The Effects of Thermal Priming on Congeneric Endangered Corals: A Warm Up Routine

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1 | Abstract

Climate change has caused sea surface temperatures to rise at unprecedented levels leading to negative impacts in tropical marine environments. Reef-building corals such as *O. faveolata* and *O. franksi* from the Flower Garden Banks National Marine Sanctuary are under threat due to increased ocean warming. This is because corals exposed to prolonged thermal stress can experience bleaching or widespread mortality, posing a major threat to overall ecosystem health and biodiversity. However, priming is shown as a potential technique to mitigate the effects of ocean warming by predisposing corals to a smaller thermal challenge, increasing tolerance to future heat stress. There is limited understanding of how priming influences endangered congeneric corals' responses to thermal challenges, and thus, the aim of this study is to better understand this phenomenon. Using pulse amplitude modulation (PAM), we show that photosynthetic efficiency significantly decreased in unprimed *O. franksi* corals compared to the control; there was no significant difference seen between the primed corals and the control. There was also a significant overall increase in red channel intensity in unprimed treatments compared to the control for both *O. franksi* and *O. faveolata*. Increased red channel intensity is indicative of a decline in chlorophyll density which demonstrates that the unprimed corals significantly bleached in response to thermal stress. This study provides insight into using priming as a beneficial conservation mechanism to mitigate the effects of increasing sea surface temperatures on reef-building corals.

2 | Introduction

Anthropogenic impacts such as burning fossil fuels, deforestation, livestock production, and urbanization have caused rapid increases in atmospheric concentrations of greenhouse gasses (Hoegh-Guldberg 2010). These greenhouse gasses trap more energy from the sun and the oceans absorb the majority of this heat, leading to a rise in sea surface temperatures (Cheng *et al.* 2019). The effects of global warming are most pronounced in tropical marine environments since these organisms have narrow heat tolerances and live near their thermal limits (Vinagre *et al.* 2016). Specifically, reef-building scleractinian corals operate so close to their thermal limits that even slight increases in sea surface temperatures can lead to severe negative effects (Hoey *et al.* 2016).

Corals host symbiotic algae in the Family Symbiodiniaceae that can provide up to 95% of their carbon requirements for growth, maintenance, and reproduction and are therefore vital for maintaining coral health (West & Salm 2003). A loss of this symbiotic relationship, which is called coral bleaching, occurs when environmental stressors such as high temperatures cause the coral to expel their algae, disrupting the symbiotic relationship (Gates *et al.* 1992). Depending on the intensity and duration of the stress, corals can recover from bleaching and regain their symbionts; however, prolonged exposure can lead to widespread coral mortality (Szmant & Gassman 1990). This poses a major problem as coral reefs are vital in maintaining ecosystem biodiversity as 25% of all known marine life are dependent on coral reefs and they provide

habitat and nursery grounds for many organisms (Sambrook *et al.* 2019). In fact, 500 million people benefit from coral reefs through food, recreation, and income from tourism and fisheries (West & Salm 2003). Thus, the rapid decline of coral reef communities calls for ways to mitigate the effects of thermal stress on corals.

Recent studies have turned to priming as a potential solution for climate change-related coral declines. Priming is a form of acclimation in which corals are exposed to a stress event that allows them to modify their response when facing a future stress event (Hackerott *et al.* 2021). Castillo *et al.* (2012) measured the growth rates of the same species of coral in both inshore and offshore locations. Their research found that being exposed to a range of temperatures allowed the inshore populations to be better acclimated to rising sea surface temperatures and as a result, they had higher growth rates than the offshore population. Safaie *et al.* (2018) conducted a similar study where they found that daily or tidal temperature fluctuations expose corals to high enough temperatures that it encourages greater tolerance to thermal stress. This resistance to bleaching occurs via physiological acclimation or adaptation through the natural selection of heat-tolerant lineages (Safaie *et al.* 2018). Other research has shown that experimental thermal priming increased the success rate of fertilization among coral species compared to their non-primed counterparts when exposed to higher-than-normal temperatures (Puisay *et al.* 2023). Thermal priming of adult coral has also been shown to increase their larvaes' ability to resist bleaching when exposed to heat stress (Jiang *et al.* 2022). Many studies have examined the advantages that come with priming corals, but little is known about how these responses vary with different species of endangered congeneric corals. To fill in this knowledge gap we have conducted a study that looks into the impacts of priming on scleractinian corals *Orbicella franksi* and *Orbicella faveolata*.

Orbicella franksi, known as boulder star coral, and *Orbicella faveolata*, known as mountainous star coral, are two important reef-building corals that are native to the Caribbean Sea and the Gulf of Mexico (Strongin *et al.* 2020). These congeneric species grow in optimal water temperatures between 23-29°C (*NOAA* 2023) and are found in the Flower Garden Banks National Marine Sanctuary (FGBNMS) along the coast of Texas and Lousianna (Green *et al.* 2014). The FGBNMS is a unique location as it has lower species diversity but more than 50% higher coral cover compared to other coral reefs (Green *et al.* 2014). *O. faveolata* and *O. franksi* have the same generalist life-history strategy and are both identified as being highly competitive and stress-tolerant (Darling *et al.* 2012). Despite their abundance in the FGBNMS, *O. franksi* is listed as vulnerable and *O. faveolata* is listed as an endangered species by the IUCN, making them interesting coral species to study (Rodriguez-Martinez *et al.* 2022; Rodriguez-Martinez *et al.* 2022). The severe population decline of the orbicella genus caused by climate change has led to reduced structural habitat complexity and thus, a decrease in the organism biodiversity of coral reefs (Rippe *et al.* 2017). Since the lifespan of both coral species lasts hundreds of years (Kemp *et al.* 2016), these corals will have to acclimatize to the effects of warming ocean temperatures within their lifetime (Drury 2019). The evolutionary pressure that these species face makes it potentially favorable to study priming as a mechanism to increase thermal tolerance.

There is an urgent need to better understand which corals are more suited to deal with the rapid effects of climate change to predict how the distributions of corals will change in the near future. Here, using *O. franksi and O. faveolata* collected from the Flower Garden Banks National Marine Sanctuary, we aim to understand whether thermal priming can mitigate the negative impacts of heat stress events and whether these corals will exhibit species-specific preferences for thermal priming. More specifically, we investigate how corals undergoing a brief thermal

stress event will perform when undergoing another, more extreme, heat challenge relative to those who were not thermally primed. This was achieved by measuring the photosynthetic efficiency and chlorophyll density of the corals across varying thermal treatments. We hypothesized that thermal priming will reduce coral bleaching in *O. franksi and O. faveolata* as primed corals will be more tolerant to future thermal challenges. Alternatively, it is possible that priming will increase coral bleaching in *O. franksi and O. faveolata* as primed corals are stressed and therefore more susceptible to future increased temperatures.

3 | Methods and Materials

3.1 | Sample Collection and Setup

Fragments from five *O. franksi* genotypes and 6 *O. faveolata* genotypes were obtained from the east Flower Garden Banks National Marine Sanctuary in the Gulf of Mexico 100 miles south of the Texas-Louisiana border (Figure 1). Each fragment was cut into three equal-sized (~3cm^2) ramets. A genet is a genetically unique colony that can be fragmented into ramets, which are single physiological individuals that are produced by clonal propagation (Drury *et al.* 2019). Thus, for this experiment, there are 15 *O. franksi* ramets and 18 *O. faveolata* ramets for a total of 33 coral ramets in the experiment. All ramets are labeled with a letter for species (K: *O. franksi*; V: *O. faveolata*) and the subsequent letter corresponds to the genet and the number after indicates the ramet (Table 1).

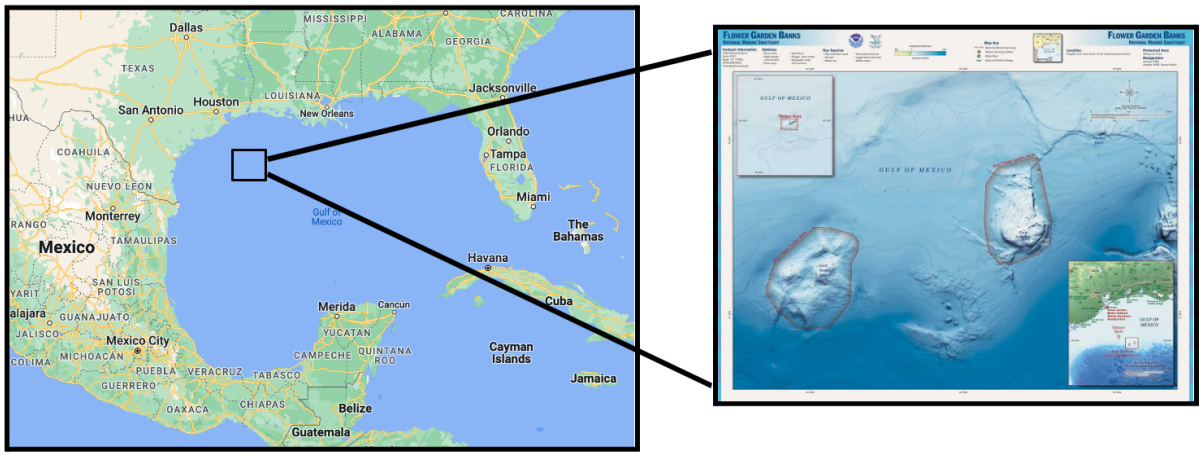

Figure 1. Map of the species collection site Flower Garden Banks National Marine Sanctuary which is located in the Gulf of Mexico. The left image was taken from Google Maps and the right image was taken from Wikipedia.

Table 1. Metadata of the individual coral IDs, the coral genet and species letters, their temperature treatments, and which tanks they were placed into after priming.

ID	Genet	Species	Tank	Treatment
VB8	B	V	2A	Control
VA15	A	V	2A	Control
MA10	A	M	2A	Control
KF4	F	K	2A	Control
VE2	E	V	2B	Control
VC12	C	V	2B	Control
KG8	G	K	2B	Control
KE6	E	K	2B	Control
VF8	F	V	2C	Control
VD8	D	V	2C	Control
KB11	B	K	2C	Control
VB10	B	V	4A	Prime-Ramp
VA17	A	V	4A	Prime-Ramp
MA12	A	M	4A	Prime-Ramp
KF6	F	K	4A	Prime-Ramp
VE4	E	V	4B	Prime-Ramp
VC14	C	V	4B	Prime-Ramp
KG10	G	K	4B	Prime-Ramp
KE7	E	K	4B	Prime-Ramp
VF10	F	V	4C	Prime-Ramp
VD10	D	V	4C	Prime-Ramp
KB13	B	K	4C	Prime-Ramp
VB9	B	V	4A	Control-Ramp
VA16	A	V	4A	Control-Ramp
MA11	A	M	4A	Control-Ramp
KF5	F	K	4A	Control-Ramp
VE3	E	V	4B	Control-Ramp
VC13	C	V	4B	Control-Ramp
KG9	G	K	4B	Control-Ramp
KE5	E	K	4B	Control-Ramp
VF9	F	V	4C	Control-Ramp
VD9	D	V	4C	Control-Ramp
KB12	B	K	4C	Control-Ramp

3.2 | Experimental Design

To test coral responses to thermal priming, three experimental treatments were tested: Control: corals were maintained at 26°C for the entire duration of the experiment; Control-ramp: corals were maintained at 26°C for the first three days after which they went through an 11-day thermal ramp with increasing temperatures by 1˚C per day for to a final temperature of 36˚C; Prime-ramp: tanks were primed on day 2 by raising temperatures from 26°C to 31°C by 1˚C per hour and then after 19 hours the next day, temperatures were reduced by 1˚C/hour back to 26˚C.

After priming, the corals went through the same thermal ramp described for the control-ramp treatment (Figure 2). Each treatment had three tanks and a ramet from every genet was represented in each treatment. The daily tasks consisted of taking temperature and salinity readings at 10:00 am, 12:00 pm, and 4:00 pm using the YSI Pro30 probe. On weekends, these readings were taken at 10:00 am and 4:00 pm. Temperature was also collected using the Onset HOBO Bluetooth Tidbit 5000 Data Logger which was placed in the sump of each system and recorded from the HOBOconnect app. Phosphate and nitrate levels were measured using the low-range handheld colorimeter, and calcium, magnesium, and carbonate levels were measured using reagent test kits.

Corals were rotated clockwise 90° every morning to prevent bias by ensuring that the ramets received equal amounts of light and water flow throughout the experiment. Current pumps were placed in the same position in the tank to keep the water flow the same. The salinity of the tanks was also maintained at a constant level of 31-34 ppt by adding deionized water daily to the sumps as water evaporated. The corals were not fed any food for the entire experiment. Lastly, all corals received a light cycle of 12 L:12 D (12:00 am-12:00 pm) that was maintained at 60-70 photosynthetically active radiation units (PAR). The PAR measurements were taken using the MQ-510: Full-Spectrum Underwater Quantum Meter.

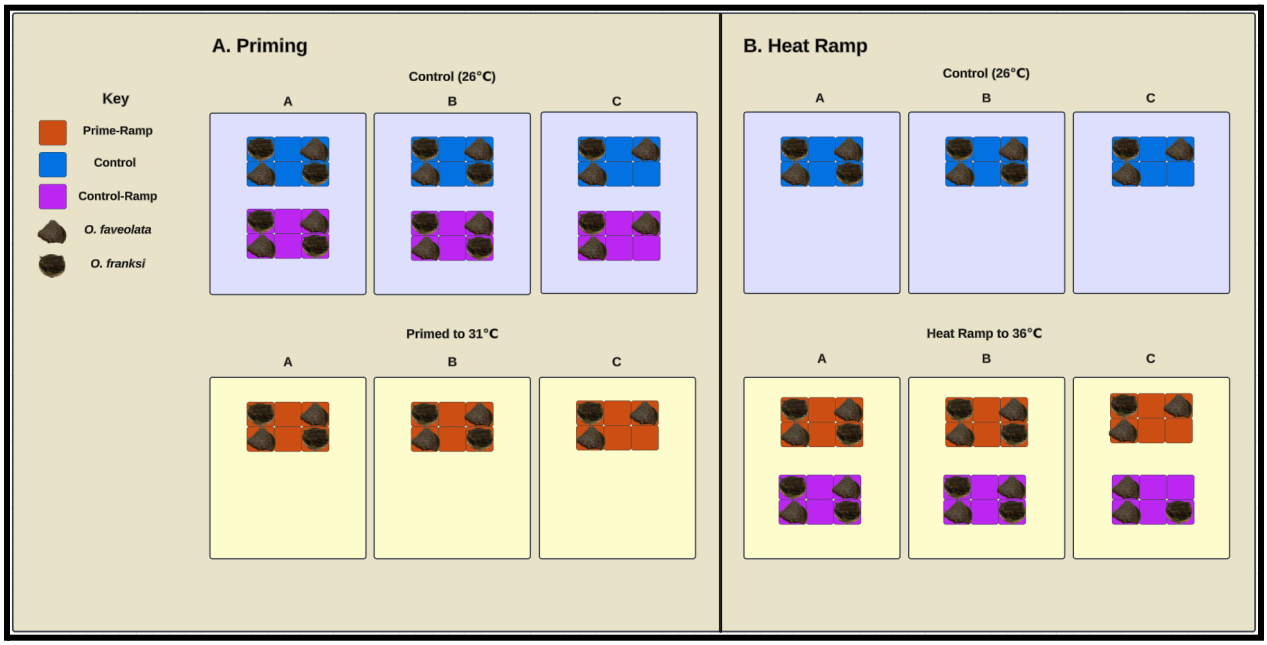

Figure 2. A schematic of the priming and heat ramp setups. The key indicates the type of treatment each tank is and the species of coral. After priming, the control-ramp corals were placed into the heat ramp tanks shown in panel B.

3.3 | PAM Analysis

After 2.5 hours of dark acclimation, Pulse Amplitude Modulation (PAM) was recorded to monitor changes in photosynthetic activity of photosystem II (Fv/Fm). Fv/Fm is the chlorophyll

fluorescence parameter and depicts the ratio of variable to maximum fluorescence after dark adaptation (Xia *et al.* 2023). The corals were dark acclimated for one hour before PAM data were collected using the JUNIOR-PAM chlorophyll fluorometer and the software WinControl-3 (Version 3.34 -rev 1206, 2023-04-27). Three Fv/Fm readings were taken at different spots on each coral ramet on each day. These points were averaged for data analysis. If Fv/Fm readings were .100 above or below the previous reading, a new reading was taken and used as a replacement to reduce error.

3.4 | Chlorophyll Density

Photographs were taken of the coral ramets on day 1, day 4 (the day after corals underwent priming), day 10 (during the heat stress event), and day 15 (the last day of the experiment after the corals underwent heating) using the iPhone 11 camera. Corals were placed on a Coral Watch coral color health chart inside a white lightbox for the photos. The iPhone camera settings, tripod height, and both spotlights were kept the same each day. Adobe Photoshop (Version 25.1.0) white-balanced photos to control for differences in lighting. Coral color was then quantified using the AnalyzeIntensity package in MATLAB (Version R2023b) and by following the Winters et al. (2009) procedure. Here, 10 points on the coral were chosen semi-randomly to obtain the red, blue, and green channel data. Focusing on the red channel values, higher values suggest coral bleaching. These RGB values were further analyzed and visualized using RStudio.

3.5 | Statistical Analysis

Using RStudio (Version 2023.06.2+561), the averages of the 3 PAM data points and the 10 red channel intensity data points were taken for each ramet. The relative change of these PAM and red channel intensity values was also calculated in RStudio by subtracting the initial mean from the final mean and dividing it by the initial mean. We conducted an ANOVA test using the relative change data to determine whether there was statistical significance in the means of our Fv/Fm readings between our control, control-ramp, and prime-ramp treatments. We then ran a Tukey's Post Hoc test to determine between which treatment groups there were observed differences in the means. We also ran an ANOVA and Post Hoc test to test the significance between the means of our red channel intensity values across all 3 treatment groups. P-values that are less than 0.05 indicate statistical significance.

4 | Results

4.1 | Water Quality

The nutrient levels on day 1 and then day 14 of our experiment were collected for each of the two systems. Table 2a shows the calcium, magnesium, carbonate, phosphate, and nitrate levels of the systems. The lighting levels for the experiment maintained a range of ~55-70 PAR from day 1 to day 14 (Table 2b).

The temperature varied throughout the experiment depending on the treatment type. Figure 3a shows that the control-ramp treatment was originally at 26°C and then increased 1°C every day starting on day 4 until it reached 36°C on day 14. The prime-ramp treatment increased to 31°C on day 2, ramped back down and rested at 26°C overnight on day 3, and then ramped up 1°C a day starting on day 4 to 36°C by the end of the experiment (Figure 3b). The control treatment tanks stayed at 26°C for the entire duration of the experiment (Figure 3c). The salinity of both tank systems steadily decreased over time from around 35 to 31 ppt (Figure 3d).

Table 2. A. The average nutrient measurements of system 2 and system 4 on day 1 and day 14. B. The lighting measurements of system 2 and system 4 on day 1 and day 14 measured in photosynthetic active radiation (PAR).

A.

Day 1			
System 2		**System 4**	
Calcium	487.5 ppm	Calcium	410 ppm
Magnesium	1275 ppm	Magnesium	1305 ppm
Carbonate	10.55 ppm	Carbonate	10.5 ppm
Phosphate	.05 ppm	Phosphate	.08 ppm
Nitrate	1.75 ppm	Nitrate	1.05 ppm
Day 14			
System 2		**System 4**	
Calcium	372.5 ppm	Calcium	370 ppm
Magnesium	1170 ppm	Magnesium	1107.5 ppm
Carbonate	10.5 ppm	Carbonate	9.1 ppm
Phosphate	.17 ppm	Phosphate	.165 ppm
Nitrate	2.05 ppm	Nitrate	1.3 ppm

B.

Day 1	
System 2	60-70 PAR
System 4	60-70 PAR
Day 14	
System 2	55-65 PAR
System 4	60-70 PAR

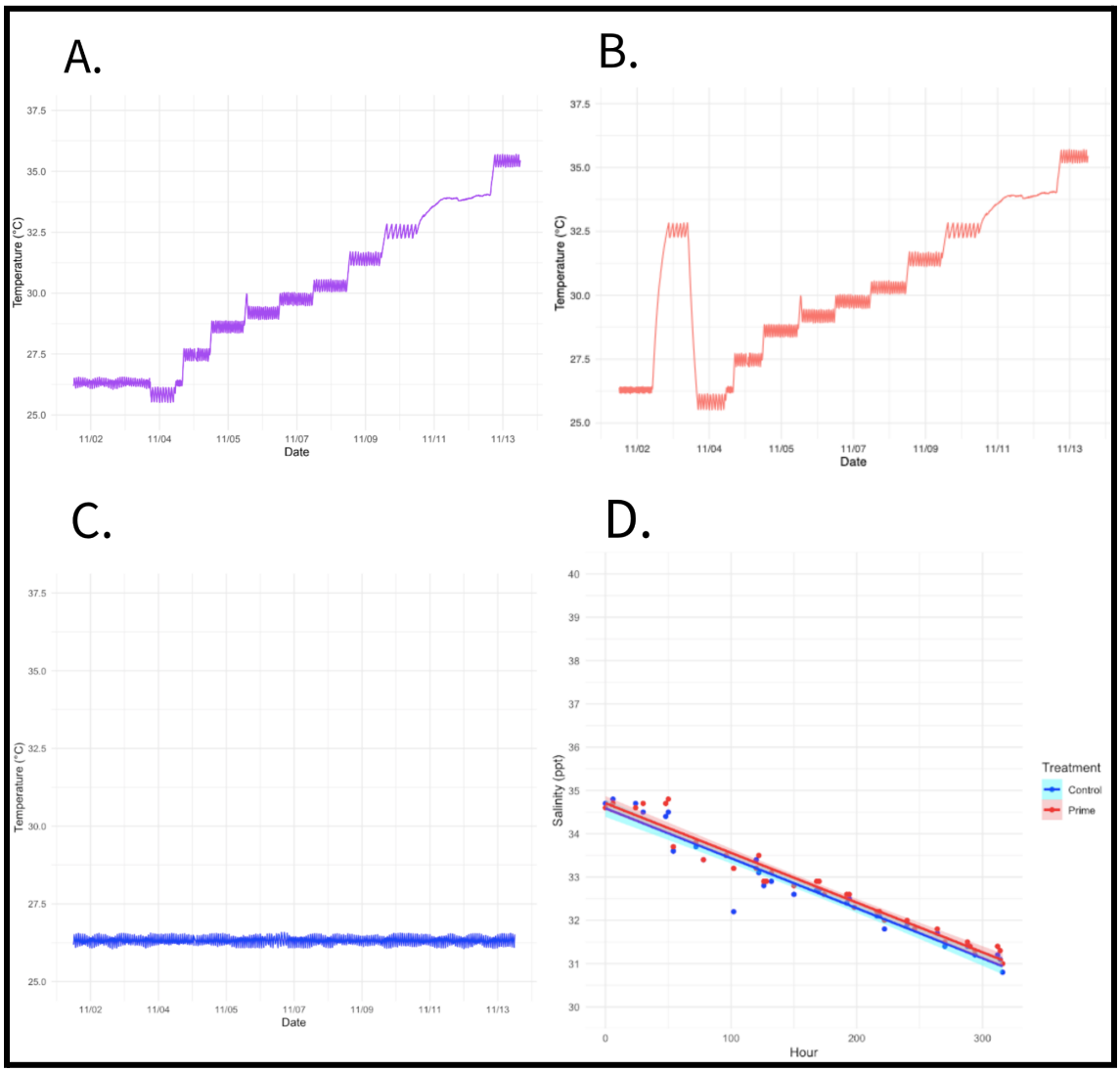

Figure 3. A. **The daily temperature of the control-ramp treatment.** The temperature was at 26 °C for the first 3 days and then increased by 1°C every day starting on day 4 until it reached 36°C on day 14. B. **The daily temperature of the prime-ramp treatment.** On day 2, the temperature increased 1°C every hour until it reached 31°C. Once it reached 31°C, the temperature decreased 1°C every hour until it reached 26 °C. On day 4, the temperature increased 1°C every day to reach 36°C on day 14. C. **The daily temperature of the control treatment tanks at 26 °C.** D. **The hourly salinity measurements in ppt.** The salinity steadily decreased from 34.5 to 31 ppt.

4.2 | Photosynthetic Efficiency

The photosynthetic efficiency of photosystem II (Fv/Fm) of corals in the control and control-ramp treatments was significantly affected over time (Figures 4c and 4d, ANOVA, p=.0044). Figures 4a and 4b display that from day 1 to day 9 of the experiment, there were consistent PAM readings ranging from .450 to .600 Fv/Fm. From day 10 to day 13, there was a steady decline in average Fv/Fm values in the control-ramp and prime-ramp treatments for both

species with a dramatic decrease on day 14 (Figures 4a and 4b). Figures 4a and 4b also show that the photosynthetic efficiency of the control treatment slightly increased on days 10 to 14 while the other treatments declined. The relative change was also analyzed to understand the extent to which the original Fv/Fm values have changed between treatments. Figures 4c and 4d show that the relative change of Fv/Fm slightly increased for the control of both species, but it was still close to 0 which indicates no change. In figure 4c, the relative Fv/Fm of *O. franksi* decreased for the prime-ramp and control-ramp as shown by the median of -.7 for prime-ramp and -1.2 for control-ramp. The relative Fv/Fm of *O. faveolata* also declined for both treatments but not as much as the median was -.5 for prime-ramp and -.7 for control-ramp.

The *O. franksi* control-ramp treatment significantly decreased over time compared to the *O. franksi* control treatment (Figure 4c, Tukey HSD, p=.0019). On the other hand, the *O. faveolata* control-ramp and control treatment were not statistically significant (p>.05). The differences in prime-ramp and control treatments were also not significant for either species (p>.05). This demonstrates that the main effect was driven by the *O. franksi* control-ramp corals having a lower photosynthetic efficiency compared to the *O. franksi* control corals.

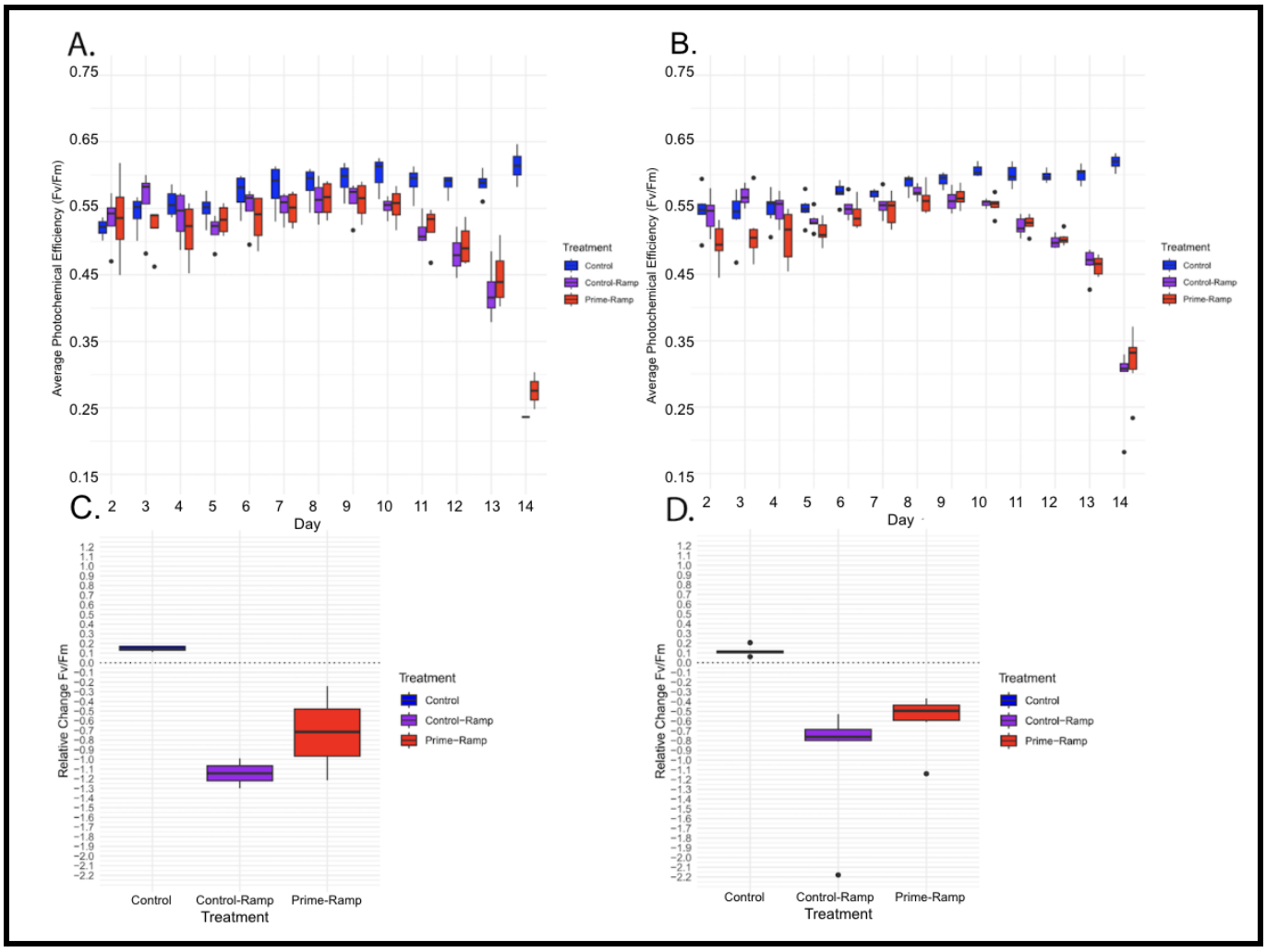

Figure 4. A. **Average photosynthetic efficiency (Fv/Fm) of *O. franksi* over a span of 14 days across control, control-ramp, and prime-ramp treatments.** Lower values indicate decreased photosynthetic efficiency in the symbiont. Dots in the figure are outliers that are outside of 3 standard deviations of the mean. B. **Average photochemical efficiency (Fv/Fm) of *O. faveolata* over a span of 14 days across different treatments.** Lower values indicate decreased photosynthetic efficiency in the symbiont. Dots in the figure are outliers that are outside of 3 standard deviations of the mean. C. **Relative change in photosynthetic efficiency of photosystem II (Fv/Fm) in *O.franksi.*** Higher values indicate a higher relative change across the experiment. 0.0 indicates no relative change in Fv/Fm. A significant difference is shown between the control and control-ramp treatment (p=.0019) as indicated by the a and b. D. **Relative change in photosynthetic efficiency of photosystem II (Fv/Fm) in *O. faveolata.*** Higher values indicate a higher relative change across the experiment. 0.0 indicates no relative change in Fv/Fm. Dots in the figure are outliers that are outside of 3 standard deviations of the mean.

4.3 | Chlorophyll Density

Using red channel intensity values as an indicator of chlorophyll density, figure 5a shows that red channel intensity increased in the control-ramp and prime-ramp treatments in comparison to the control groups for both *O. faveolata* and *O. franksi*. For *O. faveolata*, the control-ramp group increased at a higher rate than the prime-ramp group (Figure 5a). When examining the relative change in red channel values throughout the experiment, *O. franksi* had the highest median relative change in red values (2.0) in the prime-ramp treatment, and *O. faveolata* exhibited the highest median relative change (0.6) in the control-ramp treatment (Figures 5b and 5c). Figure 5b displayed that across all treatments, there was a positive relative change in red channel intensity; however, for figure 5c, only the control-ramp and prime-ramp treatments showed an increase in red channel intensity for all recorded values.

Our findings show that for both species overall, the control-ramp treatments had significantly higher red channel values than the control treatments (Figures 5b and 5c, ANOVA, p=.0398). There were marginally significant differences in red channel values between the control and prime-ramp treatments (Figures 5b and 5c, ANOVA, p=.0570). However, no significant differences in red channel intensity were observed across treatments within each species. There was an overall significant effect of treatment with prime-ramp *O. franksi* being significantly higher than control *O. faveolata* (.0047) and prime-ramp *O. franksi* being significantly higher than prime-ramp *O. faveolata* (p=.043); however, none of these pairwise comparisons within species were significant after multiple test correction. Despite this, when all of the species were pooled together, there was still a significant overall effect between the control-ramp and control treatments (p=.0398).

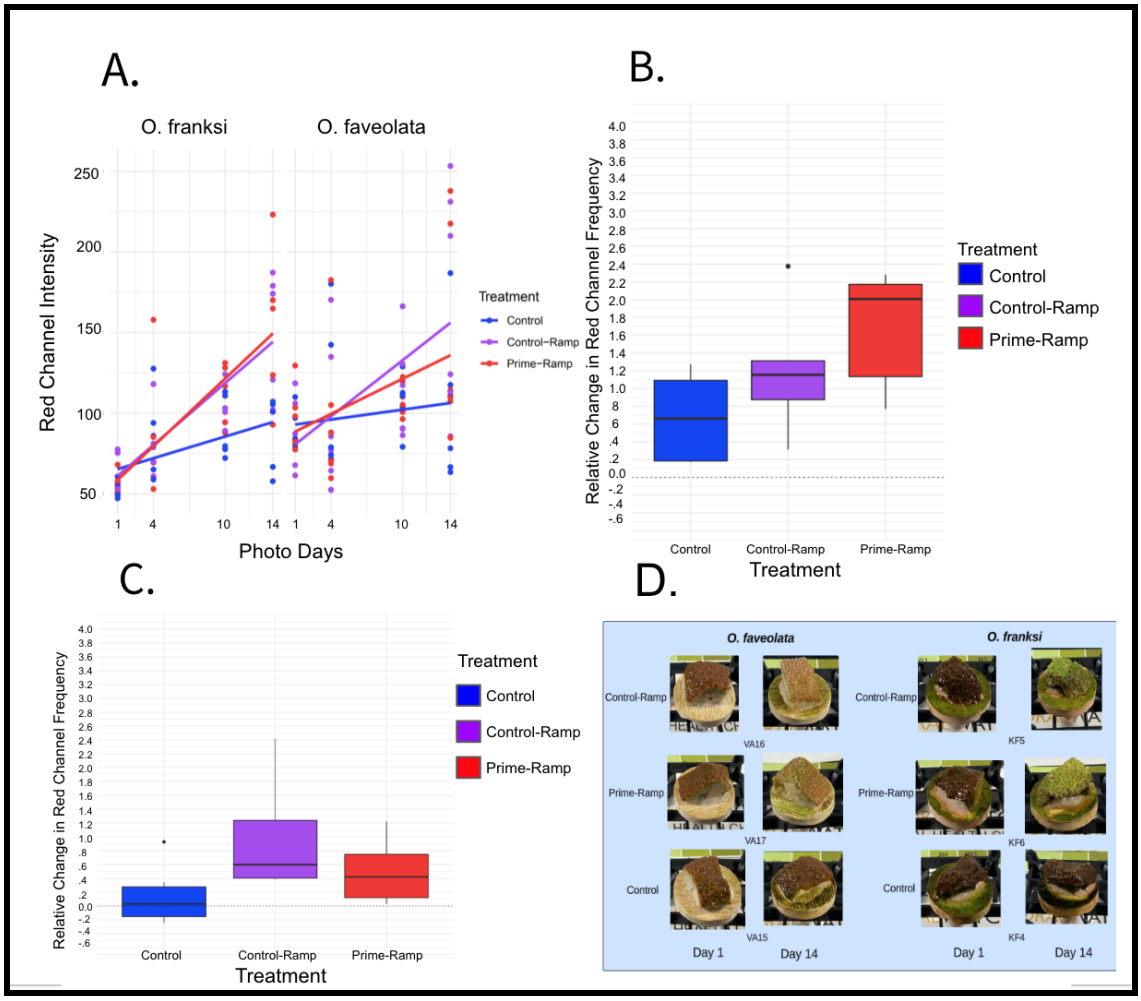

Figure 5: A. **Red channel intensity values for both species across different treatments.** Higher values indicate a lower chlorophyll concentration in the symbiont. B. **Relative change in red channel intensity values in *O. franksi* across different treatment groups.** Higher values indicate a higher relative change across the experiment. 0.0 indicates no relative change. No pairwise differences within a species were significant after multiple test correction. Overall significance between control-ramp and control is indicated by p=.0398. C. **Relative change in red channel intensity values in *O. faveolata* across different treatment groups.** Higher values indicate a higher relative change across the experiment. 0.0 indicates no relative change. No pairwise differences within a species were significant after multiple test correction. Overall significance between control-ramp and control is indicated by p=.0398. D. **Photos depicting color appearance of 3 ramets from the control, control-ramp, and prime-ramp groups for each species on day 1 and day 14 of the experiment.**

5 | Discussion

Our study aimed to discover how primed *O. faveolata* and *O. franksi* corals responded to increased thermal stress. We hypothesized that priming would reduce coral bleaching in the orbicellas since the corals will be more tolerant to future thermal challenges. We also made a second hypothesis that instead, the priming would increase coral bleaching as it would make the corals stressed and more susceptible to bleaching during the later heat challenge. Our results support the first hypothesis that there was a significant decrease in photosynthetic efficiency and chlorophyll density for the control-ramp treatment compared to the control treatment in both coral species. Thus, since the control-ramp group displayed significant bleaching, and the prime-ramp group did not compared to the control, priming was successful in making the corals more tolerant to thermal stress.

5.1 | Environmental Factors

Throughout the experiment, the nutrients remained at relatively the same concentrations (Table 2a). Some of the nutrient concentrations declined a little while others increased. These changes are of no concern as they are not drastic. The phosphate and nitrate levels are well within the coral's threshold, so they are negligible when considering other factors that could influence the coral species bleaching (Levitus *et al.* 1993; Guan *et al.* 2015). The lack of change may be due to our corals not being fed throughout the experiment. The salinity decreased from 34.5 to about 31 ppt in both systems (Figure 4d), but this is within the coral's normal range, so it would not affect the results of our experiment (Levitus *et al.* 1994; Guan *et al.* 2015). The change in salinity was due to the addition of R0 water over time, as well as the removal of salt water when Fv/Fm of the corals were quantified each day with PAM. The two system's light intensity levels were set to be at a range of 60-70 PAR, but by the end of the experiment, the light levels were recorded at 55-65 PAR for system 2 (Table 2b). These light levels are within normal limits of the corals and the change in PAR at the end of the experiment for system 2 is due to algae growth, which is not drastic enough to impact the experimental results (López-Londoño *et al.* 2022).

5.2 | Effects of Priming on Photosynthetic Efficiency

Our findings suggest that priming did aid in decreasing the effects of bleaching in *O. franksi* when exposed to a thermal challenge, as there was statistical significance between the control-ramp treatment and the control treatment (Figure 4c). Since there was no significant difference between the prime-ramp treatment and the control, this suggests that priming reduces coral bleaching in response to rising temperatures when compared to the treatment that was not primed (Figure 4c). While both experimental treatments decreased in Fv/Fm over time compared to the control, there is a trend of the primed corals doing slightly better than the control-ramp corals in both species (Figure 4a). This is because priming predisposed the corals to a smaller heat challenge and the coral's memory of the prior exposure increased their tolerance to the future thermal stress event (Hackerott 2021).

Over the 14-day period we saw a slight decrease in Fv/Fm until we reached 36°C, at that point we saw a steep decline in the species' photosynthetic ability (Figures 4a and 4b) as they were bleaching rapidly in the extreme temperatures. Higher photosynthetic efficiency (Fv/Fm) correlates with a healthier coral individual. Fv/Fm represents the maximum quantum efficiency of photosystem II which is indicative of the coral's efficiency in photosynthesizing (Wangpraseurt *et al.* 2019). For the purposes of our experiment a higher Fv/Fm, one around 0.6, correlates with a healthy individual while an Fv/Fm recording of 0.3 or less correlates with an individual that is experiencing the effects of bleaching (Figure 4).

Unlike *O. franksi*, *O. faveolata* did not have any statistical significance between treatments. Figure 4b shows that, unlike the control-ramp and the prime-ramp treatments for *O. franksi*, there was no significant difference among the experimental treatment groups over time. This could be due to a genetic difference among the species, or *O. faveolata* potentially being less thermotolerant. Wright *et al.* (2019) looked at the genetic differences between the two species and found that there weren't many as they are sympatric (Weil and Knowlton, 1994). Although Wright *et al.* (2019) looked at the genetic differences between species, they do say that this is only looking at the genetic differences relating to the effects of Hurricane Harvey. To eliminate genetic differences among species as a possible source of unsuccessful priming among *O. faveolata*, further research needs to be conducted. Dziedzic *et al.* (2019) looked at *O. faveolata* larvae when exposed to temperatures of up to 32°C. They found that the species' genetic variation allows them to have varying responses to thermal stress. This species has the ability to be successful when primed, but it has not been tested at such high temperatures in a laboratory before. The lack of exposure to such high temperatures could lead to the differing success of priming between the two species. *O. franksi* has been found to survive in temperatures of up to 35°C, at which their mortality rates plummeted to around 33°C in Bermuda and 35°C in Panama (Silbiger *et al.* 2019). In general, the Panama population was more photosynthetically efficient than the Bermuda population. These findings are consistent with the Panama population being acclimated to higher temperatures than the Bermuda population. While differences in location resulted in one population doing better than the other, that cannot be said of our study species. Both coral species were taken from the same site, indicating that there should be no difference in their thermotolerance based on the variability of exposed temperatures.

Other sources of the difference found among species responses could be due to differing *Symbiodinium*. The survival of these coral species can heavily rely on the genetic composition of the *Symbiodinium* (Rowan et al., 1997; Sampayo et al., 2008; Voolstra et al., 2009; Green *et al.* 2014). While the *Symbiodinium* seems like the likely source of differing results between coral species, Green *et al.* (2014) found that these two species have very similar proportions of *Symbiodinium* haplotypes. These findings suggest that there could be another physiological factor at play that would cause *O. franksi* to have statistically significant results and *O. faveolata* to have none.

Priming worked in this experiment due to the control of other variables such as salinity and nutrient levels. By having a lab setting we were able to eliminate any other factors that could

skew our results. Martell (2023) looked at priming on corals in a laboratory setting and found that priming increased the coral's resilience to bleaching. Although this data was found using other coral species, it supports the effectiveness of using priming to diminish the effects of bleaching. Research done in the wild such as Barshis *et al.* (2013) have found similar results in the cellular tolerance of reef-building corals and their dinoflagellate endosymbionts. Species that were exposed to levels of over 34°C had more heat-tolerant *Symbiodinium* genotypes and faster growth rates than populations of the same reef-building species found in less variable pools. Although there are varying responses of priming to heat stress, most studies find that priming mitigates the effects of bleaching, and our results support these findings.

5.3 | Effects of Priming on Chlorophyll Density

Our results display that priming corals led to *O. faveolata* and *O. franksi* being more resilient to the heat stress event overall. This is seen by the control-ramp significantly increasing in red channel intensity compared to the control treatment for both species (Figures 5b and 5c), which indicates that the corals in this treatment were more prone to bleaching. The primed treatments of both species, on the other hand, did not demonstrate a significant increase in red channel intensity compared to the control, indicating that priming increased the thermotolerance of the corals (Figures 5b and 5c).

When looking into each species, Figure 5a depicts *O. faveolata* as a more thermotolerant species than *O. franksi.* This is shown through a steeper slope in the control-ramp treatment when compared to the prime-ramp treatment for *O. faveolata*, while the *O. franksi* prime-ramp treatment and control-ramp had minimal differences in slope. However, our results show that this difference is not significant enough to conclude that the priming of *O. faveolata* led to a higher thermal tolerance and fewer signs of bleaching than in *O. franksi*, meaning further research must be conducted to investigate the species-specific responses to heat stress.

When a coral experiences an increase in red channel intensity, photosynthetic activity decreases, which lowers the coral's overall chlorophyll density and induces bleaching (Winters *et al.* 2009). Through photographic analysis, a high correlation was found between an increasing red channel intensity and lowering chlorophyll density in the coral *S. pistillata* (Winters *et al.* 2009). Another study related these factors to heat stress on *S. pistillata* in the wild, which found that at higher temperatures, the corals displayed a higher red pixel intensity as well as a lower chlorophyll density, than at medium and control temperatures (Voolsta *et al.* 2020). This suggests that the corals, which did not undergo priming, were more prone to bleaching when exposed to higher heat. This relates to our findings with increased bleaching seen in ramets under the control-ramp treatment.

5.4 | Experimental Limitations and Error

A limitation in our methodology is that all of the corals were collected from the same location: Flower Garden Banks National Marine Sanctuary. Coral reefs all have different conditions and varying temperature ranges, so the corals' response to heat stress could vary

depending on location, making it important to collect corals from various sites to get a more complete understanding of the effects of priming. Another limitation is that an unequal amount of coral ramets were collected since there were 15 *O. franksi* and 18 *O. faveolata*. Since the experiment is conducted in a lab, it does not fully reflect environmental conditions which could have an impact on the results as the sanctuary could have differing amounts of nutrient and algae growth, lighting, and acidification compared to the tanks. The temperature also changed at faster rates than what corals experience in nature as the temperature in FGBNMS is more stable and stays around 20-30°C year round (NOAA 2023). Another experimental limitation is the amount of time it could run for since we only had 14 days. Thus, we could only prime the corals for one day and we only had enough time to let the corals rest overnight after priming and ramping down, which could have caused higher-than-normal stress on the corals. A longer experiment could have caused the primed corals to be even more resilient to the thermal challenge.

Throughout the experiment, there were issues with the heater so at times the tanks did not reach or go above the desired temperature by the end of the day. For example, the primed treatment tank reached a temperature of 32.5°C instead of 31°C because the temperature listed on the heater was not accurate (Figure 3c). We tried to resolve this issue by closely monitoring the temperature, adding another heater, and adjusting the temperature as needed. Another source of error is when we rotated the coral stands, sometimes we did not place them directly under the tank light, which potentially impacted the photosynthetic efficiency of the corals. All of these factors may have influenced the experiment and thus, can be avoided in future research to yield more conclusive results.

5.5 | Future Research

Future work is required to further our understanding of thermal priming on reef-building corals. Since this experiment was short in duration, it would be interesting to see how it would differ over a longer timescale to better assess the effects of priming. This would allow for slower increases in temperature to reflect environmental conditions. Further research on priming can be done on more types of coral species, corals from different locations with varying thermal tolerances, and corals at different life stages. Since the photosynthetic efficiency decreased significantly only for *O. franksi* and not *O. faveolata* despite them being very similar physiologically, future studies can look at the changes in gene expression across species. This can be achieved by incorporating DNA and RNA sequencing to observe if genes are upregulated in primed corals to allow them to be more tolerant of heat stress. Genetic analysis can also be done on the algae symbionts to see if the host-symbiont relationship is involved in the coral's resistance to heat stress after priming. Thus, future research can build on our experimental results to further investigate if thermal priming can be used to mitigate the effects of climate change and ocean warming in coral reefs.

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