Let's be *franksi*: Divergent Thermal Challenges Elicit Unique Physiological Responses in Orbicellid Corals.

Aden Nagree, Delaney Foster, Sophia Bryan, Aby Yoon, Douglas Alvarado

Abstract

Orbicella faveolata and *Orbicella franksi* are both endangered Caribbean reef-building corals. As global sea surface temperature rises, corals experience greater and more consistent thermal stress, yet the physiological response of these species to thermal stress is relatively unknown; as is the effect of depth on thermal tolerance. This study aims to investigate the physiological impact of thermal stress on Caribbean corals, and intraspecies differences in resilience in relation to depth. In 2021, a thermal tolerance experiment was performed over 11 days on 15 coral nubbins (from 5 genets) of each shallow *O. franksi*, *O. faveolata,* and mesophotic *O. faveolata* under three treatments, hot and cold stress, and a control at 26°C. Symbiont density counts, chlorophyll, protein, and carbohydrate assays were conducted to assess physiological shifts in coral hosts and symbionts. This study found mesophotic *O. faveolata* to be the most tolerant of thermal stress and shallow *O. faveolata* to be the least tolerant. Additionally, increases in symbiont cell density and symbiont carbohydrate concentration (µg/µL) per surface area (mm^2) showed a significant correlation with a decrease in bleaching across all species. This suggests that an increase in symbiont cells could help keep up carbohydrate stores and bolster an individual's resilience. In the case of *shallow O. franksi*, it may be the case that having fewer symbionts per surface area (mm^2) was an asset in the face of increased temperatures because less symbionts were facing thermal stress and releasing reactive oxygen species. Future work should focus on the gene expression of these three populations to determine if differences in thermal resilience can be explained by differing immune responses.

Introduction:

Ocean water temperatures worldwide have been increasing at an unprecedented rate (Cheng et al., 2022). Among the many ecosystems affected are tropical coral reefs, partly due to tropical corals already existing at the upper end of their thermal optimum (Glynn, 1993). Thus, even small changes in ocean water temperatures have devastating effects on the corals inhabiting these areas. The primary result is coral bleaching, which often leads to large mortality events (Hoegh-Guldberg, 1999). Coral bleaching occurs when the host organism, while under a significant amount of stress, expels its algal symbionts (Lesser, 2011). The loss of algal symbionts leads to starvation of the host since corals receive up to 95% of their energy needs from these symbionts (Douglas, 2003). Bleaching, however, is not limited to hot temperatures, as cold temperatures also cause bleaching and increased mortality rates (Hoegh-Guldberg, Ove, et al., 2005).

In the Caribbean, bleaching and other coral stressors have led to nearly an 80% reduction in coral cover since the 1970s, and had 16% cover in 2016 (Conteras-Silva, et al., 2020). This regional trend differs from the nearby Flower Garden Banks National Marine Sanctuary (FGBNMS) located in the Northwest of the Gulf of Mexico, which has historically higher coral cover and was 57% in 2022 (Johnston et al., 2021; Johnston et al., 2022; Viehman, 2023). However, FGBNMS reefs are also at risk of bleaching, and these bleaching events have become more common in recent years (Johnston et al., 2021; Johnston et al., 2022). Reefs in FGBNMS are found primarily across two banks: East Flower Garden Bank (EFGB) and West Flower Garden Bank (WFGB). There is also approximately 20 km between EFGB and WFGB, which limits interactions between the two regions (Johnston et al., 2022).

Within FGBNMS there are three important tropical reef-building corals, *Orbicella franksi, Orbicella faveolata,* and *Orbicella annularis*, although *O. annularis* is rarer at these sites (Johnston et al., 2021; Johnston et al., 2022). Corals in the genus *Orbicella* are reef-building species and are found in shallow and mesophotic waters (Egan et al., 2021; Rippe et al., 2017). All *Orbicellids* are under threat due to global climate change and were classified as threatened under the Endangered Species Act in 2014 (Johnston et al., 2021). In the tropics, *O. franksi* and *O. faveolata* can be found up to 60 meters deep (Egan et al., 2021). These two coral species exhibit similar distributions in FGBNMS. Along with this, *O. franksi* and *O. faveolata* host the same algal symbiont (genus *Breviolum*) when on the same bank in FGBNMS (Green et al., 2014).

The thermal tolerance and response of *O. franksi* and *O. faveolata* were previously investigated by Boston University's Marine Program. A thermal challenge experiment over 11 days was performed on *O. franksi* and *O. faveolata* colonies collected from shallow depths (N=5 colonies per species) and mesophotic *O. faveolata* (N=5 colonies). 5 ramets (1 from each genet) of each species/depth were placed under one of three treatments: heat challenge (increased by 1°C per day until peaking at 35°C), cold challenge (decreased by 1°C per day until reaching 17°C), and control (maintained at 26°C). Coral color was analyzed in MATLAB as a proxy for bleaching in response to temperature stress (Winters et al., 2009). These studies found that *O. faveolata* at both depths and *O. franksi* experienced decreased color under both thermal challenge conditions (Chan et al., 2021 & Frates et al., 2021).

To develop an understanding of how bleaching changes the physiology of coral, the physiological analysis of coral nubbins, from the aforementioned experiments, will bridge the gap between the observed thermal challenge responses and the associated physiological changes. To do this, a variety of metrics including symbiont density, chlorophyll, carbohydrates, lipids, and proteins can be used to evaluate changes, as bleaching events result in decreases in symbionts, chlorophyll, or concentrations of both. The reduction in symbionts and/or chlorophyll will force corals to rely on their energy reserves (carbohydrates, proteins, lipids) instead. With depleted energy reserves and no way to photosynthesize, corals take longer to recover or have higher mortality rates (Rodrigues & Grottoli, 2007; Grottoli et al., 2006). Furthermore, as the symbiosis between the coral host and symbiont breaks down due to stress, both components are likely to exhibit integrated physiological responses (Bove et al., 2022).

This study aims to identify the physiological responses caused by hot and cold temperature challenges and whether these responses differ between *O. faveolata* from different depths (shallow and mesophotic) or between different Orbicellid coral species (*O. faveolata* and *O. franksi)*. From this, a more holistic view of coral response to thermal stress and bleaching can be constructed. We hypothesize mesophotic *O. faveolata* samples will have lower concentrations of chlorophyll and energy reserves compared to their shallow counterparts under thermal challenge; as there is less available light in the mesophotic and these corals are exposed to more stable temperatures unlike their shallow counterparts (Lesser et al., 2009). Additionally, Chan et al., (2021) suggests that *O. franksi* may be more heat tolerant since they were more successful in maintaining their color under thermal challenge. Therefore, we expect to see higher levels of chlorophyll and energy reserves in thermally challenged *O. franksi* samples compared to *O. faveolata*. Due to less severe bleaching, these corals could still receive carbon sugars from their symbionts rather than having to rely on stored carbohydrates or proteins to survive. The physiological responses are vital to understanding coral responses, but literature surrounding coral bleaching rarely focuses on the physiology. Understanding the physiological response of corals under divergent thermal conditions will add to the growing body of literature on the many effects bleaching has on corals, and aids in the conservation of these ecologically key organisms.

Species	Genotype	ID	Treatment
Shallow Orbicella franksi	B	KB17	Hot
		KB18	Control
		KB2	Cold
	C	KC2	Hot
		KC3	Control
		KC1	Cold
	E	KE3	Hot
		KE15	Control
		KE4	Cold
	F	KF4	Hot
		KF6	Control
		KF21	Cold
	G	KG25	Hot
		KG34	Control
		KG13	Cold

Species	Genotype	ID	Treatment
Shallow Orbicella faveolata	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

Species	Genotype	ID	Treatment
Mesophotic Orbicella faveolata	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

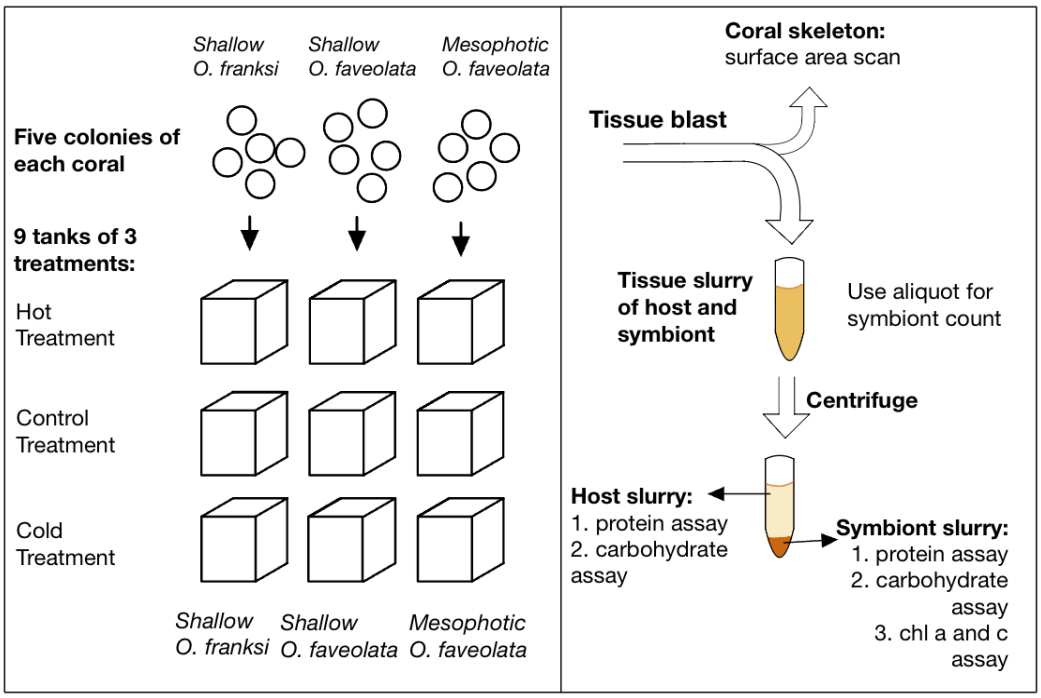

Figure 1. Experimental design summary of the previous experiments showing how each treatment was organized. Each treatment (hot, cold, and control) had three tanks with 5 nubbins in each tank, for a total of 45 samples. A ramet of each genet is represented in each treatment. The first letter of each sample ID corresponds to the sample's species: K = shallow *O. franksi*, M = mesophotic *O. faveolata*, and V = shallow *O. faveolata*. The second letter of each sample ID denotes the genet to which each ramet stemmed from. Coral nubbins were rotated counterclockwise to ensure all experienced the same conditions.

Methods:

Study Area. Five colonies of shallow *O. franksi* and five colonies of shallow *O. faveolata* corals were collected from EFGB in the FGBNMS in August 2018 (21-23 m depth; collection 1). In August 2019, five samples of mesophotic *O. faveolata* corals were collected in the same region (39-42 m depth; collection 2). All corals underwent three experimental treatments: heat challenge (raised 1˚C/day for 11 days to a maximum of 35°C); cold challenge (reduced 1˚C/day for 11 days to a minimum 17°C); and control (maintained at 26°C). All treatments had three tanks per treatment and one nubbin from each of the 15 colonies of *O. franksi* (N=5) and *O. faveolata* (N=10) were distributed into each of the nine experimental tanks (Figure 1). At the experiment's beginning and end, photographs of all nubbins were taken to assess coral color. Once the experiment concluded, all nubbins were flash frozen and stored in a -80°C freezer for future analysis.

Host and Symbiont Tissue Preparation. Frozen nubbins were removed from storage and thawed for thirty minutes. Once thawed a sterile razor blade removed tissue for RNA. Tissue was then removed from the coral skeleton with an airbrush using seawater. Samples were homogenized for three minutes, and a 300 µL aliquot was taken and stored. Symbiont cells were pelleted (4400 rpm; 3 min) via centrifugation, and a 1.2 mL sample was transferred into bead blasting tubes. Symbiont pellets were washed with seawater

and resuspended in a total of 5 mL of seawater. 1.2 mL of resuspended symbiont cells were placed in bead-blast tubes and blasted at 6 m/s for two minutes. Following bead blasting, samples were frozen until downstream analyses were performed.

Symbiont Cell Counting. 10 µL of symbiont cells were loaded from a 300 µL aliquot sample into each chamber of a hemocytometer. Five subsections were chosen randomly in each chamber of the hemocytometer and symbiont cells were counted. Symbiont cell counts were averaged by the number of subsections counted (10) to get a representative amount of symbiont cells in the 300 µL aliquot sample.

Physiological Assays. Assays for protein, carbohydrate, and chlorophyll concentrations for coral and symbionts were conducted following lab protocols. Proteins were quantified with a bicinchoninic acid protein assay (BCA: absorbance at 562 nm) and carbs using phenol-sulfuric acid (absorbance at 490 nm) were done on hosts and symbionts. Chlorophyll was extracted with acetone and then quantified spectrophotometrically (absorbance at 630 nm) for symbionts.

Skeletal Scanning & Surface Area Calculations. Tissue-blasted coral nubbins were 3-D scanned using an Einscan-SE 3-D scanner, with Einscan-S series version 2.5.0.7 software. Once coral nubbins were fully scanned, non-coral portions of the scan were deleted using the Einscan-S series. Large, non-coral portions were deleted, and then the finished coral scan was imported into Meshlab version 2020.07 to calculate the complete surface area of the scan. Non-tissue parts of the coral scan were highlighted to calculate the surface area of these parts of the coral scan. The non-tissue surface area of the coral scan was subtracted from the complete surface area scan to calculate the total surface area of live tissue of the coral nubbin. All physiological metrics were then normalized to surface area.

Statistical analysis. All data were analyzed and visualized using RStudio Version 2023.06.2+561 (RStudio). A series of box plots were made comparing the three thermal treatments (hot, cold, and control) within the species groups (mesophotic *O.faveolata,* shallow *O. faveolata,* and shallow *O. franksi*) to red channel intensity collected from previous experiments. Treatments within a species were also compared with symbiont counts and symbiont carbohydrate concentrations collected during the current experiment. ANOVAs were run on each species group to determine if there was any significance between the means of each treatment. If the ANOVA showed significance, a post-hoc test, Tukey's Honest Significant Difference (HSD), was run to determine which treatments were statistically different from each other. Correlations between all available data were found with Spearman's correlation coefficient matrices. These were broken down by the aforementioned species groups. Those with significant p-values, ($p < 0.05$) were made into linear regression models to further assess the strength of the relationship.

Results:

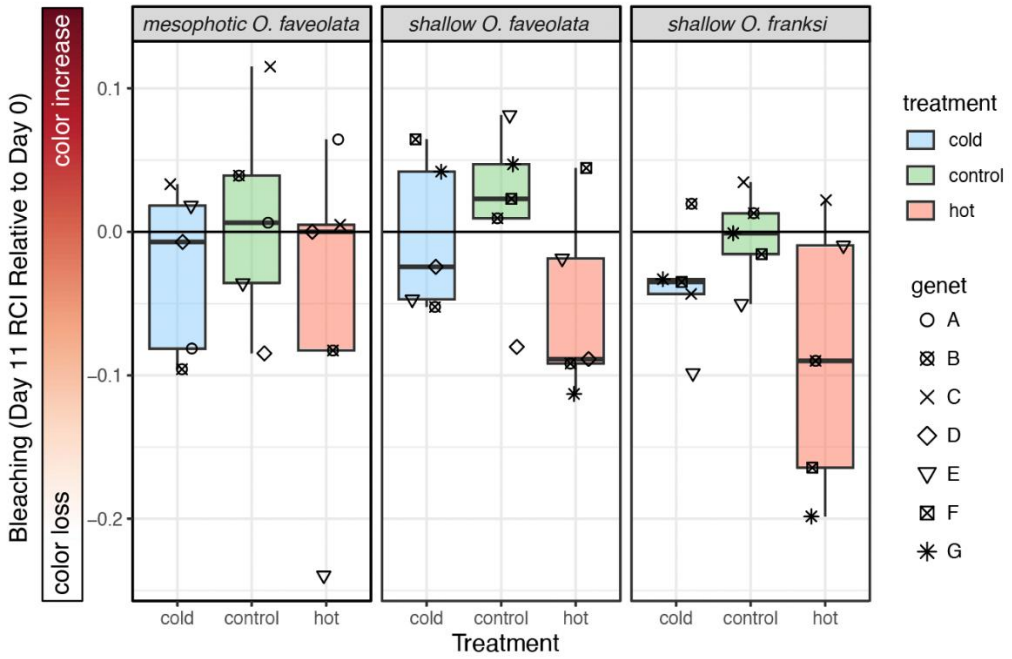

Figure 2. Change in red channel intensity (AU) on day 11 relative to day 0, separated by treatment (cold, control, and hot) and faceted by species (mesophotic *O. faveolata*, shallow *O. faveolata*, and shallow *O. franksi).* The line at 0.0 represents initial measurements from day 0. Genets are represented by different shapes indicated in the genet legend on the right, and treatments are differentiated by color as shown in the treatment legend. The red to white gradient represents red channel intensity, and is the corresponding color to the y-axis values. ANOVAs were run based on species and showed no significant difference between treatments for each species facet.

Increased bleaching under thermal stress. Color intensity data collected from Chan et al. (2021) and Frates et al. (2021) was reconfigured to plot the rate of change of red channel intensity, used here as a proxy for bleaching, on day 11 relative to day 0 (Figure 2). Values below 0 indicate a loss of color throughout the experiment, while values above 0 indicate an increase in color saturation. All thermally stressed coral populations except heat-challenged mesophotic *O. faveolata* displayed lower mean red channel intensity compared to their control treatment; the mean for mesophotic *O. faveolata* remained at 0. All control groups increased in color or remained at 0. No differences in means between treatments with a species were statistically significant.

Under the heat challenge, both shallow species of *O. faveolata* and *O. franksi* decreased in color, while mesophotic *O. faveolata*, on average, did not change. In mesophotic *O. faveolata*, two genets (A and C) gained coloring, one (D) did not change, and two (E and B) showed decreased color intensity. For both shallow *O. faveolata* and *O. franksi*, most genets showed lower red channel intensity (B, D, E, and G; B, D, F, and G respectively) (Figure 2).

Under the cold treatment, the mean red channel intensity for all three corals showed decreased color. Three genets in both the mesophotic *O. faveolata* (D, B, and A) and shallow *O. faveolata* (D, E, and B) decreased in color intensity, while two genets showed increased values (C and E; F and G respectively). In shallow *O. franksi*,

all genets under the cold challenge decreased in color intensity.

Overall, shallow *O. faveolata* displayed the greatest mean red channel intensity change relative to the control, and mesophotic *O. faveolata* exhibited the least. Additionally, the mean change of thermally challenged mesophotic *O. faveolata* was the closest to the original measurements from day 0.

Decreased symbiont density under thermal stress. Coral symbiont cell counts normalized by surface area reveal lower symbiont densities under thermal challenges (Figure 3). All three populations have lower mean symbiont densities for thermal challenge treatments compared to their control treatments; however, this difference is only significant for mesophotic and shallow *O. faveolata* ($p<0.05$). There is no statistical difference between the cold and hot treatments for any species, and for *O. franksi* there is no statistical difference between any treatment. Additionally, mesophotic *O. faveolata* has the highest control treatment average symbiont density, while shallow *O. franksi* has the lowest. Across all species, the mean symbiont cell density for the heat challenge is the lowest out of all three treatments. For shallow *O. franksi* and mesophotic *O. faveolata* the mean symbiont cell density for the cold treatment is similar to that of the heat treatment.

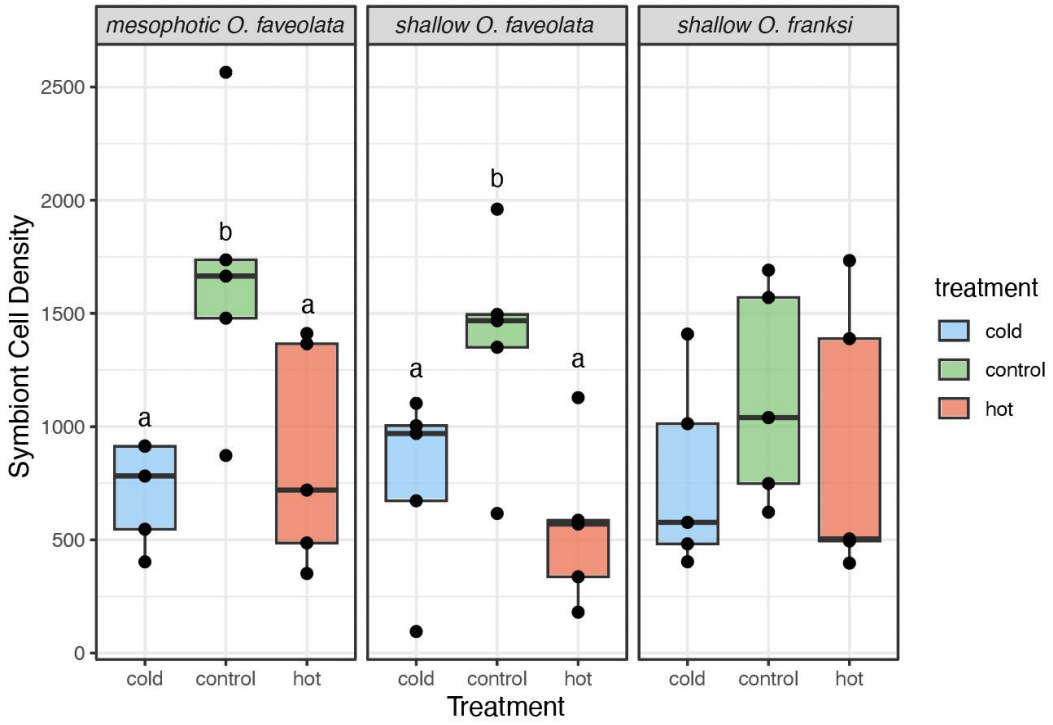

Figure 3. Symbiont cell density (number of symbiont cells per surface area (mm^2)) separated by treatment (cold, control, and hot) and faceted by species (mesophotic *O. faveolata,* shallow *O. faveolata,* and shallow *O. franksi).* Treatments are differentiated by color as shown in the legend. ANOVA tests revealed significant differences in mesophotic *O. faveolata* and shallow *O. faveolata* data. A post hoc Tukey test revealed statistically significant differences between the control treatment and the thermal stress treatments for both (represented by the different letters above; TukeyHSD $p<0.05$). The data shows no significance between any treatments for shallow *O. franksi.*

Symbiont carbohydrates under thermal stress. Symbiont carbohydrate concentration decreases under thermal stress across species. All coral species display the lowest average carbohydrate concentration under heat treatment; however, this difference is only statistically significant in shallow *O. faveolata* (Figure 4, $p<0.05$). The mean for heat treated *O. franksi* and mesophotic *O. faveolata* appear to be similar, but the mean of shallow *O. faveolata* is lower. For all species, the mean symbiont carbohydrate concentration of cold treated corals is greater than or equal to that of the species control group. Despite this, there is no significant difference between control and the cold treatment for any species. Additionally, shallow *O. faveolata* observed the lowest mean control carbohydrate concentration under the heat challenge and the highest mean concentration in the cold challenge.

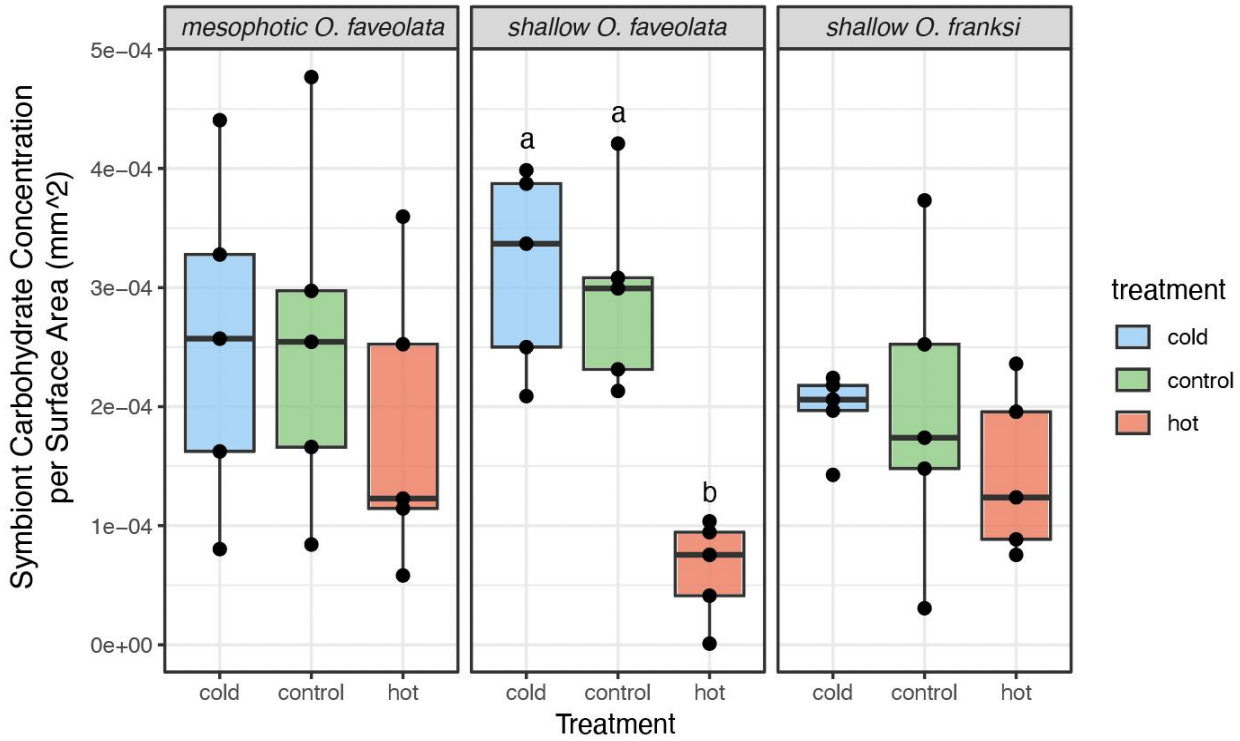

Figure 4. Symbiont carbohydrate concentration (µg/µL) per surface area (mm^2) separated by treatment (cold, control, and hot) and faceted by species (mesophotic *O. faveolata*, shallow *O. faveolata*, and shallow *O. franksi).* ANOVA test revealed a significant difference in shallow *O. faveolata* data, and a post hoc Tukey test showed it to be between the hot treatment and the cold and control treatments (highlighted by different letters above; TukeyHSD $p<0.05$). The data shows no significant difference between treatments in either mesophotic *O. faveolata* and shallow *O. franksi.*

As seen in Figure 5., Mesophotic *O. faveolata* and shallow *O. faveolata* appear to have similarities in the correlation of their physiological variables while shallow *O. franksi* looks most dissimilar from the two.

Mesophotic *O. faveolata* shows a moderately positive correlation between symbiont protein concentration per surface area and host protein concentration per surface area ($R^2 = 0.56$, $p = 0.029$) as well as symbiont cell density ($R^2 = 0.61$, $p = 0.017$).

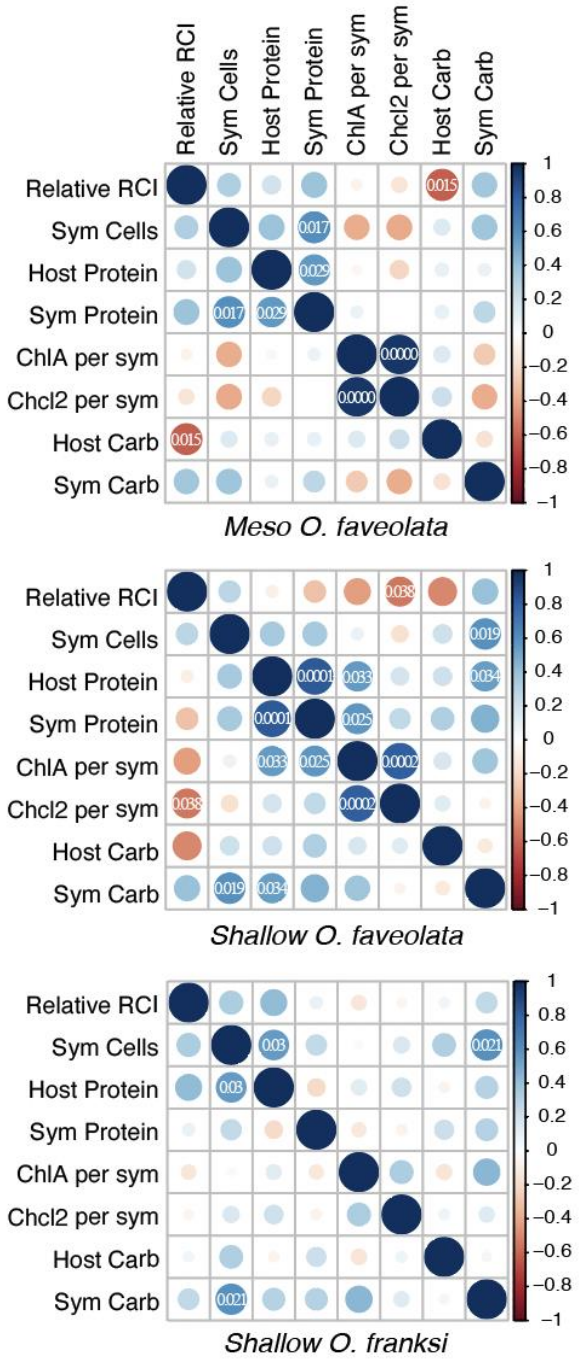

Figure 5. Correlation matrices of physiological traits for (a) mesophotic *O. faveolata*, (b) shallow *O. faveolata*, and (c) shallow *O. franksi.* Strength of R^2 values are depicted by size and color scale shown on the right; darker blue is a strong positive relationship and red is a strong negative relationship. Significant correlations are highlighted with included p-value.

The deep water corals also show a mild negative correlation between host carbohydrate concentration and relative red channel intensity (R^2 = -0.62, p = 0.015). There is also a strong positive correlation between chlorophyll A per symbiont and chlorophyll C2 per symbiont (R^2 = 0.97, p = 0.0000)

Shallow *O. faveolata* show a strong positive correlation between symbiont protein concentration per surface area and host protein concentration per surface area (R^2 = 0.83, p = 0.0001). There are weaker but still moderate correlations between chlorophyll A per symbiont cell and both host and symbiont protein concentrations per surface area (R^2 = 0.55, p = 0.033 and R^2 = 0.58, 0.025 respectively). Symbiont carbohydrate concentration per surface area is mildly correlated with symbiont cell density (R^2 = 0.60, p = 0.019) as well as host protein concentration per surface area (R^2 = 0.55, p = 0.034). Also, there is a strong correlation between chlorophyll A per symbiont and chlorophyll C2 per symbiont (R^2 = 0.82, p = 0.0002). There exists a moderate, negative correlation between chlorophyll C2 per symbiont cell and relative red channel intensity (R^2 = -0.54, p = 0.038).

Shallow *O. franksi* exhibit a moderate positive correlation between symbiont cell density and both host protein concentration per surface area (R^2 = 0.56, p = 0.03) and symbiont carbohydrate concentration per surface area (R^2 = 0.59, p = 0.021).

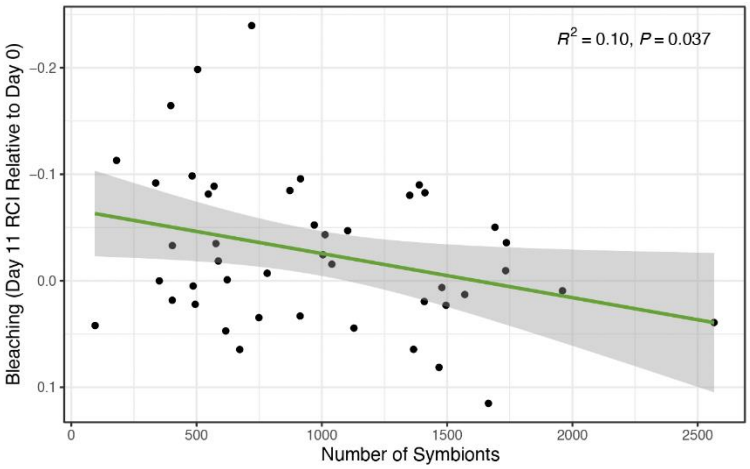

Figure 6. Linear regression of change in channel red intensity (AU) after 11 days plotted against the change in the number of symbionts per surface area (mm^2). Gray shading around the regression line represents standard error. We observe a weak negative relationship. (R^2 =0.10, p=0.037)

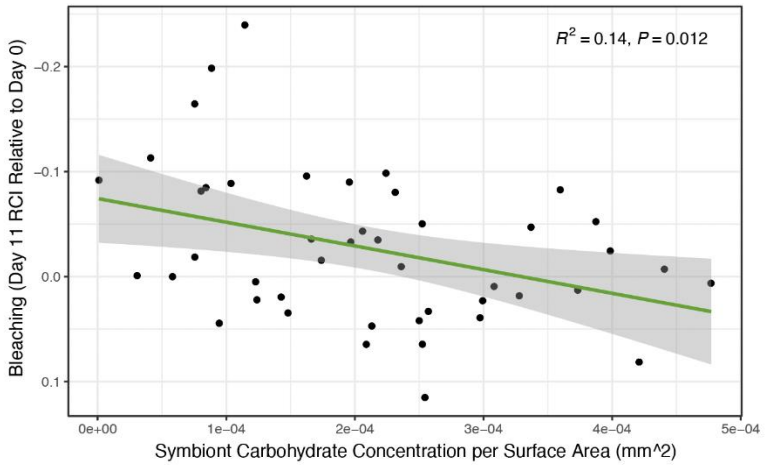

Figure 7. Linear regression of change in channel red intensity (AU) after 11 days plotted against the symbiont carbohydrate concentration (µg/µL) per surface area (mm^2). Gray shading around the regression line represents standard error. A weak negative relationship can be observed(R^2=0.14, P=0.012).

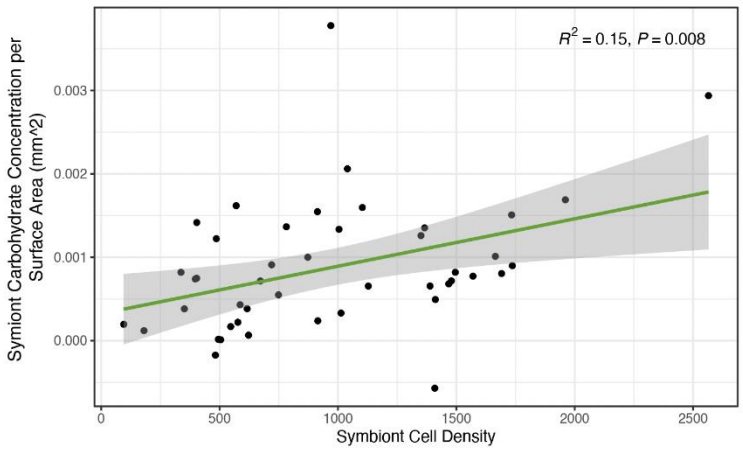

Figure 8. Linear regression between symbiont carbohydrate concentration (µg/µL) per surface area (mm^2) and symbiont cell density. Gray shading around the regression line represents standard error. A weak positive correlation is observed (R^2 =0.15, P=0.008).

By plotting a linear regression of red channel intensity, a proxy for bleaching, against the number of symbionts per surface area, we observe a weak negative relationship. Therefore, as there is less color (negative values), there are fewer symbionts present. As red channel intensity increases, and corals gain color, there are more symbionts present. A P-value of 0.037 showed significance between these two variables, but a R^2 of 0.10 means that only

10% of the variance in the data can be explained by the model (Figure 6).

A linear regression was performed to determine the statistical significance between bleaching, represented by relative red channel intensity values, and symbiont carbohydrate concentration per surface area (Figure 7). A p-value of 0.012 showed significance between the two variables. An R^2 of 0.14 showed a weak relationship and that only 14% of the variance in the data could be explained by the model.

Symbiont carbohydrate concentration per surface area was compared to symbiont cell density in the form of a linear regression. In the model, as carbohydrate concentration increases, so does symbiont cell density. The regression reveals a weak positive relationship between the two variables with an $R^2 = 0.15$; therefore, 15% of the variance in the data is explained by the regression (Figure 8). This relationship is significant, as seen by a P value of 0.008.

Discussion:

Physiological responses are vital to understanding coral responses to bleaching, and thus, rising temperatures. Investigating the impact of thermal challenge(s) on corals through a physiological lens revealed key parameters of symbiont density, along with chlorophyll, protein, and carbohydrate in symbionts and host coral. Understanding the physiological effects of changing ocean temperatures on tropical corals gives insight into the complex stress response of these animals.

Red Channel Intensity. We combined red channel intensity data from both Chan et al 2021 and Frates et al 2021 to analyze color changing data for all coral species in our study: shallow water *O. faveolata*, shallow *O. franksi*, and mesophotic *O. faveolata* (Figure 2). Color change data serves as a proxy for bleaching, allowing us to make predictions on physiological responses. Figure 2 indicates that heated shallow *O. faveolata* experienced a larger loss of color relative to the control group than *O. franksi*, suggesting *O. franksi* are more thermal stress tolerant. However, the measured physiological parameters in this study, including symbiont cell density, chlorophyll A and C2 concentrations, carbohydrate concentration, and protein concentration, serve as more nuanced indicators for overall coral health.

Coral Symbionts. Across all experimental populations, symbiont density per surface area was decreased under stress (Figure 6, $R^2 = 0.10$, $p = 0.037$). However, looking at individual species, it is observed that both shallow and mesophotic *O. faveolata* have significantly ($p < 0.05$) lower symbiont counts under stress, while *O. franksi* does not (Figure 3). Thermally stressed corals typically expel their symbiotic algae, decreasing symbiont density (Stambler, 2010). The fact that symbiont count in *O. franksi* does not significantly change under stress may suggest that this coral is more resistant to stress than their *O. faveolata* counterparts. While this difference between *O. franksi* and *O. faveolata* could be due to a number of factors, one of the more well described factors is the composition of the host coral symbiont community. The host coral symbiont community influences the physiology of the coral it inhabits (Green et al., 2014). The composition of the algae symbionts were not described in this study and could conceivably influence the outcomes in *O. franksi* and *O. faveolata*. Alternatively, while *O. frankski* did not have significant decreases in their symbiont counts, there is an observed decrease in their symbionts. One possible explanation is that these particular coral samples were collected

while undergoing active bleaching, while the *O. faveolata* corals were collected after already bleached.

Symbiont Energy Reserves. For all species, symbiont carbohydrate concentration decreased with increasing bleaching (Figure 7, R^2 =0.14, P=0.012). On a species specific level, symbiont carbohydrate concentration decreased significantly ($p<0.05$) under hot stress for shallow *O. faveolata*, and it decreased insignificantly under hot stress for mesophotic *O. faveolata* and shallow *O. franksi* (Figure 4). Decreases in carbohydrate concentrations can be used as an indicator that energy reserves are being metabolized, a stress response due to lack of nutrients (Aichelman et al, 2021). Carbohydrates are short term energy reserves, often the first to be metabolized under stress. Therefore, our findings suggest that shallow *O. faveolata* are more sensitive to heat stress than mesophotic *O. faveolata* and shallow *O. franksi*. However, a longer period than 11 days could result in more apparent energy reserve decreases. Previous studies observed that protein concentration, a longer-term energy reserve, significantly decreased under long term stress conditions (Grottoli et al., 2004). This suggests mesophotic *O. faveolata* and shallow *O. franksi* carbohydrate concentrations could decrease more significantly under a longer stress period. The short treatment period could also explain the lack of significant change in protein concentrations (Supplemental Figure 1 & Supplemental Figure 2). Furthermore, the differences in carbohydrate concentrations among species could be due to varying ratios of photosynthesis to respiration (Grottoli et al., 2004).

Symbiont Density and Carbohydrate Concentration Correlation. Both shallow and mesophotic *O. faveolata* corals had higher average carbohydrate concentrations in the control treatment than *O. franksi* corals in the control treatment (Figure 4). This is consistent with the additional finding that *O. faveolata* corals had higher average symbiont cell densities than *O. franksi* corals in the control treatments as well (Figure 3). Symbiont density per surface area is directly related to symbiont carbohydrate concentration, supporting that higher symbiont densities maintain higher symbiont carbohydrate concentrations (Figure 8, R^2 =0.15, P=0.008). This is particularly interesting as shallow and mesophotic *O. faveolata* under stress both have a significant decrease in symbiont cell density while *O. franksi* does not (Figure 3, $p<0.05$). Additionally, shallow *O. faveolata* exhibits a decrease in carbohydrate concentrations when under heat and cold stress (Figure 4). This may suggest that the presence of increased symbionts, and by proxy increased carbohydrate concentrations, is not necessarily a good predictor of how a coral will perform under stress. While more symbionts generally mean the coral will have increased energy reserves it can also be a detriment when conditions are not favorable. Symbiotic algae are known to produce reactive oxygen species (ROS) when under stress (McGinty et al., 2012). Therefore when corals are under significant stress more symbionts may lead to increased ROS and increased cellular damage ultimately leading to higher rates of mortality.

Shallow and Mesophotic *O. faveolata* elicit similar physiological responses vs *O. franksi.* Similar physiological responses are observed in *O. faveolata* regardless of original location while *O. franksi* exhibit divergent responses (Figure 5). In both *O.*

faveolata corals, positive correlations are seen between symbiont protein concentrations with host protein concentrations (Figure 5, $R^2 = 0.83$, $p = 0.0001$). This could be indicative of a shared stress response between coral hosts and symbiont algae when exposed to stressors. Additionally, both *O. faveolata* corals exhibit negative correlations between host carbohydrate concentrations and relative RCI. This is consistent with the body of literature demonstrating that increased bleaching leads to the host coral relying more on their energy reserves once symbiotic algae cell density begins to decline (Rodrigues & Grottoli, 2007). *O. franksi* exhibits less expected results. A weak positive correlation is observed between symbiont cell density and host protein concentration (Figure 5, $R^2 = 0.55$, $p = 0.034$). This correlation may suggest the physiological response in *O. franksi* is more dependent on their symbiont algae than the *O. faveolata* corals. Symbiont cell density positively correlated with host protein concentration indicates that coral host energy reserves are increased the more symbionts are present in the coral. Intuitively this relationship would be expected as the more symbionts present in the coral host, the less energy reserves the coral would need to use. However, the cryptic results of *O. franksi* corals make it difficult to definitively determine the physiologic relationship to thermal stress in this coral.

Study Limitations. In this study, we were limited by the number of specimens we had available. We received our coral samples from the previous 2021 studies, which had 15 of each group we examined (mesophotic *O. faveolata*, shallow *O. faveolata*, and shallow *O. franksi*). With a larger sample size for each species, the trends we are seeing could potentially become stronger, especially for correlations that had a p-value just larger than 0.05. Additionally, when comparing means, there could be significance between experimental treatments if there are more samples.

Additionally, the original experiments were run for a short timeframe; each study only lasted about 11 days. As a result, the effects of thermal stress on coral physiology appear to be more subtle in our study. For example, the mean relative change in red channel intensity for each treatment is similar enough to each other that there is no statistical significance between them in each species. Additionally, the means did not move far from their original measurements (Figure 2.). If the experiment were run for longer, it is possible that more extreme changes in physiological metrics could be observed between treatments and species.

Both previous experiments expressed that the health of their corals worsened by the end of their experiments. Chan et al. (2021) noted that the chlorophyll density and photosynthetic efficiency of their control treatment corals declined during their study. Also, Frates et al. (2021) witnessed a similar decline in the health of their control treatment samples by the end of the experiment. They also stated that their mesophotic coral nubbins started at a sub-optimal state. Therefore, changes in physiology could be from thermal stress or possibly from whatever caused the coral's health to decline. If all samples started off healthy and the control samples remained healthy, it could be said that changes in physiology were a result of thermal stress.

Future Studies. Despite these reservations, the observed results nevertheless highlight the complexity of the thermal stress response in tropical corals. Since the stress response of corals has been previously well characterized, a decrease in multiple

physiological parameters was expected (Bove et al., 2022). The evident decreases in key physiological parameters such as symbiont density and symbiont carbohydrate concentrations lend further credence to the well-established coral stress response. Additionally, these variable results raise questions about possible atypical thermal stress responses. For example, it has been observed that cryptic coral species may differ in their responses to thermal stress due to their genetically distinct makeup and molecular responses (Rose et al., 2021). It is thus possible that cryptic corals may have influenced this study. Future work should look at the genome of the coral fragments to identify differences in immune response. Considering the varied results of this study, further analysis should be done to identify consistent metrics on which to base the vulnerability of corals to thermal stress. Currently, conservation efforts for both *O. faveolata* and *O. franksi* are similar since they are both threatened species and in the same genus. In 2022 surveys of multiple coral species from FGB, researchers found *O. faveolata* had the highest prevalence of both bleaching and disease (Viehman et al., 2022). Because of this, conservation strategies should differ for *O. faveolata* and *O. franksi* since one species is more vulnerable to thermal stress than the other and needs more extensive monitoring. Doing so will help both threatened corals survive under climate change conditions.

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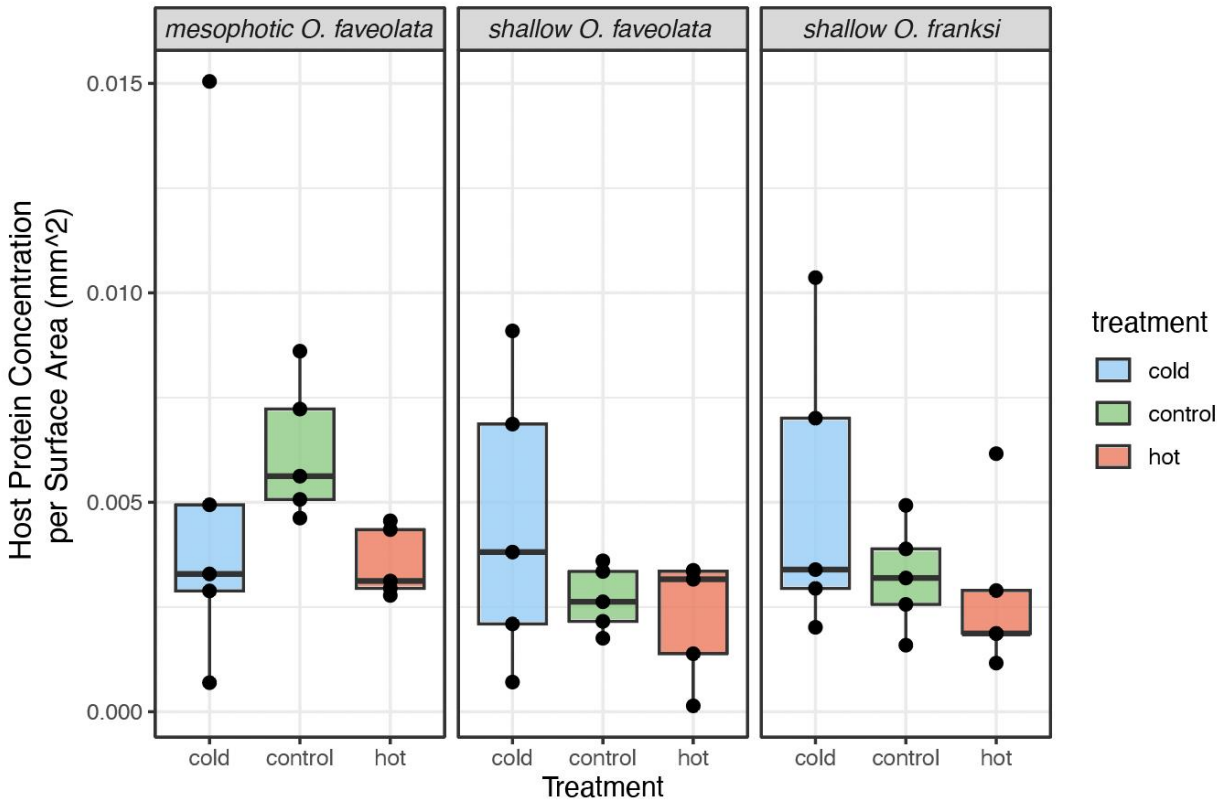
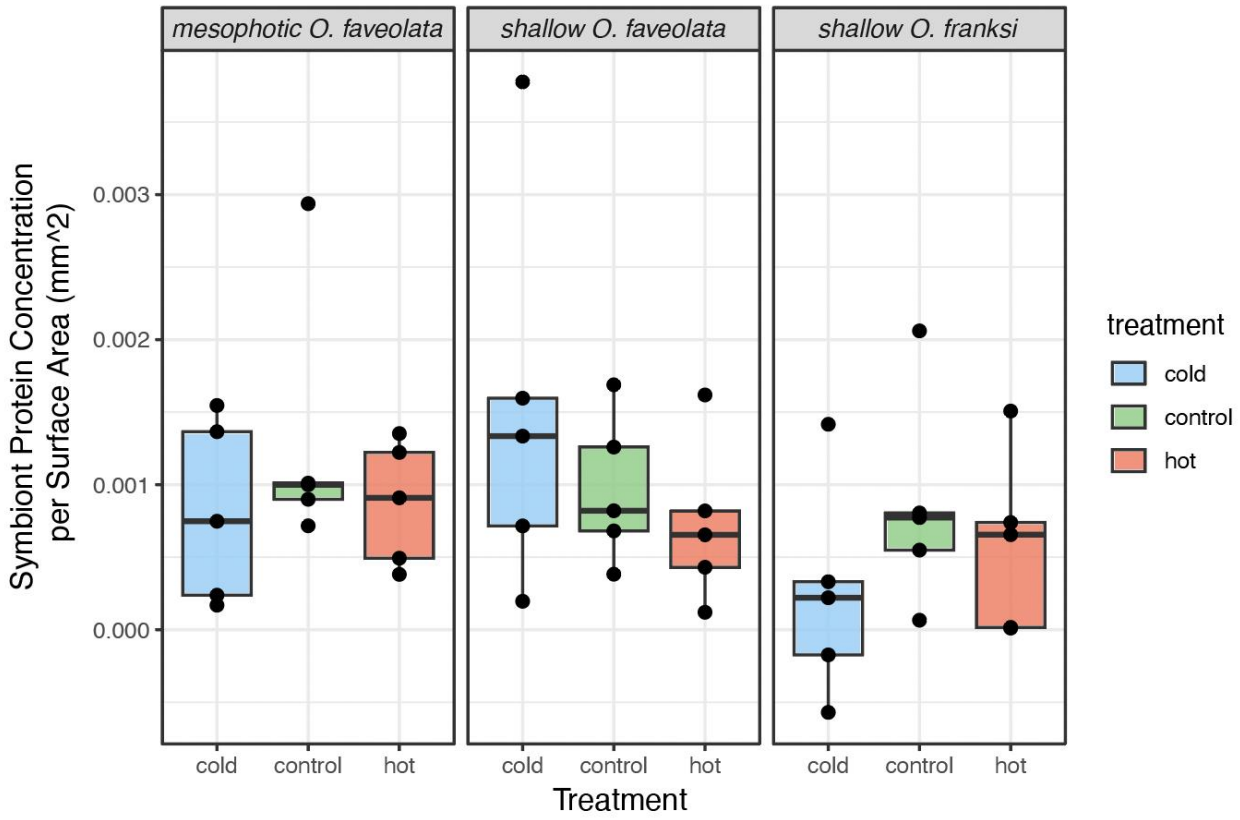
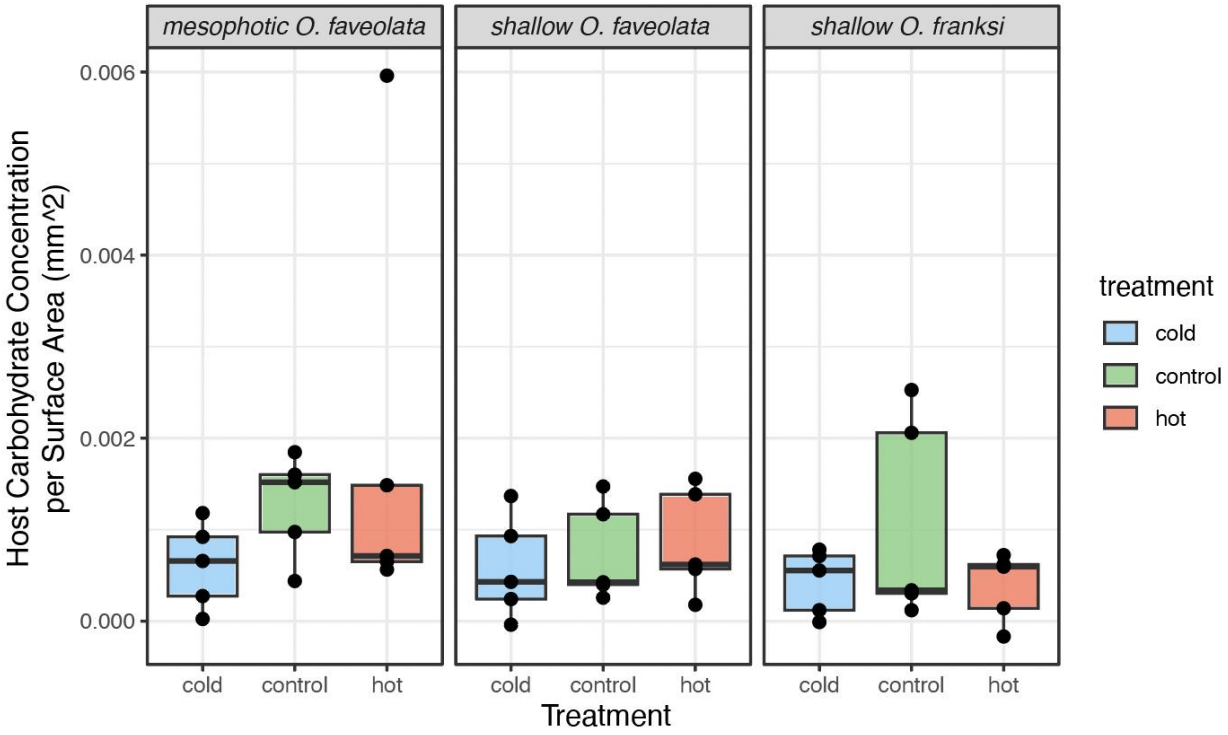
Supplemental Materials



Supplemental Figure 1. Host protein concentration (μg/μL) per surface area (mm^2) separated by treatment (cold, control, and hot) and faceted by species (mesophotic *O. faveolata*, shallow *O. faveolata*, and shallow *O. franksi*). Treatments are differentiated by color as shown by the legend. ANOVAs were run on each species, and the data shows no significance between treatments within each species.



Supplemental Figure 2. Symbiont protein concentration (μg/μL) per surface area (mm^2) separated by treatment (cold, control, and hot) and faceted by species (mesophotic *O. faveolata*, shallow *O. faveolata*, and shallow *O. franksi*). Treatments are differentiated by color as seen in the legend. ANOVAs were run on each species facet, and the data shows no significance between treatments within each species.



Supplemental Figure 3. Host carbohydrate concentration (μg/μL) per surface area (mm^2) separated by treatment (cold, control, and hot) and faceted by species (mesophotic *O. faveolata*, shallow *O. faveolata*, and shallow *O. franksi*). ANOVAs were run on each species facet to compare the means of each treatment, and the data shows no significance between treatments within each species.