Orbicella? More like *Orbi*chilly: The Effects of Cold Priming on Reef-Building Corals

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1 | Abstract

Responses of corals to anthropogenic climate change-mediated marine heatwaves have been extensive. However, climate change can also lead to severe cold spells which push corals beyond their thermal limits. Similar to heat stress, cold stress can cause the loss of symbiotic algae (coral bleaching) and mortality, damaging the biodiversity and health of tropical coral ecosystems. Despite the emergence of cold-bleaching events, limited studies have investigated how pre-exposure to cold temperatures might mitigate the corals' responses to a cold stressor by increasing their future resistance. Here we investigate if cold priming *Orbicella franksi* and *Orbicella faveolata* can improve coral physiological responses to an intense cold stressor. We analyze coral health through pulse amplitude modulation (PAM) to measure photosynthetic efficiency and perform a color analysis to determine changes in red channel intensity. With PAM measurements, we found that the photosynthetic efficiency of corals under cold stress significantly decreased. Corals pre-exposed to cold stress (primed) exhibited no variation in photosynthetic efficiency compared to corals without pre-exposure (unprimed). Primed and unprimed corals showed no significant increase in red channel intensity, indicating a lack of the chlorophyll density decline typically associated with bleaching. This study reinforces the understanding that cold bleaching can occur as a stress response to extreme cold sea surface temperatures, which are becoming more prevalent with climate change. More research is needed to further investigate if cold priming can be used as an effective conservation method to reduce the impact of ocean cold spells on reef-building corals amidst climate-induced thermal stress.

2 | Introduction

Coral reef ecosystems are experiencing escalating pressures on a global scale due to climate-driven stressors and anthropogenic disturbances (Nielsen et al., 2020). Over the last 150 years, humanity's heavy dependency on fossil fuels has increased atmospheric carbon dioxide by approximately 33%, leading to the warming of Earth through the greenhouse effect (Hardy, 2003). However, these changes in atmospheric CO_2 can also lead to more temperature extremes (Crabbe, 2008), which directly affect coral reefs. As corals reach their upper and lower temperature thresholds, they decline in calcification rates and are susceptible to coral bleaching (Crabbe, 2008). Coral bleaching is the phenomenon where, under stress, the coral host loses their symbiotic algae (Symbiodiniaceae), which is responsible for providing corals with essential nutrients (Baird et al., 2009). These bleaching events can occur under extreme high and low temperatures (Fitt et al., 2000), ultimately leading to coral mortality.

In nature, corals living in more variable environments have been shown to exhibit higher levels of bleaching resistance, associated with shifts in physiology. The difference between these corals and their counterparts in less variable environments is likely due to a mix of acclimatization and adaptation (Thomas et al., 2018). Acclimatization is a form of physiological

plasticity that allows individuals to maintain their performance across variable conditions (Thomas et al., 2018). In the case of corals from variable environments, this plasticity facilitates improved thermotolerance within their lifespan. Alternatively, adaptation is the result of the environmental selection of beneficial alleles within a population, which facilitates elevated thermal resistance across multiple generations (Barrett & Schluter, 2008). Local coral environments have been shown to have a significant impact on their thermotolerance. Corals with large ranges of daily thermal variability have been found to have enhanced tolerance to thermal stress (Schoepf et al., 2015). Even within the same reef, the zone in which the coral inhabits can have a significant impact on their ability to thermoregulate. Those in more variable zones, such as the backreef and nearshore, are more tolerant of long-term thermal changes than those in more stable temperature zones, such as the forereef (Castillo et al., 2012). These findings emphasize the importance of temperature variability on the ability of corals to tolerate the long-term thermal changes associated with climate change. Variable environments are posited to facilitate a natural equivalent of priming, where corals experience a stressor in a much smaller time frame and thus can better tolerate the stressor long-term (Thomas et al., 2018).

Studies have explored the effect of heat priming corals by exposing them to a sharp increase in water temperature, returning them to baseline conditions, and then slowly increasing the temperature to determine how their resilience may be improved. (Martell, 2023). It is important to note that coral response to stress is dependent upon the magnitude and duration of priming; if either is insufficient, the coral may not demonstrate a memory response or exhibit a decrease in resilience to stress relative to unprimed corals. Conversely, if the initial priming is too severe, it may harm the organism, which could lead to permanent cellular damage (Hackerott et al., 2021). One study found that corals that bleached in consecutive years showed reduced bleaching through time, whereas those who bleached two years apart showed no benefit from prior exposure. (Hackerott et al., 2021). Species variation in tolerance, resilience, and phenotypic plasticity could all lead to differences in optimal priming conditions (Hackerott et al., 2021). Moreover, thermal priming has been observed to improve coral fertilization success under high temperatures in some species (Jiang et al., 2023). However, priming has also been shown to reduce genetic variation in offspring, which may influence adaptive potential and long-term acclimatization (Puisay et al., 2023). Thermal priming in adult corals can also improve larval resilience to heat stress; this comes with a risk of metabolic depression in offspring which could have significant implications for coral reef survival as ocean temperatures rise (Jiang et al., 2023).

Not only do warming temperatures induce stress on coral reefs, but extreme cold events also contribute significantly to their vulnerability. Ocean currents play a crucial role in redistributing heat across the planet. Warm surface currents carry warm water from the equator toward the poles, moderating temperatures in coastal regions and preventing extreme temperature differences between the tropics and higher latitudes. Conversely, cold currents move cool water from polar regions toward the equator (Seager and Murtugudde, 1997). However, melting sea ice due to global warming is causing freshwater to be added to the seawater at the poles, making it less dense and causing ocean currents to slow. With slowing ocean currents, ocean temperatures will not be balanced, leading to extreme warm and cold temperatures in different areas (Trossman and Palter, 2001). It's hypothesized that sea ice loss weakens the polar vortex, a large area of low

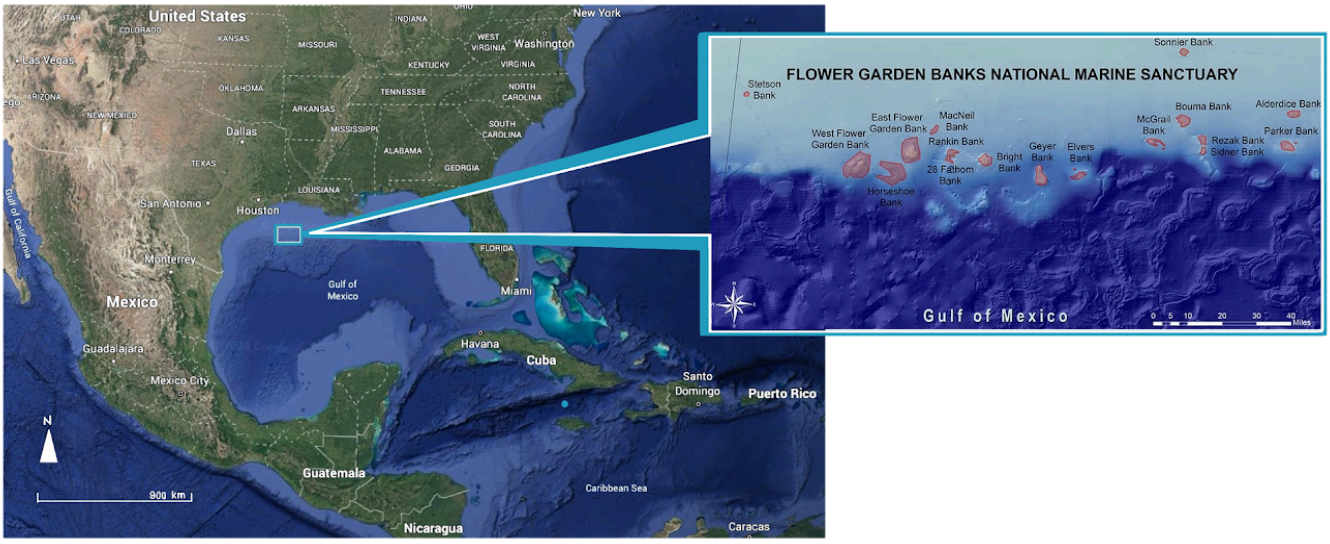

Figure 1. Map of Flower Garden Banks National Marine Sanctuary where species were collected. Left image found through Google Earth and right image found through Flower Garden Banks National Marine Sanctuary website.

pressure and cold air surrounding Earth's poles (Kim et al., 2014). A weak polar vortex can become unstable, causing it to dip further south and bring cold air to mid-latitude regions like North America, causing marine cold spells in shallower areas (Mitchell et al., 2012). Additionally, the increasing intensity of La Niña cycles and the warming Arctic can also cause more severe winter conditions in these mid-latitude areas (Cohen et al., 2014, González-Espinosa and Donner 2020). Together, these cooler waters can also negatively impact reef-building corals.

Significant cold-water bleaching events have occurred in the Florida Keys and the Gulf of California (Paz-Garcia et al., 2012, González-Espinosa and Donner, 2020). With decreasing temperatures, there is a reduction in the rate of enzymes working to catalyze the Calvin-Benson cycle, which provides corals with energy through their symbiotic algae (Saxby et al., 2003). This loss of their symbionts and reduction in photosynthetic efficiency result in cold-water bleaching, analogous to what occurs under warm-water bleaching (Saxby et al., 2003). When directly contrasting how corals respond under cooler versus warmer temperatures, it was observed that short-term exposure to cold temperatures was more damaging than short-term exposure to hot conditions, and this was reversed when corals were exposed long-term to these stressors (Roth et al., 2012). The initial cold exposure yielded a decline in coral growth. However, continued cold stress did not cause sustained damage as the corals studied were able to acclimate and change the concentrations of proteins, pigments, and enzymes involved in photosynthesis (Roth et al., 2012). These results would likely be variable by species. Another study found that the degree of bleaching was variable, with corals who were exposed to frequent cold-water upwellings having more resistance to bleaching (Paz-Garcia et al., 2012). Despite this, limited literature explores the potential for cold priming in corals. In other organisms, cold priming has been shown to enhance survival under later stress events, suggesting a potential for corals to respond similarly (Bittner et al., 2021, Wu et al., 2023).

In this study, we investigate whether pre-exposure to low temperatures can improve corals' resilience to cooler conditions and mitigate

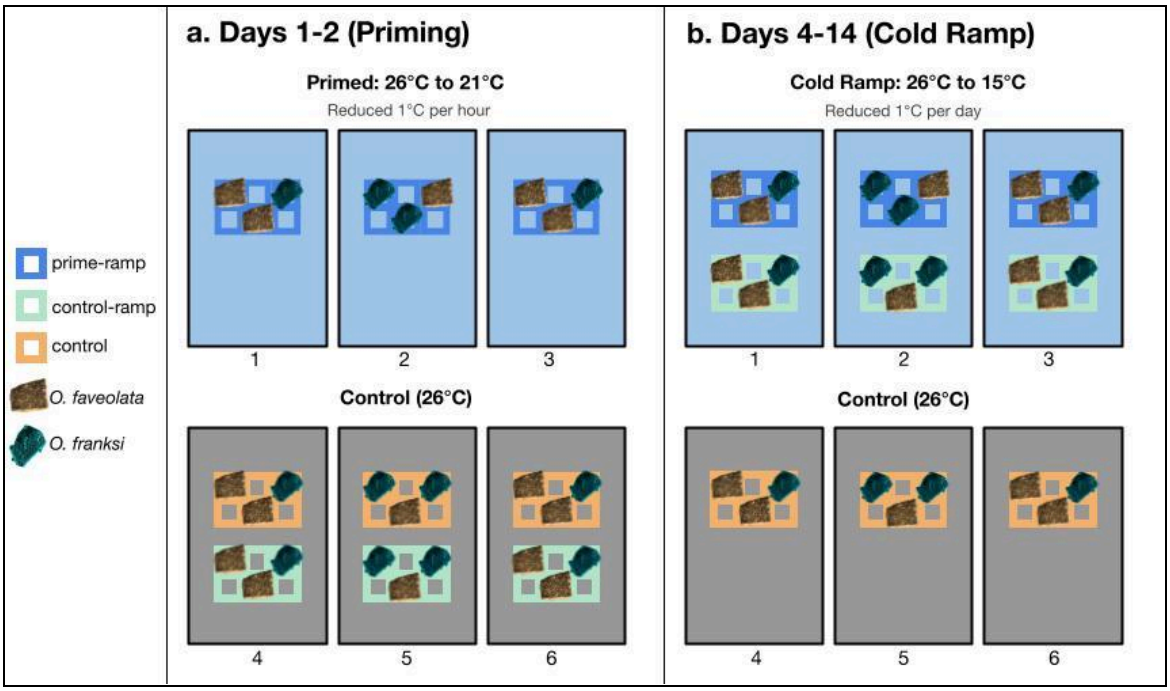

Figure 2. Experimental setup for priming and cold-ramp transition. Prime-ramp corals were cold primed in the cold system on day 1 from 26°C to 21°C, decreasing 1°C per hour Control-ramp and control corals were maintained at 26°C in the control system during this. On day 2, prime-ramp corals were brought back to 26°C, increasing by 1°C per hour, with one day to recover before the cold ramp. Control-ramp corals moved to the cold system with prime-ramp corals for days 4-14 and ramped down to 15°C, decreasing 1°C daily.

cold-water bleaching using *Orbicella franksi* and *Orbicella faveolata* sourced from the Flower Garden Banks National Marine Sanctuary of the Gulf of Mexico. As tropical reef-building corals, they live in temperatures of 23°C to 29°C (NOAA, 2023). However, in Flower Garden Banks Marine Sanctuary, temperatures can reach a low of 18°C during winter (Dias et al., 2023). As these corals exist at their thermal minima during the cold winter months, studying the impacts of a cold challenge is increasingly relevant as the water temps in the Gulf of Mexico become more variable (Del Monte-Luna et al., 2015). Here, we study the relationship between cold thermal priming, symbiont health, and bleaching. We hypothesize that cold-primed corals will experience less bleaching and be healthier than unprimed corals due to their improved thermoregulation after an experience with a cold stressor.

3 | Methods

3.1 | Sample Collection

Coral fragments from five genets of *O. faveolata* and four genets of *O. franksi* were obtained 100 miles south of the Texas-Louisiana border, in the east Flower Garden Banks National Marine Sanctuary in the Gulf of Mexico (Figure 1). Each genet was fragmented into three ramets, one for each treatment to control for genetic differences in response**.** A genet is a genetically unique coral colony, whereas ramets are biological replicates fragmented from the same colony (Drury et al., 2019). After fragmentation, there were 27 total samples, with 15 ramets of *O. faveolata* and 12 ramets of *O. franksi*. All samples were labeled with a unique ID (species, genet, ramet number). There were three species indicators: shallow *O. franksi* (K), mesophotic *O.*

franksi (M), and shallow *O. faveolata* (V). However, for this experiment, we treat shallow and mesophotic *O. franksi* as one population (Table 1).

Table 1. Metadata of coral identification, species, genet, tank placement after priming, and temperature treatment for each individual coral ramet.

ID	Species	Genet	Tank	Treatment
KB1	K	B	4	control
KB2	K	B	1	control-ramp
KB3	K	B	1	prime-ramp
KF1	K	F	5	control
KF2	K	F	2	control-ramp
KF3	K	F	2	prime-ramp
MA7	M	A	6	control
MA8	M	A	3	control-ramp
MA9	M	A	3	prime-ramp
KG5	K	G	5	control
KG6	K	G	2	control-ramp
KG7	K	G	2	prime-ramp
VE1	V	E	4	control
VE2	V	E	1	control-ramp
VE3	V	E	1	prime-ramp
VF1	V	F	4	control
VF2	V	F	1	control-ramp
VF3	V	F	1	prime-ramp
VD1	V	D	5	control
VD2	V	D	2	control-ramp
VD3	V	D	2	prime-ramp
VA12	V	A	6	control
VA13	V	A	3	control-ramp
VA14	V	A	3	prime-ramp
VC9	V	C	6	control
VC10	V	C	3	control-ramp
VC11	V	C	3	prime-ramp

3.2 | Experimental Design

Our setup consisted of two unique systems, each of which contained three tanks and a shared sump that maintained consistent water levels and salinity across the tanks. Temperatures were controlled in the sump through a heater and chiller. Corals in all tanks were exposed to a 12:12 hour light-dark UV light system at a light level of 50-60 PAR. To account for possible variability in light exposure and water flow within each tank, the coral racks were rotated 90 degrees clockwise daily. Salinity and temperature were measured twice a day and salinity readings were taken once per day using a calibrated refractometer (standardized with a reagent to 33 ppt) with a target salinity of 34–36 ppt. Freshwater was pumped into the sump to compensate for evaporative water loss. Temperature was measured from each tank, resulting in three readings per system, which were averaged to determine temperature treatments.

This study had three temperature treatments: prime-ramp, control-ramp, and control (Figure 2). Tanks 1-3 (cold system) initially held three corals each (prime-ramp). Tanks 4-6 (control system) initially held six corals each (control and control-ramp groups). After three days, the control-ramp corals were transferred to the cold tanks from the control system so that they experienced the cold ramp, not priming (Figure 2). Control corals were maintained at 26°C throughout the experiment (Figure 3c). Control-ramp corals were held at 26°C for the first three days, after which they experienced a cold ramp where temperatures gradually decreased from 26˚C to 15°C (at a rate of 1°C per day for 11 days) (Figure 3b). Prime-ramp corals underwent initial priming by decreasing the temperature from 26°C to 21°C within one day (1°C per hour), followed by a return to 26°C (1°C per hour) the next day (Figure 3a). After reacclimating to 26°C for 49 hours, on day 4, the prime-ramp corals were then ramped down to 15°C (1°C per day for 11 days) (Figure 3a).

3.3 | Photosynthetic Efficiency Assessment

To assess the photosynthetic efficiency of the coral's algae symbionts (Family Symbiodiniacaea) across the three temperature treatments, we used Pulse Amplitude Modulation (PAM) fluorometry. We used the same PAM device every day to limit variability and bias. This measurement assesses the health of the symbiosis by quantifying the photosynthetic efficiency of photosystem II (PSII) of the symbiotic algae within coral tissues. Corals were dark-adapted for 1.5 hours before measurement to ensure PSII

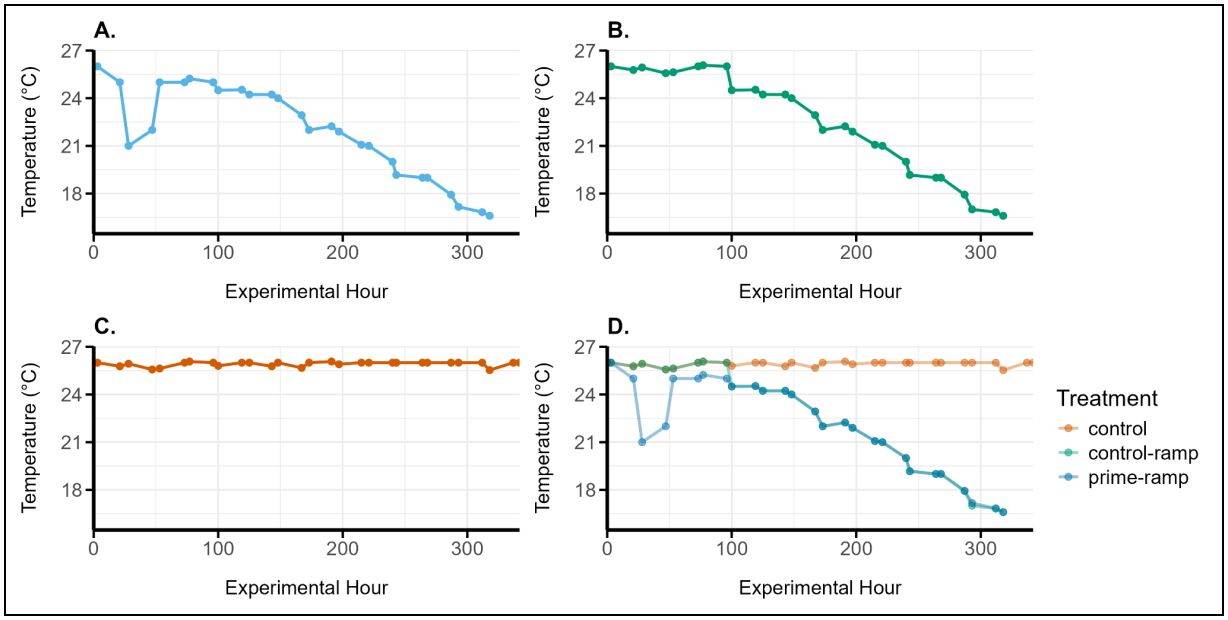

Figure 3. Daily temperature readings of A) **the prime-ramp treatment**. The temperature was initially lowered to 21°C by decreasing the temperature by 1°C an hour, then was increased by 1°C an hour the next day to 26°C, maintained at 26°C for 49 hours, and then was decreased by 1°C a day to 15°C. B) **the control-ramp treatment.** Temperatures were initially maintained at 26°C in the control system and then lowered by 1°C daily in the cold system with the prime-ramp treatment. C) **the control treatment.** Temperatures were maintained at 26°C. D) **all treatments.** The control treatment is shown in orange, the control-ramp in green, and the prime-ramp in light blue. Overlap of lines represents treatments in the same system (control or cold), with temperatures measured for the interconnected system. Dark blue represents the overlap of the control-ramp and prime-ramp, and dark green represents the overlap of the control and control-ramp.

reaction centers were fully open. Three readings per coral ramet were taken each day and these values were then averaged.

The PAM fluorescence parameter readings included minimum fluorescence (F0), maximum fluorescence (Fm), and variable fluorescence (Fv = Fm - F0), which together form the calculation of the maximum quantum yield of PSII (Fv/Fm). The Fv/Fm ratio reflects both the potential photosynthetic efficiency and the coral's possible degree of stress. Values ranging from 0.6 to 0.7 typically reflect a healthy coral photosystem, whereas a reduced value corresponds with a reduction in photosynthetic efficiency that can occur in stressful environments (Chalker et al., 1983).

3.4 | Color Analysis

On experimental day 0, a photo booth was set up to photograph each coral with a color standard. Each coral was photographed individually, and images were white-balanced using Adobe Lightroom (Version 10.0.1 45086/171). Ten points on each coral were selected in MATLAB (Version 24.2) and then averaged to obtain the red, green, and blue channel values, focusing on the red channel intensity to analyze coral bleaching (Winters et al., 2009). After 14 days, new images were taken using the same camera and lighting setup to minimize variation. *Orbicella franksi* and *O. faveolata* produced slime, causing glare in the images which was accounted for by keeping the photograph set up the same.

3.5 | Statistical Analysis

Statistical analyses were performed using RStudio (Version 2024.04.2+764). The relative change of Fv/Fm over the 14-day experiment was calculated by subtracting the mean on day four

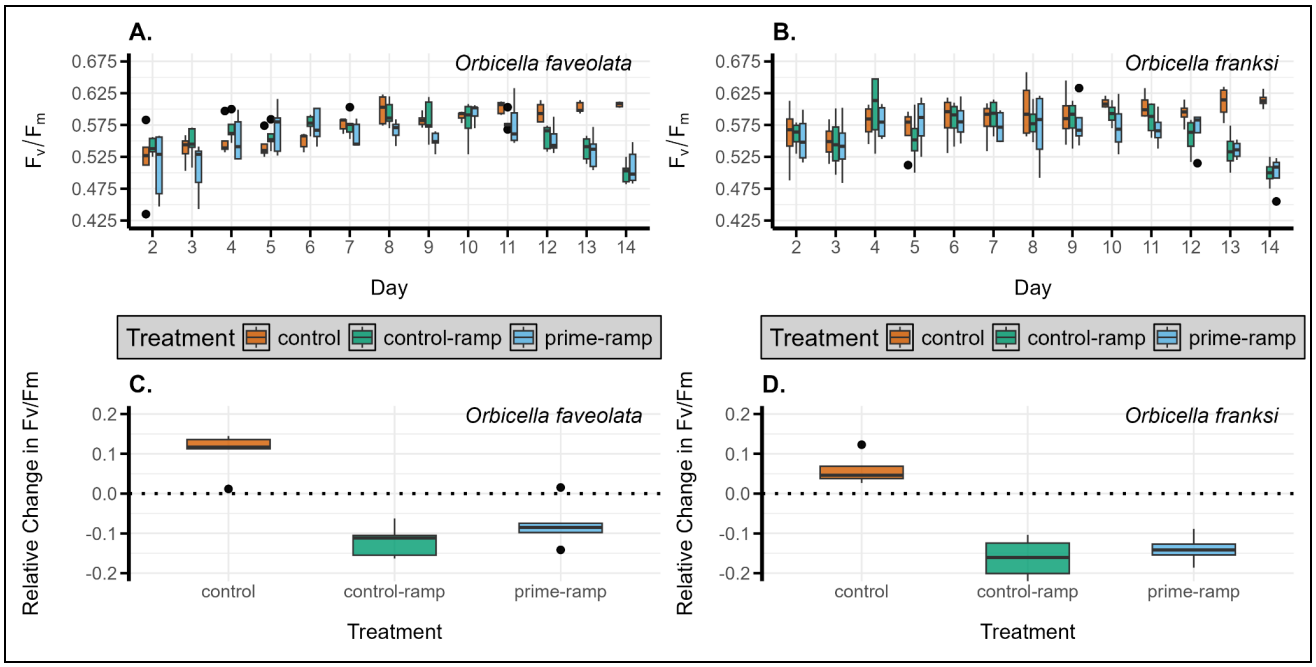

Figure 4. Change in Fv/Fm values over the experiment. Measurements of Fv/Fm were taken daily to assess coral health, with Fv/Fm as a measurement of the efficiency of photosystem II, which is involved in photosynthesis. Control-ramp and prime-ramp corals underwent a cold stressor, with temperature decreasing 1°C from day 4 to 14. Black dots represent outliers. A) **Fv/Fm of *Orbicella faveolata* from day 2 to 14.** Fv/Fm values of 0.6-0.7 indicate healthy corals. Lower values of Fv/Fm indicate decreased photosynthetic efficiency. B) **Fv/Fm of *Orbicella franksi* from day 2 to 14.** Fv/Fm values of 0.6-0.7 indicate healthy corals. Lower values of Fv/Fm indicate decreased photosynthetic efficiency. C) **Relative change in Fv/Fm from the fourth day to the final day of *Orbicella faveolata* by treatment.** Values greater than 0.0 indicate an increase in Fv/Fm over the experiment. Values lower than 0.0 indicate a decrease in Fv/Fm. 0.0 indicates no change. D) **Relative change in Fv/Fm from the fourth day to the final day of *Orbicella franksi* by treatment.** Values greater than 0.0 indicate an increase in Fv/Fm over the experiment. Values lower than 0.0 indicate a decrease in Fv/Fm. 0.0 indicates no change.

from the final and then dividing this by the day four mean for each coral ramet. Day four was used in place of the initial day of measurement (day 2) to avoid the lowered Fv/Fm values of prime-ramp during priming and the overall lower levels from acclimation. The relative change was also calculated for the red channel intensity values with the same methods, except utilizing the initial mean (day 0) and final mean. We employed Levene's test to assess the homogeneity of variances across treatment groups and the Shapiro-Wilk test to check for the normality of residuals, ensuring that the assumptions of ANOVA were met for valid results. If normality was not found, we performed a log transformation to normalize them. We then performed an ANOVA to determine whether there was a significant difference in Fv/Fm between the three temperature treatments: prime-ramp, control-ramp, and control. To determine whether different levels within a treatment were significant, a Tukey HSD (Honestly Significant Difference) test was performed. ANOVA and Tukey HSD tests were also conducted for the red channel intensity values to test for differences between treatments. P-values of less than 0.05 indicate statistical significance.

4 | Results

4.1 | Photosynthetic Efficiency

The photosynthetic efficiency of photosystem II (Fv/Fm) in *O. faveolata* and *O.*

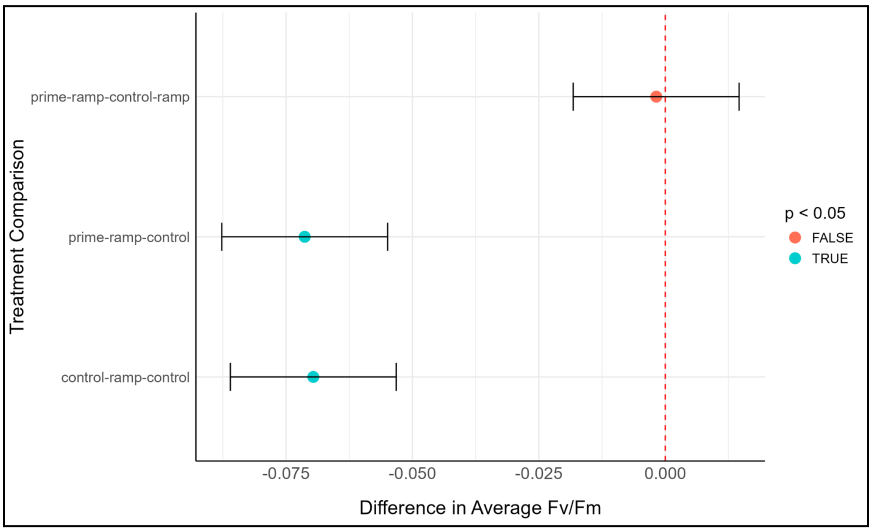

Figure 5. Difference in average Fv/Fm between treatments on the final day. Results of a Tukey HSD (Honest Significant Difference) test that compares the means of all pairs of treatments. Control-ramp and prime-ramp treatments underwent a thermal stressor through a cold ramp. Dots represent the mean difference. Error bars indicate the upper and lower bounds of the confidence interval. Red dots represent a lack of significant difference between treatments ($p \geq 0.05$), and blue dots represent significant differences ($p < 0.05$). Values less than zero indicate that the second treatment in the pair has a larger Fv/Fm value. 0.0 indicates no difference. There is a significant difference between the prime-ramp and control treatment pair and the control-ramp and control treatment pair. There is no significant difference between prime-ramp and control-ramp, indicating a lack of influence of priming.

franksi showed significant differences compared to the control group for both the prime-ramp and control-ramp groups (Figure 4). However, there were no significant differences between the two species. Control *O. faveolata* showed an initial low median of 0.527 Fv/Fm on day 2 and steadily increased to a peak of 0.609 Fv/Fm on day 14, experiencing a slight decrease on day 9 (Figure 4a). Control-ramp *O. faveolata* began at 0.538 Fv/Fm on day 2, fluctuating slightly until peaking at 0.591 Fv/Fm on day 10, and then sharply decreasing to reach a low of 0.503 Fv/Fm on day 14 (Figure 4a). Prime-ramp *O. faveolata* started at 0.529 Fv/Fm on day 2, increasing with slight fluctuations to 0.580 Fv/Fm on day 5, and then declining overall to a low of 0.498 Fv/Fm on day 14 apart from a sudden peak on day 10 at 0.602 Fv/Fm (Figure 4a).

Control *O. franksi* started at 0.568 Fv/Fm on day 2 and then dropped to a low of 0.549 Fv/Fm on day 3 (Figure 4b). They rose to a peak at 0.614 Fv/Fm on day 13 before slightly decreasing to 0.613 Fv/Fm on day 14 (Figure 4b). Control-ramp *O. franksi* fluctuated more, starting at 0.568 Fv/Fm on day 2 and peaking at 0.613 Fv/Fm on day 4 (Figure 4b). They stayed slightly below this peak before sharply declining from day 11, ending at 0.500 Fv/Fm on day 14 (Figure 4b).

Prime-ramp *O. franksi* started at 0.548 Fv/Fm on day 2 and peaked at 0.587 Fv/Fm on day 5. They remained relatively stable through day 12, after which dropping to a low of 0.509 Fv/Fm by day 14 (Figure 4b).

The median Fv/Fm of the control corals increased for both species, while control-ramp and prime-ramp corals decreased in Fv/Fm (Figure 4c, Figure 4d). Controls had slight variation in results, with *O. faveolata* increasing by 0.117 Fv/Fm and *O. franksi* by 0.0460 Fv/Fm (Figure 4c, Figure 4d). Both control-ramp groups had more variation, with *O. faveolat*a changing by -0.112 Fv/Fm and *O. franksi* by -0.160 Fv/Fm (Figure 4c, Figure 4d). Prime-ramp groups demonstrated minimal variation, with *O. faveolat*a changing by -0.0851 Fv/Fm and *O. franksi* by -0.142 Fv/Fm (Figure 4c, Figure 4d).

Figure 5 displays the results of a Tukey HSD comparing the difference in the average Fv/Fm between treatments on day 14, combining Fv/Fm values of both species. A lower Fv/Fm was exhibited for the prime-ramp averages compared to the control (-0.110), and these results were

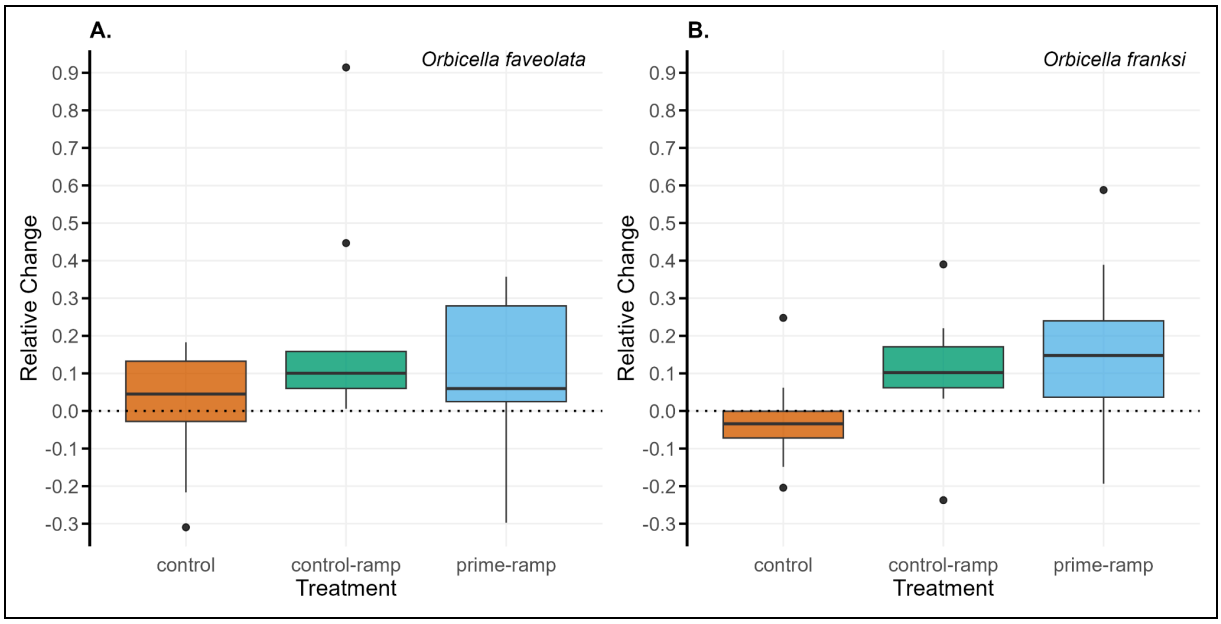

Figure 6. Relative change of red channel intensity from the initial and final days of the experiment. Control-ramp and prime-ramp corals underwent a cold ramp stressor. Higher values of relative change represent an increase in red channel intensity, which indicates the loss of the coral's symbiotic algae (Symbiodinaceae), also called cold bleaching. 0.0 represents no change. The box represents the 25th and top 75th quartile, with the line being the median. The whiskers represent the lower quartile's minimum and the upper quartile's maximum, at most 1.5 times the interquartile range (IQR = Q3 - Q1). A) **Relative change of *Orbicella faveolata.*** Relative change is the difference between the final and initial day red channel intensities, divided by the initial value. No significant difference in bleaching was found between treatments. B) **Relative change of *Orbicella franksi.*** Relative change is the difference between the final and initial day red channel intensities, divided by the initial value. No significant difference in bleaching was found between treatments.

significant (Figure 5, ANOVA, $p<0.05$). A lower Fv/Fm was also observed for the control-ramp averages compared to the control (-0.106), and these results were significant (Figure 5, ANOVA, $p<0.05$). There was little difference between the prime-ramp and control-ramp treatments in photosynthetic efficiency (+0.00433), thus insignificant (Figure 5, ANOVA, $p=0.777$).

4.2 | Coral Color (Red Channel Analysis)

Utilizing red channel intensity as an indicator of coral color which is predictive of chlorophyll content and symbiont density (Winters et al., 2009), Figure 6b displays the relative change of red channel intensity of each treatment from day 0 (initial) to day 14 (final) for *O. franksi*. The highest median relative change found was for the prime-ramp treatment (0.15), and the lowest was the median relative change of the control treatment (-0.5) (Figure 6a). The treatment with the highest variation in values was prime-ramp, with the range within 1.5 times the interquartile range (IQR) being approximately 0.6 (Figure 6a). Figure 6a displays the relative change of red channel intensity of each treatment for *O. faveolata,* in the same manner. All treatments observed a positive relative change, increasing in red channel intensity over the experiment (Figure 6b). The highest median relative change for this species was for the control-ramp treatment (0.1), and the lowest was for the control treatment (0.04) (Figure 6b). The highest variation in relative change was the prime-ramp treatment for *O. faveolata*, with the range within 1.5 times IQR being 0.65 (Figure 6b). The control-ramp values for *O. faveolata* and *O. franksi* were approximately the same (0.1) (Figure 6a, Figure 6b).

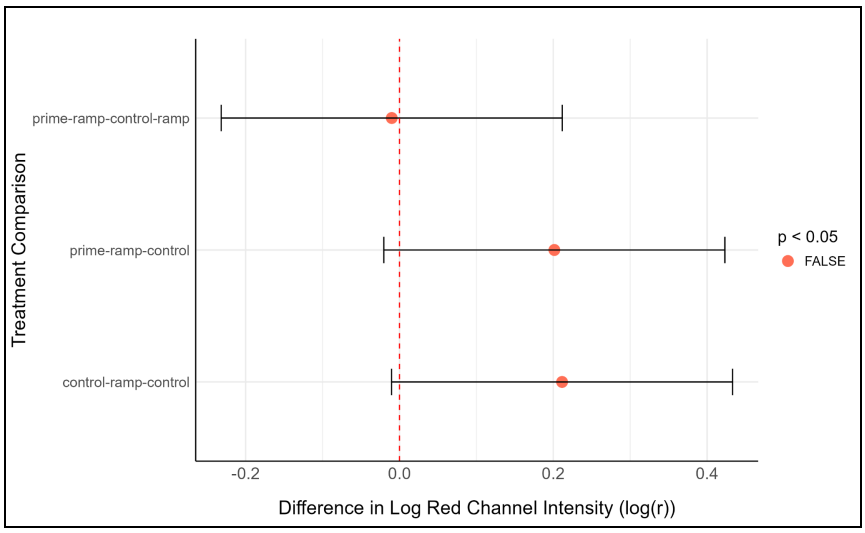

Figure 7. Overall difference in log red channel intensity (log(r)) between treatments on the final day. Results of a Tukey HSD (Honest Significant Difference) test that compares the means of all pairs of treatments. Prime-ramp and control-ramp treatments underwent a thermal stressor through a cold ramp. Values of red channel intensity (r) underwent a log transformation to normalize data for ANOVA. Dots represent the mean difference. Error bars indicate the upper and lower bounds of the confidence interval. Red dots represent a lack of significant difference between treatments, with $p \geq 0.05$. No treatment shows a significant difference in bleaching (red channel intensity) when compared to other treatments. The difference in log red channel intensity "x" indicates 10^x times higher red channel intensity of the first in the treatment pair.

Figure 7 displays the results of a Tukey HSD comparing the difference in the log red channel intensity between treatments on day 14, combining both species. A slightly larger red channel intensity was found for the prime-ramp means compared to the control (prime-ramp is 1.58 times larger), though this was not statistically significant (Figure 7, ANOVA, p=0.0800). There was also a slightly larger red channel intensity for the control-ramp than the control (1.62 times larger), but this was also not statistically significant (Figure 7, ANOVA, p=0.0637). There was little difference between the prime-ramp and control-ramp treatments in red channel intensity (prime-ramp 0.977 times control-ramp); therefore the difference was insignificant (Figure 7, ANOVA, p=0.993).

4.3 | Interesting Phenotypes Monitored

4.3.1 | Mucus Production

When comparing day 0 and day 14 images of the corals, we observed a reduction in mucus (slime) production in corals exposed to cold stress. Control *O. faveolata* and *O. franksi* produced a similar amount of slime on both day 0 and day 14 (Figure 8). In contrast, control-ramp *O. faveolata* and *O. franksi* showed decreased slime production by day 14, as did prime-ramp *O. faveolata* and *O. franksi* (Figure 8). This pattern shows a general decline in slime production over time in cold-stressed corals, regardless of the presence of priming.

4.3.2 | Mesenterial Filament Expulsion

While conducting salinity and temperature measurements on day 12, we noticed abnormalities in the phenotype of genet "F" of *O. faveolata* (Figure 9). Filaments were extruding from the polyp, initially identified as bleached tentacles. They were later identified as opaque mesenterial filaments, extending from the corals' polyps. Mesenterial filaments are thread-like structures that extend from the polyp mouths to help capture and digest foods. They contain digestive enzymes and nematocysts, aiding in feeding and defense (Coral Polyp Anatomy, n.d.). Other polyps exhibited a possibly similar phenotypic expression, but it is not clear whether they are bleached tentacles or mesenterial filaments.

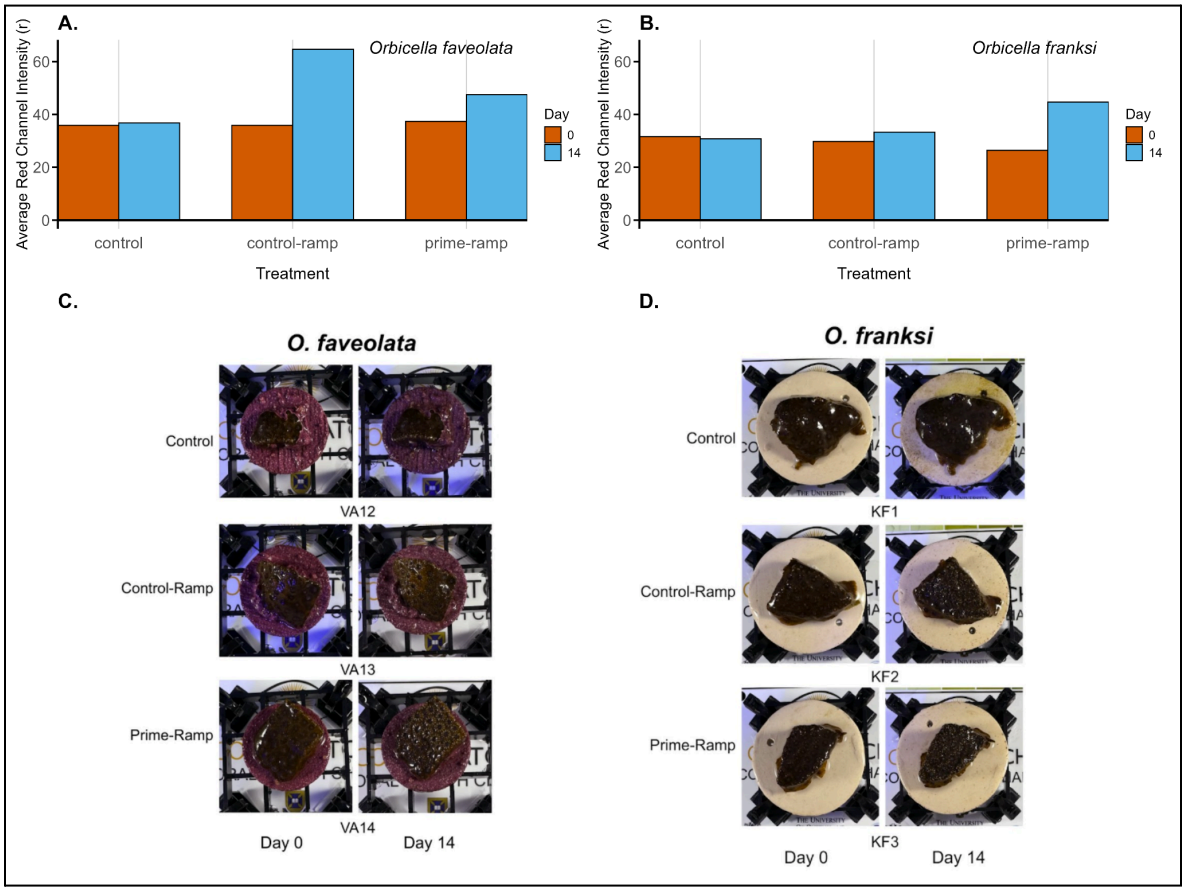

Figure 8. Comparison of *O. faveolata and O. franksi* from day 0 to day 14. Three ramets of a genet of each species were taken, VA12-14 and KF1-3. Control-ramp and prime-ramp corals underwent a thermal stressor through a cold ramp from 26°C to 15°C. A) **Average red channel intensity of *Orbicella faveolata* for genet "A" in each treatment.** Average for VA12-14 on day 0 (orange) and day 14 (blue). Red channel intensity as a measurement of chlorophyll density to represent loss of symbionts with thermal stress (cold bleaching). B) **Average red channel intensity of *Orbicella franksi* for genet "F" in each treatment.** Average for KF1-3 on day 0 (orange) and day 14 (blue) by treatment. Red channel intensity as a measurement of chlorophyll density to represent loss of symbionts with thermal stress (cold bleaching). C) **Difference in bleaching across treatments for genet "A" of *Orbicella faveolata.*** Coloration shown from the beginning of the experiment (day 0) to the end (day 14) with respect to their treatment. D) **Difference in bleaching across treatments for genet "F" of *Orbicella faveolata.*** Coloration shown from the beginning of the experiment (day 0) to the end (day 14) with respect to their treatment.

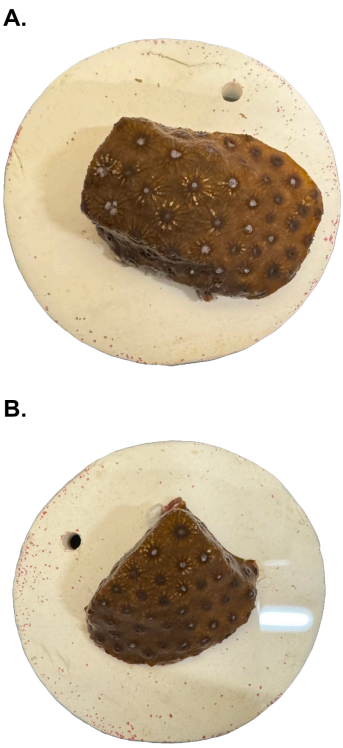

Figure 9. Images of the unique phenotypic response of genet "F" of *Orbicella faveolata*. Observed gut expulsion appearing from day 12 on for the *O. faveolata* genet "F" corals in the cold treatments. Expected to be a stress response due to the cold ramp from 26°C to 15°C. Phenotype not observed in the control coral of the same genet. A) **Image of gut expulsion of specimen VF2.** Light white spots on the coral represent mesenterial filaments (guts) expelled from polyp. B) **Image of gut expulsion of specimen VF3.** Light white spots on the coral represent mesenterial filaments (guts) expelled from polyp.

5 | Discussion

This study investigated the effect of cold priming on photosynthetic efficiency and chlorophyll density of O. *faveolata* and O. *franksi* with exposure to cold stress. We hypothesized that primed corals, that have experienced a short-term cold stressor and were then gradually ramped down in temperature, will have better photosynthetic efficiency than those who just experienced temperature ramping down. Our results did not support our hypothesis as control-ramp and prime-ramp corals had no significant differences in their photosynthetic efficiency and chlorophyll density. This suggests that cold priming did not increase the corals' tolerance to cold stress. The corals undergoing either cold treatment displayed decreases in photosynthetic efficiency and increases in red channel intensity, indicating vulnerability to cold stress. Our study provides new insights, including one of the first experimental applications of cold priming on *O. faveolata* and *O. franksi* and the novel observation that *O. faveolata* genotype "F" extends its guts in response to stress. These findings open up further avenues for understanding coral responses to environmental stressors.

5.1 | Effects of Priming on Photosynthetic Efficiency

Our analysis of photosynthetic efficiency in *O. faveolata* and *O. franksi* showed that Fv/Fm values in the control treatment increased throughout the experiment, confirming their health and suitability as a baseline for comparison. As expected, the photosynthetic efficiency decreased in the control-ramp and prime-ramp treatments, indicating a cold stress response. The Fv/Fm values began steeply decreasing after the temperature was lowered below 18°C. This is the thermal minimum for their natural habitat (Flower Garden Banks Marine Sanctuary), which is likely why going below this triggered a stress response (Dias et al., 2023). Significant differences were found between the control and cold-stressed groups, aligning with prior research showing the negative effects of cold stress on photosynthetic efficiency (Saxby et al., 2003). Our results display the danger of extreme cold on the biodiversity of coral reefs, revealing equivalent vulnerabilities to heat extremes. This highlights the need for conservation efforts focused on mitigating the negative effects of cold waters.

However, although there was a slightly smaller reduction in Fv/Fm for primed corals

compared to unprimed, this was found to be statistically insignificant. These results are a surprising contradiction to expectations that previous stress would mitigate the effect of thermal stress on Fv/Fm, as seen in other studies on priming (Middlebrook et al., 2012). This may be due to the experiment's short duration and the limited coral acclimation after being moved to the new lab, as all samples exhibited unexpectedly low Fv/Fm values during the first days of measuring Fv/Fm. Specifically, the corals only had one day to acclimate to the new light system before beginning the experiment. Exposure to rapid changes in light conditions and intensity affects coral health, leading to stress (Hoegh-Guldberg & Smith, 1989). Insufficient acclimation could have introduced stress responses that were unaccounted for in our experimental design, potentially altering results. This impact on initial Fv/Fm values could have been mitigated by conducting PAM (pulse-amplitude modulation) fluorometry on day 0, but we began measuring this on day 2. Thus, lacking this data limits our ability to establish baseline photosynthetic efficiency and fully assess the initial state of coral health before the cold treatments.

The two species exhibited some differences in their responses to the cold stressors across treatments. The median relative change of the prime-ramp and control-ramp corals differed more in *O. faveolata* than *O. franksi.* This could suggest that priming has more of an effect on this species, though these results are not significant. There is a lack of information on the differential abilities of these species to thermoregulate, so it is unclear if this is consistent with their thermotolerance in nature. Studies have found differences in thermoregulation within *O. franksi* collected from areas of different thermal regimes (Silbiger et al., 2019). This suggests a high likelihood of differences in thermotolerance within the congeneric species, though this could be due to differences in their collection location. All treatments of *O. faveolata* also had slightly greater values for relative change than *O. franksi*. Higher median relative change values may indicate that *O. faveolata* is better adapted to thermal challenges. However, early Fv/Fm data suggests otherwise. Across all treatments, *O. faveolata* began with lower Fv/Fm values on the first day of measurements, suggesting it was more negatively impacted by the stress of the new lab environment. This likely caused the observed reduction in photosynthetic efficiency regardless of the treatment applied.

5.2 | Effects of Priming on Coral Color

Red channel intensity analysis revealed that *O. franksi* and *O. faveolata* exhibited the highest median relative changes in the prime-ramp and control-ramp treatments, while the control group showed the least bleaching. Although the red channel intensities were slightly higher in the prime-ramp and control-ramp groups compared to the control, these differences were not statistically significant. Comparison between the species showed that the prime-ramp *O. franksi* had a higher median relative change in red channel intensity than *O. faveolata.* However, this difference is insufficient to conclude that *O. franksi* is a more thermotolerant species than *O. faveolata,* with further research needed to explore species-specific responses to cold bleaching.

These findings are surprising because cold priming, a process where mild stress exposure is used to enhance stress tolerance (Leuendorf et al., 2020), did not significantly affect chlorophyll density, as indicated by red channel intensity. No significant differences were observed between the cold-ramped treatments and the control, despite prior research showing that corals are susceptible to cold-water bleaching (Saxby et al., 2003). Short-term cold exposure has been shown to damage corals more than heat (Roth et al., 2013), making these results even more unexpected.

The lack of statistically significant differences suggests that cold priming may not improve the resilience of *O. franksi* and *O. faveolata* to cold stress. However, this outcome could be influenced by the sampling location. All corals were collected from the Flower Garden Banks National Marine Sanctuary, where average sea surface temperatures range from 22-26°C with occasional drops to 17.9°C in winter (Dias et al., 2023). It is possible the priming temperature, a 1°C reduction below the average, was not cold enough to elicit a meaningful priming response. Additionally, the one-day priming duration was not representative of natural conditions, and the corals did not have a sufficient recovery time before the ramp-down phase. This may have provided additional stress on the cold-primed corals, negating any potential benefits. Furthermore, the ramp-down may not have been cold enough or lasted long enough to induce a significant bleaching response in comparison to the control group.

5.3 | Future Research

To further our understanding of how cold priming affects reef-building corals' responses to temperature decreases, several areas of future research should be explored. The main limitation of our study was its relatively short duration and limited resources, which could be addressed through longer experiments with larger, more balanced sample sizes for *O. faveolata* and *O. franksi*. This would allow for more in-depth comparisons to help determine if significant changes in coral responses to cold priming occur over time. Other studies could incorporate genetic sequencing to examine variations in gene expression between cold primed, unprimed, and control corals. Seeing which genes are upregulated or downregulated in response to cold priming could provide insight into the molecular mechanisms underlying coral acclimation to temperature stress. Additionally, exploring corals at varying life stages could identify whether juvenile corals have a greater capacity to gain resistance to cold-water bleaching under cold priming compared to adult corals. It would also be beneficial to replicate these experiments with other reef-building corals to see if the findings are consistent across varying species. These studies would offer a more detailed understanding of how cold-water events and cold priming treatments influence coral health and resilience in the face of temperature stress.

Moreover, studies could focus on coral mucus production as a stress response to extreme temperatures. Investigating how different coral species modulate mucus secretion under cold or heat stress could provide important information about their adaptive mechanisms and stress responses. Understanding whether mucus plays a protective role in mitigating damage from temperature extremes may help identify potential indicators of coral resilience in the face of climate change.

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