The Microbiome Strikes Back: How *Aiptasia pallida* Combats Environmental Stress

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Abstract

Coral reef ecosystems sequester large amounts of carbon and support a high biodiversity of marine life, but rising sea surface temperatures (SST) and other environmental stressors are driving coral mortality and disease. Like corals, the model species *Aiptasia pallida* will either form symbiotic relationships with Symbiodiniacaea or rely on heterotrophy. Due to the similarities of their internal biological interactions, corals and *A. pallida* share common stress responses, making *A. pallida* a useful indicator of coral health. While most studies focus on the impact of the relationship between *A. pallida* and Symbiodinicaea, few studies investigate the role the microbiome plays in cnidarians' response to thermal stressors. Under this thermal stress, *A. pallida* produces excess amounts of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2). The excess production of ROS species leads to an over-accumulation of H_2O_2 in the cell membrane and surrounding water. This buildup of H_2O_2 can have harmful effects on the coral reef ecosystem and the individual organisms. By monitoring the photosynthetic capabilities, red channel changes, expandability, and mortality, results indicate that anemones containing both a microbiome and a symbiont retain photosynthetic capability, expandability, and survivability. These results suggest that both Symbiodiniaceae and the microbiome influence *A. pallida*'s resilience to excess ROS production under simulated thermal stress. These results contribute to the growing body of knowledge surrounding *A. pallida* as an indicator species for coral health and the importance of an intact microbiome. Considering these results, the presence of a microbiome and Symbiodiniaceae significantly influence the resilience of *A. pallida* to environmental stress events.

Introduction

Aiptasia pallida, commonly known as glass anemones, are small sea anemones that inhabit tropical and subtropical marine ecosystems. In various reef environments, *A. pallida* are characterized by their adaptability and environmental resilience (Roberty et al., 2024). One of the key features of *A. pallida* is its ability to exist in both aposymbiotic and symbiotic states, determined by the presence or absence of endosymbiotic algae (Symbiodinicaea). This relationship results in the anemone exhibiting either a brown phenotype (symbiotic) or a white phenotype (aposymbiotic). In its aposymbiotic state, *A. pallida* relies entirely on heterotrophic feeding. This allows the anemone to survive in environments with limited light or nutrient availability, where the symbiotic relationship would not be advantageous (Baumgarten et al., 2015). In contrast, when *A. pallida* exhibits a symbiotic relationship, it hosts Symbiodiniacaea. The Symbiodiniacaea provides the host with energy and in turn, the algae receives nutrition from the host (Baumgarten et al., 2015; Weis & Allemand, 2009). This symbiotic relationship is not only a key aspect of the anemone's metabolic strategy, but it also enhances the anemone's resilience to environmental

fluctuations, such as variations in light and nutrient levels (Thornhill et al., 2013; Baumgarten et al., 2015). These characteristics, along with their relatively simple anatomy and ease of cultivation in laboratory settings, make *A. pallida* a valuable model organism in marine science, especially in the study of cnidarian biology, symbiosis, and the effects of environmental stressors (Roberty et el., 2024). In particular, they offer an opportunity to investigate the complex dynamics between host organisms, their photosynthetic symbionts, and microbiomes, making them a crucial species for understanding coral health in response to climate change (Baumgarten et al., 2015). Under stressful conditions such as high temperatures or oxidative stress, *A. pallida* expels its symbiotic algae in a process known as bleaching. This leads to an overproduction of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2) (Dungan et al., 2022). ROS are highly reactive molecules generated as by-products of cellular metabolism. While they are typically produced in small amounts, excessive ROS can damage cellular structures. In response to heat stress, *A. pallida* produces and releases excessive hydrogen peroxide, which, when in surplus, can diffuse across the organism's membrane and induce apoptosis, or cell death (Dungan et al., 2022).

The anemone's ability to manage oxidative stress is influenced by both its symbiotic state and its complex and diverse microbiome, which plays a critical role in its overall health and resilience to environmental stress (McCauley et al., 2023). This microbiome consists of a wide variety of microbial species, including bacteria, fungi, and archaea, all of which are closely associated with the anemone's tissues and its algal symbionts (Curtis et al., 2023; McCauley et al., 2023). Previous studies have identified between 25 to 45 distinct microbial species in *A. pallida*, many of which contribute to the anemone's fitness and reproductive success (Jaspers et al., 2018). While the full extent of the microbiome's impact on cnidarian health is still being explored, its importance in maintaining the health of *A. pallida* is becoming increasingly clear due to its critical role in modulating the anemone's response to oxidative stress. Disruption of the microbiome, caused by the presence of antibiotics or thermal changes, influences the anemone's resilience to stressors like oxidative damage (Reshef et al., 2006). In particular, when *A. pallida* is deprived of its symbiotic algae (i.e., aposymbiotic), the removal or disturbance of the microbiome may heighten the anemone's susceptibility to oxidative stress (Weis, 2008).

In symbiotic *A. pallida*, both the microbial community and the algal symbionts may work together to buffer the effects of oxidative damage to the host. This protective effect may involve the production of antioxidants by the microbiome, which helps mitigate the effects of oxidative stress, as well as the limitation of harmful reactive oxygen species production (Weis, 2008). These interactions suggest that the microbiome not only supports the health of the anemone itself but also enhances the symbiotic relationship with Symbiodiniacaea, promoting resilience to environmental stressors like increased temperature or exposure to pollutants (Dungan et al., 2022). In this way, the microbiome acts as an essential component of *A. pallida*'s stress response, helping to sustain both the host and its symbiotic partners under challenging conditions (Sydnor et al., 2023). Understanding the role of the microbiome in mediating stress responses is important for predicting how *A. pallida* and other cnidarians will cope with climate change. By isolating the impact of oxidative stress on *A. pallida* in a controlled environment, we hypothesize that microbial communities play a crucial role in resilience. Specifically, we predict that aposymbiotic *A. pallida* treated with antibiotics to disrupt the microbiome will be more negatively impacted by mimicked environmental stressors, the H_2O_2 treatment, compared to symbiotic *A. pallida* with a microbiome present. This study aims to provide information into how microbial communities aid

in stress mitigation, potentially offering new approaches to improving the survival of marine species under environmental pressures.

Materials and Methods

Animal Rearing and Antibiotic Treatments

Individual *A. pallida* were maintained and acclimated separately in sterile 60 x 15 mm Petri dishes containing sterile artificial seawater (ASW) at 24°C in an incubator for one week. Aiptasia strains CC7 (Sunagawa et al., 2009) with 20–25 mm oral disk sizes were reared in ASW at 33 ppt salinity. They were fed 48-hour-old *Artemia salina* (hatched brine shrimp) four days before the experimental onset. The incubator was illuminated with low photosynthetic photon flux density (~50 µmol m^2 s^{-1}) under a 12:12 light-dark cycle. To disrupt the microbiome, ampicillin (Amp, Acros Organics Cat. No.: 61177), streptomycin (Strep, Alfa Aesar, Cat. No.: J61299), and neomycin (Neo, Alfa Aesar, Cat. No.: J61499) were dissolved in sterile deionized water to a working concentration of 20 mg/ml each. Rifampicin (Rif, Tokyo Chemical Industry, Cat. No.: 236-312-0) was dissolved in dimethyl sulfoxide (DMSO) at 20 mg/ml concentration. As a vehicle control, DMSO was added to seawater at the same concentration as in the antibiotic treatments (0.1% and 0.4%, v/v). The antibiotic mixture consisted of all four antibiotics, with concentrations of 200 µg/ml for Amp, Strep, and Neo, and 50 µg/ml for Rif. All antibiotics were dissolved in ASW, maintaining the same salinity used for animal care.

Experimental Design

A total of 48 genetically identical Sunagawa strain *A. pallida* anemones were used. The anemones were exposed to two treatments: a control (sterile seawater) and H_2O_2 (0.008% concentration), each set up in a six-cell well culture plate. Once the anemones were transferred to the six-cell well plates they were given 24 hours to acclimate to their new environment before any tests were performed to avoid additional stressors. Eight plates were used in total—four for the control treatment and four for the H_2O_2 treatment. Within each treatment, two plates contained symbiotic (brown phenotype) anemones, and two contained aposymbiotic (white phenotype) anemones. Within each treatment, two plates contained a microbiome (one of each phenotype) and two plates lacked a microbiome (one of each phenotype) (Fig. 1). The either plates were labeled vertically, one to six, along each cell and aligned in a grid formation under a timed light set to a 12-hour light/dark cycle with light from 11 a.m. to 11 p.m. to simulate natural sunlight. Night conditions were stimulated with a lack of light from 11 p.m. to 11 a.m.

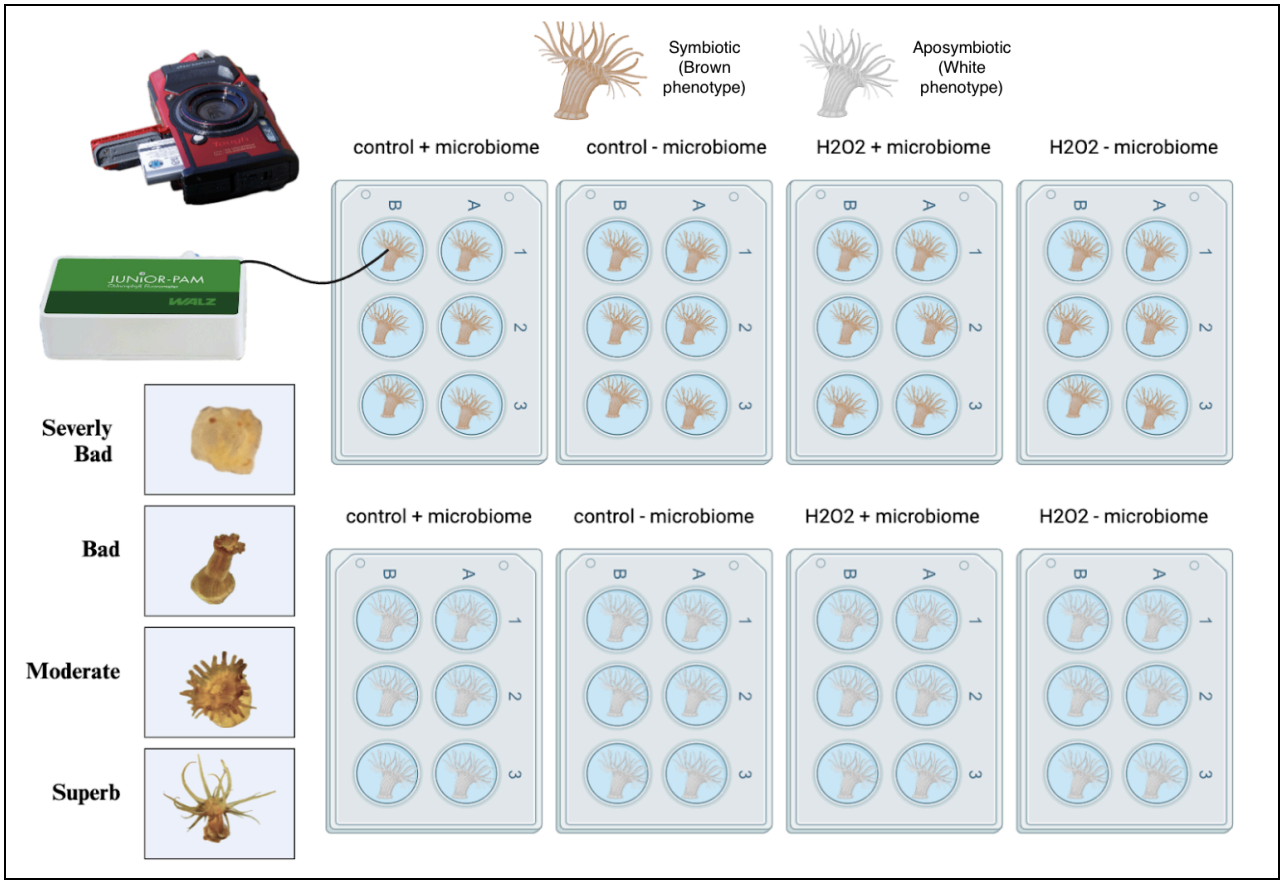

Figure 1. **Graphical Representation of Experimental Design**
Eight, six-cell well plates each contained a control treatment (sterile sea-water) or H_2O_2 treatment (0.008% H_2O_2) with *A. pallida*. The top row shows four plates containing symbiotic anemones (brown phenotype) in both control and H_2O_2 treatments with and without microbiome (+ indicates presence, - indicates absence). The bottom row shows four plates containing aposymbiotic anemones (white phenotype) in control and H_2O_2 treatments with and without microbiome. Along the left side, an expandability scale is shown, with scores ranging from "Severely Bad" (1) to "Superb" (4). A Junior Pulse Amplitude modulation (PAM) machine was used to assess photosynthetic efficiency and the TG-6 camera was used to photograph the organisms.

Feeding, Temperature, and Water Changes

Each anemone was fed daily an equal portion of 10 mL of concentrated frozen brine shrimp per feeding. Using a 10 mL pipette, one drop of solution was dispensed into each anemone's well. Approximately one hour was given in between feeding and morphological assessment. A beaker of sterile seawater kept at room temperature was placed next to the system. A thermometer was used to monitor the temperature of this water, approximating the water temperature in the system. Water changes were performed daily after observing morphological changes between 2 p.m. and 4 p.m. to ensure consistency. A pipette was used to measure 10 ml of sterile seawater or H_2O_2 solution (0.008% H_2O_2) during water changes to maintain standardization. The H_2O_2 solution was prepared using 2.4 ml of 30% H_2O_2 solution diluted in 900 ml of sterile seawater.

Pulse Amplitude Modulation (PAM)

Pulse Amplitude Modulation (PAM) fluorometry was performed once a day on each anemone, between 9:00 and 10:00 am, before the lights turned on. This schedule ensured the anemones had been in complete

darkness for at least 12 hours overnight, allowing them to acclimate to the dark. The PAM measurements were used to evaluate the maximum quantum yield of photosystem II (Fv/Fm) under dark-acclimated conditions. Three measurements were taken per anemone at different points along the oral cavity or stalk, and the three values were averaged to obtain a single Fv/Fm value for each anemone per day.

Mortality Assessment and Analysis

Mortality of the anemones was assessed using a binary scoring system: anemones that responded to external stimuli, such as movement or retraction when touched, were scored as alive (1), while those showing signs of decay (e.g., lysed) were scored as dead (0) and then disposed of. Mortality data was visualized with mortality curves in R, displaying the proportion of mortality for the six individuals across each of the 8 different experimental conditions (treatment type, phenotype, and microbiome presence) with all the control groups condensed into one curve.

Expandability Assessment and Analysis

The expandability of each specimen was assessed and ranked on a 1- 4 scale, following a method established by Dungan et al. (2022) where 1 represents the least expanded state and 4 represents full expansion (Fig. 1). A score of 0 was given to dead anemones to maintain consistency when analyzing data in R. Observers agreed on the expandability score to maintain consistency. Four bar graphs were created in R to visually differentiate the effects of the different factors on expandability. To evaluate the significance of expandability changes caused by the independent variables (treatment type, phenotype, and microbiome presence), Analysis of Variance (ANOVA) and a Tukey HSD test were conducted in R.

Fv/Fm Assessment and Analysis

Fv/Fm values were analyzed in R to assess the effects of phenotype, microbiome presence, and treatment type on photosynthetic efficiency. The difference between the final and initial Fv/Fm values was calculated for each anemone, and this difference was then divided by the initial Fv/Fm value to standardize the data. This normalization allowed for a more accurate comparison of the relative changes in photosynthetic efficiency across different conditions. Data were visualized using boxplots to illustrate the distribution of the normalized Fv/Fm values across the different treatment groups in R. The analysis was performed using a one-way ANOVA to examine the relationship between the normalized Fv/Fm values (dependent variable) and the independent factors: phenotype (symbiotic vs. aposymbiotic), microbiome presence (with or without microbiome), and treatment type (control vs. H_2O_2).

Red Channel Assessment and Analysis

For each specimen, photographs were taken to document sample details, including sample number, phenotype, microbiome presence (if applicable), well number, and day of the experiment. A second image of the specimen itself was captured against a consistent white background, using a TG-6 camera set to microscope mode with a 4x zoom. Adobe Photoshop was used to adjust the white balance to ensure accurate color representation. Red channel values were then extracted from the images using the MATLAB code AnalyzeIntensity macro for further analysis (Winters et al., 2009). Each member was responsible for the same 12 anemones for color correction and MATLAB analysis throughout the experiment to ensure consistency and reduce bias. Red channel color values are measured on a scale of 255 (indicating white) to 0 (indicating red) with pigmentation increasing as channel values decrease. The difference between the final and initial red channel colors was divided by the initial red channel color of

each anemone. This was done to ensure that the observed change in color was not caused by natural differences in the initial color of the anemone. A one-way ANOVA test was conducted in R to compare red channel variation among specimens across all comparable treatments that did not result in death between days four and five, focusing on microbiome presence and brown phenotype between control (sterile seawater) and H_2O_2-treated groups.

Results

Mortality Assessment and Analysis

In the control group, all individuals, regardless of phenotype or microbiome presence, maintained 100% survival, as indicated by the continuous "alive" status (Fig. 2). In contrast, the aposymbiotic groups exposed to H_2O_2 treatment exhibited early mortality. Specifically, the aposymbiotic groups with and without a microbiome began experiencing mortality on day 3, with all individuals dead by day 4 (Fig. 2). The symbiotic groups showed higher tolerance to H_2O_2 treatment, though this varied based on microbiome presence. The symbiotic group without a microbiome began experiencing mortality on day 4 and reached complete mortality by day 5 (Fig. 2). In contrast, the individuals in the symbiotic group with a microbiome survived the entire 13-day period under H_2O_2 treatment, as shown by the continuous "alive" status (Fig. 2).

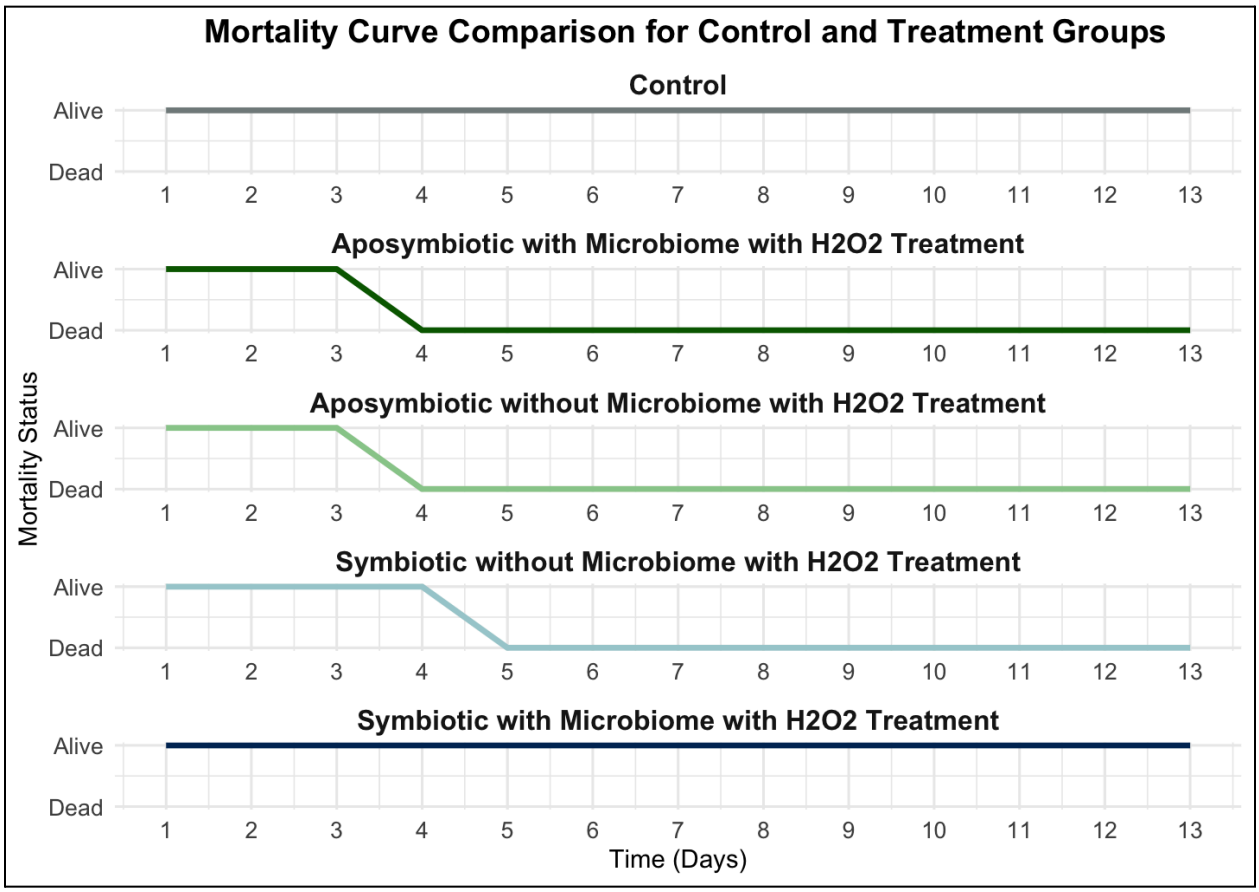

Figure 2. Mortality Curve Comparison for Control and Treatment Groups.

Mortality status of *A. pallida* over 13 days, categorized by different treatment conditions: control and H_2O_2 exposure across aposymbiotic and symbiotic states, with or without microbiome presence. The top panel represents the four combined control groups as mortality was identical throughout the experiment. Each panel represents a treatment group, with each line showing the mortality progression for the six individuals in the group. As all individuals within each treatment group exhibited identical mortality outcomes on the same day, each line effectively represents the mortality status of the entire group. Different colors denote each treatment: dark green for "Aposymbiotic with Microbiome and H_2O_2 Treatment," light green for "Aposymbiotic without Microbiome and H_2O_2 Treatment," light blue for "Symbiotic without Microbiome and H_2O_2 Treatment," and dark blue for "Symbiotic with Microbiome and H_2O_2 Treatment." Time, measured in days, is on the x-axis while mortality, measured as either alive or dead, is on the y-axis.

Expandability Assessment and Analysis

The expandability scores of aposymbiotic individuals in H_2O_2, regardless of microbiome presence, were consistent at a level of 0 after day 4 (Fig. 3). Both aposymbiotic and symbiotic individuals under control conditions, regardless of microbiome presence, exhibited consistent expandability scores of 4 throughout the experiment. In symbiotic *A. pallida*, expandability scores varied depending on microbiome presence and treatment type (Fig. 3). ANOVA test results revealed a p-value <0.001, indicating a high level of significance between the two treatment groups (H_2O_2 and control). Linear comparisons between both aposymbiotic and symbiotic *A. pallida* under H_2O_2 and control treatments, with and without microbiomes, also yielded highly significant results (p-value <0.001). However, comparisons between the same treatments (control or H_2O_2) and microbiome conditions (present or absent) within each phenotype did not show significant differences (p-value>0.1).

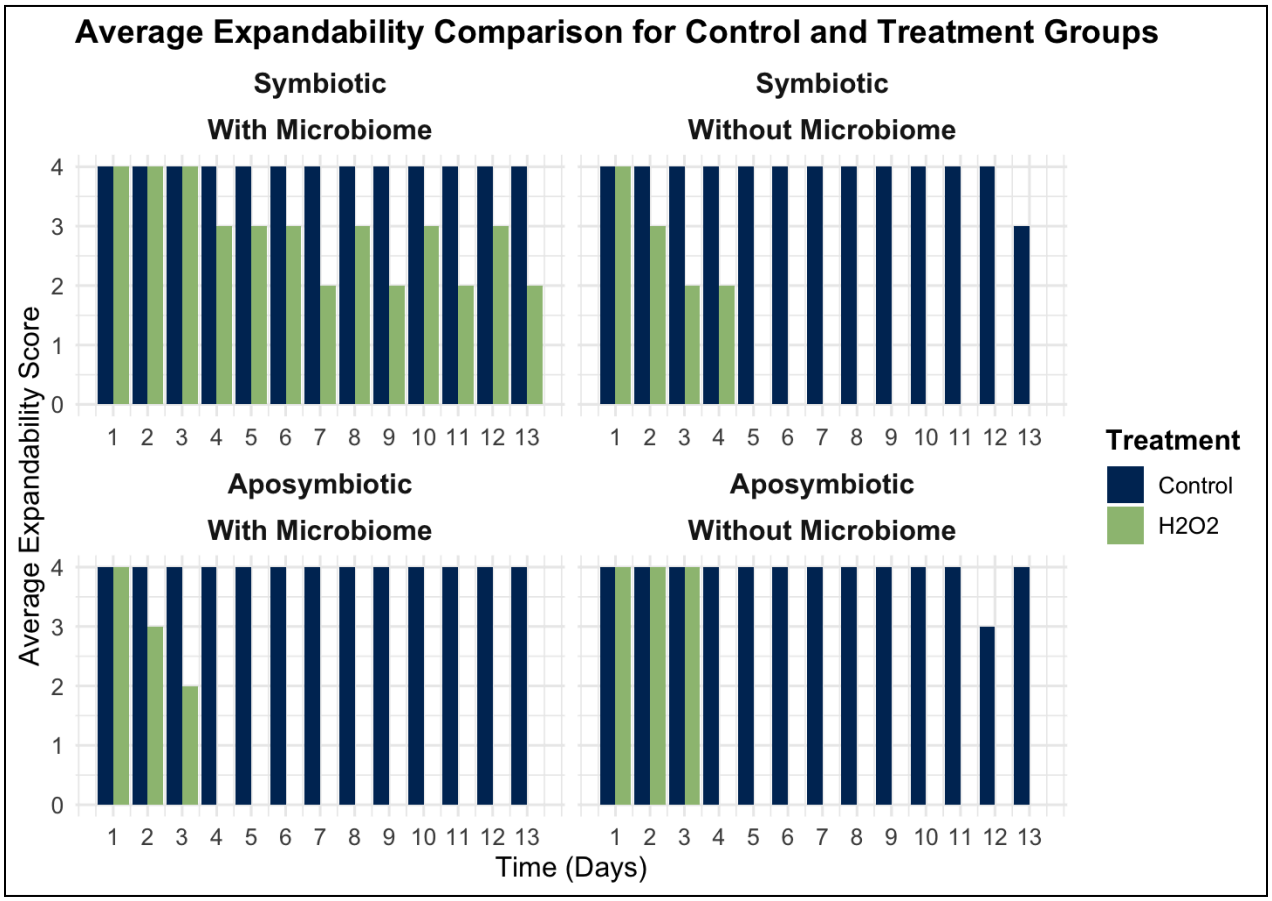

Figure 3. Average Expandability Comparison for Control and Treatment Groups.
The expandability scores (ranked 1-4 as described in Fig. 1) were taken each day after the *A. pallida* were fed. Average scores were used. The bar graphs demonstrate the relationship between expandability scores and a factor that may have influenced it (Treatment, Phenotype (symbiotic state), and Microbiome Presence). The x-axis demonstrates the time frame of the experiment and the y-axis represents the average expandability score. Each treatment (control and H_2O_2) are represented with different colors, dark blue for the control and green for the H_2O_2 treatment.

Fv/Fm Assessment and Analysis

The analysis of Fv/Fm values across different experimental conditions, control vs. H_2O_2 treatment, symbiotic vs. aposymbiotic phenotypes, and with vs. without microbiome, revealed differences in photosynthetic efficiency (Fig. 4). The H_2O_2 treatment consistently resulted in lower Fv/Fm values compared to the control group, reflecting a significant decline in photosynthetic efficiency across both symbiotic and aposymbiotic phenotypes, regardless of microbiome presence. On average, the H_2O_2-treated group exhibited Fv/Fm values approximately 23.57 units lower than the control group. This difference was statistically significant (p-value = 0.016), indicating that the reduction in Fv/Fm values was a real effect, not due to random variation. Further confirmation through Tukey's HSD post hoc test showed that the mean difference between control and H_2O_2 groups ranged from -42.42 to -4.72, a confidence interval that does not include zero, reinforcing the conclusion that H_2O_2 has a substantial effect on photosynthetic efficiency. The boxplots further support this conclusion, showing consistently lower Fv/Fm values for the H_2O_2 group across the 13-day measurement period compared to the control group (Fig. 4).

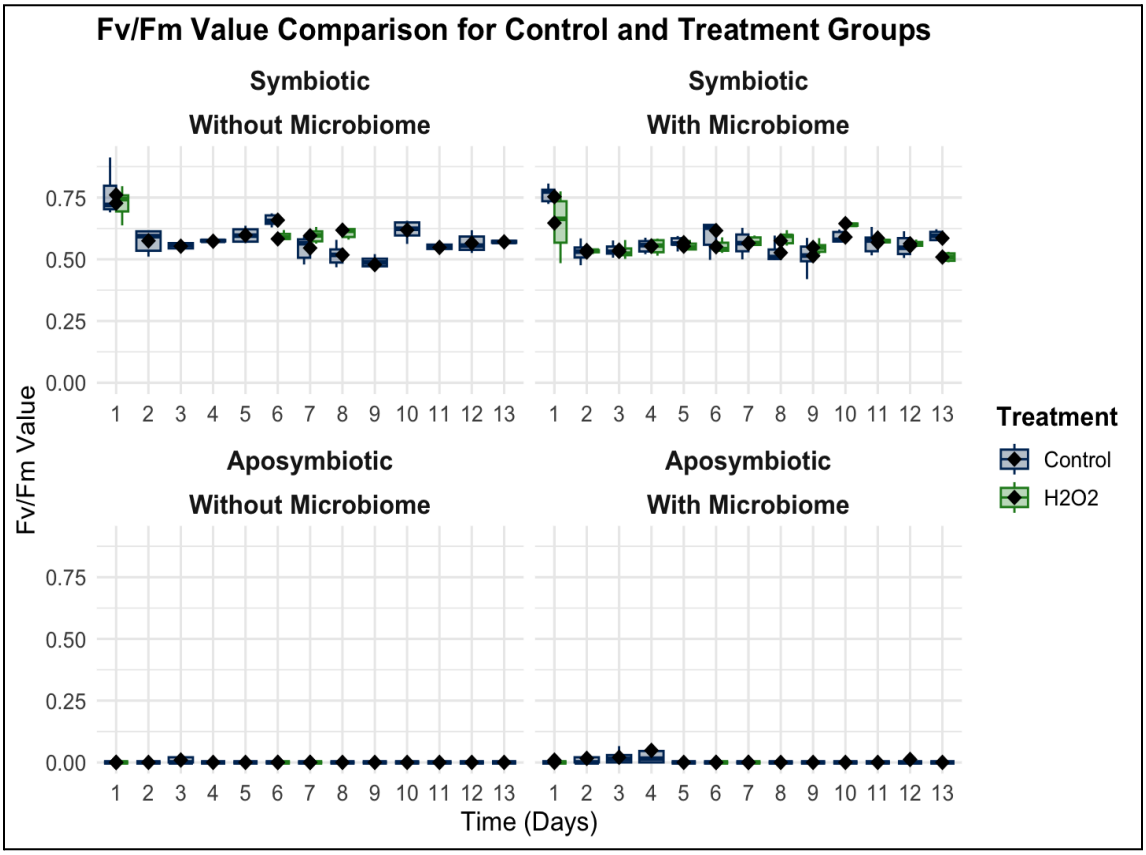

Figure 4. Fv/Fm Value Comparison for Control and Treatment Group.
Boxplots show the variation in Fv/Fm values across 13 days of measurement for different treatment groups (control and H_2O_2), faceted by phenotype (Symbiotic and Aposymbiotic), and microbiome presence (With or Without Microbiome) of *A. pallida*.

Each box represents the interquartile range, with the median indicated by the horizontal line. The x-axis represents the day of measurement, and the y-axis shows the Fv/Fm values. Treatment groups are color-coded, with dark blue representing the control and green representing the H_2O_2 treatment. Significant differences across groups were assessed using ANOVA.

Red Channel Assessment and Analysis

The average red color channel value slightly decreased over time in the H_2O_2 treatment with Symbiodinicaea and microbiomes but remained statistically insignificant (Fig. 5). Aposymbiotic *A. pallida* with and without a microbiome lysed on day 4 resulting in a red channel score of "n/a". This led to the red channel scores showing no visually consistent trends (Fig. 5). Symbiotic *A. pallida* without a microbiome lysed on day 5 resulting in a red channel score of "n/a" leading to no visually consistent trend lines (Fig. 5). Over the thirteen days, it was found that the red channel values of the symbiotic anemones with a microbiome in the H_2O_2 treatment had an insignificant decrease in red channel color value (or increase in red pigmentation) compared to that of the control group. The ANOVA test, performed on the values in the symbiotic with microbiome graph, showed $p>0.05$ indicating that the trend of decreased color value was insignificant (Fig. 5).

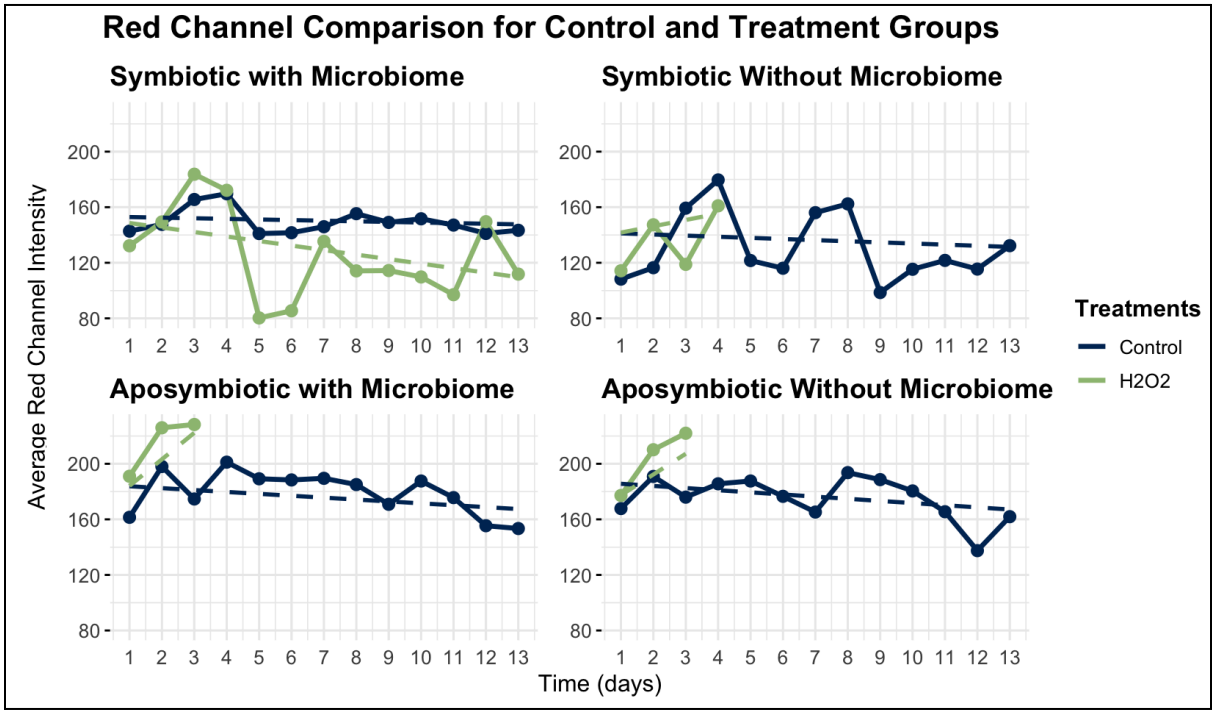

Figure 5. Red Channel Comparison for Control and Treatment Groups

Line graphs show the relation between control (sterile seawater) and treatment groups (0.008% H_2O_2) of *A. pallida*. Each point on the graph represents the average color value of the corresponding group. Each graph shows the progression of Red Channel Color Intensity across 13 days of the experiment. Blue represents the control groups and green represents the H_2O_2 control treatment groups. Each graph is positioned above as follows: Symbiotic with Microbiome (top left), Symbiotic Without Microbiome (top right), Aposymbiotic with Microbiome (bottom left), and Aposymbiotic Without Microbiome (bottom right). The dashed lines represent the line of best fit for each group. The Y-axis represents the average red channel color intensity between 255 (white) and 0 (red). The X-axis represents the time in days of the experiment.

Discussion

Our results indicate that the presence of both a microbiome and a symbiont within the holobiont creates conditions for increased resilience to induced oxidative stress. This is demonstrated through higher survival rates, maintained photosystem II functional capacity, and greater expandability scores in anemones containing both the microbiome and symbionts compared to those without. The data reveal significant differences in resilience between aposymbiotic and symbiotic individuals, with the microbiome further influencing the outcomes.

The mortality results show clear differences in resilience to oxidative stress between both aposymbiotic and symbiotic *A. pallida* under varying microbiome conditions in the H_2O_2 treatment group. This is in contrast to the control group's consistent 100% survival across all phenotypes and microbiome presence, emphasizing that neither the microbiome nor the presence of symbionts inherently affects survival and health under non-stressful conditions. However, under stressful conditions, cnidarians are known to produce a higher amount of H_2O_2 (Dungan et al., 2022), suggesting that oxidative stress exacerbates the impact of microbiome and symbiosis on resilience. The contrasting patterns of health and mortality under H_2O_2 treatment further emphasize the critical roles of both symbiosis and microbiome presence in mitigating oxidative stress.

Under oxidative stress, created by using a H_2O_2 solution, aposymbiotic *A. pallida*, regardless of microbiome presence, lysed on day 4. The complete mortality of all aposymbiotic individuals by day 4 suggests that the absence of symbiotic algae severely compromises oxidative stress defense mechanisms. In contrast, symbiotic *A. pallida* exhibited higher tolerance, with the presence of the microbiome mitigating the response to H_2O_2 as expandability scores fluctuated between 3 and 2. Symbiotic groups with a microbiome showed reduced expandability scores but maintained survival throughout the 13 days, further supporting the idea that the microbiome facilitates health and expansion under oxidative stress. Similarly, the extended survival of the symbiotic group without a microbiome, which experienced complete mortality on day 5, one day after the aposymbiotic groups, emphasizes the partial protection provided by symbiosis alone. Taken together, these findings suggest that both the microbiome and symbiosis play essential but distinct roles in enhancing resilience to oxidative stress.

The red channel results offer additional information into how the microbiome in symbiotic holobionts may mitigate the effects of oxidative stressors. As previously noted, the increased survivability observed in the symbiotic pair with an intact microbiome under H_2O_2 treatment highlights the microbiome's positive role in protecting against oxidative stress. This contrasts with the high mortality observed in other H_2O_2-treated groups without a microbiome, demonstrating how vital the microbial community is for resilience under stress (Dungan et al., 2022). While the red channel data for both the H_2O_2-treated and control groups does not explicitly confirm this finding, trends over the 13 days suggest color changes within anemones with microbiomes and symbionts exposed to H_2O_2. These enhanced color variations during the experiment imply cellular-level changes, potentially linked to the microbiome and symbionts' roles in stress responses (Johnson et al., 2007; McCauley et al., 2023). As bleaching is the result of the loss of symbionts, or rather the loss of pigmentation, noting the decrease in red color channels (or increase in red pigmentation) can be correlated with the high presence or density of symbionts within *A. pallida* (Siebeck et al., 2006). This higher presence was only associated with treatments that contained the microbiome and the symbiont (Fig. 5), alluding to the possibility that the microbiome had a minimal

impact on increased symbiont density. However, it is important to note that the red channel correlations did not have significant differences between the control and treatment groups.

Further analysis provides compelling evidence that H_2O_2 treatment significantly reduces photosynthetic efficiency in *A. pallida*, regardless of phenotype or microbiome presence. By normalizing Fv/Fm values, the relative changes in photosynthetic performance across different experimental conditions were assessed more accurately. Statistical analysis, including ANOVA, confirmed the robustness of these findings, with H_2O_2 treatment consistently leading to lower photosynthetic efficiency. These results emphasize the importance of considering both environmental and biological factors when evaluating the impacts of treatments on photosynthetic processes and overall resilience in cnidarians. This is especially relevant when considering the ability of *A. pallida* to exist in both aposymbiotic and symbiotic states, as the expulsion of symbiotic algae during stress, such as oxidative stress or high temperatures, can lead to bleaching, a process observed in previous studies (Dungan et al., 2022). The presence or absence of Symbiodiniacaea influences the phenotype of *A. pallida*, resulting in either a brown (symbiotic) or white (aposymbiotic) phenotype. The decreased photosynthetic efficiency under H_2O_2 treatment can be viewed in the context of both the stress-induced bleaching response and the dynamic relationship of the anemone and its symbionts, which may further inhibit its resilience to oxidative stress.

Future Directions

With a deeper understanding of the microbiomes within *A. pallida* and other cnidarians, future research can identify targeted strategies to enhance the conservation of marine ecosystems and mitigate the impacts of climate change. While this study demonstrated a significant increase in mortality under high H_2O_2 concentrations, it was conducted within strict time constraints. The data showed decreasing trends in the health of *A.pallida,* observed from changes in expandability and red channel scores, but a longer experiment would further support these findings and provide a better understanding of long-term effects. The red channel results were insignificant but could have indicated symbiont health if data throughout a longer period had been collected. In future studies, the effects of color within symbionts must be studied in correlation to time. Future work should extend the duration of exposure to H_2O_2, as longer treatment periods may reveal more results and information on cnidarian health and resilience. Our study focused on a single concentration of H_2O_2, but assessing the impacts of varying concentrations over time would provide a more comprehensive understanding of how cnidarians, and particularly their symbiotic microbiomes, respond to oxidative stress. Additionally, exploring the effects of a broader range of H_2O_2 concentrations would be valuable for determining a potential mortality threshold, which could inform guidelines for chemical use in marine environments. Another important area for future research is understanding how the increasing use of antibiotics, both in treating coral diseases and human infections, can impact the concentrations of antibiotics in coral reef ecosystems. This growing presence of antibiotics in the oceans may disrupt the microbiomes of cnidarians, compromising their resilience to environmental stressors. Understanding the mechanisms by which antibiotics alter these microbial communities is crucial, as the loss or alteration of beneficial microbes could weaken the ability of cnidarians to withstand environmental changes. By addressing these research gaps, we can better understand the intricate relationship between cnidarians, their microbiomes, and the broader environmental stressors they face, ultimately informing conservation strategies aimed at preserving marine biodiversity in the face of climate change.

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