 of the experiment. The lowest percent change in color was genotype CA with a 105% decrease in red channel intensity. Fragment ECA-H had the highest percent change with a 283% decrease in R value.

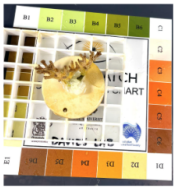
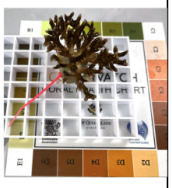
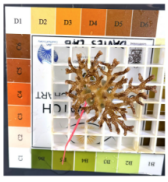
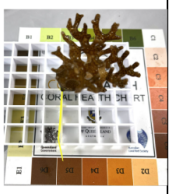
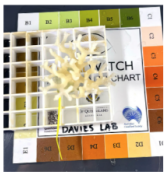
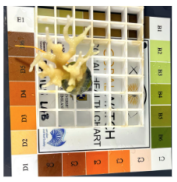
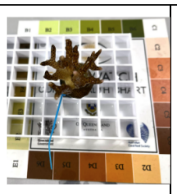
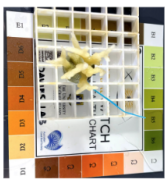
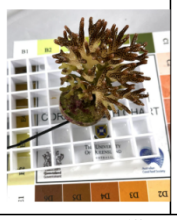
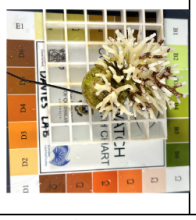
Genotype	Day 1	Day 10	% Decrease in Red channel intensity
CA			105%
ECA-H			283%
ECG			210%
ORA-G			199%
ORA-PG			214%
P			107%

Table 1. Photographs of heat challenged *Pocillopora acuta* with the corresponding genotype and percent decrease in red channel intensity (R value) between days 1 and 10 of the experiment. Photographs have been white-balanced in Adobe Photoshop to correct for lighting differences. Percent change in R value was calculated using the average of 10 random R value points for each photo.

V. Discussion

Pocillopora acuta is a tropical branching coral that is known to have wide variability in thermotolerance (Poquita-Du and Le Goh *et al*, 2020). Understanding resistance and resilience of heat stress in tropical corals is critical to help mitigate the impacts of climate change. It is unknown if the variation in *P. acuta*'s thermotolerance is primarily due to genetic factors within the coral's genome, the symbiotic algae that the coral hosts, or multiple variables. Many publications on *P. acuta* support that corals are most negatively affected by high temperatures as opposed to other variables like acidification. A previous study found that *P. acuta* corals that were exposed to heat exceeding 29.5 °C reproduced fewer and smaller planulae, and recruits had lower photosynthetic efficiency (Fv/Fm) (McRae *et al.* 2021). A different study found that acidification, ocean warming, and a combination of the two did not impact the size or abundance of *P. acuta* larval recruits. However, heating greatly influenced the subsequent survival of the recruits (Bahr *et al.* 2020). In our study we exposed fragments of *P. acuta* to heat stress and found that *P. acuta* corals that experienced heat stress displayed varying degrees of resistance to bleaching.

On day ten, the color intensity of the heat challenged fragments was visibly lower than the control fragments. Lower color intensity, used as a proxy for bleaching intensity, indicates fewer algal symbionts in the tissue of the corals. There was a high level of variability in the color intensity of the fragments (control range: -61.71 to -125.68, heat range: -70.45 to -208.45). Fragments ECG and ORA-PG in the heated system were almost completely bleached on the final day of the experiment. Fragment P had bleached substantially by the end of the experiment and had lost a majority of its tissue. However, our calculated decrease of color intensity for this fragment was one of the lowest in the heat group (107%). This is due to the fact that the points were randomized when doing the color analysis so it is likely that points were picked on the coral where dark tissue remained. Fragments ECA-H and CA had little tissue loss and had the most pigment visible compared to the other fragments within the heat challenge at the end of the experiment. However, due to its high initial color intensity, fragment ECA-H experienced the largest decrease in color intensity (283%) (Table 1). The variability in bleaching responses of these fragments from different genetic sources is supported by a previous publication which concluded that certain colonies are more resistant than others (Poquita-Du and Le Goh *et al*, 2020). Another study that tested thermotolerance in *P. acuta* found that the coral differentially expressed genes in response to heat stress, which contributes to different rates of bleaching (Poquita-Du and Huang *et al,* 2020).

After ten days of the experiment, PAM values of the corals in the heat challenge were significantly lower compared to the corals in the control system, indicating lower symbiont productivity in the heated system. The six fragments in the heated system displayed a large range of symbiont productivity (0.04-0.49 Fv/Fm), while the fragments in the control system had a much smaller range of 0.62-0.65 Fv/Fm. Fragments CA and ORA-G had high PAM values while fragments ECA-H and P had low PAM values on day 10 (Figure 6). Interestingly, while fragment ECA-H had the highest decrease in color intensity and the lowest Fv/Fm value at the end of the experiment. However, looking at its physical characteristics compared to the other bleached individuals, it still displays color and maintained the highest R channel value on day 10 of the heat challenge (Figure 7).

This large range of responses within the heat treatment indicates that there are one or more factors influencing the coral's resistance to heat stress.

We propose that the variation seen among the corals in the heated system is caused by differences in host genetics, symbiont communities, or a combination of the two. Differences within the corals' genome, or gene expression, can influence how it responds to heat stress. The variation in bleaching responses of *P. acuta* across the 10-day heat stress experiment could also be attributed to differences in the symbiont communities hosted by different fragments. The symbionts' role in thermotolerance has been described as a "nugget of hope" in the face of climate change because of its ability to increase the thermotolerance of the host coral. (Berkelmans and van Oppen, 2006). Fragments CA and ECA-H which experienced lower levels of bleaching likely hosted a more thermotolerant symbiont strain, such as *Durusdinium sp.* (Figure 7). Future genotyping of the experimental symbionts will reveal the clade hosted by each fragment. It is also possible that during the heat challenge, symbiont communities could have "shuffled", a phenomenon observed by Ros *et al.* 2021. Another study found that the microbiome of *P. acuta* can adjust to higher temperatures without disrupting physiological stability of the host, as observed in ten colonies that did not bleach during the 2016 mass bleaching event (Epstein *et al*, 2019). It will be interesting to compare the symbiont communities of each genotype from the pre-experimental corals to the post-experimental control and heat challenge corals to see if the makeup of the symbiont communities had changed during the experiment.

Another factor that could be responsible for certain coral's resistance to heat stress is the location from which it was sourced. A previous study found that corals from areas with higher seasonal temperature fluctuation are more resistant to bleaching than corals from areas with little temperature variation due to acclimation to these conditions. Corals from backreef regions, which experience higher average temperatures, more temperature variation, and greater sedimentation, were less susceptible to bleaching than corals from the forereef which experience fewer temperature fluctuations (Castillo *et al*, 2012). Overall, it is known that source locations of corals drive gene expression and symbiont behavior in corals. However, our experimental corals have been maintained long-term in the Boston University BUMP Lab and their original locations are unknown.

It is important to note that there were limitations and sources of error in this experiment. On day 2 of the experiment, the coral fragment from genet P in the control tank died from unknown causes and was replaced with another genetically identical fragment. This fragment was re-photographed on day 2, which was used as the "day 1" photo for this individual. It is also important to note that when taking PAM readings, we dark acclimated the corals for 1 hour beforehand. However, at times the door to the facilities was left slightly ajar during this acclimation period. It is unknown whether this had any effect on the PAM values recorded. Finally, there is the limitation of a small sample size. Our experiment involved only 12 total corals, which were gathered from unknown locations.

Overall, we found that the *P. acuta* fragments in the heated system had higher rates of bleaching and had lower photosynthetic productivity. Also, the heated corals bleached at different rates and showed high levels of variation. At the start of the experiment, we flash froze a third fragment from each genotype in liquid nitrogen. Next steps of this study will include genomic and protein analysis of these fragments to investigate whether coral or algal genetics are responsible for the coral's resistance to heat stress, or a combination of these factors. Further research should be conducted on innate immunity in *P. acuta*

to see if certain symbionts inhibit the NF-κB pathway, hindering resistance to bleaching, as suggested in an immunology study (Mansfield & Gilmore, 2019). Awareness of what factors make some corals more resistant and resilient to heat stress is important in understanding how rising ocean temperatures will affect coral reef communities.

VI. References

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