The effect of thermal stress and symbiotic status on host and symbiont physiologies in the temperate coral *Oculina arbuscula*

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Abstract

Anthropogenic activities since the industrial revolution have increased carbon dioxide concentrations in the atmosphere, resulting in unprecedented changes in sea surface temperatures and affecting economically and ecologically valuable ecosystems globally. One such ecosystem is coral reefs, which are currently experiencing massive global decline in response to new temperature extremes. The effects of temperature stress on tropical corals is well documented, and involves the breakdown of the symbiotic relationship between the coral animal and its symbiont in a process known as 'bleaching'. As most tropical corals exhibit an obligate symbiosis, the loss of this relationship can be fatal. While tropical coral bleaching is generally well studied, the effects of thermal stress on temperate corals that exhibit unique facultative symbiosis are not well known. Here, we considered the effects of both heat and cold thermal stress on the host and symbiont physiologies of the temperate scleractinian coral *Oculina arbuscula.* Both symbiotic and aposymbiotic fragments of *O. arbuscula* were exposed to three temperature treatments: 1) control (18°C constant), 2) heat stress (18 to 32°C increase over 15 days), and 3) cold stress (18 to 6°C decrease over 15 days). Coral host physiology was monitored over the course of the 15-day experiment by measuring growth and polyp activity, and the symbiont physiology was considered simultaneously by monitoring photochemical efficiency (Fv/Fm), pigment density, and symbiont extent. Overall, cold stress had a more negative impact on both symbiotic and aposymbiotic *O. arbuscula*, including Fv/Fm, polyp activity, and symbiont extent/pigment density. Surprisingly, this experiment seems to have missed the upper thermal limit of *O. arbuscula,* as little negative impact of heat stress was detected. Future research should extend this experiment both over a longer time frame and to warmer temperatures to gain further insight into the response of *O. arbuscula* to temperature extremes. Experiments such as this provide valuable insight into how *O. arbuscula* could respond to future extreme heat and cold temperatures that are likely to occur with increasing frequency as climate change continues to alter the Mid-Atlantic habitat of this species.

Introduction

Anthropogenic activities have increased global atmospheric CO_2 concentrations since the Industrial Revolution (Doney et al., 2009). Over 90% of this excess atmospheric CO_2 has been absorbed by the oceans, and as a result the oceans have warmed approximately 0.3°C since 1969 (Levitus et al., 2017), a trend that is projected to continue to increase (Rhein et al., 2013). These temperature increases have wide-reaching effects on species diversity and abundance in marine species (Hobday and Pecl, 2014), including tropical corals (Hoegh-Guldberg and Bruno, 2010). Most tropical corals exhibit an obligate symbiosis with endosymbiotic algae of the family Symbiodiniaceae (hereafter symbiont), which can break down under thermal stress in a process called 'bleaching'. As tropical corals are sensitive to even small increases in temperature (Baker et al., 2008), recent temperature increases associated with climate change have caused global bleaching events that are occurring with increasing frequency and severity (Hughes et al., 2017). Some tropical corals have shown shifts in temperature tolerance following a bleaching event, sometimes resulting from shuffling to accommodate more heat tolerant symbionts (Cunning et al., 2015; Hughes et al., 2017). Despite this, it is unclear how effective these coping mechanisms will be in the long term, as ocean temperatures will continue to increase into the future.

The effects of thermal stress on tropical coral species are generally well studied; however, comparatively less is known about how temperate coral species, especially those that have unique symbioses, will respond to warming temperatures. In general, observations in temperate coral ecosystems have included a "tropicalizing" effect, whereby warming ocean temperatures result in subtropical and tropical species relocating to typically cooler ecosystems (Vergés et al., 2014). Specifically, temperate coral responses to climate change stressors are variable across species, response variable and study. Several studies have demonstrated a positive relationship between temperature and coral growth in temperate corals, including in the species *Oculina arbuscula* and *Astrangia poculata* (Jacques et al., 1983; Miller, 1995, respectively). However, this positive relationship is beneficial only to a point, and has been shown to diminish at 4°C above normal maximum summer temperatures in the temperate scleractinian (i.e., skeleton-forming) coral *Cladocora caespitosa* (Rodolfo-Metalpa et al., 2008). However, another study found that for two temperate coral species (*C. caespitosa* and *Oculina patagonica*), short-term thermal stress up to 5°C above the mean summer temperature resulted in no change in symbiont density (Rodolfo-Metalpa et al., 2006). Taken together, these studies demonstrate the variable manner in which temperate corals respond to thermal stress and illustrate the need for a more comprehensive understanding of how temperate corals are likely to respond to future changes in temperature associated with climate change.

An excellent temperate coral species to ask and answer these questions in is *Oculina arbuscula,* a scleractinian coral that is facultatively symbiotic, meaning it co-exists naturally both with (symbiotic) and without (aposymbiotic) symbionts. Symbiotic (or brown) colonies of *O. arbuscula* obtain energy from the photosynthetic by-products of their symbiotic algae, while aposymbiotic (or white) colonies rely on obtaining energy through heterotrophy (i.e., eating planktonic matter out of the water column; Leal et al., 2014). *O. arbuscula* inhabits the southeastern and Mid-Atlantic US up to 200m depth

(Miller, 1995), and therefore can withstand a wide range of seawater temperatures (4–30°C; Ries et al., 2010). While *O. arbuscula* is not considered a reef-building coral, it still serves an important role in creating larval habitat for native fish and invertebrates along the western Atlantic coast, especially for important fisheries species (Deaton et al., 2010). While one previous study demonstrated that heterotrophic feeding mitigates the negative effects of thermal stress on symbiotic *O. arbuscula* (Aichelman et al., 2016), nothing is currently known about how symbiotic state modulates the thermal stress response in this species.

The aim of this study was to determine how thermal stress differentially affects the physiology of symbiotic and aposymbiotic colonies of *O. arbuscula*, both in terms of the coral host (growth and polyp activity) and its symbiont (photochemical efficiency [Fv/Fm], pigment density, and bleaching extent). We hypothesized that symbiotic *O. arbuscula* would be better able to withstand both heat and cold stress, due to the photosynthetic-derived energy these corals receive compared to aposymbiotic colonies that receive only heterotrophic energy inputs. Mid-Atlantic hard-bottom communities support economically valuable fisheries species and a variety of other economically important organisms (Deaton et al 2010). Therefore, understanding the effects of temperature stress on *O. arbuscula* has significant implications in understanding how this species, and the hard bottom communities it helps form, will change under future, more extreme ocean temperatures (Rhein et al., 2013).

Materials and Methods

Coral Collection & Experimental Design

In summer 2018, 15 genotypes of *Oculina arbuscula* were collected from Radio Island, North Carolina (34.712590°N, -76.684308°W; Figure 1). Colonies were shipped to Boston University, fragmented, attached to petri dishes using cyanoacrylate glue, and placed in control conditions (18°C) for recovery. On November 1, 2018, corals were placed in their experimental treatments, including: 1) constant temperature (control, 18°C), 2) heat stress, and 3) cold stress. These experimental temperatures were informed partly by *in situ* temperature data recorded by the NOAA buoy data closest to the collection site (Station BFTN7; Figure 1B). Temperatures in the control treatment remained at 18℃ for the duration of the study. The heat stress treatment started at 18℃ and increased by 1℃ every evening for 15 days, with a final target temperature of 32℃ (Figure 2A). The cold stress treatment started at 18℃ and decreased by 1℃ every evening for 15 days, with a final target temperature of 4℃ (however only 6°C was achieved here). In all three treatments, temperature was controlled using an Aqualogic Digital Temperature Controller. When possible, all coral genotypes were represented in all aquaria in both treatments and control and treatment tanks contained 14-15 genotypes (6 white and 9-10 brown nubbins).

Each temperature treatment consisted of three 15-gallon aquaria connected to one sump. All aquaria had a powerhead for water flow and circulation, and each sump was equipped with a filter sock and protein skimmer for water filtration. Aposymbiotic and symbiotic corals were placed on opposite sides of the same tank, and all nubbins were rotated clockwise daily. Water quality was tested daily in each tank, which consisted of measuring temperature (using a NIST-

calibrated thermometer; Figure 2A) and salinity (using a YSI meter; Figure 2B). Target salinity was between 33-34 ppm, and was maintained by mixing InstantOcean® salt with DI water. Light exposure was also monitored to ensure the corals received the same amount of light (50 µmol photons m^2sec^{-1}) and remained on a 12:12 hour, light:dark schedule throughout the experiment. Each aquaria were fed ¼ tsp (equal amounts) of reconstituted powdered brine shrimp daily.

Symbiont Physiology

Pulse Amplitude Modulation (PAM) fluorometry was used to measure the dark-acclimated photochemical efficiency of photosystem II (Fv/Fm) using a Junior PAM approximately every three days. Corals were given 8 hours of dark acclimation before Fv/Fm was measured in triplicate for each nubbin between the hours of 0800 and 1200. Additionally, fragments were photographed three times throughout the experiment, and photos were analyzed using the 'AnalyzeIntensity' macro in Matlab, following a method published by Winters et al (2009) to quantify symbiont pigment density. This script was updated by E Schlatter to also calculate symbiont extent for each fragment (updated script by E Schlatter). When quantifying symbiont pigment density, 10 symbiotic pixels were analyzed for red channel intensity on all coral photos; therefore, even in aposymbiotic fragments this metric is tracking symbiotic pigment density through time.

Coral Physiology

Coral growth was measured using the buoyant weight technique (Davies, 1989) at three times throughout the experiment (day 0, day 7, day 15). These buoyant weight measurements took place the same day that nubbin photos were taken. Any coral that fell off its petri dish and had to be re-glued was excluded from the buoyant weight analysis, as adding glue likely influenced the weight of those coral fragments. In addition, all nubbins were monitored daily for food-stimulated activity. Thirty minutes after being fed, each fragment received a polyp activity score to assess behavior following feeding on a scale of 1 – 5, similar to Burmester et al. (2018). This scale involved considering the percentage of polyps extended following exposure to the food stimulus for 30 minutes (i.e., 1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, and 5 = 100%). Each coral nubbin was scored based on a consensus, with at least two independent observers considering each coral nubbin. Polyp behavior data were collected daily. Finally, at the end of the 15-day experiment, each coral nubbin was sub-sampled for RNA for future molecular analyses to investigate the molecular underpinnings of the phenotypes observed herein.

Data Analysis

All experimental analyses and data visualizations were completed in the R v3.5.1 statistical environment (R Core Team, 2017). *Oculina arbuscula* growth and symbiont photochemical efficiency (Fv/Fm) were analyzed using a fixed-effects ANOVA, with fixed factors of treatment and symbiotic status. The mean intensity of the red channel (a proxy for photosynthetic pigment density) and bleaching extent were both analyzed using a fixed-effects ANOVA with fixed factors of treatment, symbiotic status, and date of measurement. Coral behavioral data

were analyzed using a fixed-effects ANOVA, with fixed factors of symbiotic status and temperature.

Results

Coral holobiont physiology

Growth of *Oculina arbuscula* was quantified as a percent change in buoyant weight from day 1 to day 15 of the experiment (Figure 3). *O. arbuscula* growth was significantly different based on treatment ($p = 0.003$), but not by symbiotic status ($p = 0.13$) or by the interactive effect of treatment and symbiotic status ($p = 0.22$). Within treatment, coral growth in both the cold and heat treatments was significantly greater than growth in the control treatment (Tukey's HSD $p = 0.003$ and $p = 0.033$, respectively). However, growth in the cold and heat treatments were not different from each other (Tukey's HSD $p = 0.67$). Within symbiotic status, there were no significant differences in growth between the temperature treatments.

Coral behavior as assessed following feeding was significantly different between the temperatures considered in this experiment ($p < 0.001$) and between symbiotic and aposymbiotic fragments ($p = 0.027$; Figure 4). As with growth, temperature and symbiotic status had an interactive effect on coral behavior ($p < 0.01$), and activity decreased at temperature extremes (Figure 4). The interactive effect of temperature and symbiotic state on polyp behavior seems to be driven by the divergence between symbiotic and aposymbiotic polyp activity at cold temperatures (Figure 4).

Symbiont physiology

O. arbuscula pigment density was significantly affected by the temperature treatments ($p < 0.001$; Figure 5), and were specifically different between cold and control (Tukey's HSD $p = 0.04$) and heat and cold (Tukey's HSD $p < 0.001$) treatments, but not between heat and control (Tukey's HSD $p = 0.28$). As expected, coral pigments differed between symbiotic and aposymbiotic fragments ($p < 0.001$). Coral pigments also changed over the course of the experiment ($p < 0.001$).

In addition to pigment density, photochemical efficiency of photosystem II (Fv/Fm) of *O. arbuscula* symbionts was significantly affected by treatment conditions ($p < 0.001$; Figure 6). While the corals in the cold treatment had significantly lower Fv/Fm compared to corals in both the control ($p < 0.001$) and the heat treatments ($p < 0.001$), Fv/Fm in the heat treatment was not different compared to the control ($p = 0.94$). Fv/Fm was different between symbiotic and aposymbiotic corals ($p < 0.001$), and Fv/Fm was greater in symbiotic than aposymbiotic corals (Tukey's HSD $p < 0.001$). Additionally, there was a significant interactive effect of treatment and symbiotic state on Fv/Fm ($p < 0.001$; Figure 6).

O. arbuscula symbiont coverage was not significantly different across the temperature treatments in this experiment ($p = 0.512$; Figure 7). Symbiont coverage did change throughout the course of the experiment ($p < 0.001$), and all treatments increased the percent coverage of symbionts between days 1 and 8, and then decreased between days 8 and 15 (Figure 7). As expected, symbiont coverage was different between symbiotic and aposymbiotic fragments ($p < 0.001$).

Discussion

The aim of this study was to determine the effects of extreme thermal stress (both heat and cold) on the coral and algal partners of the *Oculina arbuscula* coral holobiont. We predicted that symbiotic corals would be better able to cope with both heat and cold extreme thermal stress, and therefore maintain higher growth, symbiont pigment density, and symbiont photochemical efficiency (Fv/Fm) compared to aposymbiotic fragments. We also expected the response to the heat and cold stress to be similar, following the results of a previous study on another temperate coral, *Astrangia poculata* (MPCC 2017 class). Contrary to our original hypothesis, we found an overall more negative effect of cold stress on *O. arbuscula* in terms of both host and symbiont physiologies, and a differential response at heat and cold stress.

Differential stress response of the coral host based on symbiotic status

The metrics of coral animal fitness considered here tell contrasting stories on how symbiotic status modulates the effects of thermal stress on *O. arbuscula.* For coral growth, symbiotic status had no effect, and instead only treatment predicted coral growth over the experimental time frame considered here (Figure 3). While the growth response to temperature extremes was conserved (as predicted), the direction of change was unexpected. Instead of demonstrating decreased growth under thermal stress (e.g., Aichelman et al., 2016; Castillo et al., 2014), corals significantly increased growth in cold and heat treatments relative to the control. This pattern is likely due to the effect that corals grow slowly, and therefore 15 days was not sufficient to tease apart the patterns in growth that may become more apparent in a longer-term experiment.

Contrary to *O. arbuscula* growth, symbiotic status did play a role in coral behavior, particularly in the cold stress treatment. Symbiotic corals maintained feeding activity at colder temperatures compared to aposymbiotic fragments. However, this pattern was not uniform across all symbiotic fragments, and seems to be driven by a subset of symbiotic genotypes. Heterotrophic feeding is vital for the survival of temperate corals like *O. arbuscula,* and this is especially true for aposymbiotic fragments that receive little to no energy inputs from symbionts. This differential behavioral response at cold extremes indicates that aposymbiotic fragments will likely have a much harder time coping with cold thermal stress compared to symbiotic fragments. As both heat and cold extremes are predicted to become more severe under climate change, this result provides valuable insight into potential future responses of this species.

Differential Responses to Thermal Extremes

Contrary to our expectation of a conserved response to heat and cold stress, we observed an overall more negative impact of cold stress compared to heat stress in *O. arbuscula.* This pattern was observed in photochemical efficiency, feeding behavior, and symbiont coverage. This experiment exceeded the maximum temperature observed at Radio Island in 2017 by over 3°C, but did not reach the winter minimum of 3°C recorded at the same site and year (Figure 1B). In the context of this *in situ* temperature data, it is surprising that the heat

stress exposure here did not induce thermal stress, but overall this indicates resilience of *O. arbuscula* to warming and likely more susceptibility to cold stress.

Oculina arbuscula corals in the cold treatment maintained photochemical efficiency (Fv/Fm) similar to heat and control corals until approximately day 8 of this experiment, after which Fv/Fm of cold corals rapidly declined in both symbiotic and aposymbiotic fragments compared to the heat and control. In the tropical coral literature, declines in Fv/Fm are generally associated with elevated temperature stress (e.g., Davies et al., 2018). However, this effect has also been previously demonstrated in response to cold stress. For example, Saxby et al. (2003) observed a decline in Fv/Fm, loss of symbiotic cells and decreases in photosynthetic pigments in the tropical coral *Montipora digitata* in response to cold stress. In addition to tropical corals, *O. arbuscula* has previously been shown to host type B2 symbionts (now considered *Breviolum psygmophilum*; LaJeunesse et al., 2018), that exhibit declines in Fv/Fm at winter minimum temperatures (Thornhill et al., 2008). However, these symbionts are cold tolerant and can recover from winter minimum temperatures to regain photochemical efficiency the following spring (Thornhill et al., 2008). It is possible that Fv/Fm of the corals considered here would recover after returning to control conditions, but that consideration was outside the scope of this study. Additionally, it is difficult to interpret changes in Fv/Fm in aposymbiotic fragments of *O. arbuscula*, as these corals should have low symbiont densities and therefore little to no measurable Fv/Fm. However, it is likely representative of Fv/Fm of endolithic algae or small numbers of background symbionts.

In addition to declines in Fv/Fm at cold extremes, we also observed loss of pigment density and symbiotic extent in the second week of this experiment in the cold treatment. This loss of pigment and symbiotic extent is evidence of coral bleaching, a coral stress response that has been observed at both heat (Gates et al., 1992) and cold (Hoegh-Guldberg and Fine, 2004; Lirman et al., 2011) temperature extremes. Although there were no statistically significant differences between treatments, there is a trend in loss of symbiont extent and pigment density for corals in the cold treatment compared to the heat treatment. *O. arbuscula* considered here are likely unable to make up for this decline in symbionts and pigments (i.e., loss of photosynthetically-derived energy) with heterotrophic feeding, as we observed a decline in feeding behavior at cold temperatures compared to heat.

Previous work by Roth et al. (2012) showed that the time frame over which cold and heat stress affect the tropical branching coral *Acropora yongei* is important for its physiological response. While cold-treated *A. yongei* initially demonstrated greater declines in growth and Fv/Fm, they were able to acclimate to the cold conditions and improve their physiology after 2 to 3 weeks. In contrast to the response of *A. yongei* to cold stress, heat stress initially did not negatively affect Fv/Fm of this species, but after a delay incurred more severe damage than cold stress, including severe bleaching and loss of growth. Overall, Roth et al. (2012) conclude that cold stress is more damaging over the short term, while heat stress is more

detrimental over longer time frames for *A. yongei*. In the context of this previous work, it is certainly possible that the experiment presented here is demonstrating this short-term effect of cold stress on *O. arbuscula,* but if this experiment were extended we would eventually observe similar long-term negative impacts of heat stress.

Evidence of acclimation in O. arbuscula *symbiont pigments*

Both symbiotic extent and symbiont pigment density increased in all treatments (including the control) and both symbiotic states over the first week of the experiment (Figures 5, 7). This is likely evidence that the corals were still acclimating to the experimental aquaria conditions over this time. Although the fragments of *O. arbuscula* used in this experiment were acclimated for several months following collection and fragmentation, they were only acclimated to the aquaria used here for a few days. This shorter acclimation included a temperature decrease from 24°C (long-term holding temperature) to 18°C (experimental control conditions) in the week preceding the start of the experiment. Even though the aquaria conditions replicated the long-term holding conditions of *O. arbuscula* as closely as possible, this temperature change along with minute differences in light and more frequent feeding could contribute to these initial increases in symbiont extent and pigment density (similar effects observed in Aichelman et al., 2016; Jacques et al., 1983). Following this initial increase, both symbiont extent and pigment density decreased in the second week of the experiment, but only in the cold treatment (see discussion above; Figures 5,7). This acclimation response is additional evidence that future experiments should be extended for longer time frames.

Experimental Limitations

It is important to acknowledge that this study is limited by both experimental samples and by time. Overall, this experiment appears to have missed the upper thermal limit of *O. arbuscula,* and future experiments should push this species to warmer temperatures to fully characterize the physiological response to heat stress. It is not possible to completely disentangle the effect of symbionts on the response of *O. arbuscula* to temperature extremes, as no fragments used here were 'truly' aposymbiotic. Future studies should aim to establish fully symbiotic and fully aposymbiotic genets to attempt to disentangle these effects. Additionally, this experiment was relatively short term (15 days). As corals grow slowly, and we seem to have missed the upper thermal limit of this species, more definitive patterns would likely emerge from decreasing the rate of temperature increase, stretching the temperature change out over a longer time frame, and warming past 32°C.

Figures

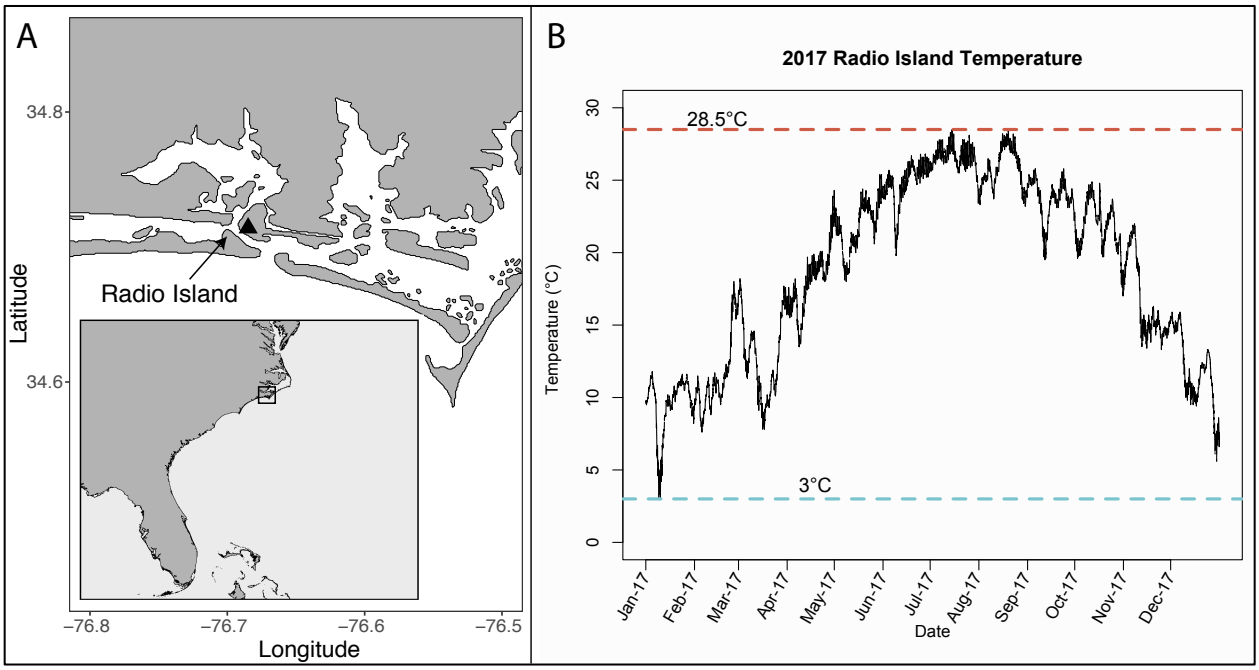

Figure 1. Collection site and *in situ* temperature of Radio Island, NC. (A) Map indicating Radio Island, North Carolina, the site of *O. arbuscula* collections in Summer 2018 (34.712590°N, -76.684308°W). (B) 2017 water temperature at Radio Island, NC as recorded by a NOAA data buoy (Station #BFTN7). The maximum temperature recorded at the site was 28.5°C, and the minimum recorded temperature was 3°C (indicated by horizontal red and blue dashed lines, respectively).

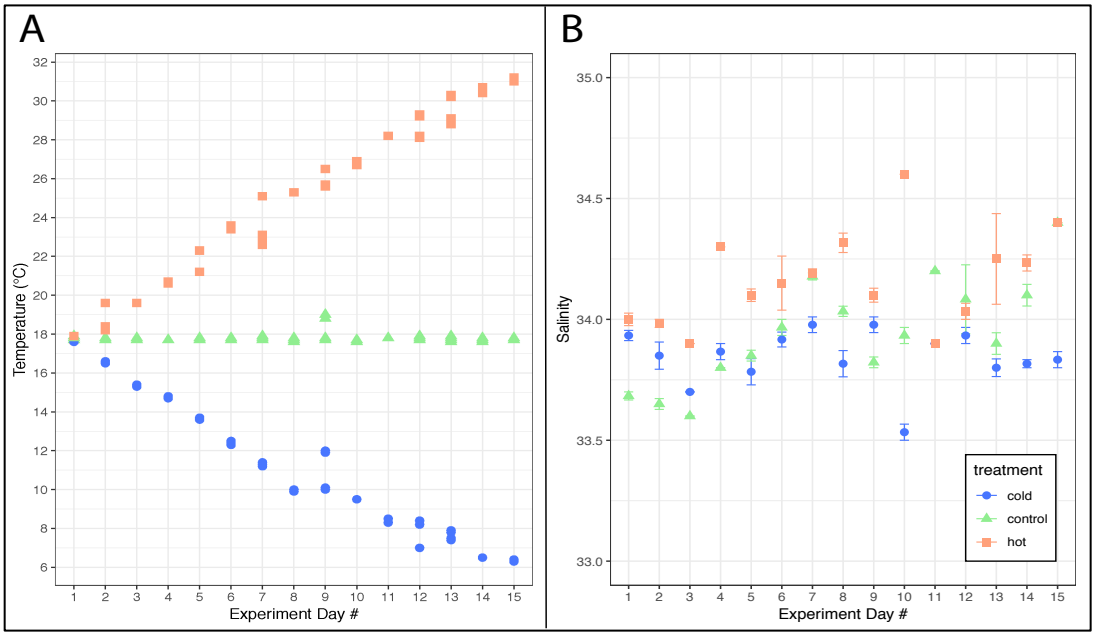

Figure 2. Experimental water quality parameters. Temperature (A) and salinity (B) were monitored at least daily in all aquaria in each of the experimental treatments (blue = cold, green =

control, red = hot). For temperature (A), each temperature measurement in all aquaria are included. For salinity (B), salinity is averaged by treatment, and error bars are standard error.

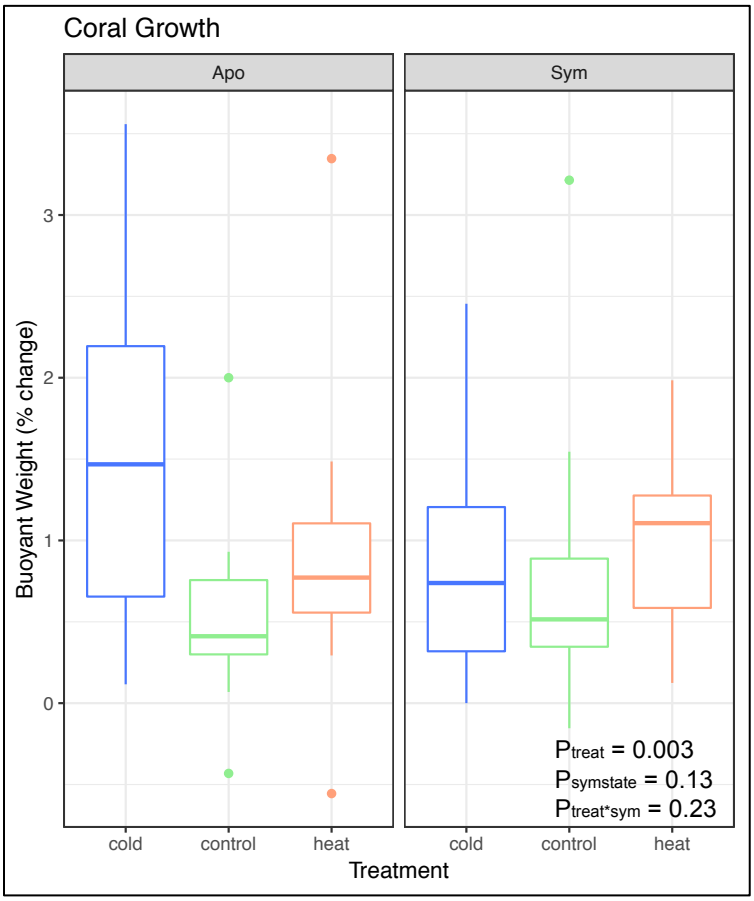

Figure 3. Percent change in *Oculina arbuscula* growth (measured via buoyant weight technique) over 15-day experimental period. Coral growth was not different across symbiotic state (p = 0.13), but was significantly affected by the temperature treatment (p = 0.003). No significant differences in growth were detected within symbiotic state. Blue = cold, green = cold, and red = heated treatments. Apo = aposymbiotic fragments and Sym = symbiotic fragments. Treatment colors and abbreviations apply to all following figures.

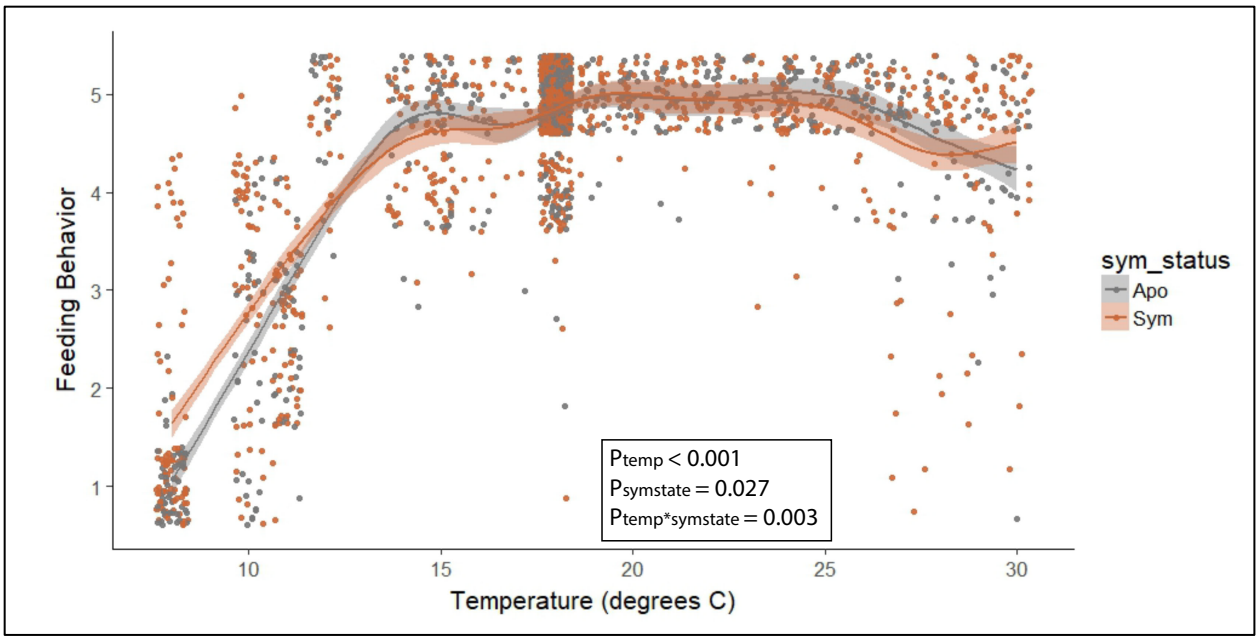

Figure 4. *Oculina arbuscula* polyp activity assessed post-feeding across temperature. Feeding behavior (i.e., polyp activity) was assessed daily in each treatment over the course of the experiment on a scale of 1 to 5, 1 being 0% polyp extension and 5 being 100% polyp extension. *O. arbuscula* polyp activity was significantly different across the temperatures considered here ($p < 0.001$), and declined sharply at cold temperatures compared to heated temperatures. Activity was also different between symbiotic and aposymbiotic fragments ($p = 0.027$), and the interactive effect of temperature and symbiotic state on polyp behavior ($p = 0.003$) seems to be driven by the divergence between symbiotic and aposymbiotic polyp activity at cold temperatures. Each point represents the polyp activity of a single coral fragment at a particular temperature.

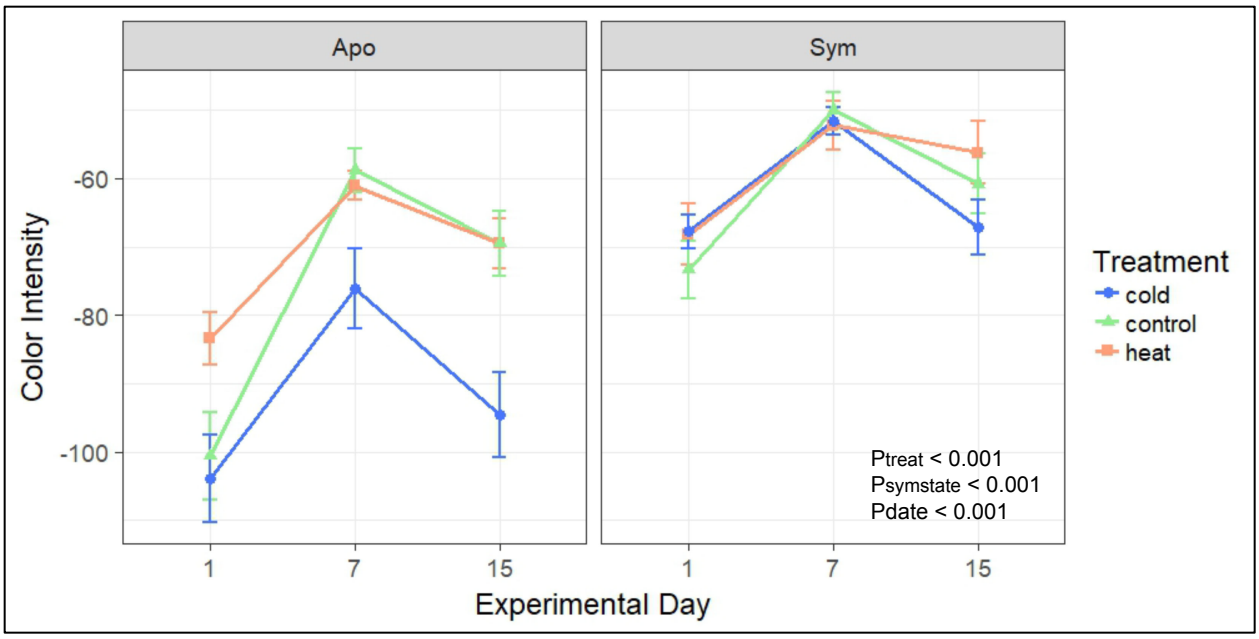

Figure 5. *Oculina arbuscula* pigment density over the 15-day experimental period. Color intensity, a proxy for symbiont pigment density, of *O. arbuscula* fragments exposed to cold (blue), control (green), and heat (red) temperature treatments. Less negative numbers (i.e., moving up the y-axis) indicates greater pigment density. Symbiont pigment density was significantly different across temperature treatments ($p < 0.001$), symbiotic status ($p < 0.001$), and through time ($p < 0.001$). All points are the average of between 16 and 29 coral fragments, and error bars are standard error.

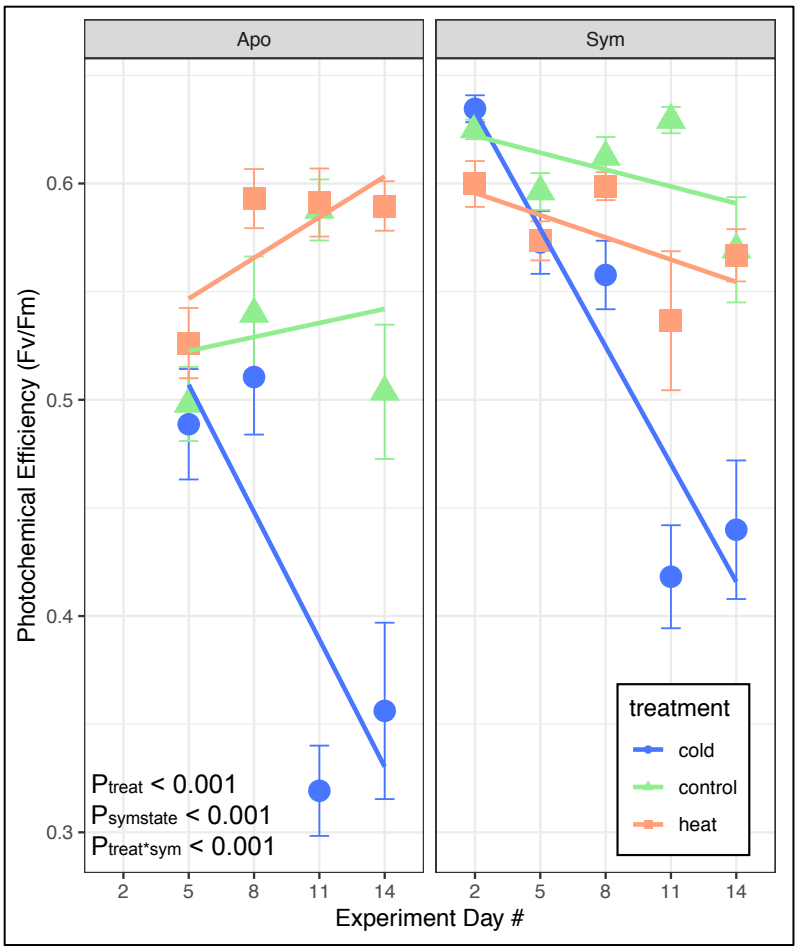

Figure 6. Change in *Oculina arbuscula* symbiont dark-adapted photochemical efficiency of photosystem II (Fv/Fm) over 15-day experimental period. Fv/Fm as measured every 3 days throughout the experiment (except no measurements were taken for aposymbiotic fragments on day 2). Fv/Fm was significantly affected by treatment ($p < 0.001$), symbiotic status ($p < 0.001$), and the interaction of the two ($p < 0.001$). Fv/Fm of corals maintained in heat and control treatments were not different ($p = 0.94$); however, corals in the cold treatment had significantly lower Fv/Fm than corals in the other treatments ($p < 0.001$). All points are the average of between 18 and 29 fragments, and error bars are standard error.

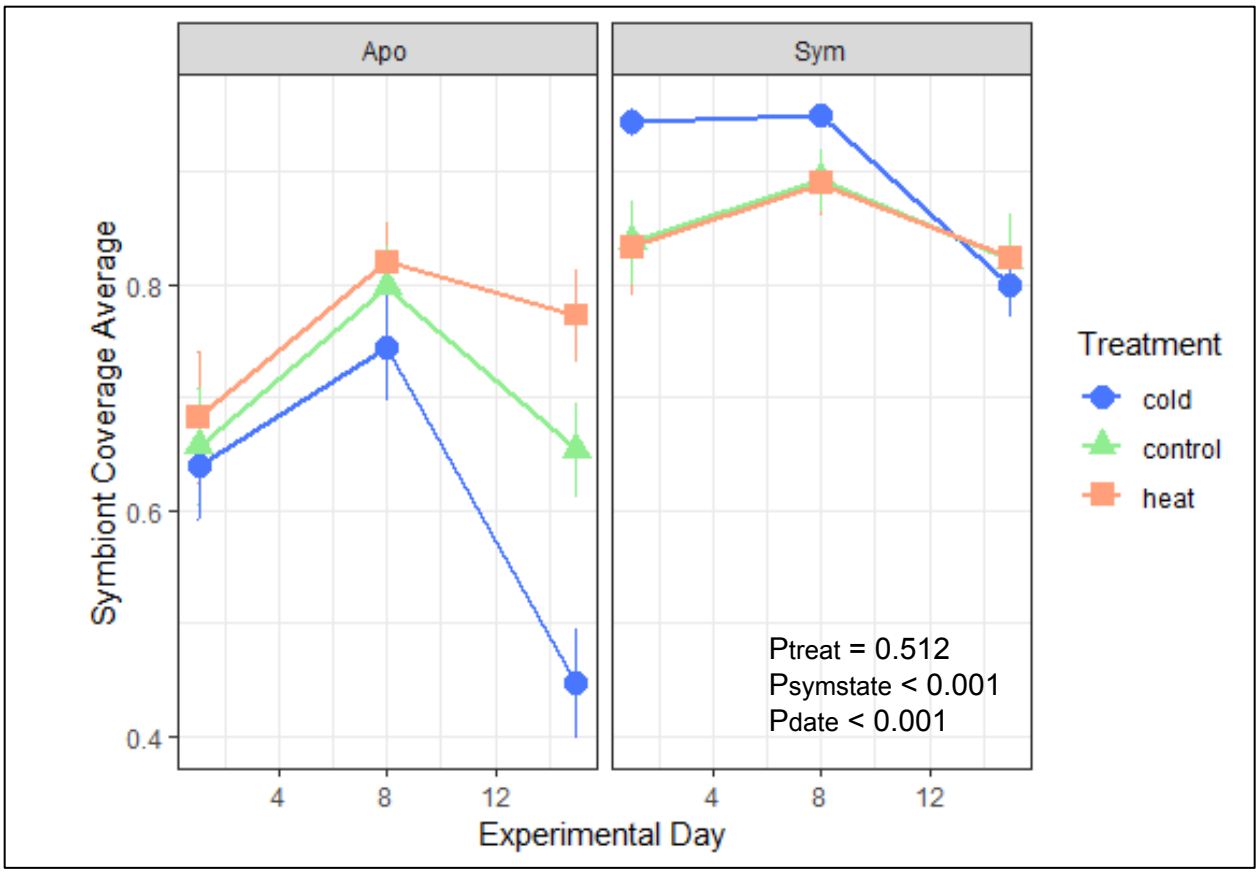

Figure 7. *Oculina arbuscula* symbiont coverage (i.e., bleaching extent) throughout the 15-day experiment. The fraction of the coral hosting symbionts (symbiont coverage area) was not different across temperature treatments (p = 0.512), but did differ between symbiotic and aposymbiotic fragments (p < 0.001) and through time of the experiment (p < 0.001). Greater symbiont coverage area is equivalent to a greater percentage of the coral fragment being symbiotic. Error bars are standard error.

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