# Too Hot to Handle: Different symbiont types fail to change thermal tolerance outcomes in *Exaiptasia pallida*

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## ABSTRACT

Since pre-industrial times, ocean temperatures have increased due to anthropogenic activity. This has given rise to coral bleaching, where corals expel their symbiotic algae needed for their nutrients in response to increased temperatures, which leads to starvation and death. Recent research indicates that the type of symbiont hosted by corals can influence their stress response, as certain symbionts may be more effective at mitigating thermal stress effects. To better understand how corals respond to thermal stress and the role of hosted symbiont, we conducted a heat stress experiment on *Exaiptasia pallida*. This species of sea anemones is renowned as a model organism for studying coral responses. We introduced two different symbionts—endogenous *Symbiodinium linucheae* and heterologous *Breviolum minutum*—into Aiptasia, as well as studied aposymbiotic Aiptasia. Starting at 26°C, we subjected them to a 1°C daily temperature increase over two weeks. We then compared their phenotypic health, photosynthetic efficiency, and mortality rates with those at a controlled 26°C. We found that aposymbiotic Aiptasia did not outperform their symbiotic counterparts. Among the symbiotic group, Aiptasia hosting *S. linucheae* did not demonstrate better resilience than those hosting *B. minutum*. This suggests that symbiont type does not influence thermal stress outcomes. However, given the short experimental period, further research is required to draw a more comprehensive conclusion. The limited knowledge regarding symbiont interactions with its host illuminates the importance of further researching the consequences of environmental stress for coral reef conservation.

## INTRODUCTION

From 1950 to 2022, sea surface temperatures have increased by an average of 0.100.01°C each decade (Johnson et al., 2023). According to the RCP 8.5 scenario set by the IPCC, if current levels of global warming continue, it is estimated that sea surface temperatures will rise another 3°C by 2100 (Cheng et al., 2019). This alarming increase has profound implications for vulnerable ecosystems, particularly coral reefs (Obura et al., 2021). Coral reefs are critical ecosystems that are being negatively impacted by rising temperatures (Guldberg, 2010). Coral have a symbiotic relationship with dinoflagellate algae in the family Symbiodiniaceae (Stanley 2006; Suggett et al., 2017). If the corals experience prolonged exposure to high temperatures, their algal symbionts are expelled from the host in a process termed coral bleaching (Weis, 2008). Because tropical corals receive the majority of their nutrients from their algal symbionts, when bleached, hosts are left in an energy deficit state until symbiosis is restored or the host dies (Hoegh-Guldberg et al., 2007**;** Wooldridge, 2010). According to Hughes et al., (2018) from 1980 to 2016, severe coral bleaching events across the world are occurring five times as frequently as they did in pre-

industrial times. Corals serve an important functional role in marine environments and losing these species will have devastating effects on global biodiversity by exposing animals to predation that otherwise use the reefs for protection (Pratchett et al., 2016; Leis et al., 1999), decrease human usage of its medicinal properties (Bruckner et al., 2002), as well as decreasing human maritime resources. Therefore, it is imperative to understand the mechanisms by which corals are affected by increases in temperature to predict future coral reef collapse. Since coral bleaching is one of the main consequences of ocean warming, studies aim to understand the underlying mechanisms behind coral bleaching in order to mitigate the effects of rising temperatures. The oxidative stress theory attempts to explain coral bleaching; it states that symbiotic algae in thermally stressed corals increase the production of reactive oxygen species (ROS), which can damage host tissue, leading to the coral host expelling their algae in an attempt to reduce ROS (Lesser., 1997). However, recent studies have shown that the type of algae hosted elicits different thermal stress responses (Yuyama et al., 2012). One experiment conducted by (Quigley et al., 2020) concluded that between *Symbiodinium, Cladocopium*, and *Durusdinium*, coral hosting *Durusdinium* exhibited the lowest rates of bleaching. Another study by Russnak et al. (2021) concluded that some strains of *Symbiodinium* are more thermally tolerant than strains of *Breviolum*. It is therefore possible that different algal strains produce different amounts of ROS, which may lead to differences in thermal tolerance. Regardless, it is clear that host-symbiont pairings can drive bleaching patterns; however, the mechanisms underlying these differences in bleaching remain unknown (Mansfield et al., 2019).

*Exaiptasia pallida*, commonly referred to as Aiptasia, has emerged as a model organism (Weis et al., 2008) for coral bleaching because they are related to corals (class Anthozoa), have symbiont hosting capabilities, and display similar phenotypic responses to increases in temperature as corals (i.e., bleaching). They are native to tropical and subtropical waters of the Western Atlantic, but are found globally (Glon et al., 2020), and can tolerate a wide range of temperatures and salinity, with temperatures up to 42°C and salinities as high as 60 PSU (Gegner et al., 2017). Aiptasia can be easily grown in laboratory settings, where it reproduces sexually (allowing for genetic studies) and asexually (allowing for genetically identical individuals) (Lehnert et al., 2012). When cut bilaterally, one individual can regenerate to create two genetically identical individuals (Burg et al., 2019). Most importantly, Aiptasia can exist in both the aposymbiotic and symbiotic state, having a facultative symbiotic relationship with algae in the family Symbiodiniaceae (Luo and Lin, 2010; Röthig et al., 2016). Aposymbiotic individuals can also be reinfected with different symbiont strains and are able to host a variety of algal symbionts from different genera (Schoenberg and Trench, 1980; Hambleton et al., 2014), making this system attractive for understanding symbioses under climate change. Given our limited knowledge regarding the relationship between symbiont and host under thermal stress, Aiptasia can uncover how hosting different symbiotic algae influences thermal tolerance, and leveraging aposymbiotic individuals allows us to ask whether there is a cost to symbiosis under warmer temperatures as predicted by the oxidative bleaching hypothesis. Here, we

test how Aiptasia (strain CC7) hosting *Symbiodinium linucheae*, *Breviolum minutum,* or no symbionts (aposymbiotic) respond to thermal challenge. It is worth noting that the CC7 strain coevolved to host *Symbiodinium* (Grawunder et al., 2015), whereas the relationship with other algal symbionts is less established. We hypothesize that 1) aposymbiotic individuals will outperform symbiotic anemones because there is little to no symbionts producing ROS and harming the host and 2) Anemones hosting *S. linucheae* will be more thermal tolerant than those hosting *B. minutum*. Our alternative hypothesis is that individuals hosting symbionts will perform better than aposymbiotic individuals because the symbionts provide nutrients to the host that could offset the effects of the stressor.

## MATERIALS AND METHODS

### *Aiptasia preparation*

From this point forward, we will be referring to *S. linucheae* as Sym A and *B. minutum* as Sym B as that is how they are displayed on our figures. The following procedures of Aiptasia husbandry were adapted from (Valadez-Ingersoll et al. 2023). Adult Aiptasia anemones of clonal strain CC7 were housed in polycarbonate tanks containing artificial seawater with a salinity of 35 psu (Instant Ocean). There were three symbiotic associations of Aiptasia used in these experiments: 1) aposymbiotic individuals (no algal symbionts), 2) endogenous Sym A, and 3) Heterologous Sym B. Aposymbiotic Aiptasia were generated through menthol bleaching at least three months prior to the experiment, following established procedures. These aposymbiotic Aiptasia were maintained in darkness, and their aposymbiotic status was verified by the absence of symbiont autofluorescence observed under fluorescence microscopy (Leica M165 FC). The anemones were fed three times a week with freshly hatched Artemia nauplii, and water changes were conducted twice weekly. The tanks were maintained at a temperature of 25 ℃ with a 12-hour light:dark cycle (if symbiotic) provided by white fluorescent light at 25 µmol photons/m2/s.

### *Experimental setup*

Twelve of each of the three symbiotic types (N=36) were individually placed in 60 ml containers filled with 50 ml of ~35 psu seawater (Fig. 1a). Half of each of the symbiotic types were subsequently positioned in two separate large 7.5 gallon water baths: one maintained at a controlled 26°C, and the other heated. Both treatments received a 7.5 h: 16.5 h light: dark cycle with white fluorescent light. The 18 jars of the same treatment were placed in a 6 by 3 grid with each symbiotic type consisting of two columns and three rows. Temperature in the heated bath was increased by 1°C daily starting at 26°C to a final temperature of 37°C after two weeks. Control conditions were maintained at 26˚C. During the experiment, Aiptasia were fed freshly hatched *Artemia* nauplii every fourth day followed by a water change after 45 minutes to prevent stagnant water. Feeding responses were recorded immediately after the Aiptasia were fed with a binary response of feeding or not. Water changes were also done midway between feeding days to

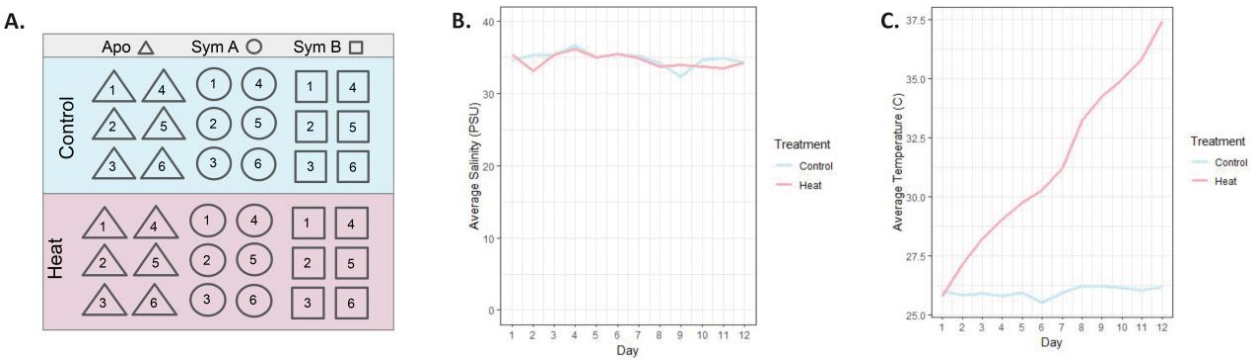


Figure 1. Experimental setup. (A) Shows the experimental design layout for the control and heat treatment. For both treatments 6 of each symbiont types were placed in a 2 by 3 grid. Triangles depict aposymbiotic Aiptasias, circles depict Symbiodinium Aiptasias, and squares depict Breviolum Aiptasias. (B) . Daily temperature across treatments taken using TidbiT MX2203. Heat treatment was set to increase by 1 ℃ daily; (C) Daily salinity across treatments taken using a refractometer. Salinity was maintained between 32 to 36 PSU.

control for increased salinity due to evaporation. Each day jars were rotated to ensure consistent light exposure.

Temperature of the water bath was measured using the TidbiT MX2203 temperature logger. Temperature measurements were taken in the morning followed by an increase of 1˚C on the water heater setting. Another measurement was taken in the evening to ensure that the temperature increased. Salinity was measured using the Brix refractometer twice a day, once in the morning and once in the evening, concurrently with temperature to maintain a salinity of 32 to 36 psu (Fig. 1b, Fig 1c).

## *Anemone Color Analysis*

Photos were taken with the Olympus SZX16 microscope and Leica microsystems computer FLEXACAM C1. For all samples, the Schott 150W ACE light source was used as the light source at a setting of 80. Aiptasia hosting Sym A and Sym B were viewed under 0.7x magnification, while aposymbiotic individuals Aiptasia were viewed under 1x magnification. Photos were taken 7 times during the course of the experiment (days 1,3, 5, 7, 9, 11, 12) concurrently with PAM. Photos were white-balanced in Adobe Photoshop to maintain equal lighting across photos. Photos were analyzed in Matlab using the Coral Color Intensity Analysis Utility 1.0 program (Winters et al. 2009) to extract red, green, and blue (RGB) values. Ten points were chosen on the tentacles randomly and the average of the RGB values were used to represent the color of the Aiptasia sample for its photo symbiont density.

Phenotype and mortality were used as a proxy for health. They were recorded using the bi-daily photos taken. Each day the photos were taken, they were analyzed using a phenotype health score chart ranging from 0-4. At score 4, the Aiptasia had their tentacles fully extended and their bodies were fully visible. At score 3, their tentacles were partially extended and you begin to see their bodies shrink in, as well. At score 2, their tentacles are mostly retracted within their bodies and begin to look like stumps. At score 1, no tentacles are visible, or it is seen fully retracted within their bodies that now begin to look like blobs. At score 0, they have disintegrated and have died.

## *Photosynthetic Efficiency*

Pulse-amplitude modulated (PAM) was used to measure the photosynthetic

efficiency of photosystem II of the symbionts using a Walz Junior PAM. PAM was conducted 7 times during the course of the experiment (days 1,3, 5, 7, 9, 11, 12). Aiptasia was dark acclimated for one hour prior to conducting PAM. Fv/Fm measurements were taken until three values within a 0.1 range were recorded, where the mean of the three values were used as a Fv/Fm value of the sample. The relative change in photosynthetic efficiency was calculated using the equation: final average value fv/fm- initial average value fv/fminitial average value fv/fm , with final and initial representing the last day and the first day of the experiment, respectively.

### *Statistical analysis*

For all statistical analyses conducted, p=0.05 or less was our standard for statistical significance. Significant P-values will be reported as ($P<0.05$), and statistically insignificant P-values will be reported as ($P>0.05$). Only photosynthetic efficiency of the final day and the relative change in photosynthetic efficiency statistics are reported in order to eliminate across-time inaccurate grouping with our limited statistical analysis knowledge. Both the final day photosynthetic efficiency and the relative change in photosynthetic efficiency were analyzed using ANOVA to determine if the individual factors (treatment and symbiont type) or the combination resulted in statistically significant results. We also used post hoc to determine where significant interactions occurred. The relative change in channel R intensity followed the same process as the relative change in photosynthetic efficiency. We used RStudio to calculate the frequency of health score, to determine whether the temperature treatments had an effect on Aiptasia health.

## RESULTS

### *Phenotype and Mortality*

Phenotype responses of all Aiptasia under control were stable and healthy with a score of 4 throughout the experiment (Fig. 2a, Fig. 2b, Fig. 2c). There was a small dip on day 8 for aposymbiotic Aiptasia, but they recovered the next day. However, we did see a declining trend in the heated treatment for all Aiptasia. 25% of the Sym A declined on

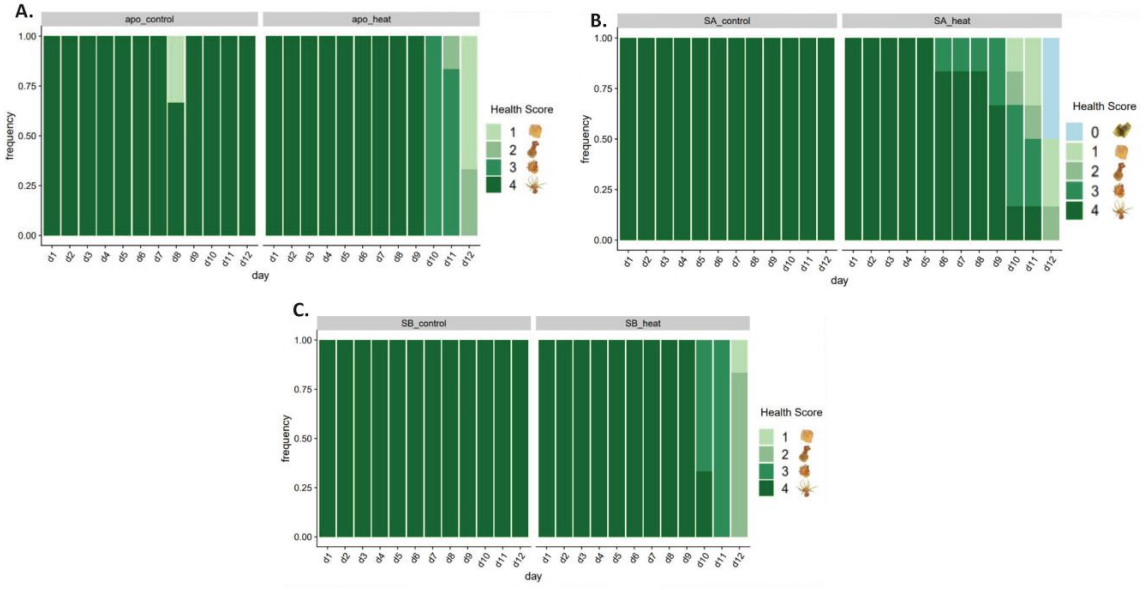

Figure 2. Behavioral response of Aiptasia to different treatments over time. (A) aposymbiotic Aiptasia, (B) Aiptasia infected with Symbiodinium, (C) Aiptasia infected with Breviolum. The legend on the right shows the basis of the score assignments. The behavioral score “0” indicates death while a score of “4” indicates a healthy Aiptasia with its tentacles fully extended. N=six/ treatment/ symbiont type. Under control treatment, almost all symbiont types maintained a score of “4”. Under heat treatment, there was a decline in health.

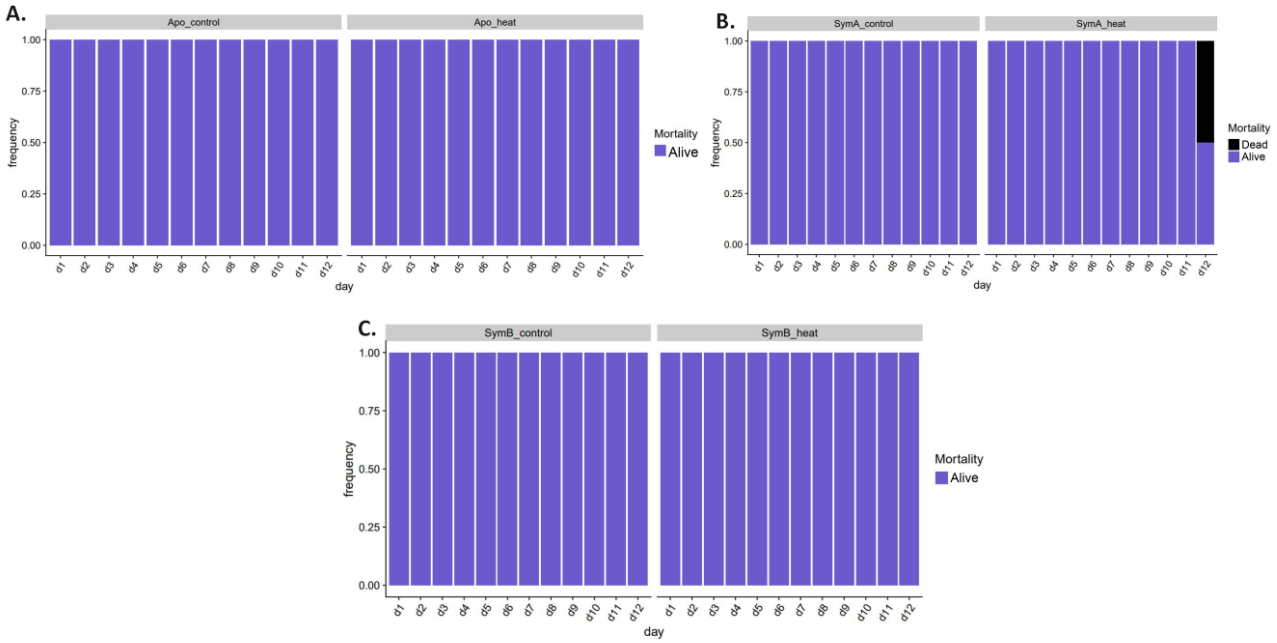


Figure 3. Mortality of Aiptasia to different treatments over time. (A) aposymbiotic Aiptasia, (B) Aiptasia infected with Symbiodinium, (C) Aiptasia infected with Breviolum. N=six/ treatment/ symbiont type. Under control treatment, all symbionts types survived. Only 3 out of 6 Sym A died on the final day.

day 6, and by day 12, 50% of the individuals died (Fig. 2b). 75% of the Sym B started to decline in health by day 10 (Fig. 3c). The heated treatment was also estimated to decline overall health an estimated 0.79 per day. There was no mortality in all Aiptasia under control treatment throughout the experiment (Fig. 4a, Fig. 4b, Fig. 4c). There was also no mortality under heat treatment for Sym B and aposymbiotic individuals throughout the experiment (Fig. 4a, Fig. 4c). Interestingly, 50% of Sym A died on day 12.

## *Photosynthetic Efficiency:*

Photosynthetic efficiency was significantly affected by temperature by the final day, with heat stressed Sym B fv/fm values being significantly less than Sym B controls ($P<0.05$), Fig. 4a), and heat stressed Sym A fv/fm values being significantly less than Sym A controls ($P<0.05$), Fig. 4a). There was no significant difference between the control and heat stressed fv/fm values for aposymbiotic individuals ($P>0.05$, Fig. 4a). There was also no significant difference between symbiont types in the heated or controlled treatments ($P>0.05$, Fig. 4a). Relative change showed a significant difference in fv/fm values by treatment ($P<0.05$) but not by symbiont type ( $P>0.05$), (Fig. 4b). Specifically, Sym A and Sym B’s controlled treatment’s relative change in fv/fm was significantly higher than Sym A and Sym B’s heat stressed counterparts($P< 0.05$), but there was no significant difference between treatments for aposymbiotic individuals ($P>0.05$, Fig. 4b).

## *Anemone color analysis*

Relative change in R intensity showed a significant difference by treatment ($p<0.05$) and by symbiont type ($p<0.05$).

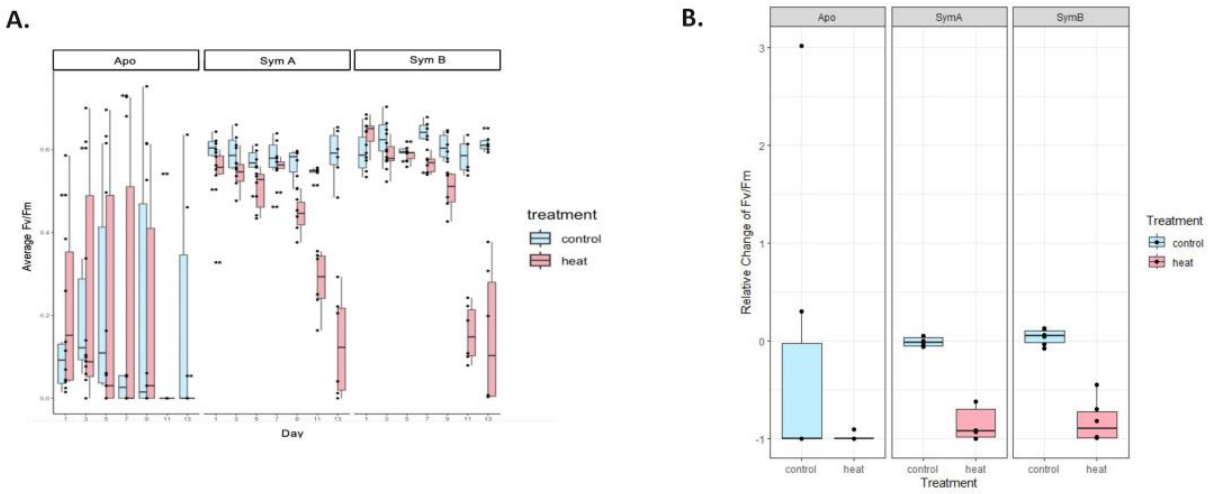


Figure 4. Photosynthetic efficiency (Fv/Fm) of algal symbionts by treatment. There was a significant decrease in Fv/Fm for all symbiont types in heat treatment compared to control. A) Changes in Fv/Fm over time.; B) Relative change ((final-initial)/ initial) in Fv/Fm between treatments by symbiont type.

Specifically, Sym A and Apo's R intensity in control were higher than their heat stressed Sym A ($p<0.05$) and heat stressed aposymbiotic individuals ($p<0.05$) counterparts (Fig. 5). There was no significant difference between Sym B's control or heated R intensity ($P>0.05$, Fig. 5). There was no significant difference in the different symbiont types' R intensities in the heated treatment ($P>0.05$), but aposymbiotic individuals showed significantly higher R intensity than Sym B ($p<0.05$) in the control treatment (Fig. 5).

## DISCUSSION

### *Photosynthetic efficiency*

As global temperatures continue to increase, it is important to understand thermal tolerance differences in hopes of mitigating its effects on corals and reef communities. Using our coral model system Aiptasia, we have determined that based on photosynthetic efficiency, there is no significant difference in performance between symbiont types as temperatures increase, suggesting that symbiont type does not influence thermal tolerance. This aligns with studies showing that Sym A and B having similar photosynthetic efficiency (Suggett et al., 2015). The two symbiont types used in this study, *S. linucheae* and *B. minutum*, have been documented to have similar photosynthetic efficiencies, PSII absorption cross-section, and PSII reaction center content (Suggett et al., 2015).

### *Anemone Color Analysis*

Our results for color analysis indicate that treatment and symbiont type were significantly different. The relative change in channel R intensity decreased for all symbiont types in the heat treatment, but the notable difference was between Sym B and Apo, with Sym B displaying lower R intensity than Apo. However, color analysis of the anemones may not be a reliable parameter for photosymbiont density since the Aiptasias shrink their tentacles when they are stressed which increases the density of photosymbionts increasing the average red value of more stressed Aiptasias. Notably, the code adapted from (Winters et al. 2009) was originally designed for corals and does not account for the high mobility and movement of the tentacles of anemones. In addition, (Johnson et al., 2007) explored the

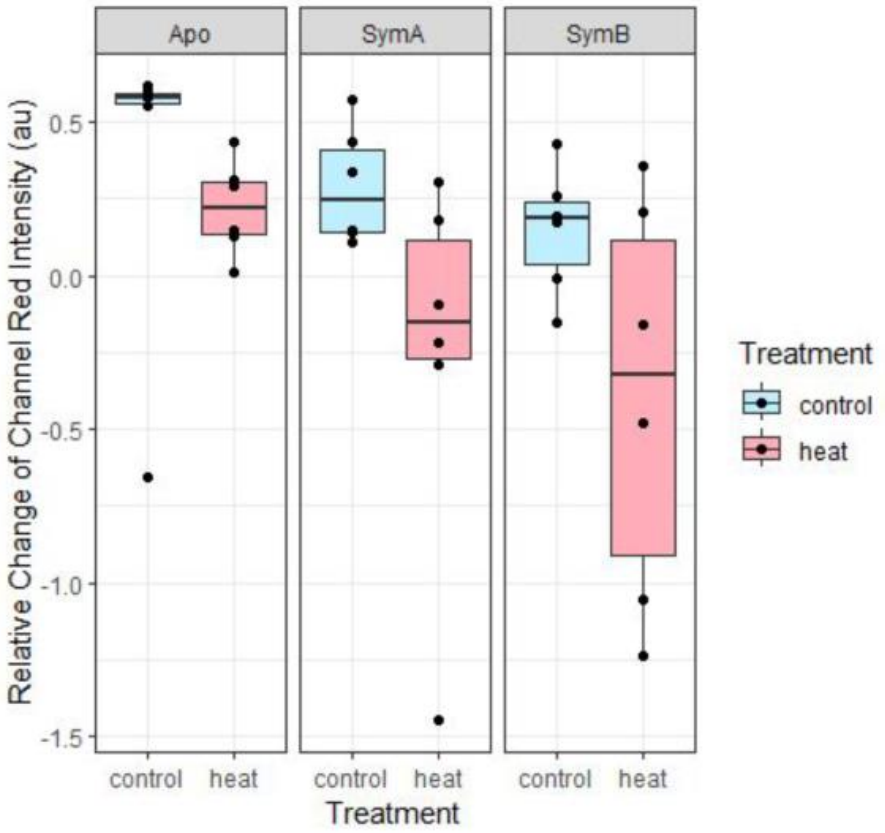


Figure 5. Relative change ((final-initial)/ initial) of channel red intensity across treatment by symbiont types. There was a significant decrease in red channel intensity for all symbiont types in the heat treatment when compared to controls. There was a significant decrease in red channel intensity for Sym B compared to aposymbiotic individuals.

correlation between color analysis and symbiont density then found that the blue values had the highest correlation to symbiont density, followed by green, and then red. (Johnson et al., 2007) also adds that using any one band risks comparisons to color morphs. Relative change in red values may instead be a useful proxy in determining the stress levels of symbiotic Aiptasias compared to subjective determination of stress levels through the phenotypic charts.

*Phenotype and Mortality*

Consistent with our other results, especially color analysis, the heat treatment negatively impacted the health of all Aiptasia, with Sym A exhibiting the earliest signs of decline on day 6, while aposymbiotic individuals and Sym B displayed health declines four days later on day 10. Notably, Sym A was the only symbiont type with mortalities, however based on the trends displayed, both aposymbiotic individuals and Sym B would've exhibited signs of eventual death had the experiment continued. More specifically, aposymbiotic individuals likely would have experienced mortality next, followed by Sym B. While this appears to show that Sym A is less thermotolerant than aposymbiotic individuals and Sym B, the observed differences are not significant enough to draw a clear conclusion.

*Future Studies and Points of Concern*

This study was conducted under a time constraint of 2 weeks and may suffer the effects of a rapid temperature increase and premature termination. The temperature of the water bath was increased by 1℃ daily with the first signs of phenotypic stress in the heat treatment appearing on day 6 at roughly 30℃. This coincides with other studies that found that Aiptasia began experiencing necrosis and apoptosis at 29.5℃ (Dunn et al., 2004). Within the same study (Dunn et al., 2004), it showed that sustaining this temperature led to increased counts of necrosis and apoptosis up to 6 days. The daily 1℃ increase makes it difficult to discern at exactly what temperature each symbiont type begins to display phenotypic stress and mortality. Furthermore, such rapid increases in temperature are unnatural in coral reefs (Guadayol et al., 2014) and could incite behaviors that will not be seen in natural conditions. From this, it is recommended that future studies increase the duration that the Aiptasias are exposed to each temperature. This would provide more insight into how types of symbionts elicit different responses in its host. Additionally, the experiment was concluded as we began to observe significant declines in health across all measurements conducted. Had the experiment continued, we might have been able to discern the limit of thermotolerance for each symbiont type or a clearer distinction between their symbiont thermotolerance. Future studies could also explore diverse symbiont species, such as Durusdinium known for its high thermal tolerance (Fabricius et al., 2004). Comparing Durusdinium with Gerakladium, which has been shown to outcompete Durusdinium (Matsuda et al., 2022), as well as comparing those more thermally tolerant species to the ones we studied here would offer a more comprehensive insight into the impact of symbionts on thermal tolerance.

Furthermore, the two symbiont types used in this study, *S. linucheae* and *B. minutum*, have been documented to have similar photosynthetic efficiencies, PSII absorption cross-section, and PSII reaction center content (Suggett et al., 2015). Future studies should test other types of *Symbiodinium* species holobionts to test for differences in thermotolerance. This experiment mainly focused on the phenotypic effects of temperature and yielded the conclusion that although the symbiont types do not have differing photosynthetic efficiencies even with varying temperature, Sym B Aiptasias experienced less mortalities than Sym A. To investigate this difference, transcriptome and metabolite testing for both the host and symbiont may be beneficial. A study by (Lenhert et al., 2012) studied the transcriptome activity of aposymbiotic Aiptasia, but no studies have been conducted on the transcriptome activity of symbiotic Aiptasia.

In conclusion, our results demonstrate that within Aiptasia, Apo, Sym A, and Sym B all do poorly under heat and none was more thermally tolerant than the other. This is particularly important because corals are hugely dependent on the energy contribution from their symbionts and finding more thermally tolerant symbionts could mitigate the effects of climate change on corals and reef communities. A longer experimentation period is needed to understand the full effect of temperature on symbiont types. This work highlights the importance in perseverance of such studies to better understand the complex dynamics of symbiont type and host under thermal stress. Future works leveraging how symbionts play a role in thermal stress can

contribute to creating better methods for coral conservation.

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