Orbicella faveolata: Shallow Versus Mesophotic Coral Responses to Temperature Change

Erin Frates[1], Annabel Hughes[1], Anne Randall[1]

[1]Boston University Marine Program, Boston, MA

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Abstract:

Orbicella faveolata was added to The Endangered Species Act as threatened in 2014 due to anthropogenic influences in the wider Caribbean. As sea surface temperatures rise, the specific thermal tolerance of these populations at different depths remains unknown. Characterization of mesophotic corals is lacking in current literature in regard to both temperature range and thermal tolerance. This study aimed to analyze and compare the thermal stress responses of *O. faveolata* populations collected from both shallow-water and mesophotic reefs in East Flower Garden Bank. We treated coral samples collected from two depths: a shallow reef at a depth of 20 meters and a mesophotic reef at a depth of 43 meters. Two tank systems were used to subject corals to either an incremental increase or decrease in temperature, while a third system remained at 26ºC over the course of the experiment. Pulse-amplitude modulation (PAM) fluorometry and photographic surveys of color intensity were used to assess coral health by measuring photosynthetic efficiency of photosystem II and chlorophyll content/symbiont density, respectively. After 11 days, corals from both shallow and mesophotic depths exhibited a decrease in the photosynthetic efficiency and symbiont density, regardless of treatment. Mesophotic corals experienced more dramatic changes, suggesting that colonies from this depth are relatively less tolerant to thermal stress. Given the significant decline in heat-treated coral health and activity, *O. faveolata* appears more resilient to decreasing temperatures. This experiment provides insight into the comparative thermal stress responses of shallow and mesophotic corals, which broadens our understanding of coral responses to climate change and increasing sea surface temperatures.

Introduction:

Anthropogenic influences on our oceans are increasing average annual sea surface temperatures and lowering average pH values, effectively changing general reef structures throughout the tropics (Bongaerts et al., 2010). Smaller, fast-growing corals such as *Porites* spp. begin to dominate these areas as coral communities as we know them face physical degradation by human-induced climate change (Rippe et al., 2017). In 2014, *Orbicella* spp. were added to The Endangered Species Act as threatened, and this decline in *Orbicella* spp. is lowering structural complexity and biodiversity of important reef-dwelling fish (Rippe et al., 2017; Aronson et al., 2008). *Orbicella faveolata*, commonly known as the mountainous star coral, is a critical framework species in the wider Caribbean (Egan et al., 2021). *O. faveolata* is a broadcast spawning reef-building coral that demonstrates characteristic slow growth and long life spans (Page et al., 2018) which make their declining populations a great threat to Caribbean reef diversity.

In this study, *O. faveolata* colonies from both mesophotic and shallow depths were analyzed to better understand their thermal tolerance and provide insight into the potential future for this species as oceans warm. The 'deep reef refugia' hypothesis (DRRH) suggests that reefs at further depths are naturally protected from the disturbances affecting shallow-water corals, and therefore can provide the necessary reproduction to support shallow reef survival (Bongaerts et al., 2010). Shallow-water coral reefs lack the inherent depth buffer to anthropogenic activity and environmental change that mesophotic reefs have and consequently experience more variable conditions. We aim to obtain results that suggest how well *O. faveolata* fits into the DRRH as there is limited information available about how mesophotic corals respond to increasing sea surface temperatures (Gould et al., 2021). It remains unclear how shallow-water corals' natural tolerance to changes in environmental conditions differ from that of mesophotic corals.

This experiment was conducted with corals collected from the Flower Garden Banks National Marine Sanctuary (FGBNMS), which experiences a fairly wide range of temperatures. Shallow-water reefs, which are found from 0-30m, are exposed to temperatures ranging from 17.4ºC to 32.3ºC, while mesophotic reefs, which are found 30-150m, are exposed to temperatures that span from 19.3ºC to 30.3ºC (Watanabe et al., 2019; NOAA). FGBNMS is mildly secluded

from the rest of the Caribbean and therefore may host organisms with unique characteristics. For example, throughout the Caribbean, *Orbicella* spp. show a history of hosting mixed populations of Symbiodinium (Manzello et al., 2019), but studies specifically characterizing *Orbicella* spp. from FGBNMS show their symbiont populations to be dominated by *Breviolum minitum* (Lajeunesse et al., 2018; Green et al., 2014). Studies examining the holobiont of mesophotic corals are extremely limited.

Here, we examine how *O. faveolata* fragments collected from two different depths respond to incremental increases and decreases in water temperatures over an 11-day period to test the thermal tolerance of these corals and determine whether mesophotic reefs may serve as a refugium for shallow-water populations under climate change.

The goal of this experiment is to better understand mesophotic reef systems to fill the gap in the current literature regarding thermal tolerance as well as characteristics of hosted *Symbiodinium* populations by comparing them to well-studied shallow-water reef corals of the same species (Gould et al., 2021).

Due to the greater thermal variability experienced by shallow-water corals, we hypothesized that the *O. faveolata* fragments collected from the shallow reef would respond more favorably to the induced thermal fluctuations than those from the mesophotic reef. Given the temperature ranges withstood by *O. faveolata* in situ (NOAA), we anticipate that populations from both depths will have a greater rate of survival when treated to gradually decreasing temperatures, albeit with lowered productivity. Meanwhile, corals treated to increasing temperatures are suspected to have greater productivity, but a lesser rate of survival by the end of our experimental period.

Methods:

Experimental collections

In August 2018, corals were collected on a research cruise aboard the R/V Pelican from East Flower Garden Bank (Figure 1), one of 17 banks located within Flower Garden Banks National Marine Sanctuary (FGBNMS) in the northwestern Gulf of Mexico. Eight fragments of *Orbicella faveolata* were collected by divers from a depth of 20 meters. In August 2019, fragments from mesophotic *Orbicella faveolata* colonies were collected from the East Flower Garden Banks from a depth of 43 meters. These samples were collected via

Remotely Operated Vehicle (ROV) *Yogi* aboard R/V Manta, where they were maintained on deck in flow-through seawater until relocated to the Boston University Marine Invertebrate Research Facility. Colonies from both depths were later fragmented into multiple different ramets per genet and secured to ceramic dishes, and maintained at 26ºC until experimentation (Strader et al., 2020).

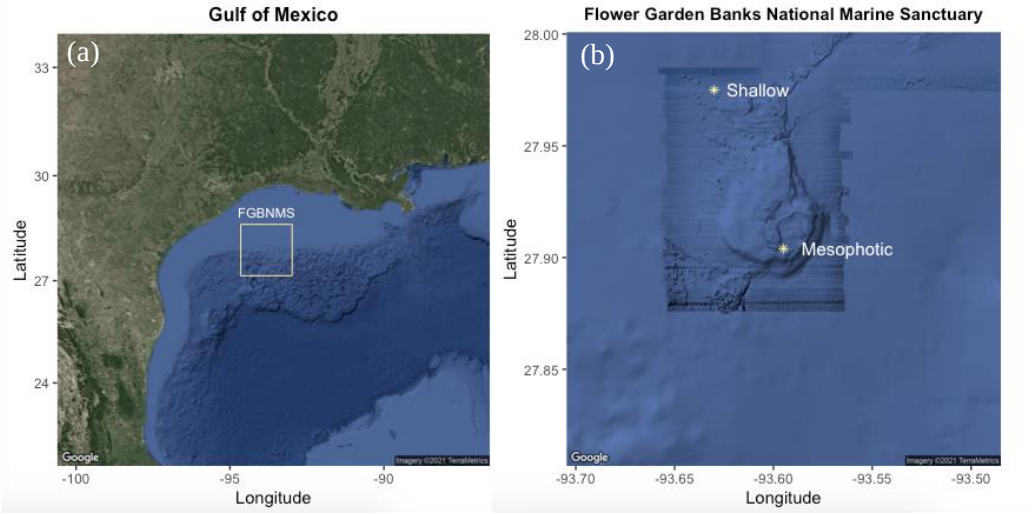

Figure. 1 Map of *Orbicella faveolata* sample collection sites. **(a)** Corals were collected from Flower Garden Banks National Marine Sanctuary (FGBNMS) in northeastern Gulf of Mexico. **(b)** Two sites were sampled to obtain *Orbicella faveolata* fragments from both shallow (20m) and mesophotic (43m) depths.

Common garden thermal stress experiment

Three experimental treatments, each consisting of three replicate tanks, were prepared with 26ºC seawater with salinities of approximately 33-35ppm. Nitrate levels in each system were recorded at the start and end of the experiment, and the temperatures and salinities were monitored three times daily throughout the experiment. Five *O. faveolata* colonies were selected from each depth. One representative fragment from each colony was placed in each of the three treatments, for a total of 15 shallow-water coral fragments and 15 mesophotic coral fragments (N=5 genets/depth; Table 1). All corals were submerged in Flatworm Rx for five minutes in order to remove flatworms and brittle stars, brushed to remove excess algae, and placed into the experimental tanks (Figure 2). In order to prevent unintentional bias that may arise from their location within the tank, the arrangement of the corals was

rotated counterclockwise daily. Corals were fed with brine shrimp every other day, cleaned to remove excess algal growth as needed, and were subjected to 12-hour light cycles with photosynthetically active radiation (PAR) set to 60, using AquaIllumination AI Hydra 32 HD LED.

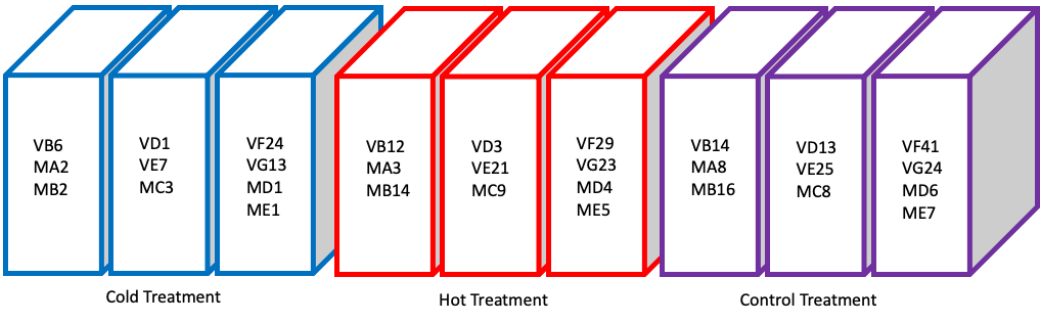

Figure 2. Experimental design showcasing how each treatment had three experimental tanks with coral fragments divided so that a ramet from each genet was represented in each treatment. Fragment IDs are shown in their respective tank assignments. VX# = shallow-water corals; MX#= mesophotic corals.

Community	*Genotype*	*ID*	*Treatment*
Shallow	B	VB12	Hot
		VB14	Control
		VB6	Cold
	D	VD3	Hot
		VD13	Control
		VD1	Cold
	E	VE21	Hot
		VE25	Control
		VE7	Cold
	F	VF29	Hot
		VF41	Control
		VF24	Cold
	G	VG23	Hot
		VG24	Control
		VG13	Cold
Mesophotic	A	MA3	Hot
		MA8	Control
		MA2	Cold
	B	MB14	Hot
		MB16	Control
		MB2	Cold
	C	MC9	Hot
		MC8	Control
		MC3	Cold
	D	MD4	Hot
		MD6	Control
		MD1	Cold
	E	ME5	Hot
		ME7	Control
		ME1	Cold

Table 1. *Orbicella faveolata* fragments divided by collection depth and genotype with the ID and respective treatment of each fragment provided.

In order to assess the physiological responses of *O. faveolata* from different depths to thermal stress, temperatures of two treatments were changed incrementally over the course of 11 days. The heat treatment began at 26ºC and was increased by 1ºC per day until reaching 35ºC; cold treatment began at 26ºC and decreased by 1ºC per day ultimately dropping to 17ºC (Figure 3). Temperature changes were conducted gradually and manually controlled using ApexFusion. The control system was maintained at 26ºC throughout the duration of the experiment.

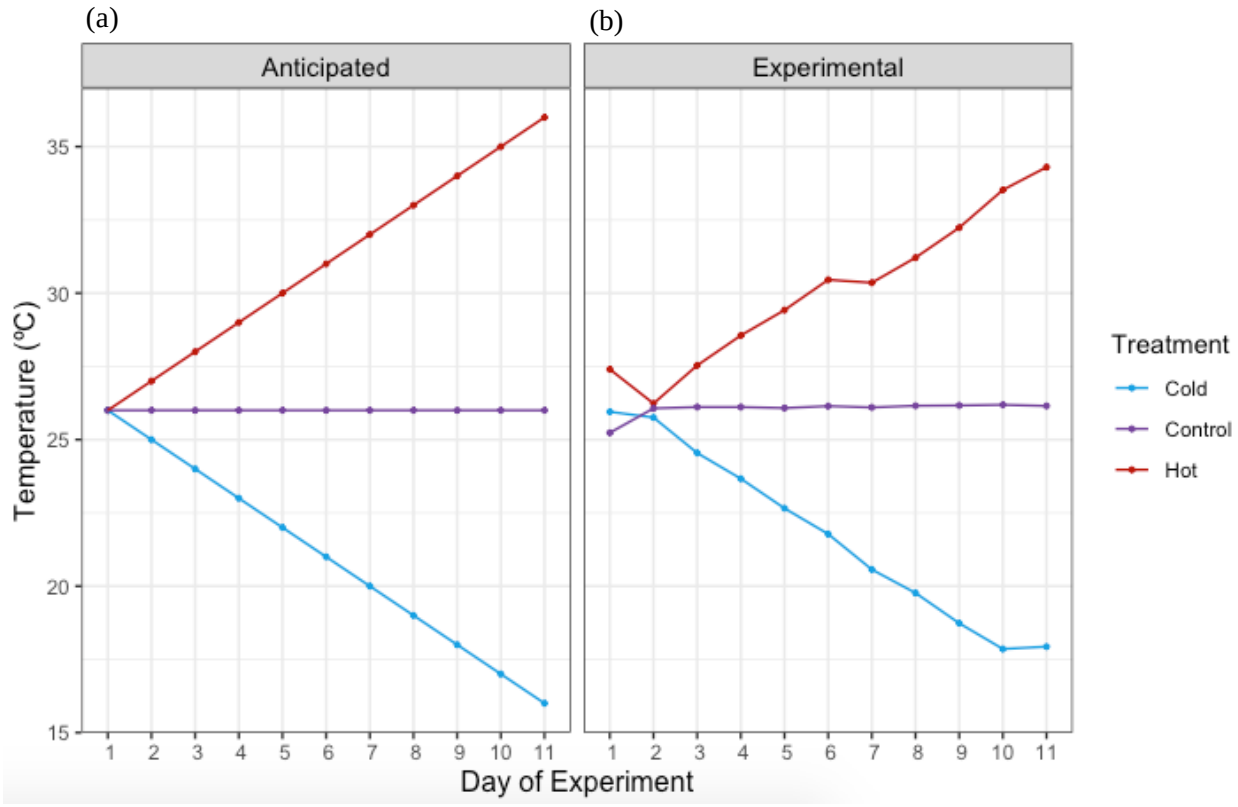

Figure 3. Daily set temperature across treatments. **(a)** Temperature ramps for each treatment according to the experimental plan. Hot and cold tanks were set to increase or decrease respectively by 1ºC daily. **(b)** True daily temperature patterns throughout the experiment due to heater and chiller malfunction.

Quantifying algal health

The change in photosynthetic efficiency of photosystem II (F_v/F_m) and a proxy for chlorophyll content/symbiont density were used to monitor coral health. Pulse-amplitude modulation (PAM) fluorometry was used to measure photosynthetic efficiency of photosystem II over the course of the experiment. PAM measurements were taken in terms of the chlorophyll fluorescence parameter F_v/F_m, which is the ratio of variable to maximum fluorescence after dark adaptation. These measurements were collected every other day using Junior-PAM, and these data were analyzed and visualized using RStudio Version 1.4.17.17 (RStudio Team, 2021). To determine chlorophyll content/symbiont density, photographs were taken of corals on days 1, 5, and 11 of the experiment. Coral color was then quantified using MATLAB according to Winters et al. (2009) and further analyzed in RStudio. Data was inverted in order to represent darker colors as larger, less negative numbers.

Statistical analysis

Significance between variables was tested in RStudio with analysis of variance (ANOVA) calculations. For factors containing more than 2 levels (ex. Treatments), a post-hoc test was run. In particular, we used Tukey tests, or Tukey's Honest Significant Difference tests in order to determine significance. P-values less than 0.05 were considered significant and p-values less than 0.1 were noted as trends.

Results:

Photosynthetic Efficiency of Photosystem II

After analyzing the PAM fluorescence data at the end of the experiment, we can conclude that the mesophotic corals had a higher F_v/F_m overall compared to the shallow-water corals (Figure 4). Steep declines in F_v/F_m were observed on day 11 in populations from both depths. For shallow-water colonies, every day's measurements differed significantly from the others except for day 6 compared to day 4 and day 8 ($p<0.005$). For the mesophotic colonies, every day differed significantly from day 11 ($p<0.0009$). Shallow-water colonies also present trends wherein hot treatment F_v/F_m values remained lower than control treatment values throughout most of the experiment ($p<0.07$).

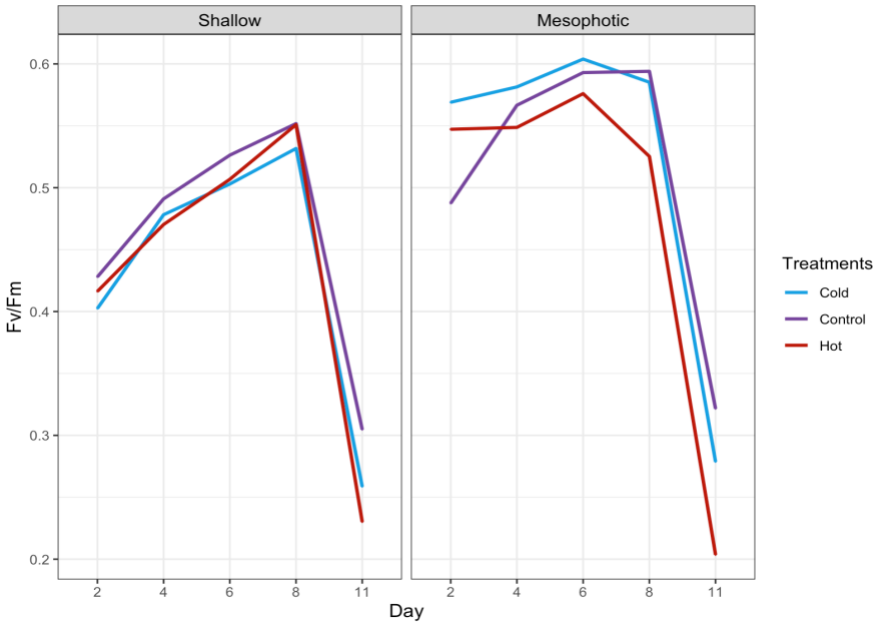

Figure 4. Change in photosynthetic efficiency of photosystem II (F_v/F_m) over the 11-day study of hot, cold, and control treatments. *Orbicella faveolata* collected from both 20m (Shallow) and 43m (Mesophotic) reefs on the Flower Garden Banks National Marine Sanctuary (FGBNMS) experienced a steep decline in Fv/Fm between days 8 and 11 of the experiment, regardless of treatment.

On day 2, F_v/F_m for both populations did not differ significantly across treatments (Figure 5a). It is still notable here that mesophotic corals exhibit higher F_v/F_m. On day 11, there is a parabolic F_v/F_m response across treatments from both populations, and the hot treatment shows significant differences from the control treatment for both shallow-water and mesophotic corals ($p<0.02$) (Figure 5b).

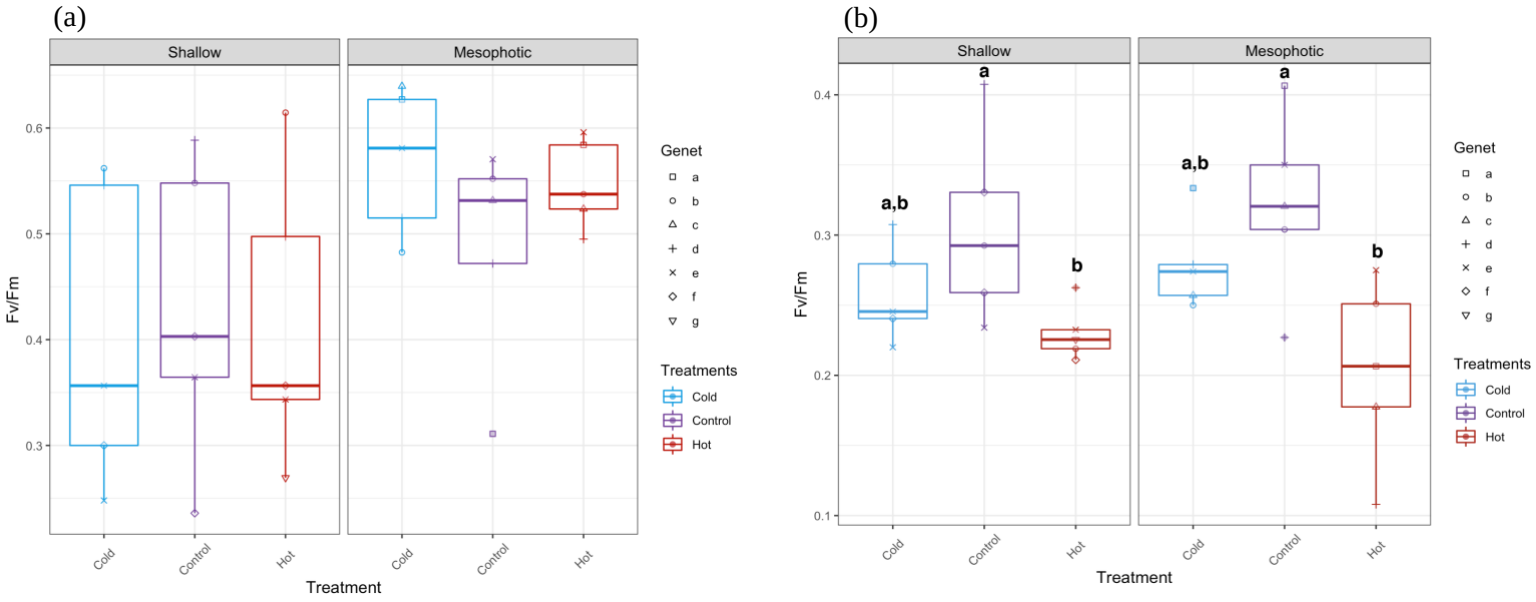

Figure 5. Photosynthetic efficiency of photosystem II (F_v/F_m) on (a) Day 2 and (b) Day 11 of the study separated by treatment (cold, control, and hot) and population depth (shallow or mesophotic). Colonies (genet) are indicated by shape. Letters indicate significance.

Relative change in F_v/F_m was analyzed to understand the cumulative differences between populations according to each treatment (Figure 6). Every treatment for both shallow-water and mesophotic corals resulted in an overall decreased F_v/F_m, with the mesophotic population being more affected than the shallow-water. There is yet again a parabolic trend in relative change of F_v/F_m for both populations across treatments. For the mesophotic colonies, we also noticed that there is a trend for the hot treatment to yield lower F_v/F_m values than the control treatment ($p<0.07$). This is not the case for the relative change across treatments for the shallow-water colonies.

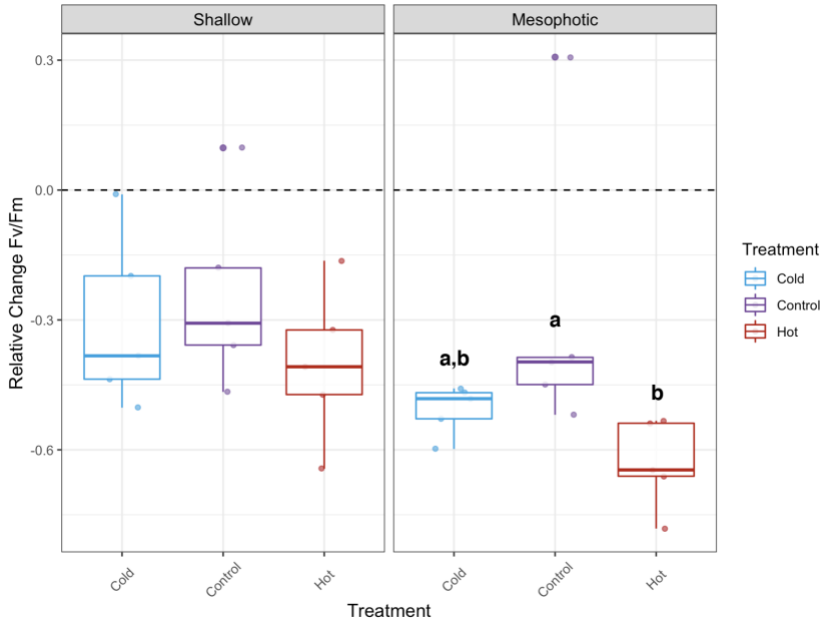

Figure 6. Relative change in photosynthetic efficiency of photosystem II (F_v/F_m) differed according to treatment (cold, control, or hot) and population depth (shallow or mesophotic). Letters indicate significance. Horizontal line indicated zero change.

Chlorophyll Content/Symbiont Density

Color intensity quantified from photo analyses in MATLAB is being used as a proxy for chlorophyll content and symbiont density within coral fragments (Figure 7). There is a parabolic response across treatments for populations from both depths (Figure 8). Though there is visual evidence of a decline in color intensity for the shallow-water corals, there is no significant difference between treatments on any day of data collection. On the other hand, the mesophotic corals show a significant difference between both the cold and hot treatments and the control treatment on the final day of data collection (day 11) ($p<0.04$).

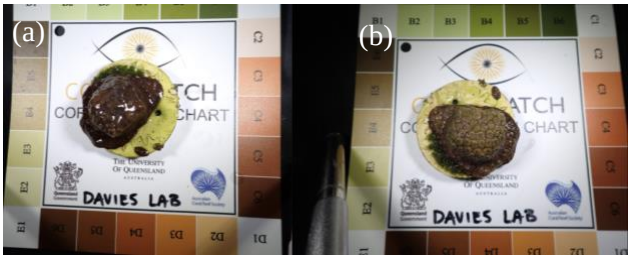

Figure 7. Photos of heat-treated *Orbicella faveolata* fragment VE21 for color analysis. Comparison of photos from (a) day 1 of experiment and (b) day 11 of experiment reflect the decline in health after subjecting coral to thermal stress.

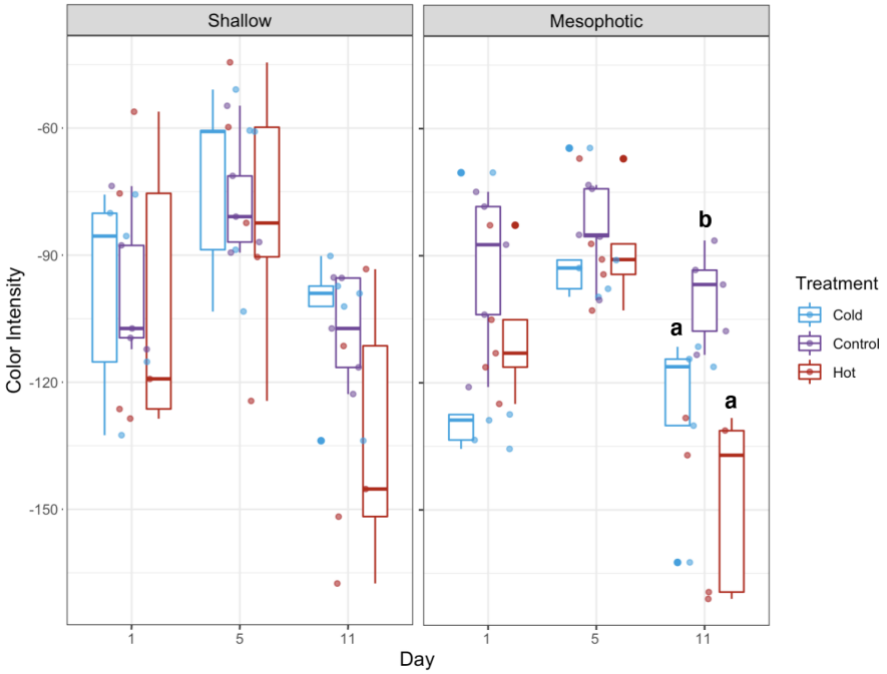

Figure 8. The change in color intensity of corals at three time points (Start: day 1, Mid-Point: day 5, End: day 11) throughout the experiment. Differences were observed according to treatment (cold, control, or hot) and population depth (shallow or mesophotic). Letters indicate significance.

Discussion:

Shallow-Water and Mesophotic Coral Response to Thermal Stress

The primary goal of this study was to examine how the thermal stress response of *Orbicella faveolata* from mesophotic reefs differs from that from shallow-water reefs. We anticipated that shallow-water corals would demonstrate a greater thermal tolerance than mesophotic corals of the same species, due to the considerable flux in environmental conditions at shallow depths. The initial data collected through PAM fluorometry suggests that there is little difference in the thermal stress response of corals regardless of the depth at which the coral originated, as the change in F_v/F_m according to variations in temperature produced similar patterns in both shallow-water and mesophotic corals (Figures 4, 5). However, upon comparing the relative change in F_v/F_m between populations, it becomes apparent that mesophotic corals reacted more negatively to increased temperatures than shallow-water corals (Figure 6). This is corroborated by data collected through color intensity analyses, which were used to assess symbiont density

and chlorophyll content. Here, it was determined that mesophotic corals exhibited a significant decline in symbiont density and chlorophyll content after 11 days of both temperature treatments. This outcome was not observed in shallow-water corals (Figure 8), suggesting that colonies from greater depths are more sensitive to thermal stress.

Interestingly, mesophotic corals demonstrated a greater F_v/F_m than shallow-water corals (Figure 4). Regardless, more work is needed to explore the implications that mesophotic coral activity could have for the rehabilitation or recovery of shallow-water coral reefs, which are expected to face more imminent environmental changes of a relatively greater magnitude. Given the sensitivity of mesophotic corals to thermal stress, it remains unclear whether mesophotic reefs have the potential to function as a refuge for threatened populations.

Orbicella faveolata response to hot and cold stress

This study also aimed to investigate how increasing and decreasing temperatures individually influence *Orbicella faveolata* populations. We expected that corals would exhibit greater resilience when treated to increasingly cold temperatures, given that the range of temperatures tested in the cold treatment resembled the in-situ temperature range experienced at FGBNMS more closely than the temperatures used in the heat treatment. Additionally, we hypothesized that the heat-treated corals would exhibit greater F_v/F_m compared to cold-treated corals. Results from both PAM measurements and color analyses suggest that cold-treated corals maintained higher F_v/F_m values, symbiont densities, and chlorophyll content throughout the course of the experiment (Figures 5, 8). This data supports the notion that corals were more tolerant of cold temperatures. While much of the data collected from cold-treated corals lacks significant difference from either the control or heat treatment corals, the latter differed significantly from controls (Figures 5, 6). This pattern also follows the distinct, albeit inconclusive trends observed in changing F_v/F_m and color intensity over time. As such, it is likely that heat-treated corals were prone to greater bleaching than cold-treated corals. Further work is necessary to determine with greater certainty whether, and if so, how cold stress and heat stress affect *O. faveolata* differently.

Limitations & Error

Throughout the experiment, there were complications with the automatic thermal

regulators used in both the hot and cold treatment tanks. The heater for the hot treatment tank and the chiller for the cold treatment tanks were both replaced during the course of the experiment due to mechanical failures. On the 6th and 7th days of the experiment, the heater failed and caused the temperature to slowly decrease from 30.8ºC to 30.2ºC over a 24 hour period, which interrupted the scheduled temperature ramping for the corals. The cold treatment system also had issues with thermal regulation as the chiller failed multiple times throughout the experiment; this is especially evident during days 10 and 11, where the system temperature increased from 17.7ºC to 18ºC. These issues with temperature regulation forced the experiment to be extended for an additional day so the systems could meet their target temperatures of 35ºC and 17ºC (Figure 3).

It should also be noted that the corals in our control tanks experienced a drastic decline in health towards the conclusion of our experiment, similar to that of temperature treated corals (Figure 4). This is likely due to the poor health of the fragments from which our corals were initially selected. Additionally, only a small number of coral fragments were available for this work, and despite efforts to control for their initial health, the fragments, particularly those from mesophotic colonies, began the experiment in a sub-optimal state. As a result, the sample size of our experiment was fairly limited. This work was also conducted over a robust yet brief experimental period, thus placing constraints on the time available to treat our corals. As a result, substantial changes to water temperature occurred frequently, and data was collected over fewer time points than was ideal.

Future Work

Due to the aforementioned time constraints on this study, further work is required in order to assess the effect of gradual temperature change on *O. faveolata* with higher resolution. Over a longer experimental period, temperatures could be increased or decreased by smaller increments, and corals could be afforded greater adjustment periods after each alteration.

Multi-stressor experiments investigating the effect of decreased pH on corals could provide insight into the tolerance of *O. faveolata* to ocean acidification and temperature rise. While this study provides insight regarding the impact of anthropogenic stress on coral health, further work is required to examine coral growth under such conditions.

A gap in knowledge regarding the community composition of algal symbionts of *O. faveolata* still remains, especially among mesophotic colonies. In order to address this, future work will be conducted sequencing the symbionts hosted by the corals present in this study. This work will not only identify and characterize symbionts hosted by *O. faveolata*, but will also allow for further comparisons of shallow-water and mesophotic coral reefs.

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