"Bringing the Heat": Thermal stress responses of *Pocillopora damicornis*

Rebecca Campbell, Alexandria Sangermano, Aryanna Volk

Boston University Marine Program, Boston, MA 02215

Abstract

As the rate of climate change accelerates due to anthropogenic global warming, coral reef ecosystems are experiencing greater threats. Elevated ocean temperatures and carbon dioxide concentration pose unprecedented risks to the highly biodiverse ecosystem. While the importance of the study area is on the rise, it is hard to quantify the social, ecological, and economic consequences. The study investigates the heat stress response of *Pocillopora damicornis*, a highly resilient scleractinian coral with a wide geographic range. Specifically, the study observed heat stress effects on photosynthetic efficiency and color. Corals were given one day to acclimate and then split between the control system, which was maintained at 26°C, and the heat treatment system which rose 2°C daily until it reached the desired 32°C threshold. Coral color in the treatment tank was visually changing, but we found no significant difference in either photosynthetic efficiency or in the color analysis. The results of the study indicated no statistical significance in any heat-stressed colony, but *P. damicornis* photosynthetic efficiency and color were variable under extreme 32°C temperature. Ultimately, we concluded that *P. damicornis* was a highly resilient coral with the capability to endure consistent short-term heat stress.

Introduction

Coral reefs, which support thousands of different species, are one of the most biodiverse marine ecosystems on the planet (Roberts *et al.*, 2002). While this highly complex system accounts for nearly one-third of the world's marine species, their importance goes beyond significant species richness and productivity (Roberts *et al.*, 2002; Cunning, *et al*, 2018). Reefs provide social and economic value for coastal communities as they create jobs, sustain recreational uses, and promote a sense of livelihood (Stat *et al.*, 2008). However, the rapid increase in human population size has, directly and indirectly, caused unprecedented degradation to coral reef ecosystems (Moberg *et al.*, 1997). Recent work has established that rampant carbon dioxide emissions associated with fossil fuels are causing temperature increases in the ocean to be the most prominent factor (Hoegh-Guldberg *et al.*, 2007).

The diversity of corals in shallow oligotrophic waters can be largely attributed

to their symbiotic relationships in the genus Symbiodiniaceae,where nutrients are exchanged between the two partners for survival (Stat *et al.*, 2008; Pearse and Muscatine, 1971). Individual coral polyps host many algal cells in specialized structures called symbiosomes, and these algae photosynthesize and provide the corals with carbon sugars (Stat *et al.*, 2008). Rising ocean temperatures disrupt this mutualistic relationship in a process called "coral bleaching" (Hoegh-Guldberg *et al.*, 2007). During bleaching, corals expel their algae and begin to lose their characteristic coloring, and if the stressor the caused the bleaching does not subsist, the coral will die (Hoegh-Guldberg *et al.*, 2007).

Previous studies observing the heat responses of *P. damicornis* have different experimental setups limited by time and remote location of the field study (Wicks *et al.*, 2010). Experimental limitations were also demonstrated by restricting each setup to one tank, posing the issue that any complication could negatively affect the entire experiment. A common procedure in similar studies is to immediately expose corals to heat stress, rather than allow for acclimation and progression of temperature increase in treatment systems (Hill *et al.*, 2014).

In this study we are testing how acute heat stress affects the symbiotic relationship of *Pocillopora damicornis*, which is a common reef-building coral that exhibits fast growth rates (Cunning, *et al.*, 2018). We selected *P. damicornis* as our study coral for a number of reasons. This coral has a wide geographic distribution across the western Indo-Pacific as well as the eastern tropical pacific, which means demonstrates it is exposed to a range of temperatures across this range. As a study subject of many previous heat stress studies, *P. damicornis* has shown to be a good indicator of responses of temperature change and subsequent bleaching. In the present study, *P. damicornis* nubbins will be subjected to a 16 day period of heat stress. The temperature in our treatment system will be increased by 2°C per day until the water temperature reaches 32°C, where the temperature of the water will remain for the remaining duration of the experiment. Our goal is to gain a better understanding of how increased water temperature can affect coral bleaching as global warming becomes a greater threat to all coral reef ecosystems.

Methods

Heat Stress Experimental Design

To investigate the effects of thermal stress on *Pocillopora damicornis,* two coral nubbins were taken from 13 distinct colonies for a total of 26 nubbins. Each colony was randomly assigned to a position in one of three tanks within a closed seawater system and there were two seawater: control and heat stress treatment. Nubbin placement was replicated so that both representatives of each colony would have identical locations in each system, resulting in 13 nubbins in the control system and 13 in the treatment system (Fig. 1).

Temperature (℃) and salinity (ppt) were recorded at 10 AM and 3 PM each day using a glass thermometer and

refractometer. The temperature in the control system was maintained at 26°C, while the heat-stress treatment experienced increases in temperature by 2°C per day from 26℃ to a final temperature of 32°C. After four days the temperature of the treatment system reached 32°C, where it was maintained for the remainder of the 16-day study (Fig. 2B). Salinity levels were maintained between 32 and 35 ppt (Fig. 2A). Light level and flow rate were equalized between all control tanks, with lights remaining on for 12 hours. The nubbins were rotated counterclockwise within each system to normalize light and water flow. A 10% water change was conducted weekly and included the removal of algae from the tanks and nubbin dishes.

Photochemical Efficiency data collection

The photochemical efficiency (Fv/Fm) of the corals' algal symbionts was measured using-- Junior Pulse Amplitude Modulated (Junior-PAM) fluorometry at the beginning (Day 1), middle (Day 10), and end of the study (Day 15). Junior-PAM measurements were taken after all the corals dark-acclimated for one hour and were used to assess bleaching. Photosynthetic efficiency was measured by initial fluorescence (Fo) and final fluorescence (Fm) and calculated where Fv = Fm-Fo. Three values were recorded per nubbin, each with a maximum difference of 0.100 units allowed.

Color change Data collection

Pictures of each nubbin were taken daily for the entirety of the experiment using. A lightbox was set up for each coral nubbin which were placed inside on a Coral Watch card. Adobe Photoshop was used to calibrate the white balance of each photo, ensuring consistent color comparison between photos. Using MatLab, color intensity was analyzed using the procedure outlined in Winters et al., 2009. Ten points were selected on each coral in order to measure RBG (Red/Blue/Green) color values. All values were recorded, but the mean red color value was utilized to represent change.

Statistics

All of the collected data was placed into R for statistical analyses and graphs (R core team, 2016). Single-factor one-way ANOVAs were run to determine any statistical differences in either photochemical efficiency or coral color between the control and heat treatments.

Results

Effects of Thermal Stress on Photosynthetic Efficiency

Both control and treatment coral photosynthetic efficiency had a nonsignificant change ($p = 0.421$) in response to temperature changes. Heat-treated corals generally had higher photosynthetic efficiency than control corals, however, the differences were not statistically significant (Fig. 3A). Photosynthetic efficiency had variable responses between control and heat-treated corals from the same colony (Fig. 3B).

Effects of Thermal Stress on Phenotype

Corals in both the control and heat-stress systems did not show significant ($p = 0.451$) changes in color throughout the experiment. Corals in the treatment system had a decrease in color intensity compared to corals in the control system, however, the variation was nonsignificant (Fig. 4A). When comparing corals from the same colony, the change in color intensity was nonsignificant between the control and treatment corals (Fig. 4B)

Discussion

Effects of Thermal Stress on Photosynthetic Efficiency

Photosynthetic efficiency exhibited variable responses to heat yet showed no statistical significance between the control and heat systems. Both the middle (day 10) and end (day 15) PAM measurement values were taken with the heat treatment at 32°C, and not all *Fv/Fm* values were reduced (Fig. 3). Three heat-treated coral nubbin *Fv/Fm* values unexpectedly increased compared to their corresponding genotype in the control. These genotypes show variability in how *P. damicornis* responded to heat stress and perhaps were more resilient colonies. This would correlate with the P7, P8, and OP genotypes responding higher to stressors beyond the heat variable. The *P. damicornis* heat nubbins that experienced a reduction in photosynthetic efficiency over time supported our hypothesis, as we expected to find a decline as a response to the heat stress.

Effects of Thermal Stress on Color

Our results indicate that *P. damicornis* did not have significant color change differences between the control and heat-stress treatment. Corals in both systems experienced decreases in color intensity, signifying a bleaching event occurred (Gates, *et al.,* 1992). However other corals showed an increase in color intensity, indicating an increase in the concentration of symbionts within the nubbins. These results did not support our hypothesis that there would be significant bleaching in the heat-stress system when analyzed against the control corals (Hill, *et al.*, 2008). Bleaching was observed in the control tank with P7, P8, and OP colonies and the source was never determined. This may be due to fluctuations in the desired conditions which are salinity, water flow, and air exposure. However, these characteristics would have also disrupted their corresponding genotype in the heat tank. Colonies P7 and OP also showed signs of bleaching within the heat-stress treatment, however, the decrease in color intensity was not significant when compared to the bleaching that occurred in the P7 and OP colonies within the control. The heat-stress P8 nubbin had an increase in color intensity over the course of the study, indicating that the P8 colony may be a more resilient genotype, with the exception of an error that occurred in the control system.

Limitations and Sources of Error

Bleaching unexpectedly occurred in the control nubbins throughout the study.

Multiple coral nubbins from colony P7 experienced extreme bleaching in the control system. Due to the unknown cause of the bleaching, P7 was removed from the experiment in order to ensure that it would not affect the surrounding nubbins and so as not to cause more P7 nubbins to bleach. Control nubbins from the colonies P8, OP, EG, and PG also experienced extreme bleaching and were replaced by other nubbins from the same colonies.

In order to determine the cause of the unexpected bleaching, light levels were decreased and the hours of light were changed from 12 to 8 hours for both control and treatment systems. Due to the number of nubbins that bleached in the control system, and their location within the control system, the left-most tank was removed. The control system was then drained, taken apart and washed with RO (reverse osmosis) water to combat the continually unexpected bleaching within the control system. The left-most tank was also cleaned and replaced back into the system.

Conclusions

Our findings indicate that *P. damicornis* can survive under short-term heat stress and the coral nubbins exhibited resilience to extreme temperature change. Experimental stressors, namely salinity, water flow, and air exposure, might have influenced the results. It is possible that these factors may have attributed to the bleached control corals. Bleaching occurred more slowly than we expected once the thermal maximum was reached. The daily variability in the desired condition may also imitate natural occurrences. The short-term duration of the experiment made it inherently hard to determine the effects of thermal stress on each colony. For future studies, we would extend the duration of the experiment and assess bleaching under a long-term timescale. A long-term timescale study would allow the study to explore the potential recovery of heat stress corals, especially if the temperature was gradually ramped back down. We would also broaden the scope by examining more genotypes, perhaps in different geographic areas with similar environmental conditions. We infer that if *P. damicornis* would respond differently under long-term heat treatment and would present better assess coral response. Associated studies might also consider improving communication and public conversation about the economic and ecological goods and services that coral reef communities provide.

Acknowledgments

Our team would like to extend our deepest gratitude toward Nicola Kriefall and Sarah Davies. To Justin Scace who graciously provided us with a workplace, and to Julia Hammer for guiding our academic interests. And also to our peers in Marine Physiology and Climate Change who endured the grueling weekend hours.

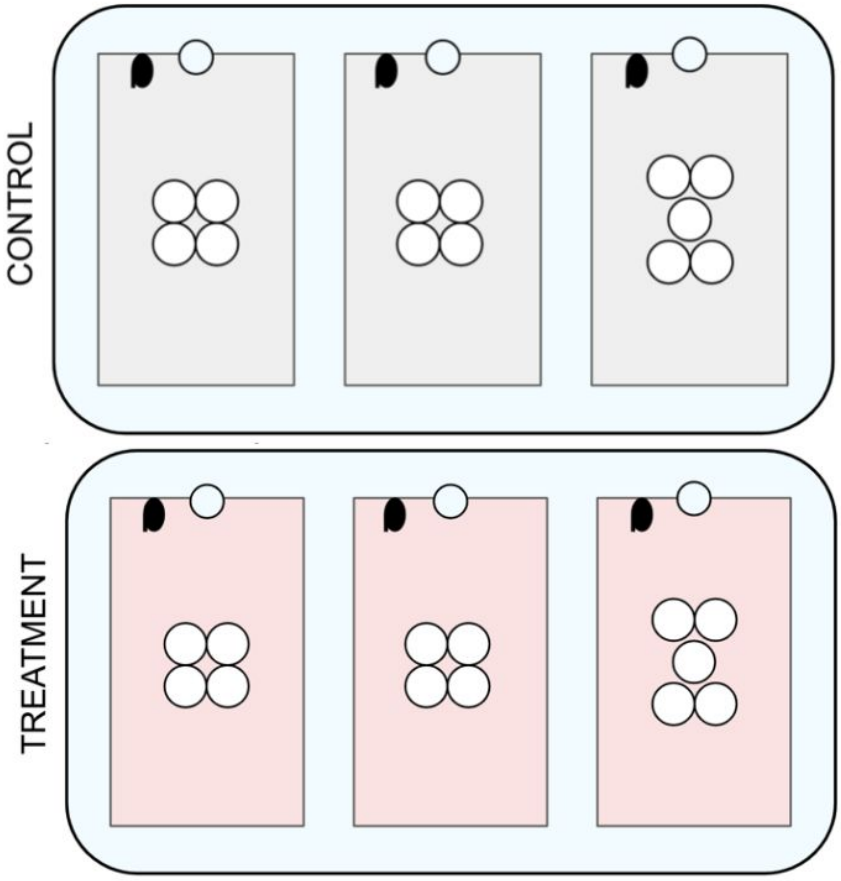

Figure 1.

Experimental tank design. 26 nubbins (white circle) were randomly assigned within the control and treatment systems so that each genotypic pair was placed in the corresponding location within either the control or treatment system. Powerheads were located in the upper left corner of each tank within the system along with water intake and outtake tubing. 5 nubbins were placed in each right-most tank, and 4 nubbins were placed in the left-most and middle tanks for a total of 13 nubbins per system.

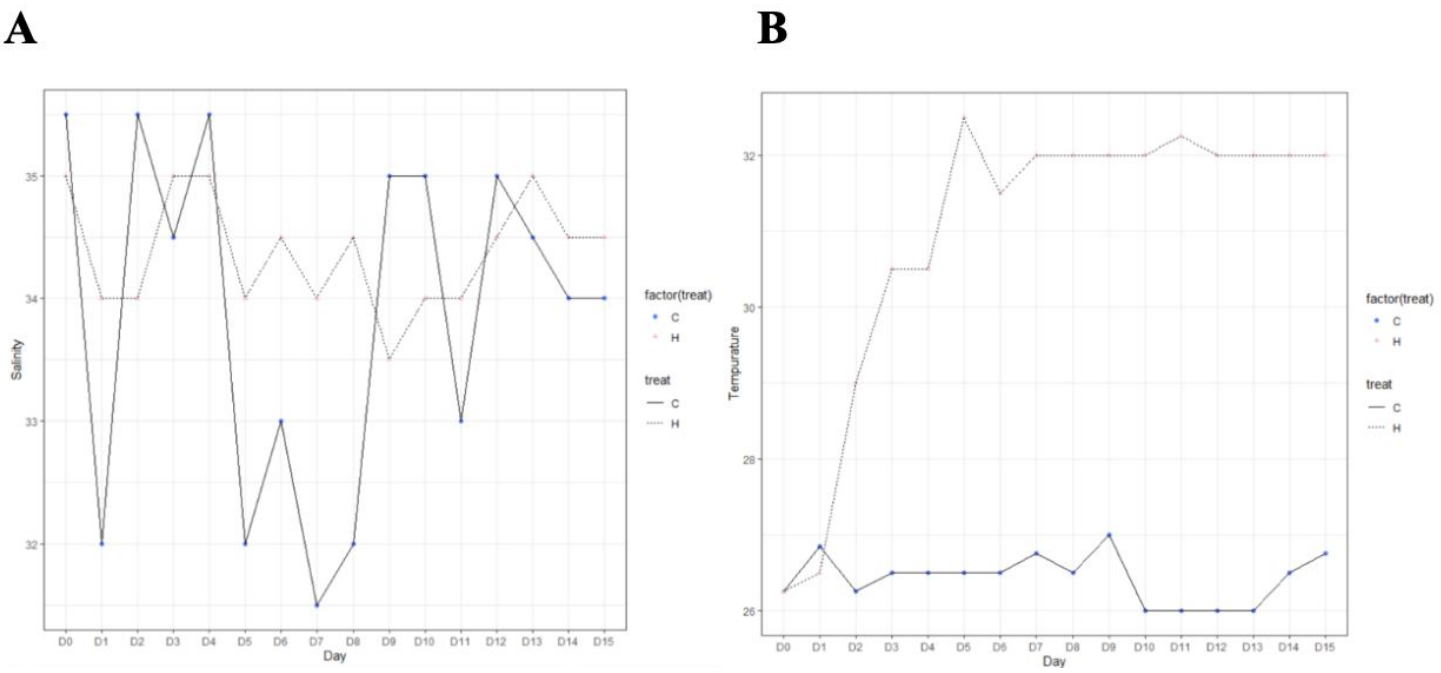

Figure 2. Daily water quality recorded for the duration of the study in control and heat tanks. (A) Graph of salinity (ppt) fluctuations and (B) displays the temperature (℃) profile.

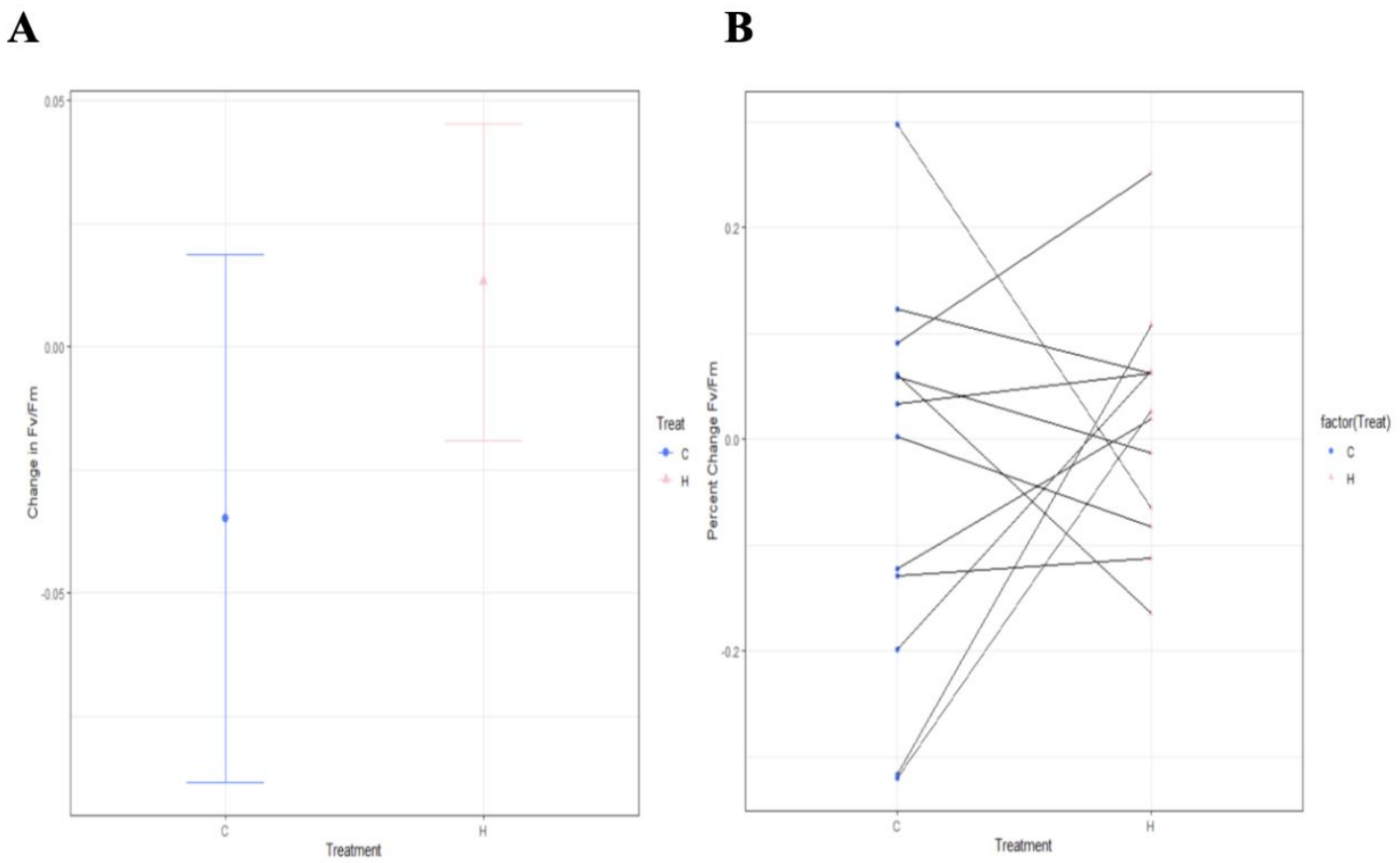

Figure 3. Effect of thermal stress on photosynthetic efficiency on each experimental day. (A) (Left) Comparison of change in photosynthetic efficiency (measured as chlorophyll fluorescence Fv/Fm) between the control and the heat treatment (Mean c=0.014, mean h=0.0235; control standard deviation c =0.0536; heat standard deviation=0.032; p=0.451). (B) Plots 13 *P. damicornis* phenotypes variable responses to heat stress over time.

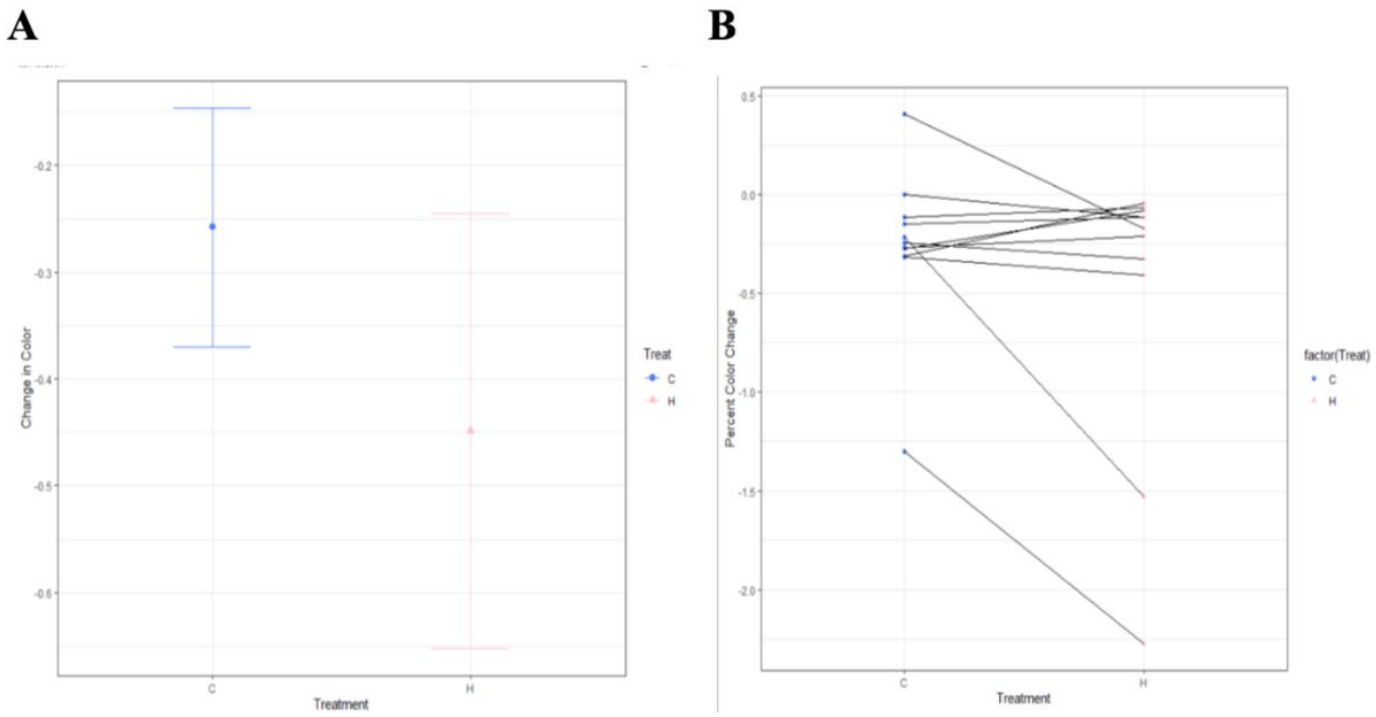

Figure 4. Effects of thermal stress on *P. damicornis* color change. (A) Comparison between the control and the treatment over the duration of each experimental day (Control mean =0.2188; heat mean=0.3247; control standard deviation=0.1118112; heat standard deviation= 0.2040009; p=0.421). (B) Plots variable phenotypic responses where the lines connect how each nubbin changed compared to its corresponding coral colony in the control system. Coral change was minimal and hardly noticeable. P7 observed the most bleaching in both.

Literature Citations

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