The effects of heat stress on growth and photosynthetic efficiency on the temperate coral *Astrangia poculata*

Xuqing Chen, Laura DiRoberts, Kalli Richmond

Abstract

Rapid increases in sea surface temperatures and carbon dioxide concentration pose a great threat to the health and diversity of coral reefs across the globe. While the effect of thermal stress on tropical corals has been well studied, little is known about the response of temperate corals to warming ocean temperatures. *Astrangia poculata*, a temperate scleractinian coral, lives across a wide range of temperatures and exists naturally with and without its symbionts, making it a model organism. This study investigated the effect of thermal stress on photosynthetic efficiency, color change, calcification change, and polyp behavior of *A. poculata*. Control treatments were kept at 18℃ and thermal stress treatments started at 18℃ and were increased by 1℃ daily for fourteen days, reaching a maximum of 30℃. Photosynthetic efficiency of symbiotic control corals at the end of the experiment were significantly higher when compared to aposymbiotic control corals and all thermal stressed corals. Symbiotic corals were significantly darker than aposymbiotic corals in both treatments, but differences between treatments were not significant. Coral growth was significantly greater in the heat treatment than in the control treatment. Symbiotic control corals had significantly more polyp activity than symbiotic heat corals. The only exception to these findings were heat stressed colonies of genotype S, which experienced complete tissue death by the end of the experiment. These results indicated that *A. poculata*'s photosynthetic efficiency and color density were unaffected by temperatures up to 30℃, and growth benefited from warmer temperatures.

Introduction

The relationship between coral health and ocean conditions has become clear over the last few decades. Globally, oceans are responsible for more than 25% of the total uptake of anthropogenically generated carbon dioxide (Sett *et al.* 2014). As humans increase CO_2 emissions, mainly through the burning of fossil fuels, oceans are being pushed to take up increased CO_2 every year. According to the Mauna Loa observatory in Hawaii, the annual average of atmospheric CO_2 has increased from ~315ppm in 1958 to 406.5 ± 1 ppm in 2017 (Dlugokenchy *et al.* 2018). Additionally, increased CO_2 leads to increases in trapped atmospheric heat, 93% of which is absorbed by the ocean (Levitus *et al.* 2012). Studies have shown that coral calcification is reduced by 20% when pCO_2 levels are greater than 700 ppm and temperatures rise by 3°C (Kornder *et al.* 2018). With corals supporting more than 30% of all marine species, global climate change is an extreme threat to the survival of these ecosystems (Coles *et al.* 2018).

Coral reefs are known for their high productivity and economic importance in fisheries, tourism and coastal protection (Hoegh-Guldberg 1999). Scleractinian corals, also known as stony corals, are well known for their symbiotic relationships with autotrophic dinoflagellates (Baker 2018). These

relationships are very sensitive to changes in the environment such as salinity, light and temperature (Hoegh-Guldberg 1999). Elevated temperature can cause mass coral bleaching (the loss of symbiosis between corals and photosynthetic *Symbiodinaceae*) resulting in reduced growth, calcification and mortality (Hoegh-Guldberg 1999; Hoegh-Guldberg *et al.* 2007; Donner 2009). With global CO_2 concentration and temperatures increasing at an unprecedented rate, annual bleaching of coral reefs could be seen globally by 2040; for Caribbean and tropical western Pacific corals, this could be by 2025 (Van Hooidonk *et al.* 2013). Effects of heat stress on tropical corals have been well documented (i.e., Lesser 1997; Hughes *et al.* 2010; Doney *et al*. 2012). However, few studies have focused on how temperate corals perform under warming scenarios and results have demonstrated a high variability of responses to thermal stress (Aichelman *et al.* 2016; Movilla *et al.* 2016).

Tropical corals typically harness much of their energy for reef-building through their symbionts. Temperate scleractinians, however, also have symbionts but can rely on heterotrophic carbon available in more nutrient rich waters. This type of symbiosis is called facultatively symbiotic and one such temperate scleractinian is *Astrangia poculata*. This species exists naturally with and without its algal symbiont *Breviolum psygmophilum*, named as such because it is a "cold-loving" species (Dimond and Carrington 2007; LaJeunesse *et al.* 2012, 2018). In *A. poculata,* polyps appear brown with symbionts, white without symbionts, and they also exhibit a mixed state with partial symbiont cover. Although *A. poculata* are facultatively heterotrophic and can sequester nutrients through polyp food capture, they have been shown to exhibit symbiont densities as high as tropical corals and can harnesses nutrients through photosynthetic products from these symbionts (Cohen *et al.* 2002; Szmant-Froelich and Pilson 1980). *Astrangia poculata* is commonly found along rocky shorelines stretching from the southside of Cape Cod to the Gulf of Mexico and have been observed to survive temperature ranges from -2℃ up to 23℃ (Peters *et al.* 1988; Cohen *et al.* 2002).

This broad range of thermotolerance begs the question of how temperate corals are able to withstand such broad thermal profiles. Of the studies on temporal coral responses to thermal stress, one study showed that *A. poculata* exhibited the highest wound healing rate at 24℃, which is outside of its natural thermal range (Burmester *et al*. 2017). Another study demonstrated that exposure to 28℃ (4℃ above summer maxima) for three weeks led to tissue necrosis in the temperate coral *Cladocora caespitosa* (Rodolfo-Metalpa *et al.* 2005). In contrast, no impact was found when *C. caespitosa* was thermally stressed at 28℃ for eleven days and 29℃ for four days (Kersting *et al.* 2015). In addition, heterotrophy was found to provide the temperate coral *Oculina arbuscula* with enough energy to reduce bleaching under thermal stress (Aichelman *et al.* 2016). Lastly, another study investigating the effects of carbon dioxide and nutrient enrichment on *A. poculata* suggested that increased levels of CO_2 at ambient nutrient levels had a negative effect on calcification, and that elevated nutrient levels had the potential to offset the negative impacts of increased CO_2 (Holcomb *et al.* 2010). This offset is likely

because when the coral is not nutrient limited, it has a greater capacity for CO_2 fixation (Holcomb *et al.* 2010). It seems that coral responses to environmental stressors are extremely species specific, making it difficult to predict the responses of one species based off research of another species.

The aim of this study is to investigate the physiological responses of the temperate coral *A. poculata* to heat stress. Measurements will include photosynthetic efficiency, buoyant weight, color change, and behavior changes to quantify responses to thermal stress. Both aposymbiotic and symbiotic corals will be in each treatment, allowing us to observe any possible differences relative to symbiotic state.. Being an important member of temperate and subtropical coastal ecosystems, *A. poculata* is a great model organism; this study could allow us to gain insight into how *A. poculata*, and possibly other temperature corals, can potentially withstand warming oceans.

We hypothesize that heat will have a positive effect on both symbiotic and aposymbiotic *A. poculata* in terms of photosynthetic efficiency, calcification rate, levels of polyp activities and consistent color intensity until it hits its tolerance limit, estimated to be ~23°C. We expect that once this limit is surpassed trends for all variable will reverse.

Methods

Experimental Design

In November 2017, twenty *Astrangia poculata* colonies were collected from Woods Hole, MA (Fig 1). Colonies were fragmented and then separated into symbiotic (brown) and aposymbiotic (white) groups. Each nubbin was assigned a specific ID according to genotype (labeled A through T) and glued to a 60mm petri dish which was etched with a code to identify the coral. A total of twenty genotypes were used in this experiment: seven colonies with four nubbins, six colonies with three nubbins, six colonies with two nubbins, and one colony with five nubbins totaling to sixty three experimental subjects. Each nubbin was assigned randomly to one of two treatments, each treatment containing three tanks. No tank had more than one of each genotype. Three of tanks, designated thermal stress tanks, had nineteen symbiotic and twelve aposymbiotic treatment corals divided between them. Temperatures began at 18℃ and were raised 1℃ at the end of every day for fourteen days, ending at 30℃ (Fig 2a). The three control tanks had nineteen symbiotic and thirteen aposymbiotic corals divided between them and were maintained at 18 ± 0.5℃ for the duration of the experiment (Fig 2a). Aposymbiotic corals and symbiotic corals were separated within each tank; each nubbin was arranged randomly and rotated clockwise every day to avoid bias due to difference in flow and light position. Aposymbiotic and symbiotic corals were also switched from one side of the tank to the other every third day corresponding with PAM measurements to avoid within tank effects. Corals were illuminated on a 12:12 h light:dark cycle. One 25% water change was completed on the ninth day of the experiment. Salinities of both treatments were monitored and recorded twice daily (Fig 2b).

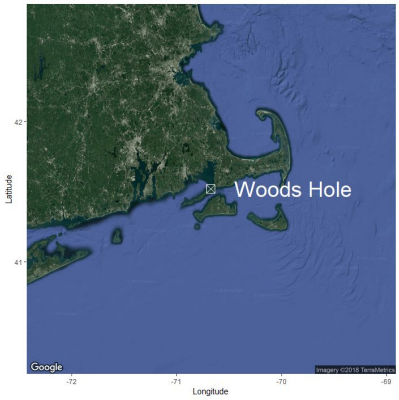
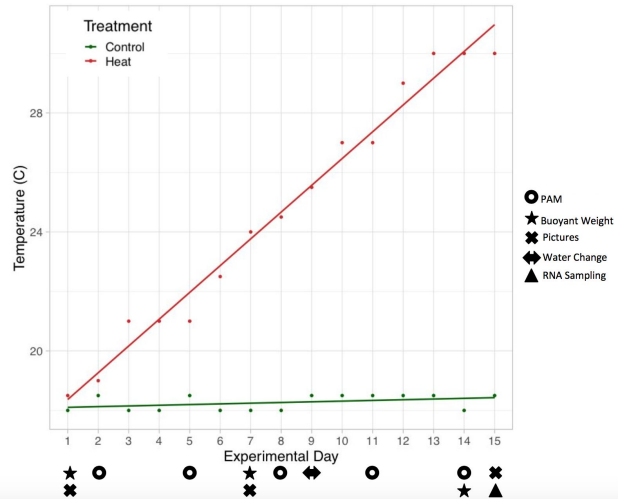


Figure 1. Satellite map of the shoreline of Massachusetts marking the collection site.

A



B

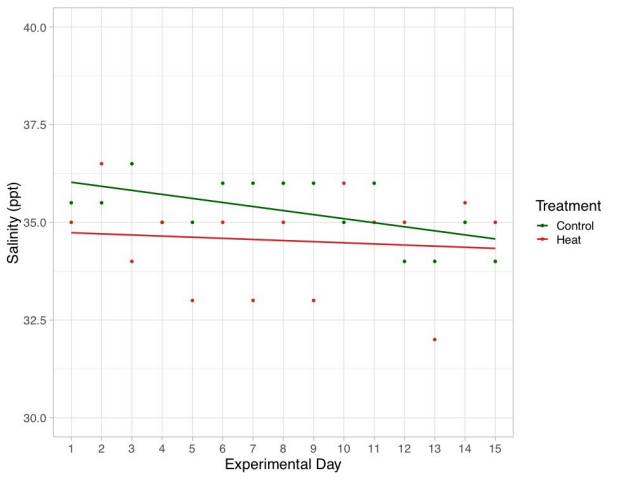


Figure 2. Experimental timeline. (A) The green line represents the controls and the red line represents the thermal stress treatments. Control temperatures were set for 18℃ throughout the experiment. Thermal stress temperatures started at 18℃ and were increased by 1℃ daily. PAM measurements were taken every three days and photos and buoyant weights were taken at the beginning, middle, and end of the experiment. One 25% water change was completed on day 9. RNA sampling was conducted on the final day. (B) Salinities throughout the duration of the experiment were held relatively constant around 35 ppt for heat and control treatments.

Photosynthetic Efficiency

To estimate photosynthetic efficiency, pulse amplitude modulated (PAM) fluorometry was used on each colony using a junior PAM fluorometer in the last few hours of their dark cycle to ensure dark acclimation. Red headlamps were utilized so that photosynthetic efficiency was not stimulated, and colonies remained submerged in their tanks during testing. Initial fluorescence (Fo) and final fluorescence (Fm) were measured and photosynthetic efficiency (Fv/Fm) was then calculated as where Fv = Fm-Fo. Multiple polyps from colony were measured until three readings within 0.100 were recorded. The mean of these three measurements was used as a proxy for the photosynthetic efficiency of the whole colony. Measurements were conducted on days two, five, eight, eleven, and fourteenth of the experiment.

Color Change

Corals were photographed on days one, seven, and fifteen using an Iphone 8 camera. Each coral nubbin was placed into a light box on a coral watch health card with its identifying etching at the top of the card. Each photo was calibrated using Adobe Photoshop to standardize the white balance. To analyze coral color intensity, the Macro "AnalyzeIntensity" from Winters *et al.* (2009) was used in MATLAB. This script uses ten points selected

by the user to measure color values in RGB (red/green/blue). Points for analysis were selected from different places on the colony to best represent the whole. On mixed colonies, only polyps corresponding to the assigned symbiotic state were selected. Red values were recorded, and the mean color value for all points used to represent the whole colony. Lower symbiont count is correlated with higher red intensity, therefore lower symbiont density is reflected in higher red values.

Buoyant weight

Buoyant weight was also measured on days one, seven, and fifteen immediately following being photographed. Approximately four gallons of control seawater was put in a five-gallon tank and a scale with a hook at bottom was fixed on top of the tank with duck tapes. A 10cm hook was made with wire and colonies were suspended under water via a 0.5cm diameter hole which had been drilled in each petri dish. Algae on petri dish and coral skeletons were brushed off with a toothbrush prior to each weighing. The scale was calibrated with the wire hook attached before each measurement. Each nubbin was weighed three times and the mean was taken.

Feeding and Polyp Behavior

Corals were fed daily at 4pm throughout the experiment. Each tank received one level ¼ teaspoon of "Two Little Fishies ZoPlan" dried zooplankton diet, which was mixed with seawater from the corresponding sump in a 400mL beaker. Tanks were isolated for thirty minutes without water flow and polyp behavior was assessed on a 1-5 scale (see Table 1) to determine behavioral changes associated with increasing temperature. After behavioral observations, tanks were recirculated.

1	No polyp extension
2	25% polyp extension
3	50% polyp extension
4	75% polyp extension
5	100% polyp extension

Table 1: Visual feeding assessment of coral behavior. Nubbins were assigned a behavior score based on overall percentage of polyp extension after feeding.

Statistical Analysis

All statistical analyses and graphs were plotted in R (R core team, 2016). For all analyses, one-way ANOVAs and Tukey's HSD tests were run to determine any statistical differences between treatments, symbiotic states, or genotypes.

Results

Temperature and Salinity

Although we aimed to reach 32°C in heat treatments, temperatures never reached above 30°C (Fig 2A) due to ambient air temperature. Salinities for the heat treatment fluctuated between 32 ppt and 36 ppt. Salinity for the control treatment fluctuated between 34ppt and 36 ppt (Fig 2B).

Effects of Thermal Stress on Photosynthetic Efficiency

Photosynthetic efficiency by treatment showed only a few significant variations over duration of the experiment (Fig 3A). When comparing treatment types, day fourteen control corals were significantly higher than day two

control corals (p=0.0056), day two heated corals (p=0.042), day eleven control corals, (p=0.00042), day eleven heated corals (0.015), and day eight heated corals (p=0.035). No other differences were significant.

Differences within treatment by symbiotic state showed some additional significant relationships. Within the control treatment, only day fourteen symbiotic corals showed any significant differences (Fig 3B). This group was significantly higher than symbiotic and aposymbiotic corals on day eleven (p=0.0042, p=0.0016), aposymbiotic corals on day two (p=0.002), and nearly significantly different from day two symbiotic corals (p=0.064). In contrast, there were no significant differences by day and symbiotic state for any of the heat treated corals (Fig 3C).

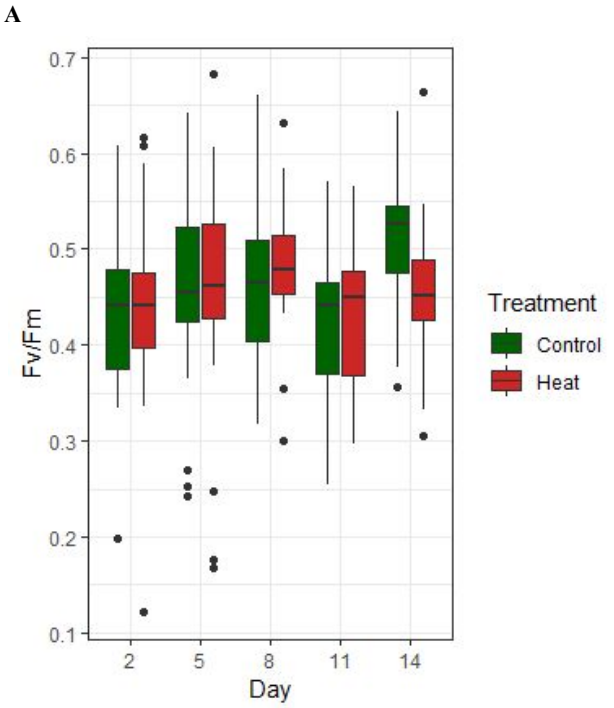


Figure 3. Photosynthetic Efficiency: A) Comparison between standard temperature and heat stressed corals by day. Values were significantly higher for control corals on day fourteen than corals from both treatments on days two and eleven. Values were significantly lower for control corals on day eleven than heated corals on day eight. B) Comparison between symbiotic states for standard temperature corals. Symbiotic corals on day

fourteen were significantly higher than than both symbiotic and aposymbiotic corals on days two and eleven. C) Comparison between symbiotic states for heat stressed corals. No significant differences were found ($p < 0.05$).

Effects of Thermal Stress on Color Change

All symbiotic colonies were significantly darker than aposymbiotic colonies ($p=0.00$) in both the control and heat treatments. Symbiotic and aposymbiotic colonies did not vary in color intensity by treatment. It appeared that symbiotic corals got darker over the course of the experiment in both the control and the heat stressed treatments, but differences were determined to be not significant. Aposymbiotic coral color was found to be darker in heat treatment from day 7 to day 14 ($p < 0.05$) (Fig 4). Complete tissue death was observed in two individuals on the final day of analysis (AS3 and AS6), and partial tissue death was observed on another individual (AI2). All colonies that lost tissue were heat stressed, and the two completely dead ones were of the same genotype. Another heat treated colony (AH1) changed symbiotic state from white to brown in the latter half of the experiment.

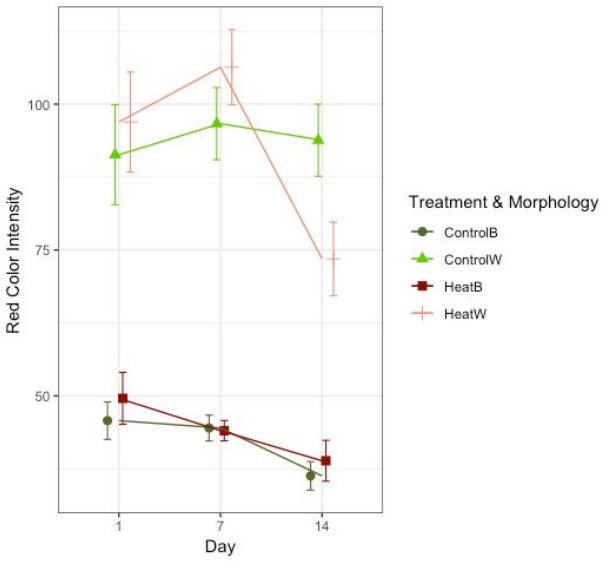


Figure 4. Red channel intensity as a measure of brightness of symbiotic and aposymbiotic *A. poculata* in control and heat treatments. Differences between symbiotic states in both treatments are significant (One-way ANOVA: $p=0.00$). Differences within each symbiont state over time were only found in aposymbiotic *A. poculata* in heat treatment from day 7 to day 14 . Differences between treatments of the same state are not significant. Error bars stand for SE for mean color intensity ($n = 63$).

Effects of Thermal Stress on Weight Change

When exposed to increasingly warmer temperatures, corals reared in the heat treatment had a significantly higher percent gain in calcification when compared to corals in the control treatment ($p=0.0025$). When comparing weight gain between different days of the experiment, no statistically significant difference was found. There was also no significant difference between weight gain of differing symbiotic states within treatments or between different colony genotypes. Heat treatment corals appeared to have a higher mean weight gain than corals in the control treatment. Control treatment corals experienced more weight loss than heat treatment corals (Fig 5).

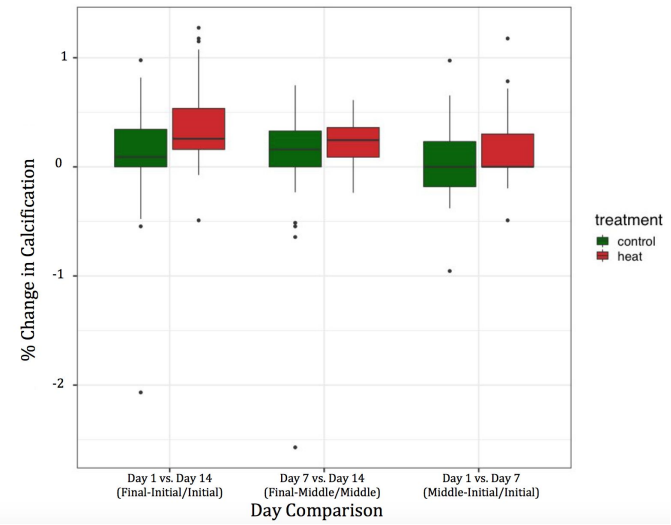


Figure 5. Percent change in calcification of *A. poculata* in both heat and control treatments. Calcification is significantly higher in heat treatments (p=0.0025). Comparisons between days one, seven, and fourteen are shown. No statistical difference is found for comparisons of different days (p=0.161).

Effects of Thermal Stress on Polyp Behavior

Among all treatments, symbiotic *A. poculata* in control treatment had the highest level of polyp activity ($p < 0.05$). For symbiotic *A. poculata*, level of polyp activity was significant higher in control than heat treatments ($p < 0.05$). At day 13 (30°C), corals in heat treatment starting to exhibit a significant drop in level of activity comparing to the first five days ($p < 0.05$). At day 14 (30°C), corals in heat treatment dropped significantly to 3.5 (62.5%) comparing to all other temperatures ($p < 0.05$) (Fig 6A). For aposymbiotic *A. poculata*, no significant differences were found between treatments (Fig 6B).

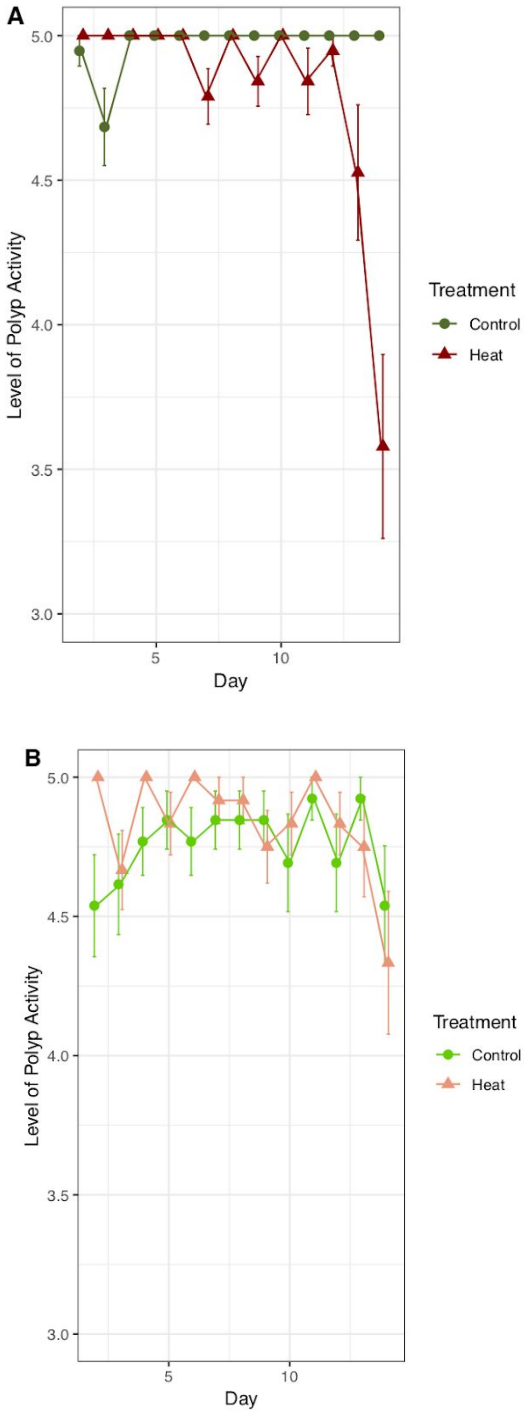


Figure 6. Level of polyp activity of *A. poculata* scored 1 (0%) through 5 (100%) over 14 days with (A) control and heat treatments of symbiotic corals. Significant differences were found between treatments using one-way ANOVA ($p < 0.05$). Error bars stand for SE for mean score (n = 38) and (B) control and heat treatments of aposymbiotic corals. Error bars stand for SE for mean score (n = 25).

Discussion

Effects of Thermal Stress on Photosynthetic Efficiency

Photosynthetic efficiency is a good predictor of symbiont health and density. As such, we expect to see more efficiency in symbiotic than in aposymbiotic corals. For the most part, however, this was not the case. There were no significant differences between the two treatments of symbiotic corals by day. Additionally, controls symbiotic corals seemed to fare the best, performing significantly higher at the end of the experiment. This suggests that under laboratory conditions, symbiotic corals will increase their photosynthetic efficiency over time. This is likely due to the corals having the energy for symbiont cell replication in a well fed, well lit, predator free environment. Since heated corals did not significantly change their efficiency over time it is possible that heat stress was sufficient to negate the benefits of other favorable conditions, but not reverse them. These results were similar to a recent study by Aichelman (2018), which found that 18°C was optimal for the photosynthetic efficiency of *A. poculata*, and that symbiotic colonies to be more efficient that aposymbiotic colonies. This mirrors our results, where symbiotic corals in control (kept at 18°C) were significantly more efficient.

Effects of Thermal Stress on Color Change

No bleaching was observed in heat stressed treatments for either symbiotic or aposymbiotic corals, indicating that *A. poculata* might have a higher threshold for bleaching than we expected. Moreover, we observed an increase in photosynthetic efficiency in heat treated aposymbiotic colonies from day 7 (24℃) to day 14 (30℃). These findings differ from previous finding in Kenkel e*t al.* (2013)'s study which observed that the tropical coral *Porites asteroides* was significantly brighter after six weeks of heat treatment. It is possible that exposing *A. poculata* to high temperatures over a longer period would give us a more complete picture of its response to heat stress.

Some tissue death was observed in photographs during color analysis. All colonies of genotype S in the heat treatment died. One individual (AI2) experienced about 25% tissue loss localized to one section of the colony. No other individuals of genotype I were affected. Only three colonies out of 95 experienced tissue death. One heat treated colony (AH1) switched from aposymbiotic to symbiotic between the day seven and day fourteen. This suggests that thermal tolerance varies among genotype. It also suggests that while heat stress may benefit symbionts in the short term, there are outliers that experience more extreme positive and negative reactions.

Effects of Thermal Stress on Weight Change

When *A. poculata* nubbins were exposed to heat stress, they had a higher percent calcification increase relative to corals in the control treatment. Our analysis indicates that this difference in growth between treatments is statistically significant. These results suggest that for *A. poculata*, warming sea surface temperatures (SST) increase calcification rate. These findings are consistent with those of Dimond and Carrington (2007); the field study found that *A. poculata* growth increased with increasing temperatures. Temperatures below 6.5℃ were found to stunt calcification,

indicating that *A. poculata* is limited by cold temperatures. Another *A. poculata* study found that above 15℃, symbiotic coral growth exceeded normal growth rates; it was suggested this was due to the ability of *A. poculata* to regulate metabolic rate at high temperature ranges thus allowing more energy to be allocated towards coral calcification (Jacques *et al.* 1983). Similar findings were reported for *Porites* coral colonies (Lough and Barnes 2000; Bessat and Buigues 2001). If the present study had been carried out over a longer period of time and the heat treatment continued to increase in temperature, we may have reached a thermal optimum for growth, beyond which calcification would decrease, as observed in *Porites* (Cooper *et al*. 2008). Further research is needed to find the upper thermal limit of *A. poculata* relative to calcification.

Effects of Thermal Stress on Polyp Behavior

We found that the average level of polyp activity in symbiotic *A. poculata* in control treatment is higher than aposymbiotic *A. poculata* in both treatments. It has been found that facultative symbiotic coral *Oculina arbuscula* depended on different food source based on its symbiotic status (Leal *et al*. 2014). It is possible that in our experiment, symbiotic *A. poculata* were able to acquire more energy through its symbionts and be more active than aposymbiotic *A. poculata* that relied solely on feeding. Polyp behavior remained consistent for symbiotic *A. poculata* in both treatments from day 2 to day 12. Starting at day 13, level of polyp activity of symbiotic *A. poculata* in heat treatment dropped significantly, suggesting that 30°C may be a tipping point in polyp activities of symbiotic *A. poculata* with coral death in some colonies. Our results correspond with a previous study on hawaiian corals which coral death were observed in *Pocillopora damicronis*, *Montipora capitata*, and *Lobactis scutaria* after their exposure to 31.4°C for 13, 15 and 17 days (Coles *et al.* 2018). Levels of polyp activities in aposymbiotic *A. poculata* were not affected significantly by treatment, which could be explained by the ability of corals to cope with thermal stress with heterotrophy (Towle *et al*. 2015; Aichelman *et al.* 2016).

Conclusions

Our results show evidence that *A. poculata* can thrive under elevated temperatures. Its growth under thermal stress increased significantly compared to the control corals throughout the duration of the experiment. Photosynthetic efficiency was not hindered by thermal stress, but was enhanced when not exposed to temperatures up to 30°C. Color change was not a significant factor between treatments, although, symbiotic corals in both the control and heat treatments appeared to become darker in color throughout the experiment, indicating that most symbiotic *A. poculata*, a least for a brief time period, can live in higher temperatures up to 30°C and show no signs of bleaching (with the exception of genotype S). As a temperate coral, *A. poculata* in nature undergoes annual fluctuations of temperature and may be adapted to severe yet short term temperature shifts. The scope of this study is limited by its timeframe and temperature maximum. A longer study or a study with a higher maximum temperature could show the limits of the subject's thermal tolerance in ways this study did not. Symbiotic corals in the control treatment were the most

active overall. Control corals in general continued to be very active throughout the experiment. Polyp activity of symbiotic thermal stress corals decreased significantly on day 13 of the experiment, suggesting an approach of a possible thermal threshold around 30°C. No significant differences were found for aposymbiotic corals between treatments for any variables tested. This suggests that observed declines in symbiotic *A. poculata* polyp activity and the lack of increase of photosynthetic efficiency in heat treatments is likely related to symbiotic state. It is possible that the cold loving nature of *B. psygmophilum* makes it energy costly to maintain at higher temperatures. This could indicate an upper thermal breaking point for the algal symbiont rather than the coral itself.

Acknowledgments

We would like to thank Dr. Sarah Davies, Nicola Kriefall, Justin Scace, Brooke Benson, and Matt Kanke for their support and guidance throughout this research. We also thank our fellow Marine Physiology and Climate Change classmates for their collaborative efforts.

Literature Cited

Aichelman, H. E. (2018) Local Adaptation Signatures in Thermal Performance of the Temperate Coral *Astrangia poculata*. Master of Science (MS), thesis, Biological Sciences, Old Dominion University

Aichelman, H. E., Townsend, J. E., Courtney, T. A., Baumann, J. H., Davies, S. W., Castillo, K. D., (2016) Heterotrophy mitigates the response of the temperate coral *Oculina arbuscula* to temperature stress. Ecol Evol, 6(18), 6758-6769

Baker, D. M., Freeman, C. J., Wong, J. C. Y., Fogel, M. L., Knowlton, N. (2018) Climate change promotes parasitism in a coral symbiosis. The ISME Journal, 12, 921-930

Bessat, F., Buigues, D. (2001) Two centuries of variation in coral growth in a massive Porites colony from Moorea (French Polynesia): a response of ocean-atmosphere variability from south central Pacific. Palaeogeography, Palaeoclimatology, Palaeoecology, 1-4, 381-392

Burmester, E. M., Finnerty, J. R. Kaufman, L., Rotjan, R. D. (2017) Temperature and symbiosis affect lesion recovery in experimentally wounded, facultatively symbiotic temperate corals. Mar Ecol Prog Ser, 570, 87-99

Cohen, A. L., Owens, K. E., Layne, G. D., Shimizu, N. (2002) The effect of algal symbionts on the accuracy of Sr/Ca paleotemperatures from coral. Science, 296(5566), 331-333

Coles, S. L., Bahr, K. D., Rodgers, K. S., May, S. L., McGowan, A. E., Tsang, A., Bumgarner, J., Hoon Han, J. (2018) Evidence of acclimatization or adaptation in Hawaiian corals to higher ocean temperatures. PeerJ, 6(5347)

Cooper, T.F., De'ath, G., Fabricius, K. E., Lough, J. M. (2008) Declining coral calcification in massive *Porites* in two nearshore regions of the northern Great Barrier Reef. Global Change Biology, 14(3), 529-538

Dimond, J., Carrington, E. (2007) Temporal variation in the symbiosis and growth of the temperate scleractinian coral *Astrangia poculata*. Mar. Ecol. Prog. Ser., 348, 166-172

Dlugokenchy, E. J., Hall, B. D., Montzaka, S. A., Dutton, G., Muhle, J., Elkins, J. W. (2018) Atmospheric composition [in "State of the Climate in 2017]. Bull. Amer. Meteor. Soc., 99 (8), S45-S49

Donner, S. D. (2009) Coping with Commitment: Projected Thermal Stress on Coral Reefs under Different Future Scenarios. PLoS ONE, 4(6), e5712

Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, J. P., Chan, F., English, C. A., … Talley, L. D. (2012) Climate Change Impacts on Marine Ecosystems. Annu. Rev. Mar. Sci. 4, 11-37

Ellis, J., Solander, D. (1786) *Astrangia poculata.* The Natural History of many curious and uncommon Zoophytes, collected from various parts of the Globe. Systematically arranged and described by the late Daniel Solander. 4.(Benjamin White & Son: London): 1-206, pls 1-63

Hoegh-Guldberg, O. (1999) Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research, 50, 839-866

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., … Hatziolos, M. E. (2007) Coral Reefs Under Rapid Climate Change and Ocean Acidification. Science, 318(5857), 1737-1742

Holcomb, M., McCorkle, D. C., Cohen, A. L. (2010) Long term effects of nutrient and CO2 enrichment on the temperate

coral *Astrangia poculata* (Ellis and Solander 1786). Journal of Experimental Marine Biology and Ecology, 386(2010), 27-33

Hughes, A. D., Grottoli, A. G., Pease, T. K., Matsui, Y. (2010) Acquisition and assimilation of carbon in non-bleached and bleached corals. Mar. Ecol. Prog. Ser., 420, 91-101

Intergovernmental Panel on Climate Change (2007) Fourth Assessment Report: Climate Change 2007: The AR4 Synthesis Report. Geneva: IPCC

Jacques, T. G., Marshall, N., Pilson, M. E. Q. (1983) Experimental ecology of the temperate scleractinian coral *Astrangia danae*. Mar Biol, 76(2), 135-148

Kenkel, C. D., Goodbody-Gringley, G., Caillaud, D., Davies, S. W., Bartels, E., Matz, M. V. (2013) Evidence for a host role in thermotolerance divergence between populations of the mustard hill coral (*Porites astreoides*) from different reef environments. Molecular Ecology, 22, 4335-4348

Kersting, D. K., Cebrian, E., Casado, C., Teixidó, N., Garrabou, J., Linare, C. (2015) Experimental evidence of the synergistic effects of warming and invasive algae on a temperate reef-builder coral. Sci. Rep., 5, 18635

Kornder, N. A., Riegl, B. M., Figueiredo, J. (2018) Threshold and drivers of coral calcification responses to climate change. Global Change Biology, 24, 5084-5095

LaJeunesse, T. C., Parkinson, J. E., Reimer, J. D. (2012). A genetics-based description of *Symbiodinium minutum* sp. nov. and *S. psygmophilum* sp. nov. (Dinophyceae), two dinoflagellates symbiotic with cnidaria. J. Phycol., 48, 1380-1391

LaJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R., Santos, S. R. (2018) Systematic revision of symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Current Biology, 28(16), 2570-2580

Leal, M. C., Ferrier-Pagès, C., Calado, R., Brandes, J. A., Frischer, M. E., Nejstgaard, J. C. (2014) Trophic ecology of the facultative symbiotic coral *Oculina arbuscula*. Mar Ecol Prog Ser, 504, 171-179

Lesser, M. P. (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs, 16, 187-192

Levitus, S., Antonov, J. I., Boyer, T. P., Baranova, O. K., Garcia, H. E., Locarnini, R. A., … Zweng, M. M. (2012) World ocean heat content and thermosteric sea level change (0-2000 m), 1955-2010. Geophysical Research Letters, 39, L10603

Lough, J. M., Barnes, D. J. (2000) Environmental controls on the growth of the massive coral Porites. J. Exp. Mar. Biol. Ecol., 245(2), 225-243

Movilla, J., Calvo, E., Coma, R., Serrano, E., López‐Sanz, A., Pelejero, C. (2016) Annual response of two Mediterranean azooxanthellate temperate corals to low‐pH and high‐temperature conditions. Mar. Biol. 163, 135

Peters, E. C., Cairns, S. D., Pilson, M. E. Q., Wells, J. W., Jaap, W. C., Lang, J. C., Vasleski, C. E., St. Pierre Gollahon, L. (1988) Nomenclature and Biology of *Astrangia poculata*. Proc. Biol. Soc. Wash., 101(2), 234-250

Rodolfo‐Metalpa, R., Bianchi, C. N., Peirano, A., Morri, C. (2005) Tissue necrosis and mortality of the temperate coral *Cladocora Caespitosa*. Italian Journal of Zoology, 72:4, 271-276

RStudio Team (2016) RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/

Sett, S., Back, L. T., Schulz, K. G., Koch-Klavsen, S., Lebrato, M., Riebesell, U. (2014) Temperature modulates coccolithophorid sensitivity of growth, photosynthesis, and calcification to increasing seawater pCO2. PLOS one, 9(2), e88308

Szmant-Froelich, A., Pilson, M. E. Q. (1980) The effects of feeding frequency and symbiosis with zooxanthellae on the biochemical composition of *Astrangia danae* Milne Edwards & Haime 1849. J. exp. Mar. Biol. Ecol., 48, 85-97

Towle, E. K., Enochs, I. C., Langdon, C. (2015) Threatened Caribbean Coral Is Able to Mitigate the Adverse Effects of Ocean Acidification on Calcification by Increasing Feeding Rate. PLoS ONE 10(4): e0123394.

Van Hooidonk, R., Maynard, J. A., Planes, S. (2013) Temporary refugia for coral reefs in a warming world. *Nature Climate Change,* 3, 508-511

Winters G, Holzman R, Blekhamn A, Beer S, Loya Y. (2009) Photographic assessment of coral chlorophyll contents: implications for ecophysiological studies and coral monitoring. *Journal of Experimental Marine Biology and Ecology* 380: 25-35