Symbiotic status mediates quiescence, photosynthetic efficiency, and growth of the facultative coral, *Astrangia poculata*, under thermal stress

Brown CM, Pelose GE, and Wuitchik DM

Boston University Marine Program, Boston, MA 02215

Abstract

Thermal stressors associated with climate change affect organisms on multiple levels of biological organization, from cellular stress to behavioural thermoregulation. Climate change is often a colloquial equivalent to global warming, but regionalized cooling patterns also may emerge as global circulation patterns shift, subjecting organisms to hot and cold thermal extremes. *Astrangia poculata* is a temperate stony coral that lives subtidally on the East Coast of the United States and experiences large temperature ranges throughout the year (-1C to 25C). Unlike commonly studied tropical reef building corals, *A. poculata* exists in a facultatively symbiotic state with the algae Symbiodiniaceae. In symbiotic colonies, feeding occurs primarily via autotrophy and in aposymbiotic colonies, feeding occurs primarily via heterotrophy. We conducted a common garden thermal stress experiment comparing how different symbiotic states of *A. poculata* (N = 20) respond to cold and hot thermal stressors. We observed a parabolic response in polyp extension behaviours which was further influenced by symbiotic state. Symbiont densities were also influenced by temperature extremes and symbiotic status. Temperature impacted growth rates, but had surprisingly no effect on photosynthetic efficiency. These results highlight the importance of symbiotic status in conferring thermal resistance to coral performance under hot and cold stress associated with a continually changing climate.

Introduction

Anthropogenic warming is causing average global temperatures to increase at a rate of 0.2°C (±0.1°C) per decade; in 2017, warming was 1°C (±0.2°C) above pre-industrial levels (IPCC, 2014). While this temperature increase may seem small, such changes are predicted to have widespread ecological consequences (Hoegh-Guldberg & Bruno, 2010). Coral reefs are living habitats particularly sensitive to thermal changes. In response to these thermal anomalies, worldwide destruction of these reefs has occurred in the Anthropocene (Hughes *et al.,* 2018). The cumulative effects of physical, chemical and biological stresses correlated with warming, are estimated to cause the decline of 40% to 60% of reefs worldwide (Lesser, 2004) which demands a more thorough investigation into the continued existence of our world's reefs.

As the foundational species for one of the most diverse ecosystems on the planet, corals are of utmost importance. Despite only covering 0.2% of the global oceans, coral reefs are home to 25% of all marine species (Chen *et al*., 2015). Reefs are nursery habitats for commercially important species, making them critical to fishery replenishment. Their nearshore presence allows them to easily supply food to over 100 million people globally (Lesser, 2014). Coral reefs are typically found in tropical oligotrophic waters, so the high productivity and resulting biodiversity in these regions is unexpected. Corals thrive in these conditions due to the

symbiotic relationship between the coral host and microscopic algae in the family Symbiodiniaceae, known hereafter as "symbiont". Through photosynthesis, the symbiont produces carbon sugars for the coral host and this relationship is pivotal to the success of the entire reef ecosystem. Thermal stressors associated with climate change lead to the breakdown of this symbiosis in a phenomena known as coral bleaching (Hoegh-Guldberg *et al*., 2008). Understanding if this symbiosis can survive a changing climate of more extreme thermal stressors is paramount to conservation efforts.

Coral bleaching research has traditionally focused on tropical coral species because of their ecological and economic importance; however, it is predicted that the strongest ocean warming will occur in the subtropical regions of the Northern Hemisphere (IPCC, 2014) and little is known about how coral from these regions may respond to contemporary climate change. Climate change has become synonymous with warming, but regionalized cooling patterns may emerge, such as the documentation of more intense cold weather events due to anthropogenic climate change shifting the natural cycles of the North Atlantic Oscillation (Cattiaux *et al*. 2010). Furthermore, tropical corals have an obligate relationship with their symbionts and cannot survive long term in a bleached state. This vital relationship makes it difficult to separate the effects of stress from a changing environment from the stress of the breakdown of the obligate symbiotic relationship.

Astrangia poculata (previously known as *Astrangia danae*) is a temperate stony coral that can exist in a facultative symbiotic state; in symbiotic colonies, feeding occurs primarily via autotrophy, and in aposymbiotic colonies, feeding occurs primarily via heterotrophy (Sharp *et al*. 2017). In addition, *A. poculata* experiences large temperature ranges throughout the year (-1°C to 25°C) (Dimond & Carrington, 2007). This considerable temperature range indicates that metabolic rates in *A. poculata* vary with the seasons and reducing metabolic activity during cold weather may be energetically favorable (Sharp *et al*. 2017). Periods of reduced activity when polyps and their tentacles are not readily extended are defined as quiescence (Burmester *et al*. 2018). This flexibility in symbiotic state and ability to withstand such large temperature variation makes this coral an ideal model system for understanding the relationship between coral hosts and algal symbionts and how this relationship responds to thermal extremes.

The present study aims to untangle how symbiotic status affects coral performance under hot and cold stress. To investigate this question, we present a comprehensive study exploring the responses of symbiotic and aposymbiotic *A. poculata* to hot and cold thermal extremes. Holobiont response to thermal stress was quantified through maintenance of symbiotic state, calcification rate and photosynthetic efficiency. Taken together, this experiment will shed light on the critical aspects of the algal-host symbiosis and how this relationship is expected to be maintained under contemporary climate change.

Methods

Experimental setup

Colonies of *A. poculata* (N=20) were collected from Woods Hole, MA (Figure 1) in November 2017 and were transported to Boston University's BUMP laboratory. Based on color, colonies were classified as

aposymbiotic (white) or symbiotic (brown). Colonies were fragmented by genotype into roughly equally sized "nubbins" and were randomly split into one of three experimental treatments: heated, cooled and control, creating a common garden experiment (Moloney *et al*., 2009). The cooled treatment started at 18°C and was cooled by 1°C each day to a final temperature of 4°C. In contrast, the heated treatment was warmed by 1C daily to a final temperature peak of 30°C. The heated treatment was maintained at 30°C for the final two days of experimentation as our heater could not reach the previously planned higher temperature of 32C. Control treatments were maintained at 18°C across 15 days of experimentation (Figure 1).

A 12:12 light-dark cycle was maintained and colonies were systematically moved throughout experimentation to control for the confounding effects of uneven light exposure and/or water flow. Water quality was monitored daily for temperature and salinity to ensure consistent conditions outside of temperature manipulations. The salinity of all systems was maintained at 34 (∓1) ppt. Corals were fed daily, with each replicate treatment receiving .25 grams of freeze-dried plankton dissolved in 400 mL of ambient seawater.

Performance Metrics:

All metrics were taken at multiple intervals throughout the experiment to determine overall holobiont performances (Figure 1). RNA was extracted from all colonies at the end of the experiment for further analysis at a later date. Measurements of Pulse Amplitude Modulation (PAM) were collected using a junior-PAM chlorophyll fluorometer to quantify the photosynthetic efficiency of photosystem II (PS II). Measurements were conducted every third day starting on the second day of the experiment. Corals were acclimated to darkness for at least an hour prior to PAM measurements. Measurements were conducted in light devoid environments using red headlamps to ensure that we recorded the dormant efficiency of PS II. Due to the physical properties of light penetration in water, red light dissipates first so deploying red headlamps does not affect photosynthetic rates. Each nubbin was measured in triplicate and replicate values within .100 of each other were deemed acceptable.

Overall coral growth was estimated using the buoyant weighing technique (Castillo *et al*. 2014). Corals were weighed in triplicate and data was collected on the first, seventh and fourteenth days of experimentation. Growth between sampling dates was then determined with the following formula:

$$(weight_{\_} - weight_{\_})/weight_{\_}$$

When corals are placed under stress, they can become less active, this period of inactivity is referred to as quiescence (Burmester *et al*. 2018). In quiescence, polyps are less likely to extend their tentacles. Quiescence and polyp activity levels were measured daily by scoring polyp behaviour 30 minutes after feeding (Table 1, following Wuitchik et al., in prep).

Table 1. Common practice of scoring behaviour based on polyp extension as done by Burmester *et al*. 2018 following protocol established in a pilot study by Wuitchik *et al*., in prep.

Score	Percent of polyps extended
1	0%
2	25%
3	50%
4	75%
5	100%

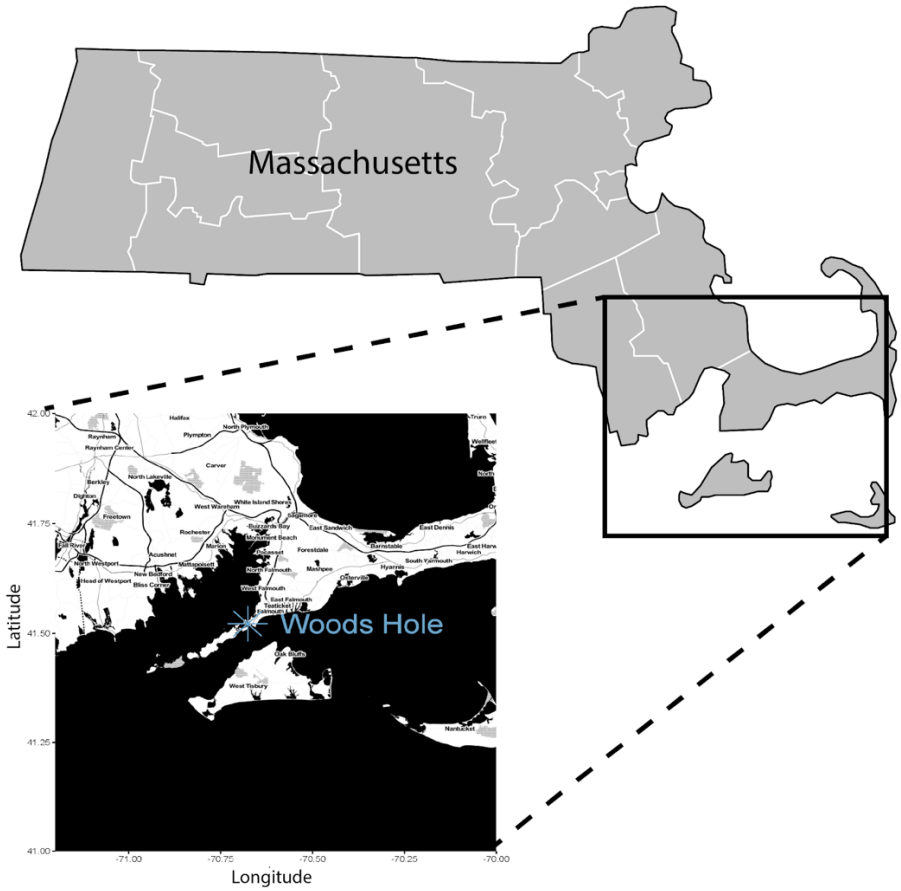


Figure 1. Map of collection site with inset map of Massachusetts. Blue star indicates collection site of *A. poculata* colonies from Woods Hole, MA that were sampled in November 2017.

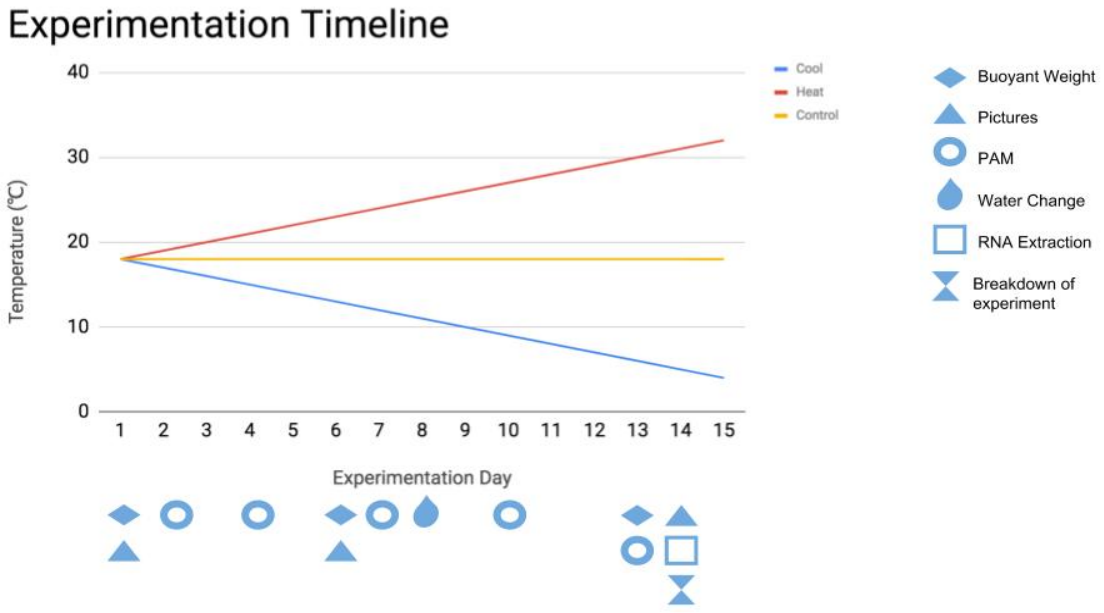


Figure 2. Experimental design and sampling timeline of *A. poculata* across the 15 day experimentation period. Each thermal treatment is denoted with a colored solid line. Various symbols indicate timeline of sampling techniques for determining performance metrics of *Astrangia poculata* which include buoyant weighting, symbiont density, Pulse Amplitude Modulations (PAM) and RNA extraction.

Each nubbin was photographed on the first, seventh and last day of the experiment to estimate bleaching. Images included a Coral Watch Coral Health reference card as a standard for exposure (Davies *et al.,* 2018). White balance was standardized across all images using Photoshop. Ten polyps representative of the designated symbiotic state of the colony were randomly selected and measured at the center of the polyp to standardize across images. Averages of the ten polyps red channel values were used as a proxy for symbiont presence using a custom matlab script (Winters *et al*. 2009) to determine changes in pigmentation, which is a proxy for symbiont density.

Statistics

All statistical analyses were implemented in R (RStudio Team, 2016). Analysis of variance tests (ANOVA) were used to test how buoyant weight, polyp behaviour and symbiont density were impacted by the interaction between symbiotic state and temperatures. The following represents the models used for each analysis:

$$Y \sim Temperature + Symbiotic\ State + Temperature*Symbiotic\ State$$

As it does not make sense to test photosynthetic efficiencies on aposymbiotic coral, only symbiotic coral were tested and an ANOVA with the following model was run:

$$Y \sim Temperature$$

A post hoc Tukey's HSD test was used to determine the specific comparisons that were different. Equal variance and normality were assessed by plotting residuals against the mean and q-q plots. Behaviour was not normally distributed, various transformations were applied and had no impact towards transforming to a normal distribution. Since ANOVAs are robust against deviations from normality no transformations were ultimately applied.

Results

Pulse Amplitude Fluorometry Modulation:

Only symbiotic coral were observed for photosynthetic efficiencies as it was assumed that aposymbiotic coral had no meaningful sugars provided through symbiosis. For symbiotic coral there was no influence (Figure 3, one-way ANOVA, df = 6 , F = 0.444, p = 0.847) of temperature on the photosynthetic efficiencies of photosystem II as measured by PAM.

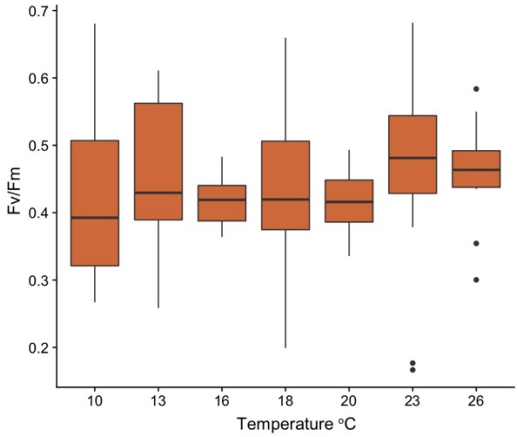


Figure 3. Symbiotic *A. poculata* variable fluorescence (F) / maximum fluorescence (F) which is a proxy for photosynthetic efficiencies plotted across temperature

treatments. There was no relationship between temperature and photosynthetic efficiency (one-way ANOVA, df = 6, F = 0.444, p = 0.847).

Buoyant Weight Analysis:
Over the course of the experiment, six corals detached from their plates and were subsequently reglued; therefore, their weights were disregarded in this analysis. Percent change in calcification rate, a proxy for growth, did vary when compared across different time points. Differences in growth were not consistent across all time points. The change in calcification rate from the midpoint to the initial point was significantly lower than the change from the final point to the initial point (Figure S1, one-way ANOVA, df = 2, F = 3.62, Tukey HSD adjusted p-value =.0413). There was no significant difference between symbiotic status and growth (Figure 4, one-way ANOVA, df = 1, F = 0.994, p = 0.322). There was no interaction between symbiotic state and treatment (Figure 4, one-way ANOVA, df = 2, F = 1.07, p = 0.204). Treatment affected growth rate, with the growth rate being significantly higher in the heat treatment (Figure 5, one-way ANOVA, df = 2, F = 2.59, Tukey HSD adjusted p-value = .0361)

Feeding and Behaviour:
There was a parabolic trend of polyp extension in relation to temperature (Figure 6, one-way ANOVA, df = 26, F = 123, p = .2 e-16). At 5°C, polyp extension was at its lowest for both brown and white corals. The proportion of polyps extended increased along with temperature, with nearly 100 percent of polyps extended at 13°C. Corals maintained high levels of polyp extension until 25°C, when they became less active as temperatures increased to 30°C. Aposymbiotic corals were more active at higher temperatures (Figure 6, one-way ANOVA, df = 1, F = 11.5, p = .000727). At approximately 25°C, the percent of polyp extension of brown corals began to decline at a faster rate than white corals. From 13°C to 25°C, the brown corals had a slightly higher percentage of polyp extension than the white corals.

Overall, aposymbiotic coral had significantly lower (Figure 6, one-way ANOVA, df = 1, F = 524 p = 2.00e-16) symbiont densities than symbiotic coral. Furthermore, temperature had a significant effect (Figure 6, one-way ANOVA, df = 6, F = 5.13, p = 5.27e-05) on overall symbiont densities where aposymbiotic coral appear to have greater variation. At 32°C the symbiont densities increased in the aposymbiotic coral relative to other temperatures. There were no interactions between temperature and symbiotic state (Figure 7, one-way ANOVA, df = 6, F = 2.01, p = .0653).

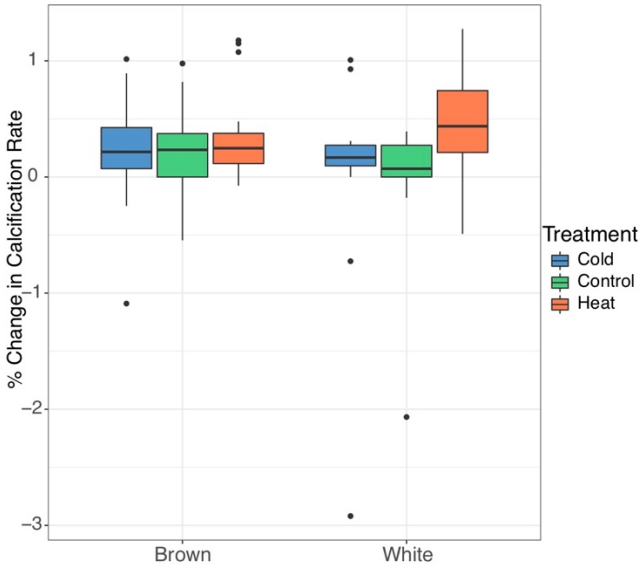


Figure 4. Effect of symbiotic state on calcification rates across thermal treatments. *A. poculata* colonies were designated as white or brown. Calcification rates were calculated using the equation: *(weight$_{initial}$-weight$_{final}$)/weight$_{initial}$)*. There was no interaction between symbiotic state and treatment (one-way ANOVA, df = 2, F = 1.07, p = 0.204).

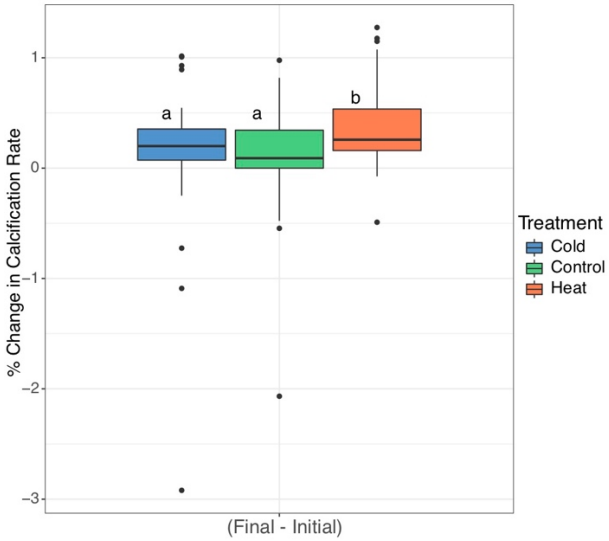


Figure 5. Change in calcification rates of *A. poculata* across thermal treatments. Calcification rates were calculated using the equation: *(weight$_{fin}$-weight$_{in}$)/weight$_{in}$*). Nubbins in the heat treatment had a higher rate of calcification than nubbins maintained at 18°C in the control treatment (one-way ANOVA, df = 2, F = 3.86, p = 0.0223). Letters show significant pairwise comparisons with a Tukey HSD test.

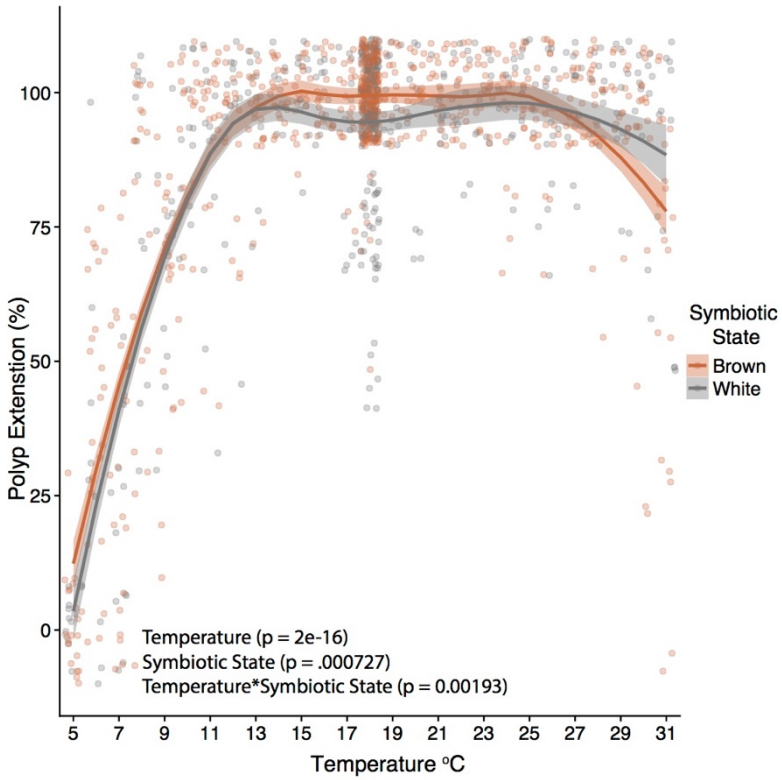


Figure 6. Polyp extension behaviours of brown (symbiotic) and white (aposymbiotic) states of *A. poculata* across temperature. There was an effect of temperature (one-way ANOVA, df = 26, F = 2.01, p = .00193), symbiotic state (one-way ANOVA, df = 1, F = 11.5, p = .000727) and an interaction between state and temperature (one-way ANOVA, df = 26, F = 123, p = .200 e-16).

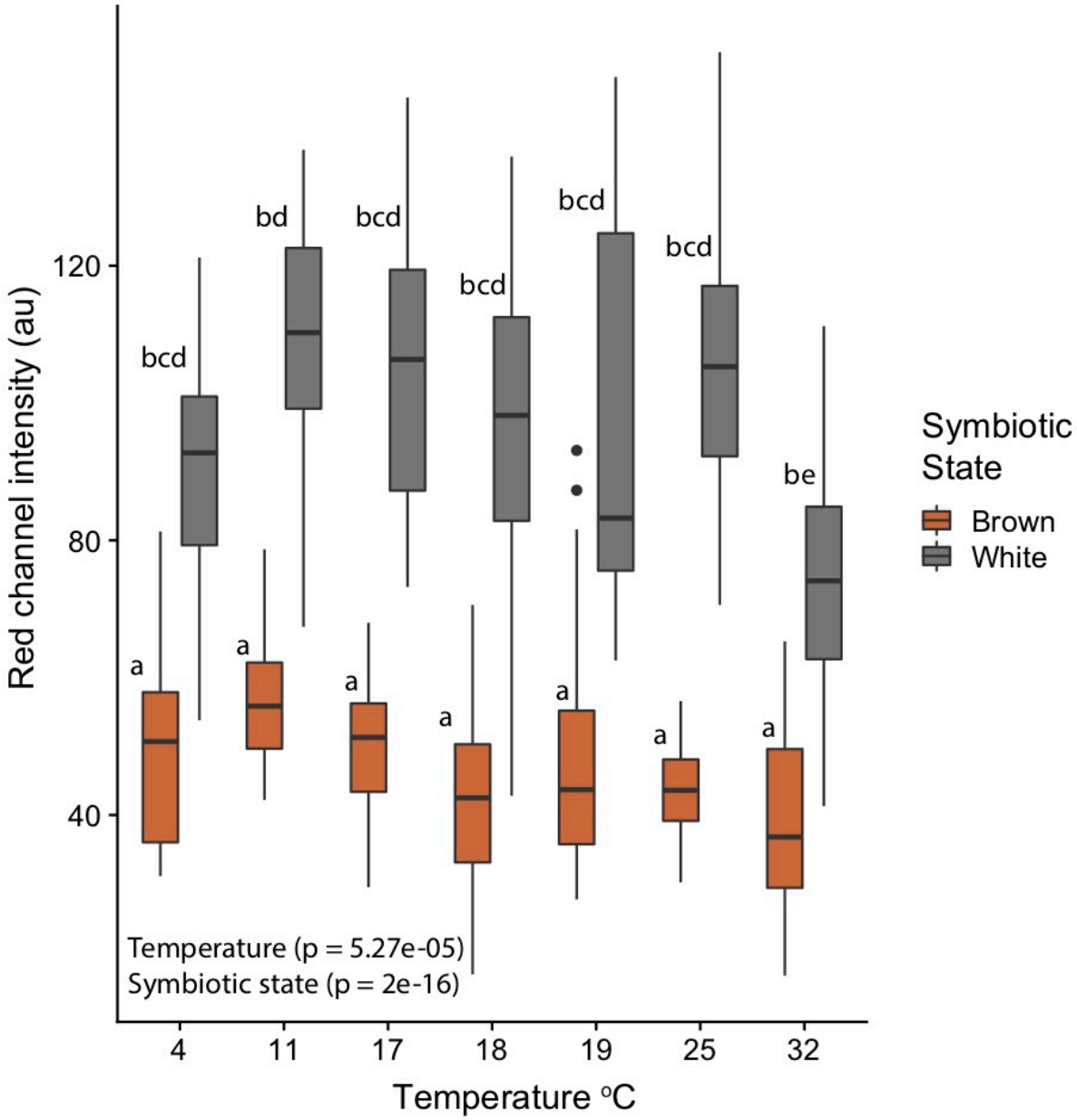


Figure 7. Intensity of red channel values from photograph analysis in arbitrary units, increased values denote lower symbiont density across temperatures and symbiotic states. Temperature (one-way ANOVA, df = 6, F = 5.13, p = 5.27e-05) and symbiotic state (one-way ANOVA, df = 1, F= 524 p = 2.00e-16) had significant influences on symbiont density as calculated by a one-way ANOVA. Letters show significant pairwise comparisons with a Tukey HSD test.

Discussion

The present study aimed to untangle how symbiotic status affects coral performance under hot and cold stress. This is of particular interest in light of a changing climate, and the results presented here help us to better understand the responses of temperate corals. Interestingly, there was no relationship between temperature and photosynthetic efficiency in symbiotic colonies of *A. poculata*. This is contrary to research using tropical corals (Warner, Fitt, & Schmidt, 1999) (Ainsworth *et al*. 2016) where increased temperatures have negatively impacted the functioning of symbionts. *A. poculata* are known to host different species of symbiont than tropical obligate corals (Thornhill *et al*. 2008), it is therefore possible that the symbionts in *A. poculata* have higher and lower thermal tolerances. It would be prudent for future research to explore mechanisms of symbiont performance, and establish what the costs/benefits are for the establishment of symbiosis in this system.

Though symbiotic state was classified by color, PAM measurements of aposymbiotic colonies showed a background level of photosynthesis (results not presented here). The dividing line between symbiotic states in *A. poculata* is defined by symbiotic corals having a symbiont density greater than 10^6 cells/cm^2 (Burmester *et al*. 2017) therefore, aposymbiotic corals may have low concentrations of symbionts present. It is possible that these measurements come from endolithic algae or photosynthetic bacteria, which was observed on the exposed coral skeleton. Samples were collected from wild populations which can introduce native algae to tank systems. Just like symbionts, some bacteria photosynthesize (Sato-Takabe *et al*. 2012), perhaps leading to observed background levels of photosynthetic efficiency. With this in mind, there is a difference in algal metabolism at different temperatures leading to potentially different photosynthesis confounding across treatments.

Temperature impacted the overall growth in our experiment; however, it is important to clarify that it is the *percent* difference in calcification rates, not the calcification rates themselves, that are used as proxies for growth. Percent differences in calcification rates were not constant across all time points in the experimentation period. Buoyant weights were sampled at three times throughout the experiment providing an initial, middle and final comparison point. Rates between the middle and initial time points were significantly lower than between final and initial time points. The middle buoyant weight measurements were taken closer to the beginning of the experiment, where experimental temperatures were closer to the control temperature of 18°C. These specific *A. poculata* colonies have been reared in holding tanks at 18°C since November 2017, so they are likely to be acclimated to temperatures within a close range of 18°C.

Growth was significantly increased in heated treatments than control treatments. Calcification rates in reef-building corals generally increase with higher temperatures; since these corals have a large latitudinal natural range (Thornhill *et al*. 2008), their maximum calcification rates coincide with a thermal maximum of 25°C - 28°C (Howe & Marshall, 2002). This trend of increasing calcification rates at elevated temperatures is present in *Plesiastrea versipora*, another temperate non-reef building coral similar to *A. poculata* (Howe & Marshall, 2002). A 1983 study of calcification rates of *A. poculata* in light and dark environments found that calcification increased as temperature increased for both symbiotic

states (Jacques et al. 1983). Calcification was diminished for symbiotic colonies below 15°C due to lower photosynthetic rates with lower temperatures (Jacques et al. 1983). Calcification does not simply depend on coral metabolism, but also relies on concentrations of necessary minerals. Including calcium, alkalinity and magnesium parameters in water quality analyses could more carefully be controlled in order to help to avoid confounds in the results. Endolithic algae may also have contributed mass to buoyant weight measurements across all treatments. The short snapshot of this experiment was not enough to capture biologically significant growth or calcification. If this experiment was extended it would give the coral more time to grow and allow for further analysis on if temperature affects calcification rates.

Polyp activity is necessary for heterotrophy in *A. poculata* and this activity was influenced by thermal stress. It has been demonstrated that heterotrophy mitigates calcification in response to temperature stress in *Oculina arbuscula* which is a facultative coral (Aichelman et al., 2016). Our results show an interesting parabolic trend in polyp activity at thermal extremes. We suspect that polyp retraction is a defense response as individuals appeared to enter quiescent-like states (Burmester *et al*. 2018). This convergent physiological response to opposite thermal extreme is interesting, and future research should explore whether the underlying mechanisms behind these responses are the same. A longer experimentation period could have examined the full parabolic response of corals to thermal stress on upper and lower extremes.

Climate change is expected to increase SST seasonally, creating warmer summers, therefore it is important to track coral recovery in the Anthropocene. For corals to persist, they not only must survive thermal extremes, but be capable of recovering at elevated temperatures. A longer experimental period which returns corals to the control range of 18 °C could track coral mortality and survivorship post thermal stress.

The initial methodology was to increase the heat treatment by 1°C/day to a maximum of 32°C. Due to mechanical limitations, the heater was only capable of reaching a maximum of 30°C, which was maintained the final two days of treatment. With this constraint, we examined a narrower portion of the parabolic response of *A. poculata* to increased temperatures. Further studies could expand on this temperature curve and track the full parabolic response to identify an optimum temperature.

Symbiont density are shown to be influenced by both temperature and symbiotic state. Unsurprisingly aposymbiotic coral have fewer symbionts than their symbiotic counterparts. What is surprising however, is that aposymbiotic coral demonstrated increasing symbiont density at very warm temperatures. This suggests that symbiotic state might be regulated by temperature for certain coral genotypes. This corroborates the findings of Dimond *et al*. (2007) who found that symbiotic state fluctuates with seasonal changes in temperature. Further studies that extend temperature treatments may better ascertain how symbiotic states may be regulated through temperature differences.

Conclusions

Thermal stressors associated with climate change affect organisms on multiple levels of biological organization, from cellular stress to behavioural thermoregulation. *A. poculata* exists in different facultatively symbiotic states and this experiment demonstrates that symbiotic state influences how the organism responds to temperature differences. This sets

the stage for further work to explore the trade-offs of symbiosis and highlights the importance of symbiotic status in conferring thermal resistance to coral performance under hot and cold stress associated with a continually changing climate.

Acknowledgements

We would like to thank Dr. Sarah Davies, Nicola Kriefall and Brooke Benson for their intellectual contributions as well as for laboratory resources. A special thank you to Justin Scace for building our treatments from the floor drain up and the enjoyable conversations. Thank you to fellow classmates who helped collect data and maintained water quality. No thanks to Hannah the *Oculina* goddess, Greg's gorgeous lady friend, and Dan's beautiful wife.

Literature Cited:

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 arbusculato temperature stress. Ecology and Evolution, 6(18), 6758-6769. doi:10.1002/ece3.2399

Ainsworth, T. D., Heron, S. F., Ortiz, J. C., Mumby, P. J., Grech, A., Ogawa, D., . . . Leggat, W. (2016). Climate change disables coral bleaching protection on the Great Barrier Reef. Science, 352(6283), 338-342. doi:10.1126/science.aac7125

Burmester, E., Finnerty, J., Kaufman, L., & Rotjan, R. (2017). Temperature and symbiosis affect lesion recovery in experimentally wounded, facultatively symbiotic temperate corals. *Marine Ecology Progress Series*, 570, 87-99. doi:10.3354/meps12114

Burmester, E. M., Breef-Pilz, A., Lawrence, N. F., Kaufman, L., Finnerty, J. R., & Rotjan, R. D. (2018). The impact of autotrophic versus heterotrophic nutritional pathways on colony health and wound recovery in corals. *Ecology and Evolutio*n. doi:10.1002/ece3.4531

Castillo, K. D., Ries, J. B., Bruno, J. F., & Westfield, I. T. (2014). The reef-building coral *Siderastrea siderea* exhibits parabolic responses to ocean acidification and warming. *Proceedings of the Royal Society*, 281(20141856). Retrieved November 18, 2018.

Cattiaux, J., Vautard, R., Cassou, C., Yiou, P., Masson-Delmotte, V., & Codron, F. (2010). Winter 2010 in Europe: A cold extreme in a warming climate. Geophysical Research Letters, 37(20). doi:10.1029/2010gl044613

Chen, P., Chen, C., Chu, L., & Mccarl, B. (2015). Evaluating the economic damage of climate change on global coral reefs. *Global Environmental Change*, 30, 12–20. http://doi.org/10.1016/j.gloenvcha.2014.10.011

Davies, S. W., Ries, J. B., Marchetti, A., & Castillo, K. D. (2018). Symbiodinium Functional Diversity in the Coral Siderastrea siderea Is Influenced by Thermal Stress and Reef Environment, but Not Ocean Acidification. *Frontiers in Marine Science*, 5. doi:10.3389/fmars.2018.00150

Dimond J, & Carrington E. (2007a). Temporal variation in the symbiosis and growth of the temperate scleractinian coral *Astrangia poculata*. *Marine Ecology Progress Series*. 348, 161-172. http://doi.org/10.3354/meps07050

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., … Hatziolos, M. E. (2008). Change and Ocean Acidification. *Science*,

318(December 2007), 1737–1742. http://doi.org/10.1126/science.1152509

Hoegh-Guldberg, O., & Bruno, J. (2010). The impact of climate change on the world's marine ecosystems. *Science*, 328, 1523–1529. http://doi.org/10.1367/1539-4409

Howe, S. A., & Marshall, A. T. (2002). Temperature effects on calcification rate and skeletal deposition in the temperate coral, *Plesiastrea versipora* (Lamarck). *Journal of Experimental Marine Biology and Ecology*, 275(1), 63-81. doi:10.1016/s0022-0981(02)00213-7

Hughes, T. P., Anderson, K. D., Connolly, S. R., Heron, S. F., Kerry, J. T., Lough, J. M., …Wilson, S. K. (2018). Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science*, 359(6371), 80–83. http://doi.org/10.1126/science.aan8048

IPCC, Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (2014). *Climate Change 2014 : Synthesis Report*. IPCC, Geneva, Switzerland: Cambridge University Press.

Lesser, MP (2004). Experimental biology of coral reef ecosystems. *Journal of Experimental Marine Biology and Ecology*, 300, 217-252. http://doi.org/10.1016/j.jembe.2003.12.027

Moloney KA, Holzapfel C, Tielbörger K, Jeltsch F, Schurr FM. (2009). Rethinking the common garden in invasion research. *Perspectives in Plant Ecology, Evolution and Systematics*,11(4), 311-320. doi:10.1016/j.ppees.2009.05.002

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

Sato-Takabe, Y., Hamasaki, K., & Suzuki, K. (2012). Photosynthetic characteristics of marine aerobic anoxygenic phototrophic bacteria *Roseobacter* and *Erythrobacter* strains. *Archives of Microbiology*, 194(5), 331-341. doi:10.1007/s00203-011-0772-z

Sharp, K. H., Pratte, Z. A., Kerwin, A. H., Rotjan, R. D., & Stewart, F. J. (2017). Season, but not symbiont state, drives microbiome structure in the temperate coral Astrangia poculata. *Microbiome*, 5(1), 120. http://doi.org/10.1186/s40168-017-0329-8

Thornhill, D. J., Kemp, D. W., Bruns, B. U., Fitt, W. K., & Schmidt, G. W. (2008). Correspondence between cold tolerance and temperate biogeography in a western Atlantic Symbiodinium (Dinophyta) lineage. *Journal of Phycology*, *44*(5), 1126–1135. http://doi.org/10.1111/j.1529-8817.2008.00567

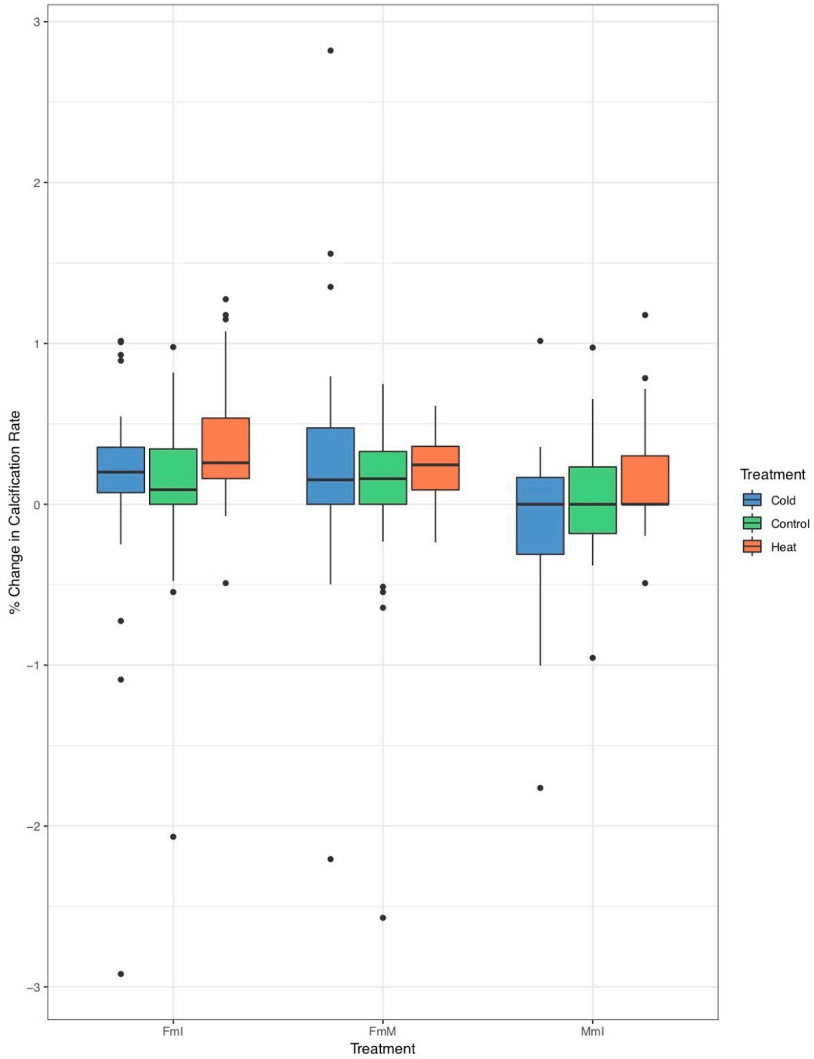
Warner, M. E., Fitt, W. K., & Schmidt, G. W. (1999). Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. *Proceedings of the*

National Academy of Sciences, *96*(14), 8007–8012. http://doi.org/10.1073/pnas.96.14.8007

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.

Wuitchik D, Almanzar A, Benson B, Brennan S, Chavez D, Liesegang M, Reavis J, Schniedewind M, Trumble I, Davies SW. (2018, in prep) Genomic basis of convergent phenotypic responses to thermal extremes in a temperate coral.

Supplementary Figures



S1. Differences in percent calcification rate across thermal treatments and 15 day experimentation period. Buoyant weights were sampled at three time points providing initial, mid and final weights. Calcification rates were calculated using the equation: *(weight$_{...}$ - weight$_{...}$)/weight$_{...}$*). Rates were

calculated for Final - Initial (FmI); Final - Mid (FmM); and Mid - Initial (MmI). (one-way ANOVA, $df = 2$, $F = 3.62$, $p = 0.0281$)