Investigation of heat and light tolerance in *Astrangia poculata*

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Abstract

Industrialization and human disturbance have led to increasing amounts of atmospheric carbon dioxide (CO_2), causing a rise in sea surface temperatures, and introducing stressors to coastal ecosystems. Coral reef systems are a significant source of biodiversity and economic growth on a global scale, making them an important topic of study in the context of changing climatic conditions. There have been multiple studies addressing the decrease in resiliency of corals associated with increased temperatures, however, few studies have addressed the effects of changing light availability. Here, we address how varying light availability and increasing temperature conditions affect both symbiotic and aposymbiotic individuals of the facultative reef-building coral *Astrangia poculata* in a 14-day experiment with six total treatments of differing light and temperature regimes. Through the investigation of photosynthetic activity, feeding behavior, and color variations we found that light was the most significant factor in establishing coral and symbiont health, however heat was marginally more significant in determining color intensity. All samples responded the worst, photosynthetically and behaviorally, in treatments with no light availability, and the best in treatments with normal light conditions (40 mv). Additionally, we found that aposymbiotic samples were better equipped for no light conditions, whereas symbiotic individuals were better adjusted to high light conditions. This research shows how varying light availability has a significant impact on photosynthetic efficiency and health of corals regardless of facultative symbiont state, however symbiotic state does allow for increased resilience to certain conditions. This analysis is suggesting that light availability may be a more significant factor than previously considered in terms of coral health and resilience.

Introduction

The rise in atmospheric carbon dioxide (CO_2) has presented a number of environmental concerns including but not limited to a rise in ocean and atmospheric temperatures, subsequently followed by reduced resiliency in a number of marine ecosystems and organisms (Hoegh-Guldberg & Bruno, 2010). Responses to these human-induced changes in climate include decreased productivity, changing species distribution, altering food web dynamics, greater susceptibility to disease, and weakened physiological function (Hoegh-Guldberg & Bruno, 2010). The introduction of heavily dense human populations to coastal regions can be attributed with rises in pollution on both local and global levels, putting organisms–especially those who are sedentary and cannot move–at risk for disease and mortality.

One sedentary organism that is particularly sensitive to increased ocean temperatures are reef-building corals (Carpenter *et al.* 2008). These corals have a symbiotic relationship with dinoflagellate algae of the family *Symbiodiniaceae* where, in exchange for a productive habitat in the coral, the algae return organic carbon and oxygen to the corals through photosynthesis, aiding in calcification and growth of the corals (Roth, 2014). As atmospheric CO_2 levels increase causing temperatures to rise, corals experience a stress response causing reduced growth and reproduction rates, increased vulnerability to disease, and mortality (Brown *et al.* 2002). This leads to the loss of the symbiotic relationship between algae and coral in a process called coral bleaching. When the algae leave the coral tissue, the coral no longer receives key nutrients from the algae, causing it to turn white and experience increased chances of mortality (Brown *et al.* 2002). Corals act as significant members in marine ecosystems by providing habitat for a number of organisms. Additionally, corals act as a crucial resource for coastal ecosystems by providing food, acting as a barrier system, and bringing in tourism. According to Hughes *et al*. (2003), 60% of coral reefs are expected to be lost by 2030, and a large portion are already in decline. This makes studying coral resilience and thermal tolerance imperative for the advancement of coral reef restoration and management.

Studying tropical corals that maintain the role of obligate symbiotic corals (meaning they require zooxanthellae for survival) proves difficult due to the highly probable chances of mortality after a coral has lost its symbiont. Facultative symbiont corals, however, are able to survive via heterotrophy, meaning they do not require an algal symbiont for survival. This relationship provides a good model for understanding interactions between multiple stressors, and how these interactions affect the symbiotic algae-coral relationship.

One example of a facultative symbiotic coral is *Astrangia poculata*. *A. poculata* is a reef-building coral species ranging from Canada to Florida, temperatures varying greatly throughout. Areas around the northern habitable limit for this species have highly variable temperatures ranging from -2ºC to 26°C (Wuitchick *et al*. 2021). This illustrates the unique relationship that *A. poculata* has with sea surface temperature. In fact, rising temperatures have been seen to increase chlorophyll densities in *A. poculata*, while lowering them had a negative effect on photosynthetic efficiency (Dimond & Carrington 2007).

A. poculata is very resilient in changing temperatures because of its large habitable temperature range. However, their

symbionts, *Breviolum psygmophilum,* are not as resilient to increasing temperatures, leading to decreasing photosynthetic efficiency which can lead to bleaching of the symbiotic coral (Chan *et al*. 2021). This difference in resiliency to heat stress causes harm to the corals as they become more vulnerable to disease caused by bacteria and viruses (Chan *et al*. 2021). *A. poculata* is also affected by light availability due to its wide range of depth. A dissertation conducted in the Rotjan Lab (Speroff, 2022) observed the effects of changing environmental factors on coral stress levels, including light availability. The study observed that light treatment didn't impact photosynthetic activity, but that symbiotic corals were more likely to be stressed by a decrease in light than the aposymbiotic, since these didn't rely on light for harnessing energy. Additionally, Brown *et al.* (2002) has found that differences in solar radiation exposure has an effect on resiliency in *Goniastrea aspera* studied in Phuket, Thailand; corals exposed to higher levels of radiation exhibited a smaller change in algal density and chlorophyll *a* content. This shows that changes in light intensity may affect the photosynthetic abilities of symbionts in corals, or allow for acclimation to increased temperatures. Although solar radiation differs from the type of light our corals will be under, the effects of it may be similar.

For these reasons, we expect to see the greatest decline in photosynthetic efficiency, color intensity, and healthy feeding behavior in the systems with the highest temperature and no light, especially for symbionts in this system, across the 13 day experimental period. Brown *et al.* (2002) shows that higher radiance may be associated with greater resilience to ocean warming, so if there is no radiance paired with ocean warming, we will expect corals to respond negatively. Aposymbiotic corals that are maintained with no light will be more resilient when exposed to heat stress because they primarily get their energy from heterotrophy symbionts will be at greater risk due to the algal symbiont's dependence on light for photosynthesis, as explored by Speroff (2022). We also predict that over the course of 14 days, the coral nubbins exposed to the highest temperature and highest light levels will experience a great loss in photosynthetic efficiency and higher rates of bleaching. A rapid increase in temperature, paired with a tenfold increase in light intensity as compared to our control light systems, will introduce greater thermal variations than the corals and symbionts are able to handle, as supported by Chan *et al.* (2021). An alternative hypothesis to this notion is that the systems that experience increases in temperature may perform better photosynthetically, as supported by the ideas discussed in Dimond and Carrington (2007), suggesting that *A. poculata* might be better adapted for warmer conditions (Wuitchik *et al.* 2022).

Here, we tested the effects of increased temperatures and varying light irradiances on aposymbiotic and symbiotic *A. poculata* corals in six different treatments to determine how different light levels influence the coral stress response to increased temperature. Multiple studies have outlined the effect of thermal stress and variation on coral resilience and symbiont relationship (Kriefall *et al.* 2022; Drury 2019), however this study aims to address how photosynthetic efficiency, feeding behavior, and color intensity changes in response to multiple stressors - addressing the impacts of light availability. There is not much research determining the relationship between light availability and coral health in *A. poculata*; for this reason, we aim to discuss how light may have an impact on

photosynthetic efficiency, polyp feeding behavior, and color intensity in corals that occupy multiple types of environments. This study discusses the importance that light has on the health of *A. poculata*: a coral that occurs across a large latitude and therefore experiences numerous temperature and light regimes, as well as stressors due to the presence of urbanized coastlines.

Methods

Coral collection and acclimation

40 colonies of *Astrangia poculata* (N= 20.5 aposymbiotic, N= 19.5 symbiotic) were collected from Great Harbor, Woods Hole, MA (Figure 1) on October 13, 2022 from 20-25 feet in depth. Colonies were transported to Boston University Marine Invertebrate Research Facility and were maintained at 18˚C under 40mV light conditions for three days after which each colony was cut in half and glued onto a labeled dish (N= 79 fragments). Colonies were allowed to recover for two weeks after which one fragment from each colony was placed into one of six treatments, ensuring that each of the colony pairs were present in an 18˚C control and heat challenge conditions.

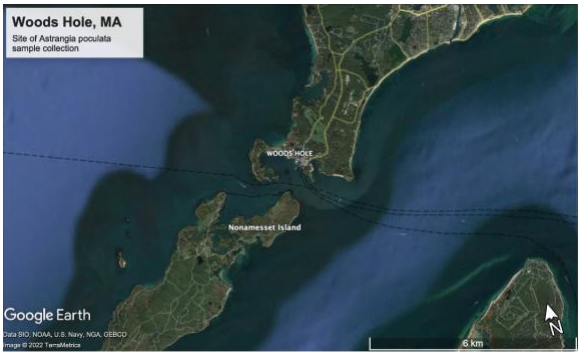

Figure 1. Map of collection site for *Astrangia poculata* samples used in this study (Google Earth Pro).

Experimental Design

There were a total of six treatment conditions, each of which had three tanks for a total of 18 experimental tanks. Three of the systems were set to be heat challenged: the temperature in each tank increasing by 1˚C every day for the duration of the experiment from 18°C to 31°C. The other three systems were temperature control tanks maintained at 18˚C. Then within each temperature regime, each system was maintained at one of three different light conditions:
1. high light experiencing 400 millivolts (mV) 2. control light experiencing 40 mV and 3. no light where tanks were completely blacked out with black paper. All tanks that had light experienced 12:12 hour light: dark conditions daily.

Water quality parameters maintained throughout the experiment include temperature and salinity, which were measured using a YSI probe. Measurements for both parameters were taken 2-3 times daily. Temperatures were checked once a day using a more accurate glass thermometer as well, as the YSI probe was not properly calibrated on a number of experimental days. Each morning, temperatures of the heat challenge tanks were raised by 1˚C. In order to maintain the salinity in a range of 32-34 ppt, each system operator (SUMP) was refilled with deionized water (DI) daily. If the range fell below 32 ppt, SUMPs were refilled with saltwater instead.

Once every three days, cleaning of the SUMP filters took place. The protein skimmers were cleaned by taking the collection cup out and rinsing all the bubbles out with a spray faucet before putting the collection cup back into the rubber O-ring. Then the two filter bags in each SUMP were taken out, turned inside out, and sprayed with the spray faucet. Then they too, were put back in their place in the SUMP.

Coral Feeding & Behavioral Assay
Corals were fed everyday around 4:00 p.m. and feeding behavior was reported every 4 days by the same two people every time to account for observer bias. Approximately 55 milliliters of saltwater from each system was mixed with about 0.15 cubic centimeters each of Reef Chili and ReefRoids, into a squeeze bottle for feeding. Once flow was off in each tank, corals were fed by ejecting the contents of the bottle equally among all the corals over each polyp. When in the water, the squeeze bottles were continually pressed upon to provide a constant stream of food to layer over each nubbin, while not allowing water to refill the bottle in order to keep the concentration of food in the solution stable. The feeding times of each system were recorded, and after 30 minutes, circulation was turned back on, except for on the days behavior was observed. On these days, the tank circulation was kept off, and one person rated the amount of tentacles extended from the polyps on a scale from 1-5 (0-100%), while the other person recorded the data. After the behavior for each coral was reported, the systems could be turned back on.

Coral Color Analysis
Photos of every coral were taken on days 1, 10, and 13 of the experimental period in a black photo box using an iPhone 13 camera. Photos were calibrated in Adobe Photoshop using the white eyedropper tool (Image-Adjustments-Levels) to ensure RGB channel values for each image were all at 255. All photos were saved as JPEG files and loaded individually into MatLab. An "AnalyzeIntensity" code provided by Winters *et al* (2009) selected 10 points (point dimension: 25 pixels x 25 pixels) on the coral for analysis of red, green, and blue channels. Corals were organized by symbiotic state and average values of the red channel of each coral were then explored for each treatment using a net change formula (Red_Day13 - Red_Day1 / Red_Day1). Data were visualized using ggplot2 (Wickham, H., 2016). ANOVA and TukeyHSD tests were performed to determine significant differences in results and strength in relationships between variables.

Photosynthetic Efficiency
Using a pulse-amplitude modulated fluorometer (PAM, JuniorPAM - Waltz), data of the photosynthetic efficiency of photosystem II (Fv/Fm) of all 79 experimental *A. poculata* nubbins were taken on Days 6, 10, and 13. PAMing began every morning at 9:30 a.m., before the tank lights turned on to ensure that corals were dark-acclimated. Three replicate Fv/Fm values were recorded for each coral, with each replicate being collected from a different polyp. Gloves were used to handle the nubbins and both the magnetic leaf-clip and coral were kept underwater for polyp measurement. After each system, the magnetic leaf-clip was washed with deionized water. Red headlamps were used for illuminating the experimental area as red light is quickly scattered in water because of its large wavelength, and corals are assumed to be unable to respond to red light.

Data Analysis
All data were cleaned in Google Sheets, and analyzed in RStudio version 2021.09.2+382.pro1. Measurements from PAM were first checked for a normal distribution and good residuals, and analyzed with analysis of variance (aov() in RStudio) and the Tukey's HSD test. PAM measurements were then used to display responses in aposymbiotic versus symbiotic samples. These tests provide information about which factors have statistical significance, from which a correlation model between light intensity (mv) and photosynthetic efficiency was performed

using ggscatter(). In order to display a box and whisker plot of maximum photosynthetic yield (Fv/Fm) for aposymbiotic and symbiotic corals, the averages of three PAM measurements per sample were taken using a code provided by Dr. Sarah Davies (Davies, 2022). Analysis of variance and Tukey's HSD was run for food behavior as well, and code provided by Dan Wuitchik was used to create polyp activity bar plots using R Studio.

For temperature (ºC) and salinity (ppt), we calculated averages in Google Sheets and displayed temperature in a time series plot using the package ggplot2 in R Studio (Figure 2), and salinity along with nutrient parameters (measured and provided by lab technician Kian Thompson from BUMP) in Table 1. For feed behavior, we used a code provided by Dan Wuitchick and the ggplot2 package in RStudio to create frequency bar graphs, showing the frequency at which each polyp activity score had occurred for each day measured. This was done for heat, light, and state variables.

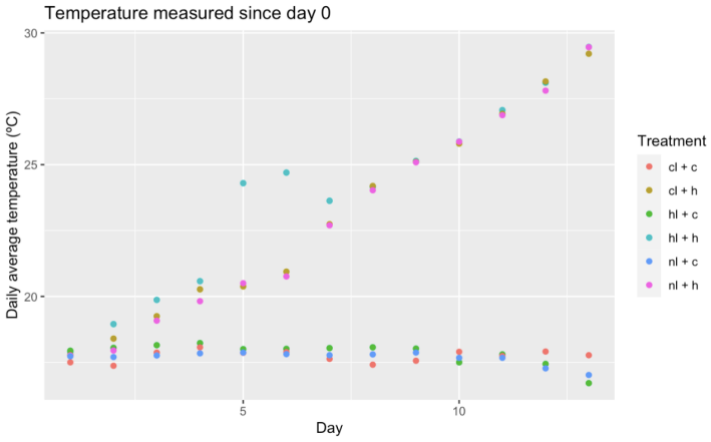

Figure 2. Plot of the daily average temperatures in ºC for each treatment (nl = no light; c = control temp; cl = control light; hl = high light; h = heat) throughout the experimental period. Overall, the temperatures were consistent and were linear both in increasing heat stress temperatures and the constant control temperatures. Error observed in system 1 on days 5-7 as the chiller stopped working and temperatures quickly increased and did not drop for a couple of days. The drop in temperature of system 2 on day 13 was due to the heater of the tank breaking down.

Table 1. Water quality parameters for each treatment are given below. Salinity was maintained between 32.0-34.0 ppt throughout the experimental period; controlled temperature was expected to be 18ºC, heated treatments were expected to end at 32ºC. Temperature and salinity were taken every day in the morning, afternoon, and evening. Averages were taken using Google Sheets; nutrient concentration (nitrate and phosphate) were given by BUMP lab technician Kian Thompson on 11/17.

Treatment	Mean salinity (ppt)	Phosphate (ppm)	Nitrate (ppm)	Mean temp. day 13 (ºC)
High light + high temp	33.35	0.00	0.20	29.47
High light + control temp	32.89	0.00	0.10	16.71
Control light + high temp	32.33	0.00	0.00	29.21
Control light + control temp	32.95	0.02	1.80	17.77
No light + high temp	32.99	0.10	1.00	29.46
No light + control temp	33.06	0.14	0.50	17.02

Results

Analysis of variance test results indicated no significant relationship between heat and photosynthetic efficiency ($p=0.90$), however a significant relationship exists between Fv/Fm and light, Fv/Fm and state, and state and light ($p < 0.05$). Alternatively, there was no significant relationship between aposymbiotic and symbiotic samples in no light treatments ($p = 0.93$ for heated treatments; $p=0.79$ for control

temperatures). Tukey's HSD test showed that the most significant relationships exist between samples of high light/no light and control light between the same symbiotic state (p=0.00), however there is no significant relationship between high light and no light systems. This understanding can be emphasized in Figure 3, which illustrates the negative relationship that generally exists between light intensity (mv) and photosynthetic efficiency (Fv/Fm). This figure reveals that the control light treatments, maintained at 50 mv, yielded the greatest photosynthetic efficiency overall.

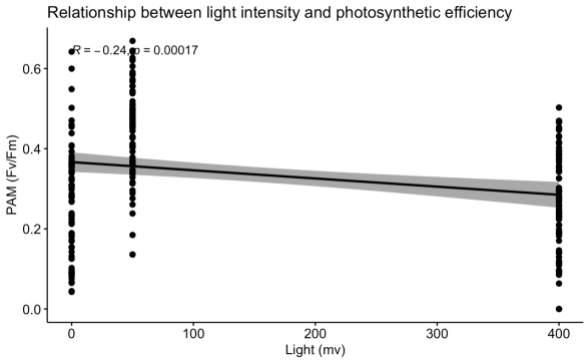

Figure 3. Linear regression model of photosynthetic efficiency used from PAM values for each sample, and light intensity used for each of the three different light treatments; ran using R Studio (R^2 = -0.24; p = 0.00017; 95% CI shown by shading). This shows a relatively weak negative relationship between light intensity (mV) and photosynthetic efficiency (Fv/Fm), meaning as light intensity increases, photosynthetic efficiency generally decreases. This interpretation could suggest an ideal light intensity for photosynthesis (between 0-100 mV, possibly around 40 mV).

After graphing the results of our PAM measurements, we observed that symbiotic individuals have the lowest photosynthetic efficiency in no light regimes ($M_{nl+h;sym}$ = 0.27 ± 0.01 (SE) Fv/Fm, $M_{nl+c;sym}$ = 0.239 ± 0.02 (SE) Fv/Fm) and highest in control light and heated systems ($M_{hl+h;sym}$ = 0.58 ± 0.005 (SE) Fv/Fm). Aposymbiotic individuals performed the worst photosynthetically in high light regimes ($M_{hl+h;apo}$ = 0.183 ± 0.03 (SE) Fv/Fm, $M_{hl+c;apo}$ = 0.255 ± 0.04 (SE) Fv/Fm), but the best in control light and heated systems as well ($M_{cl+h;apo}$ = 0.433 ± 0.04 (SE) Fv/Fm) (Figure 4). Tukey's HSD had confirmed a significant difference between aposymbiotic and symbiotic individuals (p<0.01), and further analysis of PAM data also suggests that aposymbiotic individuals had overall performed better in dark conditions as compared to symbiotic individuals, regardless of temperature regime (Figure 4). This does support our hypothesis that aposymbiotic samples would perform better in lower light conditions.

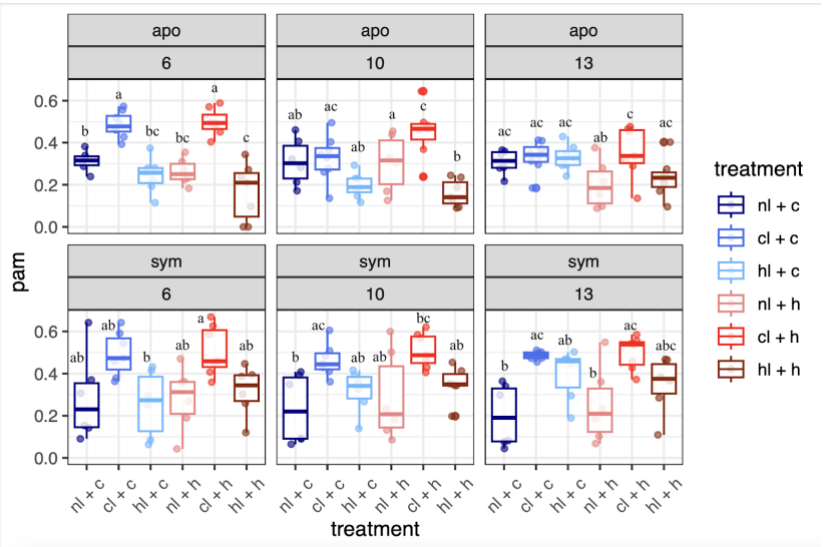

Figure 4. Box and whisker plots depicting the photosynthetic efficiency of aposymbiotic (apo) versus symbiotic (sym) coral samples in each of the six treatments for all 79 samples. Measurements were taken using a JuniorPAM on *A. poculata* and across different treatments (nl = no light; c = control temp; cl = control light; hl = high light; h = heat) for days 6, 10, and 13. Using Tukey's HSD test, there was a significant difference (p<0.01) in Fv/Fm between each treatment (indicated by different letters) for apo and sym. A box plot depicts the median in the middle of the box, the 25th and 75th percentiles on the bottom and top of the box, 95% confidence intervals, and outliers depicted as circles.

Polyp activity scale (Table 2) was used to monitor feeding behavior, and results indicate that light intensity was a major factor in the feeding behavior of *A. poculata*. There was a decrease in frequency of high scores as the experiment proceeded, especially in no light tanks (Figure 5A). Thermal variation, however, did not produce a major difference in polyp activity after feeding (Figure 5B). This figure also illustrates that aposymbiotic coral samples perform marginally better across the board in terms of feeding behavior, however this pattern is more pronounced in varying light intensities rather than temperature. Tukey's HSD of polyp activity data reveals a p-value of 0.00 between no light and control light treatments as well as high light and no light treatments. There was no statistical significance for neither the relationship between polyp behavior and heat nor state.

Polyp score	Percentage of tentacles observed
1	0%
2	25%
3	50%
4	75%
5	100%

Table 2. Polyp activity scale, which is applied by determining how many tentacles are outstretched on each polyp about 30 minutes after feeding, 3 times throughout the experiment. This was determined by the same person each time in order to eliminate bias.

Color intensity analysis shows minimal differences in color change between the high, control, and no light treatments for the apo and sym coral nubbins in both control and heated systems. ANOVA test results show that varying light and temperature treatments had statistically insignificant impacts on the changes in color intensity for both aposymbiotic and symbiotic corals ($p > 0.05$, Figure 6). Further statistical analysis showed insignificant relationships between

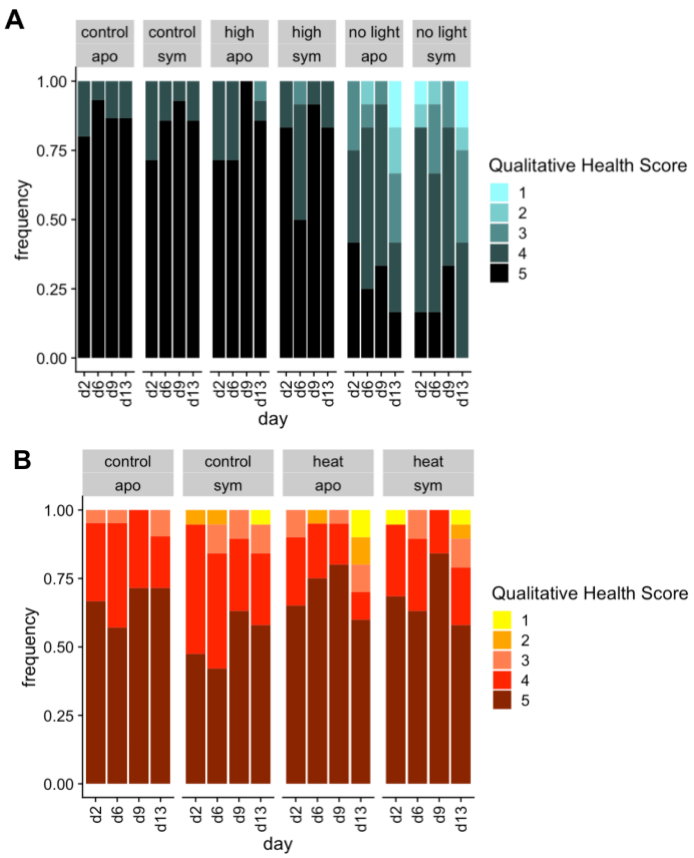

Figure 5. Polyp behavior scale graphed as frequency bins for different light regimes (panel A) and heat treatments (panel B) applied to 6 treatment groups for 79 samples of symbiotic or aposymbiotic *Astrangia poculata*, collected from Woods Hole, MA (graphed in R Studio). Panel A shows that the control light system had the most encouraging response in food behavior, and the corals in no light conditions worsened as the experiment proceeded. Additionally, this plot illustrates how aposymbiotic individuals tend to do slightly better than symbiotic corals in all light regimes. Panel B illustrates that there is not much of a difference in feed behavior between heated and controlled temperature systems as the experiment proceeds, however aposymbiotic appears to perform marginally better.

the majority of the variables, however temperature and light were shown to have the strongest relationship with a value marginal to the 95% confidence level ($p = 0.0857$). Although both values are far from the 0.05 confidence value, temperature is calculated to have a stronger significance

than light on change in color intensity (p = 0.1889, p = 0.4812, respectively).

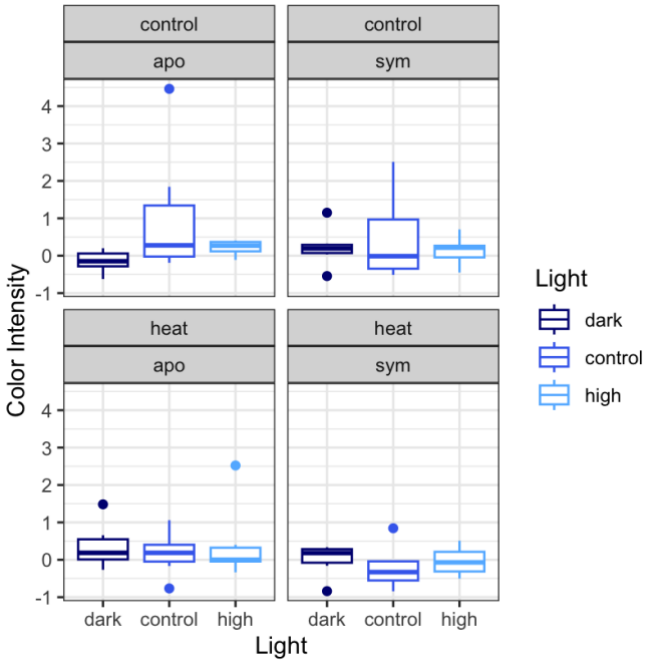

Figure 6. Box and whisker plots displaying the calculated change (Day13 - Day1 / Day1) in average red color channel values (color intensity) in control temperature (18˚C) and heat (up to 31˚C) conditions for aposymbiotic (apo) and symbiotic (sym) corals in high, control, and no (dark) light treatments. The line in each box plot represents the average color intensity value for each of the light treatments, the whiskers show minimum and maximum values, and the dots are outliers. No statistically significant p values were found (all p > 0.05), so overall results show no difference between treatments. A lower p value showed that temperature had a greater influence on change in color intensity than light.

Discussion

The purpose of this study was to determine the effect that multiple stressors (heat and light) had on the photosynthetic efficiency, feeding behavior, and color intensity of both aposymbiotic and symbiotic members of the facultative symbiont coral *Astrangia poculata*. We discovered that the conditions that lead to the strongest decline in *A. poculata* samples are conditions of high light and no light, which means that light is a significant variable when it comes to establishment of the symbiotic relationship between algae and coral host. There was no significant relationship between temperature variability and photosynthetic efficiency (p=0.90), which debunks the previously understood relationship established between thermal stress and coral resilience. This data suggests that heat may have an effect on color intensity, but light intensity is a more significant factor in determining photosynthetic yield and polyp activity.

This phenomenon has been seen in other studies with *A. poculata* where increasing temperatures did not negatively impact their health (Brown *et al.* 2018) and could even result in slower rates of bleaching (Harman 2020) and lower stress responses (Wuitchik *et al.* 2021), suggesting that *A. poculata* are better adapted to live in warmer waters. *A. poculata* may be able to display resilience by being able to physiologically regulate a high metabolic rate at high temperatures (Jacques *et al.* 1983), maintaining homeostasis. This also could explain the expansive temperature range that *A. poculata* are found, living in cold waters at their upper geographical limit and warm waters at their lower geographical limit. These individuals were taken from areas at their upper geographical limit, in Woods Hole, MA, so putting them in a higher temperature environment may be more normal for them, as they experience higher temperatures during the summer months compared to the average yearly temperature. Also it has been found that in their northern ranges, *A. poculata* experience large seasonal variation, so they may be adapted to a thermal challenge (Wuitchik *et al.* 2021). In the face of future warming due to climate change, *A. poculata* could be quite resilient to elevated temperatures (Harman 2020) and possibly even thrive under the new conditions. Our experimental time

period of fourteen days may have been too short to be able to see the harmful effects of increased temperatures. In the short term, biological activity may increase under higher temperatures, making it easier to reach activation energies, but a long-term chronic temperature study with *A. poculata* could yield different results.

We found that the *A. poculata* under high light levels (400 mV) had a significant reduction in photosynthetic efficiency compared to the control (50 mV) counterparts, as well as a reduction in polyp activity score (Figures 4 & 5, respectively). The same is true for no light systems as compared to normal light systems, but there was not a statistically significant difference between the high and no light corals for both photosynthetic efficiency and color intensity. These results differ from other work on temperate corals where it has been found that increasing light levels actually helps corals as they absorb and utilize more light (Iglesias-Prieto *et al.* 2005). These other corals did this through a process of multiple scattering via its coral skeleton, a mechanism that *A. poculata* may not have or may not do well. It seems that instead, *A. poculata* when under higher light levels, cannot redistribute the extra light in an effective manner and instead respond by expelling zooxanthellae due to unhealthy over-productivity. It has been found that different species of corals respond disparately to light stress (Kuanui *et al.* 2020). *A. poculata* may be one of the species that responds strongly to light, as it was affected by both no light and high light.

The corals in the blackout tank showed lower polyp activity compared to both the control and high light treatments. By day 13, system 5 consisted of the lowest average feeding score for both aposymbiotic (M_{apo} = 2.17) and symbiotic (M_{sym} = 2.83) samples, supporting our hypothesis that the systems with the highest temperature and no light would respond the worst. This decrease in observable feeding behavior could be due to reduced feeding rates of coral during nighttime conditions in the absence of light (Kuanui et al, 2016) as is often observed in many different types of corals. It is interesting that, in terms of polyp activity after feeding, aposymbiotic individuals in the high heat and no light conditions performed marginally worse than symbiotic individuals in the same system. We had expected apo symbionts to do better in no light conditions because their dependence on heterotrophy excludes the need for light; however, evidence suggests that the presence of symbiotic algae may provide greater energy storage (Burmester, 2017).

Following the analysis of PAM data, however, we found that coral samples without algal symbionts performed better in no light treatments regardless of temperature. This allows for the understanding of the relationship between light intensity and photosynthetic efficiency/resiliency in *Astrangia poculata*, suggesting that as light availability decreases and the presence of stressors magnifies, aposymbiotic individuals may be better equipped to handle these stressors, and remain resilient. Conversely, data analysis revealed that symbiotic samples had performed better in high light treatments than aposymbiotic samples for both high and control temperature regimes. This provides the implication that symbiotic individuals may be more resilient if solar radiation increases with time, supporting the ideas provided by Brown *et al.* (2002). Additionally, we discovered that both aposymbiotic and symbiotic corals had performed best in the control light systems, and marginally greater in the higher temperature treatment. This supports the

results produced by Wuitchik *et al.* (2021), which found that *A. poculata* in heated treatments produces lower stress responses compared to those in colder treatments through differentially expressed genes.

We found that elevating temperatures did not have a significant effect on the photosynthetic efficiency of *A. poculata* ($p=0.90$), but it did have an effect on changes in color intensity. This may be attributed to the length of the experiment, however it supports the understanding that elevated temperatures are a significant factor in the rate of bleaching (Carpenter *et al.* 2008). The highest value for color intensity is seen for control light and control temperature tanks (Figure 6), which makes sense when we can understand this response in the context of *A. poculata* life cycle. To prepare for colder and darker environments experienced in Northern winters, *A. poculata* will collect chlorophyll in order to increase photosynthetic availability. In warmer seasons, however, corals will get rid of unnecessary chlorophyll (Dimond & Carrington, 2007). This trend can be observed for heated tanks, which showed reduced color intensity as compared to the temperature control tanks (Figure 6), signifying the collection of chlorophyll in order to prepare for colder temperatures and less light availability that is observed during the winter (Dimond & Carrington, 2007). Any observed change in color intensity can be compared to the changes occurring due to seasonal variation of *A. poculata*, where light intensity decreases during late summer into early fall, causing the corals to collect more chlorophyll in order to get the same amount of productivity from lower light conditions (Dimond & Carrington, 2007). These trends were not seen in the blackout tanks because there was an absence of light, so photosynthesis could not occur even with higher chlorophyll concentrations.

During the experiment, both the heater and chiller broke on two separate occasions causing system 1 to reach temperatures that were too hot for that point in the experiment, and system 2 to reach temperatures under the control temperature at the end of the experiment. The corals in these systems may have experienced a reduction in photosynthetic efficiency as a result of this rapid increase in heat, causing a higher change in stress than any of the other heated systems. However, the median value for photosynthetic efficiency during the days of higher temperatures were consistent with those taken later, (Figure 4) suggesting that the rapid increase in temperature did not significantly impact the corals within the system. The decrease in control temperature in system 2 occurred right before extracting RNA on experimental day 13, so it may affect the gene expression of the corals in the system by showing more of a response to the drop in temperature instead of being in the presence of high intensity light. However, this drop in temperature happened at the end of the experiment, after all data had been collected, so it did not impact any of the results. The temperatures reported in Figure 2 were all approximately 1°C lower than they should have been due to error caused by an uncalibrated YSI probe. This however, did not affect the results as all temperatures were consistent to each other throughout the experiment, and replicate measurements taken with a thermometer to determine the accuracy of the probe.

RNA sequencing could be incorporated into a further study in order to address changes in gene expression based upon varying heat and light stresses as was observed in this experiment. A process similar to what was done in Kenkel *et al.* (2013) can be done to identify which genes are upregulated in these *A. poculata* that allows them to endure

heat stress. A possible further study could incorporate acidification into the experiment to find how it affects coral productivity and bleaching rates along with light availability and heat to find the resilience of *A. poculata* in environments that more closely reflect oceans today. This same experiment could also be done focusing on the effects of cooling at different light levels instead of heating, resulting in a better understanding of how temperatures and light affect coral productivity and bleaching rates.
Before adding any additional stressors, it is more important for extensive research to be done with *A. poculata* and its response to light in order to solidify the relatively novel results seen in this experiment. For this reason, repeating the experiment with either a broader range of light intensities or differing light intensities may lead to the solidification of the trends observed in this study.

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