Investigating the effects of thermal stress on the physiological responses and symbiont density of *Oculina arbuscula*

Nicole Haftel, Julia Russo, E Schlatter

“*Oculina arbuscula* Freeze”

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**ABSTRACT:** Globally, the output of CO$_2$ into the atmosphere is increasing at an exponential rate from various anthropogenic causes, leading to atmospheric trapping and elevations in temperatures. While tropical corals often can live within only 1 or 2 degrees of their normal habitat temperature, temperate corals such as *Oculina arbuscula* are adapted to live in a range of temperatures through facultative symbiosis, existing in both symbiotic and aposymbiotic states. Current studies focus on temperature elevations, but rarely focus on temperature reductions. Through investigation of the effects of thermal stress on both sides of the temperature spectrum on physiological responses and symbiont density of *O. arbuscula*, we gained a deeper understanding of the mechanisms behind resilience and bleaching. Over a 14-day period, 17 genotypes of *O. arbuscula* underwent daily 1°C increases and decreases in separate treatments. Photosynthetic efficiency, calcification, symbiont density, and polyp behavior were measured. Photosynthetic efficiency linearly declined in cold treatment nubbins across symbiont state likely due to a reached cold threshold. There was no significant change in calcification, likely due to the time constraint of the test period. Symbiont density demonstrated a partial parabolic response with an increase in color intensity and symbiont coverage. Symbiont density likely increased at first due consistent feeding and upkeep and decreased as a cold threshold was reached. Genotypic variation in symbiont density was observed suggesting some genotypes were more thermally tolerant than others. Polyp behavior was found to be lowest in the cold treatment with aposymbiotic nubbins demonstrating less behavior compared to symbiotic nubbins. One possible explanation for polyp behavior is that symbiotic corals could rely on symbionts for energy.

**Introduction**

Coral reefs are highly productive, species diverse ecosystems that provide humans with important services, delivering approximately one-eighth of the world’s total fish harvest (Munro, 1984). They support more species per unit area than any other marine environment, including 4,000 species of fish, 800 species of hard corals, and an additional 1 to 8 million undiscovered species (NOAA 2018). Corals play a crucial role in these ecosystems by creating the physical habitat structure upon which other species rely. An important feature of corals is their role as host in a symbiotic relationship with algae in the family Symbiodiniceae. The coral host provides protection and nutrients, while the algae aids in detoxification of the coral, produces oxygen, and supplies the coral with glucose and amino acids then used to by the coral for growth and metabolism (Barnes, R.D., 1987; Barnes, R.S.K. and Hughes, 1999; Lalli and Parsons, 1995; Levinton, 1995; Sumich, 1996). This relationship promotes the nutrient recycling between the algae and coral that is the fundamental force behind the productivity and development of coral reefs (Barnes, 1987; Levinton, 1995).

Climate change poses a significant threat to corals and their ecosystems. Increased atmospheric CO$_2$ is bringing higher means and variability in temperatures to the world’s oceans. These changing conditions affect coral physiology: corals exposed to thermal stress exhibit reduced calcification and often lose their symbionts through the process of bleaching (Hoegh-Guldberg et al., 2007). Bleaching has been observed in response to cold as well as heat stress. In a 2003 study, samples of the tropical coral *Montipora digitata* exposed to moderate cold stress (16°C) displayed photoacclimatory responses, but corals exposed to severe cold
stress (12°C) showed photodamage, bleaching, and increased mortality (Saxby et al., 2003). A coral’s ability to withstand and/or recover from these bleaching events has a threshold that once passed means resilience is lost. In the face of increasing frequency and severity of these events, the capacity of organisms to adapt or acclimatize to thermal extremes will be essential for their future survival. A deeper understanding of thermal stress thresholds for different coral species will help drive efforts to restore their valuable ecosystem processes (Mumby et al., 2007).

To fully understand thermal stress responses, it is necessary to disentangle the contributions of the coral host and its symbionts. Some temperate corals are being used as models for studying this problem because they can rely on heterotrophic carbon and may exist with or without their symbionts (facultative symbiosis; Piniak, 2002). The temperate compact ivory bush coral, *Oculina arbuscula*, is one such facultatively symbiotic species. A member of the order *Scleractinia* with a broad range extending down the east coast of North America, *O. arbuscula* is also economically and ecologically important, providing necessary hard-bottom habitat for fishes and invertebrates (Aichelman, 2016). Understanding the drivers of resilience to temperature stress in this species will be beneficial to species-specific conservation efforts, but will also provide insights that are generalizable to other temperate corals and potentially to tropical corals as well. Previous work with *O. arbuscula* has shown that under conditions of cold stress as low as 10°C, the photosynthetic efficiency of symbionts is reduced but symbiont density is unaffected (Thornhill 2008). In these circumstances, the role of heterotrophy becomes more important as corals rely on external sources for the nutrition their symbionts can no longer provide (Leal et al., 2014). It is unknown whether, at temperatures below 10°C, *O. arbuscula* might exhibit a bleaching response like that seen at extremely high temperatures. Also unknown is the extent to which corals may be able to use heterotrophy to compensate for reduced photosynthetic efficiency at extremely low temperatures.

In this study, we will investigate the upper and lower limits of thermal tolerance in *O. arbuscula* to determine thermal thresholds and the effect on physiological processes of the holobiont. We will quantify the effect of individual variability and symbiotic status on the physiological response to cold and heat stress. We hypothesize that *O. arbuscula* will exhibit decreased photosynthetic efficiency and symbiont prevalence under cold and heat stress because symbionts will enter a state of quiescence. This reduction will be greater in symbiotic corals as compared to aposymbiotic corals who lack photosynthesizing symbionts. Additionally, thermally stressed individuals will exhibit decreased polyp behavior because they will become fatigued. Symbiotic individuals will display more strongly decreased polyp behavior than aposymbiotic individuals because they can rely on their symbionts for subsistence and thus have less need to feed heterotrophically. Finally, thermally stressed samples will have a lower calcification rate than control samples, due to a lack of available energy. This effect is hypothesized to be strongest in aposymbiotic individuals since symbionts will be unable to provide additional energy.

**Methods**

**Study site and organism**

Colonies of *O. arbuscula* were collected in May 2018 by SCUBA from Radio Island, Morehead City, NC, (34.715530, -76.684988, Fig. 1). Colonies were shipped to Boston University, fragmented into experimental nubbins, glued onto labeled Petri dishes and maintained in
Figure 1. Panel A displays a map of Radio Island, NC. A triangle marks the Oculina arborescens collection site. 17 genotypes were collected. Panel B is a line graph of January 2017 - January 2018 annual temperatures at Radio Island, NC. The maximum temperature reached was 28.5°C and the minimum temperature reached was 3°C.

Figure 2. Temperatures (A) and salinity (B) measured twice daily in each treatment (blue circle=cold, green triangle=control, and orange square=heat) over the course of the experiment. Temperature was lowered by 1°C in the cold tank, raised by 1°C in the heat tank, and maintained at 18°C in the control tank. Salinity was kept at approximately 33.5 ppt - 34.0 ppt.
common garden conditions for five months at 25°C.

**Experimental design**

A common garden experiment was used to test the effects of increasing or decreasing temperature on coral physiological response. 141 coral nubbins (105 symbiotic and 36 aposymbiotic) across 17 genotypes were studied. These were separated evenly into three treatments; a cold, hot, and control treatment. The experiment ran for 15 days, with a temperature decrease from 18°C to 4°C, and a temperature increase from 18°C to 32°C through 1°C daily increment decreases or increases respectively. The control system was kept at a constant room temperature (18°C). To control for environmental bias, the position of the samples in each tank was rotated daily. Water quality, salinity and temperature, was tested twice daily. One water change (20%) was performed midway through the experiment.

**Physiological measurements**

Responses of corals to cold stress were quantified in four ways:

1. **Photosynthetic efficiency.** Pulse amplitude modulation (PAM) was conducted on symbiotic and aposymbiotic *O. arbuscula* using a Junior-PAM chlorophyll fluorometer. In the case of mottled coral fragments (those with a mix of symbiotic and aposymbiotic tissue), measurements were taken from areas corresponding with the fragment’s classification. For instance, on fragments classified as symbiotic, all measurements were taken from brown areas (i.e. areas with symbionts). Additionally, three measurements were taken for each nubbin and then averaged per PAM. Fv/Fm measurements were taken using the PAM five times at equal intervals throughout the experiment and PAM was conducted in the dark to eliminate any lab light bias which would skew photosynthetic readings on the nubbins.

2. **Calcification.** Growth was examined three times during the experiment using the buoyant weight method. A scale was placed across the top of a weighing tank filled with salt water. Samples were suspended in the water from a hook underneath the scale by a hole in the petri dish. Weights were taken in triplicate for each sample, and the average value was used. Salinity, temperature and water volume in the weighing tank were kept constant for all time points.

3. **Symbionts.** Polyps containing symbionts are brown due to the presence of algal symbionts, while polyps without symbionts are white; thus, polyp color can be used as a proxy for symbiont presence (Winters et al. 2009). To measure the extent of symbiosis, the percent of brown polyps was calculated from photographs using Matlab software. To estimate symbiont density, red-channel color intensity was measured in Matlab at 10 randomly selected locations on each sample. The average value of the 10 measurements was used as a proxy for symbiont density, with higher color intensity values indicating whiter colors and fewer symbionts.

4. **Polyp behavior.** All samples were fed daily. Each system was given ¼ teaspoon of dried brine shrimp. 30 minutes after feeding, polyp behavior was recorded observationally using Wuitchik’s scale: 1- 0% of polyps out, 2- 25% of polyps out, 3- 50% of polyps out, 4- 75% of polyps out, 5- 100% of polyps out (Wuitchik et. al, in prep).

**Data analysis**

Data were recorded by hand and then entered in Excel. After quality control, all data were converted to CSV files for analysis in R. We
used a linear mixed model with temperature treatment, date, and symbiotic status as fixed effects and genotype as a random effect to examine changes in photosynthetic efficiency, buoyant weight, and symbiont quantity over the course of the experiment. An ANOVA and Tukey’s post-hoc test were used to test significance of results. The relationship between temperature and polyp behavior was visualized in R using a scatterplot and polynomial regression. Additional statistical techniques will be necessary to determine the significance of these results.

Results

Photosynthetic efficiency
Photosynthetic efficiency declined with temperature in samples exposed to the cold treatment; this was true for symbiotic and aposymbiotic groups. Responses of corals in the heat treatment over the course of the experiment did not differ significantly from the control: both increased slightly over time in the aposymbiotic groups, and decreased slightly in the symbiotic groups.

Calcification
Calcification, measured as percent change in buoyant weight, was positive for all samples. Neither treatment nor symbiotic status had a significant effect on growth. For the aposymbiotic group, the difference between the controls and individuals exposed to the cold treatment was borderline significant (p=0.056).

Symbionts
Color intensity, a proxy for symbiont density, was darker in symbiotic than in aposymbiotic corals (p=.013). Aposymbiotic corals differed significantly in color intensity among the three thermal treatments (p<0.0001), but symbiotic corals did not. In all treatments, color intensity increased from Day 1 to Day 8 of the experiment. This increase was significant overall (p<0.0001) and for all treatment/symbiotic state combinations except aposymbiotic corals exposed to heat. Color intensity decreased significantly from Day 8 to Day 15 only for symbiotic corals in the cold treatment (p=0.014).

Symbiont coverage was significantly different over the course of the experiment (p=0.00362). In all treatments, symbiont coverage increased from day 1 to day 8 and decreased from day 8 to day 15 of the experiment. On day 15 of the experiment, symbiont coverage was the lowest in corals in the cold treatment (~66.5%) and highest in corals in the heat treatment (~80.4%).

Figure 3. Photosynthetic efficiency during exposure to three temperature treatments: cold (shown in blue), control (green) and heat (orange). Aposymbiotic and symbiotic groups of samples are shown separately. Each point represents the mean of observations for a treatment/symbiotic status group on a single day. Linear regression lines are drawn for each group. Error bars indicate standard error. Photosynthetic efficiency decreased under the cold treatment, while no significant change occurred in the control and heat treatments.
Figure 4. Calcification, as measured by percent change in buoyant weight, over the course of the experiment. Aposymbiotic and symbiotic groups are shown separately, and colors indicate thermal treatment (blue=cold, green=control, and orange=heat). No significant differences were present among treatment groups or between symbiotic and aposymbiotic samples.

Figure 5. Changes in coral color intensity during the experiment. Y-axis values are red-channel measurements multiplied by -1, with less negative values (higher on the axis) indicating darker colors. Aposymbiotic corals are shown on the left and symbiotic corals on the right; thermal treatments are separated by color (blue=cold, green=control, and orange=heat). Error bars indicate standard error.

Figure 6A. Average changes in symbiont coverage across treatments (blue circle=cold, green triangle=control, and orange square=heat). Determined through Matlab software analysis of coral images taken at experimental day 1, 8, and 15 (x-axis). The y-axis demonstrates the average proportion of dark to light colors, lower values indicate a greater proportion of light colors. Error bars indicate standard error.

Figure 6B. Average changes in symbiont coverage across aposymbiotic (left; Apo) and symbiotic (right; Sym) state within treatments (blue circle=cold, green triangle=control, and orange square=heat). Determined through Matlab software analysis of coral images taken at experimental day 1, 8, and 15 (x-axis). The y-axis demonstrates the average proportion of dark to light colors, lower values indicate a greater proportion of light colors. Error bars indicate standard error.
Symbiont coverage was significantly different between aposymbiotic and symbiotic corals ($p < 2e-16$). Additionally, symbiont coverage among aposymbiotic and symbiotic corals was significantly different between treatments ($p = 4.44e-05$). Aposymbiotic corals in the cold treatment on day 15 had the lowest symbiont coverage (~44.6%). Symbiotic corals in the cold treatment on day 1 had the highest symbiont coverage (~94.4%).

Figure 6C. Average changes in symbiont coverage across genotypes (17 genets labeled A-R) within treatments (blue circle=cold, green triangle=control, and orange square=heat). Determined through Matlab software analysis of coral images taken at experimental day 1, 7, and 15 (x-axis). The y-axis demonstrates the average proportion of dark to light colors, lower values indicate a greater proportion of light colors. Error bars indicate standard error.

Symbiont coverage varied significantly across the 17 coral genotypes ($p=2.2e-16$). Genet B, I, J, Q, R did not show significant change across the treatments. Genet A, E, K, and O had a higher symbiont coverage average in the heat treatments (~84.2%, 80.2%, 87.5%, and 94.3% coverage on day 15 respectively) than in the control or cold treatments over the course of the experiment. Genet L had a higher symbiont coverage average in the cold treatment than in the heat treatment over the course of the experiment (~86% coverage on day 15). Genet A, C, E, F, H, M, N, O, and P had lower symbiont coverage averages in the cold treatment compared to the heat and control treatment (~ 38.6%, 66.5%, 51.7%, 77.4%, 61.5%, 47.1%, 19.7%, and 34.1% average coverage on day 15 respectively).

**Polyp Behavior**

Figure 7A. Coral feeding behavior in response to temperature. Each point represents one daily observation of a fragment from the heat, control or cold treatments. Its x-axis position is determined by the temperature in its tank on the date of observation, and the y-axis position indicates feeding behavior on a scale of 1 to 5 (with 5 meaning all polyps fully extended, and 1 meaning all polyps retracted). Symbiotic individuals are shown in red and aposymbiotic in gray. Polynomial regression lines and shaded 95% confidence intervals are plotted for each symbiotic state. Point positions have been jittered to improve visibility.
Figure 7B. Feeding behavior in response to temperature for 17 different genets. All genets showed a decline in polyp activity at low temperatures; some also showed a decline at high temperatures (most strongly C, D, F, H and Q).

At temperatures between 15 and 25 degrees C, symbiotic and aposymbiotic samples displayed polyp behavior near the maximum value of 5. Above 25 degrees, a slight decrease in polyp activity was evident for both groups. Below 15 degrees, polyp behavior decreased sharply. This decrease was stronger in the aposymbiotic group. Genets varied in their behavioral response to temperature variation. The temperatures at which declines in polyp activity began, and the steepness of these declines, are potential indicators of the resistance of genets to cold or heat. Although we do not develop the statistical methods here to characterize these responses, some trends are visually evident. Certain genets were unusually resistant (e.g., genet R) or sensitive (genet F) to both cold and heat, while others were sensitive to heat but resistant to cold (genet B) or vice versa (genet A).

Discussion

*Photosynthetic efficiency exhibited a linear reduction in the cold treatment:*

Across symbiont state (aposymbiotic and symbiotic), photosynthetic efficiency was greatly reduced in *O. arbuscula* samples in the cold treatment (Fig. 3). This is likely due to thermal stress debilitating the Calvin-Benson cycle (carbon fixation), the enzymes within this cycle are temperature dependent and outside of their threshold, the rate of catalyization becomes reduced (Jones et al. 1998, Hoegh-Guldberg 1999). Corals under stress therefore becomes susceptible to photoinhibition and symbiont cellular damage. Previous studies on *O. arbuscula* support this discovery and have found that under conditions of cold stress, photosynthetic efficiency of symbionts was greatly reduced (Thornhill 2008). Damaged photosynthetic components lead to an inability to process photons due to the production of toxic oxygen species and an overall impediment of photosynthesis (Osmond 1994).

Corals did not exhibit the same reduced photosynthetic efficiency in response to heat stress as they did to cold stress. Both the heat and control groups increased slightly over time in the aposymbiotic groups, and decreased slightly in the symbiotic groups (Fig. 3), but neither increase or decrease was significant. We can interpret this as meaning that this experiment did not reach the upper limit of thermal tolerance for *O. arbuscula*’s symbiotic relationship, or that temperatures above that upper limit were not maintained for a sufficient period of time for measurable effects to occur.

It was expected that a reduction in photosynthetic efficiency would be greater in symbiotic corals across treatments as compared to aposymbiotic corals. This was not observed in the results. Residual algae present on the nubbins may have caused biased PAM results as
this would have given a photosynthetic read on aposymbiotic corals. Other bias may have come from the presence of symbiont polyps on aposymbiotic nubbins. *O. arbuscula* undergoes facultative symbiosis and the symbiont state of the nubbin was determined by a great presence or a lack of symbionts, therefore, no coral nubbin nor genotype existed in purely one symbiont state or another.

**Calcification did not significantly change:**
In our study, we observed the net calcification of the *Oculina* nubbins after 14 days (Figure 4). Calcification was measured by percent change in buoyant weight from the beginning to end of the experiment. Nubbins from all three treatments increased at the final buoyant weight measurement. For the symbiotic coral nubbins, there was a higher increase in net calcification from those in the control and heat treatments, while the aposymbiotic corals in the cold treatment conversely had a higher percent growth, however there was no statistical significance between the buoyant weight measurements taken at the beginning and end of the experiment. In contrast, a previous study from Cooper et. al (2007) showed that at higher and lower temperatures, calcification, one of several parameters measured, declined by approximately 15 percent per degree celsius change.

The discrepancy between our study and the results of Cooper et al. could be due to the length of our experiment. The temperature in each treatment only remained constant for a single day before increasing or decreasing by a degree. In turn, the coral nubbins did not have time to appropriately adjust before being pushed farther out of their range. The coral did not exhibit significant amount of calcification that we expected and we expect this is because the length of the study was so short that we might not have the ability to observe patterns related to treatment because the ability of a coral to calcify significantly in that time is extremely limited.

A potential reason that there was not a statistical significance related to calcification could be due to the fact that being a temperate coral, *O. arbuscula* was still living within its general range, despite the fact that it was pushing its outermost limits.

Another possible explanation for overall net increase in calcification could be related to algal growth on the nubbins in the control system and in the heat treatment. Measures were taken to remove algae by hand however it was not possible to assure that it was all removed when buoyant weight measurements were taken.

Although not statistically significant, an unusual pattern observed in the cold treatment was that the aposymbiotic nubbins had the greatest overall percent calcification increase between any of the systems. In order to determine the causation of this observation, further research is needed.

**Symbiont density demonstrated a partial parabolic response with genotypic variation:**
Across treatments and symbiont state, symbiont density was partially parabolic in nature with a steep increase, optimum point, and steep decrease (Fig. 5, 6A, and 6B). With only 3 data points, an optimum point cannot be clearly identified. However, the partially parabolic nature of the results suggests that *O. arbuscula* reached a cold thermal threshold in this experiment, an optimum point occurred roughly around experiment day 7 with an approximate cold temperature of 11°C within the cold treatment (Fig. 5). Symbiont coverage was significantly different over the course of the experiment, again hinting at a parabolic nature with 11°C as the cold threshold (Fig. 6A). Previous studies have shown that *O. arbuscula*’s cold stress threshold occurs at 10°C (Thornhill
Another possible explanation for the initial increase in symbiont density is the daily feedings corals received as part of the experiment, which had previously not been part of their care regimen. The extra energy gained heterotrophically may have allowed corals to increase their symbiont density. Additionally, because symbiotic density was measured through the use of a photograph of the nubbin, shadows on the coral may have skewed the reading, resulting in the overall higher symbiont density measurements on Day 7.

Symbiont density and coverage were greater in symbiotic than in aposymbiotic corals, which was expected as symbionts are brown and aposymbiotic polyps are white. Across the three treatments, aposymbiotic corals differed significantly in symbiont density, but symbiotic corals did not (Fig. 5). This may be due to bias from the length of the experiment. The rate at which symbiotic corals dispel symbionts varies across species and is unknown for *O. arbuscula*. In order for a significant difference to be observed across treatments, the experiment may need to run longer than 15 days.

Because photosynthetic efficiency and symbiont density are both quantifications of algal activity, we might expect them to covary. In general, this was the case: in control and heat treatments, both of these measures remained relatively constant throughout the course of the experiment, while declines were observed in the cold treatments.

**Polyp behavior displayed a partial parabolic response with genotypic variation:**
In our study we observed polyp behavior to exhibit a partial parabolic response, with strongly reduced polyp activity occurring at the lowest temperatures and some reduction in activity also present at the highest temperatures. We hypothesized that the symbiotic coral nubbins would exhibit decreased polyp behavior in response to thermal stress, because their reliance on symbionts would decrease their need to gain as much energy from heterotrophic feeding. In contrast, we observed in the cold treatment that the aposymbiotic nubbins exhibited more reduced polyp activity in comparison to the symbiotic nubbins. Thus, symbiosis does not appear to mitigate the need for heterotrophic feeding. Reduced behavior in the aposymbiotic nubbins in the coldest temperatures may mean that they did not have the energy required to feed due to lack of symbionts.

The variety of responses of polyp behavior to temperature across genets suggest that heat and cold tolerance may vary independently of one another. Some genets appeared to exhibit higher-than-average tolerance to both heat and cold, while others were particularly tolerant to one extreme but not the other. This indicates that the mechanisms underlying thermal tolerance may be different for heat and cold, although the observed bleaching response at both ends of the temperature continuum, observed in this and other studies, is similar.

**Individual variation among genotypes is important:**
Intraspecific differences in thermal tolerance, as seen in both polyp behavior and symbiont coverage, suggest a potentially important role for individual genetic variation and symbiont community in determining resilience. Findings from a 2017 study on intraspecific differences in molecular stress responses and coral pathobiome in *Acropora millepora* suggest that diseases may arise from weaknesses in holobiont physiology, instead of the virulence of the diseases themselves (Wright et al., 2017). This would suggest that oxidative stress varies across corals and symbiodinium. This is further supported by Díaz-Almeyda et al.’s study on intraspecific and
interspecific variation in thermotolerance and photoacclimation in symbiodinium dinoflagellates. To evaluate effects of temperature and light on physiological stress, this study investigated three strains with varying degrees of thermotolerance (tolerant, intermediate and susceptible) under five light intensities (65, 80, 100, 240 and 443 μmol quanta m-2 s-1) and two temperatures (26°C and 32°C). It was found that high irradiance worsened the effects of high temperature, particularly in strains from thermally sensitive species (Díaz-Almeyda et al., 2017), which supports that thermotolerance varies significantly between species and strains of symbiodinium. Such results in an energy cost to the coral host (Lesser & Shick 1989). As a result, the symbionts are expelled from the coral to prevent further oxidative stress (Lesser 1997).

Conclusion
Photosynthetic efficiency linearly declined in nubbins exposed to the cold treatment; this was true for symbiotic and aposymbiotic groups. It may have significantly decreased due to a cold threshold being reached in the experiment. Photosynthetic efficiency was not significant across heat and control treatments. There was no significant change in calcification, likely due to the time constraint of the test period. Symbiont density demonstrated a partial parabolic response with an increase in color intensity and symbiont coverage. Symbiont density likely increased at first due consistent feeding and upkeep and decreased as a cold threshold was reached. Genotypic variation in symbiont density was observed suggesting some genotypes were more thermally tolerant than others. Polyp behavior was found to be lowest in the cold treatment with aposymbiotic nubbins demonstrating less behavior compared to symbiotic nubbins. One possible explanation is that symbiotic corals had more energy from the photosynthesis of their symbionts and therefore were more active than aposymbiotic corals in the cold temperature. The heat treatment followed the trend of the control treatment and was not significant. Future studies could replicate this experiment with O. arbuscula over a longer test period, on a scale of months. Instead of 1°C daily changes, temperature could be changed by 1°C over weeks. Such an experiment would better identify parabolic trends in data over more than just 3 points and is likely to show greater changes in calcification.

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