Assessing the effects of cold thermal stress on *Astrangia poculata* quiescence: analyzing photosynthetic efficiency, calcification rate, polyp behavior and *Symbiodinium*

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Abstract:

Astrangia poculata is a model organism due to its tolerance to both cold and warm water and its facultative relationship with its *Symbiodinium*. Understanding how this temperate coral tolerates such a wide range of temperatures, with and without symbionts, can help us understand what will happen in the future as the climate becomes more variable and the world experiences both higher high temperatures and colder cold temperatures. We conducted a common garden laboratory experiment in order to test the impacts of cold thermal stress on *A. poculata*. Data from photosynthetic efficiency, calcification rate, polyp behavior, and *Symbiodinium* were used to analyze the decreasing health of the coral as it approached and ultimately entered quiescence. We found no significant difference in calcification rates between the controls at 22°C and the experimentals at decreasing temperatures. We also found a decreasing trend in photosynthetic efficiency in experimental corals and a decrease in polyp behavior as a result of cold stress. Our results show that *A. poculata* is very resilient, quickly recovers from thermal stress and enters dormancy at about 7°C.

Introduction:

From spikes in global temperatures to adapted migration patterns of organisms, the Earth is changing rapidly due to global climate change. This is particularly problematic for coral reefs, comprising sensitive, slow growing organism, with 30% of corals already damaged and close to 60% predicted to be lost by 2030 (Wilkinson, 2008). However, corals have shown great intraspecific variability in tolerance to climate change stressors (Hughes et. al., 2003). For example, *Astrangia poculata* is a particularly resilient temperate coral native to the Western Atlantic Ocean and the Caribbean and, due to its latitudinal range, it naturally withstands a wide range of water temperatures (Dimond & Carrington, 2007).

Most coral species exhibit obligate symbiosis, meaning that they will eventually die if they experience an extended amount of time without their *Symbiodinium*, a single celled algae living in the coral (Muscatine & Porter, 1977). The symbiotic state of coral is defined by the density of *Symbiodinium* in its tissues (Sharp et. al., 2017). Little is known about the mechanisms of symbiosis, however, research has shown that under stressful conditions the symbiosis between corals and their *Symbiodinium* is lost in a process called bleaching (Gates et. al., 1992). Unlike other corals, *A. poculata*, has a much more complicated relationship with its *Symbiodinium*, as it is facultatively symbiotic

(Dimond & Carrington, 2008). This form of symbiosis means that each colony can be symbiotic (with *Symbiodinium)*, aposymbiotic (without *Symbiodinium*), or a mixture of both. The zooxanthellae found in *A. poculata* *Symbiodinium* are relatively cold tolerant, however they lose photosynthetic efficiency and the host enters a state of quiescence, or inactivity, at temperatures below 10°C (Dimond et. al., 2012). In cold temperatures, when *A. poculata* becomes dormant, the polyps retract and become unresponsive, feed less, and allocate less energy to growth and repair (Sharp et. al. 2017). In the spring this process reverses as *A. poculata* reemerges from dormancy (Sharp et. al., 2017).

Since *A. poculata* has such a unique life history, both with its cold temperature tolerance and its facultative symbiosis, it can potentially serve as a good model organism to better understand overall coral resilience and symbiosis. In this common garden experiment, the effects of reduced temperatures on New England *A. poculata* health was investigated over a period of 15 days to assess the effects of continually cooling waters on *A. poculata.* The effects of cold thermal stress on the coral nubbins as they enter quiescence was analyzed, with emphasis on their photosynthetic efficiency, calcification rate, polyp behavior and *Symbiodinium*. The objective of this study was to determine what happened when *A. poculata* enters quiessence and at what temperature. Because *A. poculata* operates with a lower metabolism in the colder seasons when compared to the warmer seasons (Gori et. al., 2016), we hypothesized that *A. poculata* would enter quiescence at around 10°C, the average winter temperature of coastal New England waters. We predicted that as *A. poculata* becomes more cold-stressed, reductions in *Symbiodinium* would be observed. Coral health was quantified using four parameters: calcification rate via weight gain, chlorophyll performance, polyp behavior, and symbiotic state .

Results from this study will further our understanding of how corals are affected by decreased temperature ranges, and may help elucidate the effects of changing temperatures on coral stress and resilience.

Methods:

Experimental Set-up: In October 2017, nine *Astrangia poculata* coral colonies were collected from Woods Hole Bay, MA (Fig. 1), shipped to Boston University and maintained at 16°C for three weeks. On November 17, 2017, all colonies were fragmented and and each nubbin was assigned a unique ID based on genotype and glued to a 60mm labeled petri dish using Ethyl Cyanoacrylate Gen Aquarium Frag Glue.

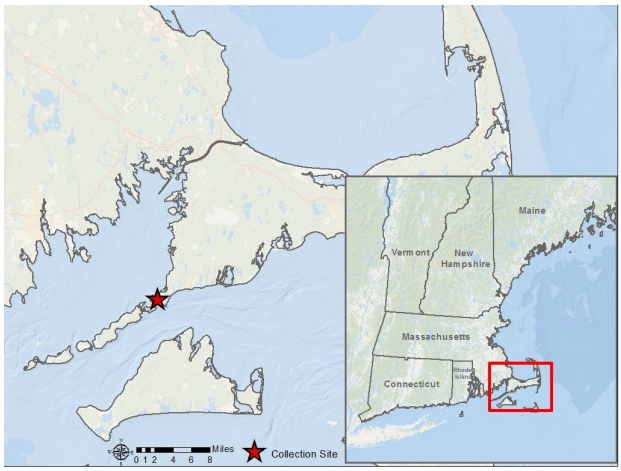

Figure 1: Location of the collection of the *A. poculata* used in the experiment. Samples were collected from Woods Hole Bay on November 5, 2017.

Corals were then acclimated in an aquarium tank at 16 °C and fed daily for 24 days. Six experimental tanks were set up with inlet hose, powerhead, drain, light, chiller, and filter. Light in all tanks was quantified using an MQ-500 Full-Spectrum Quantum Flux Meter and ranged between 6-12 µmol m^{-2} s^{-1}. All tanks were held on a 12-hour light-dark cycle. Three tanks were assigned controls and temperatures were held at 22°C, as this was what the acclimation tanks were incorrectly measured at. Three other tanks were assigned as experimental treatment tanks. Treatment tanks were started at 22°C for the first two days and then temperatures were decreased by 2°C each day until the tanks reached 10°C. The temperature was then lowered 1°C each day until they reached 6°C, a common winter temperature for the water in the area, where they were held for the remaining four days of the experiment (Fig. 2a).

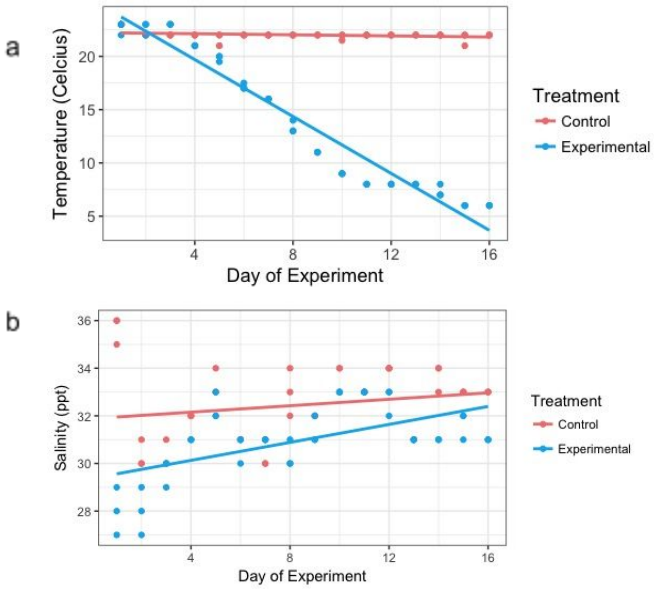

Figure 2: Water quality conditions in (a) water temperature in degree C and (b) salinity in ppt of experimental and control tanks throughout 16 day experiment.

Each tank had seven randomly selected nubbins of *A. poculata* from nine different genotypes, where each genotype was represented at least once in control and once in treatment tanks. Colonies with more or less than 6 nubbins were randomly placed using a number generator. Petri dishes were rotated through predetermined locations (Fig. 3) every two days to account for uneven light and water flow within a tank. Corals were fed cubes of juvenile brine shrimp three times a week. Two frozen cubes of baby brine shrimp were dissolved in 150ml of water and each coral was fed via pipette 3ml of liquid to ensure each nubbin got an equal amount. Petri dishes and tank walls were cleaned with a toothbrush twice weekly to remove algal growth and a 10% water changes was conducted midway through the experiment. Twice-daily temperature and once daily salinity measurements were taken throughout the experiment to ensure proper water quality (Fig. 2).

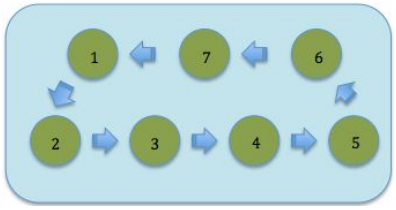

Figure 3: Placement and rotation pattern of corals samples On petri dishes every two days.

Coral Calcification Rate: Buoyant weight of corals were taken at the beginning, middle, and end of the experiment to quantify the calcification rate throughout the experiment. Temperature, salinity, water levels, and scale position were kept constant for all measurements. Three replicates per coral were taken at each time point and these values were averaged to calculate the weight of the coral.

Calcification rate was then calculated as $((weight_f - weight_i)/weight_i)$.

Photosynthetic Efficiency: Pulse Amplitude Modulation (PAM) measurements were taken four times throughout the experiment to measure photosynthetic efficiency of photosystem II using a Junior-PAM chlorophyll fluorometer. PAM measurements were taken at the start of the experiment to establish a baseline. Further PAM measurements were taken at day 6, day 11, and day 14 after two hours of dark-adaptation. Three independent PAM measurements were taken for each nubbin at each time-point and averaged.

Polyp Behavior and Symbiotic State: Polyp behavior was recorded observationally every morning before any stimulation to determine the phenotypic response to cold stress on a 1-5 scale as follows:

1- 0% of polyps out
2 - 25% of polyps out
3 - 50% of polyps out
4 - 75% of polyps out
5 - 100% of polyps out

Symbiotic state of the polyps was also recorded based on visual observations at the beginning, middle, and end of the experiment to infer the influence cold stress has on relationships with *Symbiodinium*.

Statistical Analyses: Statistical analyses were completed in R (R Core Team, 2017). Linear models were run to determine significance of calcification rates and photosynthetic efficiency results. Polyp behavior and symbiont status over time were plotted in R to qualitatively assess the effect of cold stress on *A. poculata*.

Results:

Effects of decreased temperature on calcification rate in A. poculata:

There was no significant difference in calcification rate by treatment between the *A. poculata* in control or treatment conditions (p=0.88, Fig. 4). However, graphical trends show that the control nubbins show more variation in calcification rates than the experimental nubbins. The experimental nubbins had a higher average calcification rate than the control nubbins (Fig. 4).

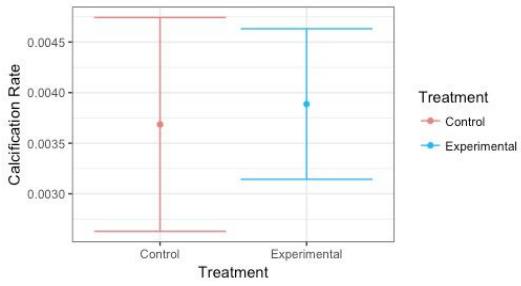

Figure 4: Calcification rates of *A. poculata* in control conditions and in low temperature conditions. Calcification rate is not significantly affected by treatment.

There is not a clear, significant relationship between genotype and weight gain (p=0.24). There was no significant difference in the standard error in genotype between the control or experimental nubbins by genotype (p=0.65, Fig. 5).

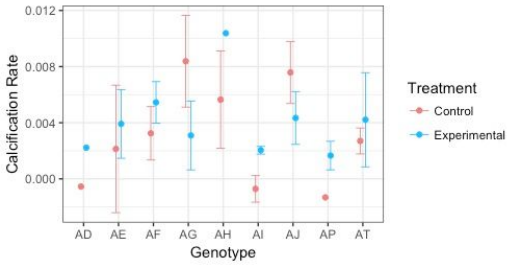

Figure 5: Calcification rates of *A. poculata* by genotype in control conditions (red) and cold stress conditions (blue).

Effects of decreased temperature on photosynthetic efficiency in A. poculata:

Temperature did not have a significant effect on photosynthetic efficiency of *A. poculata* (p=0.08, Fig. 6), but there was a strong trend showing that control corals had higher photosynthetic efficiencies than treatment corals. The control corals, contrary to what was predicted, decreased in photosynthetic efficiency initially. After day 6, photosynthetic efficiency began to increase again until they leveled off. The experimental corals increased in photosynthetic efficiency from day 1 to day 6 and then decreased to day 11 and levelling off. The experimental tanks were at 22 °C on day 1, 13 °C on day 6, 7 °C on day 11, and 6 °C on day 15. Control tanks were always at 22°C

Figure 6: Average photosynthetic efficiency in *A. poculata* on days 1, 6, 11, and 14 of both control and experimental conditions.

Genotype does not appear to influence photosynthetic efficiency differently in treatment or control conditions.

In the experimental tanks, there appears to be more variation among genotypes with no genotype appearing to be inherently more or less adapted to the cold water, however this is not statistically significant (p=0.99, Fig. 7). There is no significance among individual genotypes across either control or experimental tanks, although they do mimic the trends seen in Figure 6.

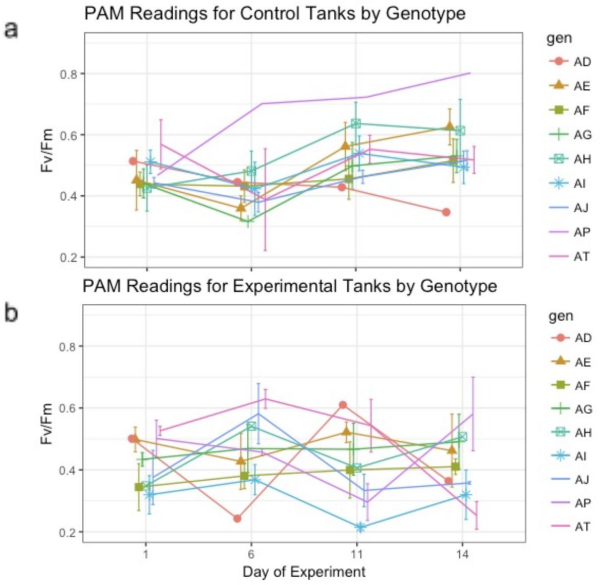

Figure 7: Photosynthetic efficiency in *A. poculata* on days 1, 6, 11, and 14 by genotype a) in control tanks, which all show the same trends except for genotypes AP and AD, and b) in experimental tanks, which show much more variation and generally lower Fv/Fm values.

Polyp behavior and symbiont status as a result of lowered temperature:

The observed polyp behavior trend indicates a difference by treatment. Control corals experienced a decrease in percent of active polyps in the first few days but showed recovery in the latter half of the experiment.

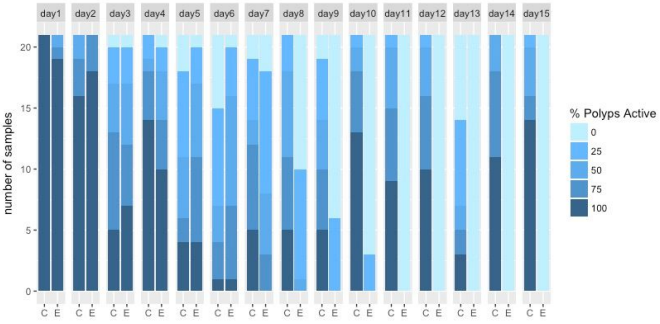

Figure 8: Polyp Behavior in *A. poculata* on each day of the experiment. Polyp Behavior was visually assessed by determining what percent of polyps were extended.

The experimental corals displayed a slow decrease in activity which sharply dropped off at day 8. Polyp activity was predicted to decrease to zero at 10°C, but *A. poculata* retained some active polyps until day 11 when they reached 7 °C and became dormant (Fig. 8).

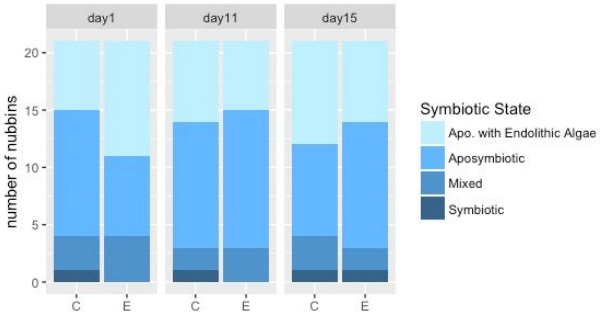

Figure 9: Symbiont status of nubbins throughout the 15 day experiment. There are very few symbiotic nubbins throughout. Complete bleaching is not seen in experimental nubbins

There was no trend in symbiont status or endolithic algae content in either control or experimental corals. Some nubbins gained symbionts in the experimental conditions while other nubbins in the same conditions lost symbionts. Endolithic algae increased slightly in control corals but remained largely unaffected in experimental corals (Fig. 9). Significant bleaching in the cold water corals was expected but not observed.

Discussion and Conclusion:

Thermotolerance and recovery seen in control A. poculata:

The trend seen in the photosynthetic efficiency calculations and polyp behavior show a pattern of stress and recovery in the control corals. The control tank, at 22°C, was much warmer than water in the acclimation tank which was at about 16°C. The decrease in photosynthetic efficiency of control corals, as well as the decrease in polyp activity, can likely be attributed to initial heat stress. After the second photosynthetic efficiency measurements, control nubbins seem to have acclimated to higher temperatures and recovered for the remainder of the experiment. This trend is mirrored in polyp behavior. During the first days of the experiment, decrease in corals with 100% active polyps was observed until day six (Fig 8). After day six, corals appeared to recover and polyp activity increase in control nubbins overall.

Thermotolerance seen in treatment A. poculata:

In the first three days of the experiment, control corals and cold treated corals had the same polyp behavior decrease (Fig. 8). From day three to day seven, the experimental corals increased their polyp activity, quickly exceeding control polyp activity. This is likely due to all corals, including the control nubbins, being heat stressed at the start of the experiment. Polyp activity in the treatment tanks increased initially as the temperature dropped back into *A. poculata's* prefered range. By day eight, the treatment corals passed their tolerance threshold for cold temperature and the polyp activity decreased sharply as the coral nubbins began to enter quiescence, finally becoming fully dormant on day 11 at 7°C. A previous study conducted a similar experiment on cold stressing three temperate corals (*Montastraea faveolata*, *Porites astreoides*, and *Siderastrea siderea*) (Kemp et. al., 2011). When exposed to decreasing temperatures from 20°C to 16°C to 12°C, all temperate coral species showed

similar reactions in polyp behavior and lower photosynthetic output (Kemp et. al., 2011).

Short term thermal cold stress has no significant effect on calcification rate:

Over the 15 day experiment, there was no statistically significant change in calcification rate between genotypes within a treatment, or between treatment and control. *Astrangia poculata* is a slow growing coral so we did not expect to see a change (Jacques et. al., 1980). A study conducted in 2011 aimed to measure the calcification rate of *A. poculata* at two seperate temperatures, 24°C and 16°C. The net weight of corals in both groups increased, but it was not the temperature that had an effect, it was the symbiont status; aposymbiotic corals calcified faster in cold water (Holcomb et. al., 2011). Most of the the colonies in our experiment were aposymbiotic. Had the experiment been conducted for a longer period of time, we may have seen colder temperatures have a larger role in the calcification rates of the corals. However, Halcomb et. al only tested to 16°C, where we dropped the temperature much lower. Future research is needed to see if these trends would have held at the low temperatures used in our experiment.

Short term thermal cold stress creates trend of correlation to photosynthetic efficiency:

While there is a negative trend in photosynthetic efficiency with cold stress, it is not significant. However, in only 15 days with only 42 nubbins, there was 92% confidence in the trend. It is hypothesized that this decrease is a result of expulsion of symbionts or decrease in activity in symbionts present (Thornhill et. al, 2008). Since most corals in our study were aposymbiotic, future studies should be conducted to determine what is causing aposymbiotic corals to lose photosynthetic efficiency; it may be beneficial to consider the endolithic algae present on many of the nubbins used.

Symbiont status is not affected by storm term temperature treatment:

There was no significant change in symbiont status for corals at control temperature or corals in the cold water. Literature has suggested that hosts containing certain thermo tolerant *Symbiodinium* strains are more tolerant than their aposymbiotic counterparts (Thornhill et al., 2008). This suggests that, had the experiment contained a more even distribution of symbiotic to aposymbiotic colonies, there would have been an observed difference in photosynthetic efficiency, and perhaps fitness between treatments. Since we did not see bleaching in cold stressed *A. poculata,* it would be beneficial to investigate what effect different *Symbiodinium* species abundance have on low temperature survival in future studies.

In conclusion, there were several promising trends in accordance with our hypotheses. *A. poculata* became dormant at 7°C rather than the 10°C we had expected. Photosynthetic efficiency does appear to decrease with temperature even though we did not see significant bleaching in the cold treatment tanks. Future genomic testing may shed light on the roots of these trends. Further research is needed with longer experimental time and more symbiotic nubbins to determine the

effects of symbionts or endolithic algae on *A. poculata* resilience to cold stress.

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