

# It's Getting Hot in Here: Coral host genotype interacts with symbiont associations to determine thermal stress responses

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## ABSTRACT

The unprecedented rise of anthropogenic carbon dioxide emissions has resulted in a sea surface temperature rise of nearly 1°C since the mid-19<sup>th</sup> century, severely impacting tropical reef-building corals as they exist primarily within the upper threshold of their thermotolerance. With coral reefs under significant duress in the face of climate change, research has largely focused on the independent effects of coral species and symbionts on organismal thermotolerance. In this study, we aimed to determine to what extent host genotype and symbiont type interact to modulate thermotolerance levels within the tropical coral species *Pocillopora acuta*. To do so, we leveraged different combinations of *P. acuta* genotypes and symbiont types in a common garden experiment to study individual responses to heat stress. The results of our study suggest that the photosynthetic efficiency of host Genotype Y is most resilient under thermal stress, regardless of the symbiont type hosted. We also found that corals hosting *Durussdinium* most effectively avoided bleaching while host-symbiont pair Z-C bleached most significantly. However, the significant tissue sloughing observed only in *Durussdinium*-hosting corals suggests variation in cell signaling between hosts harboring native vs. non-native symbionts. These findings suggest that both coral host genotype and symbiont type influence the holobiont's plasticity in response to thermal stress. The findings of this study offer new insights into the relationship between coral hosts and symbionts when subjected to thermal stress with applications for future research surrounding the holobiont and climate change.

## 1 | INTRODUCTION

Carbon dioxide emissions produced by changes in human industry and land use (Arneth *et al.* 2017) have presented a threat to the world's oceans that has not been recorded historically. Analysis of ice core data shows that present-day atmospheric carbon dioxide levels of 419.41 ppm (Keeling Curve 2023) are unprecedented over at least the past 420,000 years (Petit *et al.* 1999). Not only is the concentration alarming, but rates of carbon dioxide increase are now two to three times greater than ever observed (Hoesch-Goldberg *et al.* 2007), with concentrations nearly doubling since as recently as 1740 (Keeling *et al.* 2001). With 65% of this rise in emissions being attributed to the rise of global economies (Canadell *et al.* 2007), the upward trend shows no signs of slowing in the near future. Paleoclimate records have shown that seawater temperature increases of approximately 0.74°C (Hoesch-Goldberg *et al.* 2007) have occurred since the mid-19<sup>th</sup> century on account of Earth's enhanced greenhouse effect (Abram *et al.* 2016). This increase in long-term average temperature, along with the increasing frequency of marine heatwaves (Smith *et al.* 2013) can exacerbate the deleterious effects of local stressors (Gissi *et al.* 2021), increase metabolic demands, create energy deficits, reduce reproduction, alter protein structure, and stunt growth for a plethora of marine organisms (Smith *et al.* 2013) — especially at low latitudes where coral reefs are found (Abram *et al.* 2016).

Scleractinian corals in particular — which are major framework builders of coral reefs — suffer from elevated sea surface temperatures, as their symbiosis with Symbiodiniaceae breaks

down (Mayfield *et al.* 2018). Coral holobionts rely on their photosynthetic algal symbionts to produce carbon sugars, but thermal stress causes polyps to expel their symbionts in bleaching events that ultimately reduce the performance of the coral host (Pandolfi *et al.* 2011). Elevated sea surface temperatures are associated with the unprecedented increase in the frequency, scale, and severity of coral bleaching events since 1870 (Glynn 1993). In fact, of all stressors associated with climate change, coral health has been shown to be most sensitive to increased temperatures (Davies *et al.* 2017, Takahashi & Kurihara 2013, Wall *et al.* 2014). Even week-long spells of sea surface temperatures just 1-2°C above localized baselines can induce traumatic bleaching to tropical corals (Castillo *et al.* 2012). Wide-scale coral bleaching episodes known as “mass bleaching events” are associated with high mortality, depressed colony growth, and decreased reproduction amongst survivors (Mendes & Woodley 2002). This is particularly concerning in the tropics because most reef-building corals live near the upper threshold of their thermotolerance; as such, even slight increases in ocean temperatures will have severe consequences on the health of tropical coral reefs (Castillo *et al.* 2012). In addition to bleaching, increases in infectious diseases amongst corals have been linked to rising sea surface temperatures as thermal stress may suppress immune responses. (Mydlarz *et al.* 2010). With world economies losing between \$4 billion and \$24 billion annually from climate change-induced coral loss (Chen *et al.* 2015), there is an urgent need to understand coral resilience to prevent heat-induced extinction events and total loss of tropical reef ecosystem services.

Motivated by this accelerated degradation of coral reef ecosystems under climate change, the thermotolerance of coral systems has been extensively investigated over the past few decades. Different genotypes within *Porites astreoides* and *Pseudodiploria clivosa* corals exhibit distinct thermotolerance capabilities, even when harboring a similar composition of endosymbiotic Symbiodiniaceae (Kenkel *et al.* 2013, Klepac *et al.* 2023). *Cladocopium* symbionts are stress-sensitive and thus survive best in the tropic and subtropic regions where there is relative thermal stability (Yuyama & Higuchi 2014). *Durusdinium* symbionts, on the other hand, are currently understood to be stress-tolerant and capable of surviving high and low thermal fluctuations which allows them to survive in temperate regions of the ocean (Lien *et al.* 2007, Oliver & Palumbi 2011). A comprehensive global coral-symbiont network analysis also suggests that thermal resilience in the coral holobiont is primarily determined by the phylogeny of the coral host, rather than Symbiodiniaceae phylogeny or transmission mode (Swain *et al.* 2021). Yet, although the coral holobiont is comprised of the coral host and its symbiotic partners, most existing literature focuses on how associations with thermally tolerant Symbiodiniaceae, especially *Durusdinium* spp., enhance their resilience to thermal stress (e.g. Glynn *et al.* 2001, Berkelmans & Van Oppen 2006, Cunning *et al.* 2015).

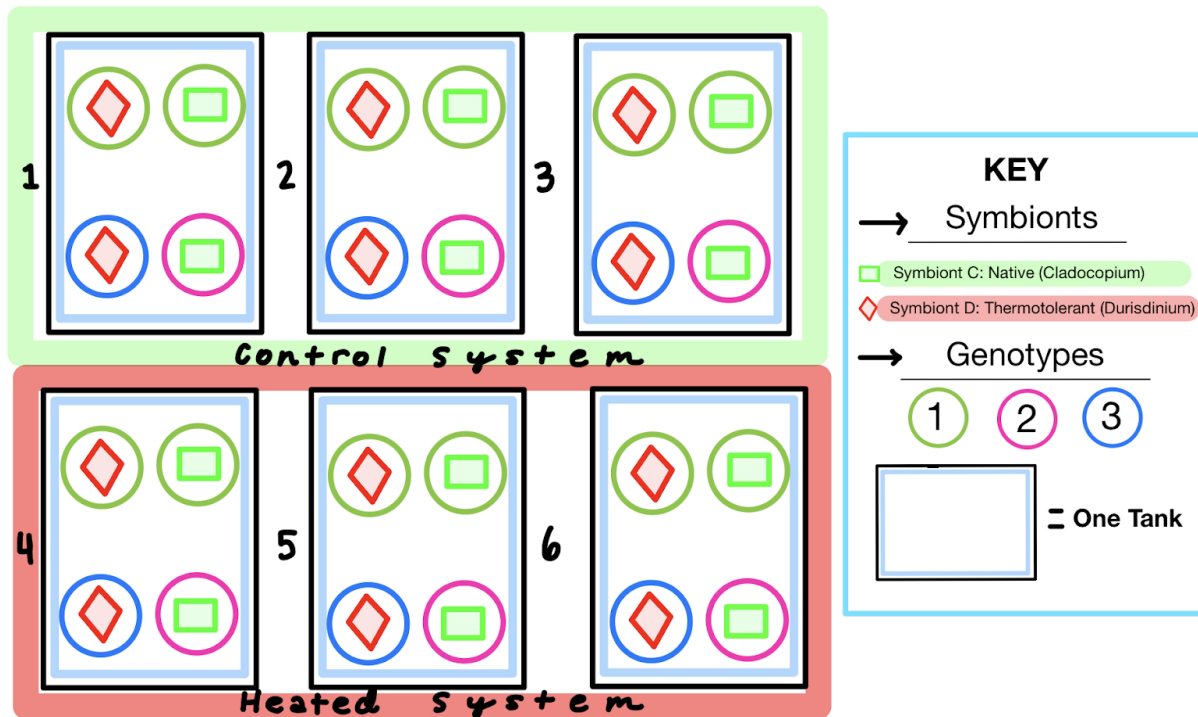
To disentangle the specific contributions of coral host genotype and symbiont type in shaping holobiont thermotolerance, we used *Pocillopora acuta* as a scleractinian coral model system within a common garden experiment to investigate the collective heat stress response. *P. acuta* is a widespread Indo-Pacific scleractinian coral species, known to form symbiotic associations with both *Cladocopium* and *Durusdinium* symbionts (Turnham *et al.* 2021, Poquita-Du *et al.* 2020). We utilized four distinct combinations of host genotype and symbiont type identified in prior investigations: X-D, Y-D, Y-C, and Z-C. One of the *P. acuta* genotypes, genotype Y, demonstrates the flexibility to host either *Cladocopium* or *Durusdinium* sp. while the other two *P. acuta* genotypes are constrained to host one of these Symbiodiniaceae genera. We hypothesized that the coral host genotype and symbiont type interact to shape the holobiont thermotolerance. The results of this study are expected to provide insights into the individual and

synergistic roles of the coral host and symbiont in determining holobiont resilience to escalating seawater temperatures.

## 2 | MATERIALS & METHODS

### 2.1 | Experimental Design

We conducted a common garden experiment with two experimental treatments, each of which had three tanks: Control: maintained at 26°C for the duration of the experiment, and Heat Treatment: temperature was ramped incrementally each experimental day from 26°C to 32°C and then maintained at 32°C for four days. All tanks experienced a 7.5:16.5-hour light-dark cycle with a light intensity of 85-92 photosynthetically active radiation units (PAR) and identical flow conditions were maintained via powerheads. The heated treatment was increased by 1°C each day until noticeable tissue sloughing was observed. Every tank held one fragment of each of the four *P. acuta* host-symbiont pairs, resulting in n=12 fragments per treatment; n=24 total. The host-symbiont pairs were sequenced and identified prior to the start of the experiment; sample IDs of the four unique combinations were as follows: X-D (host genotype 1, *Durusdinium*), Y-C (host genotype 2, *Cladocopium*), Y-D (host genotype 2, *Durusdinium*), and Z-C (host genotype 3, *Cladocopium*). Each nubbin was labeled using a colored zip tie to establish which of the four host-symbiont combinations the nubbin belonged to, and every zip tie was notched according to the number replicate tank in which the coral would be placed. The *P. acuta* fragments utilized in this study were acclimated in the Boston University Marine Program facility at 26°C and salinity of 33-35 parts per thousand (ppt) for roughly 10 years prior. Nutrients (nitrate, phosphate) and pH were tested at the beginning and end of the experiment. Salinity and temperature were measured multiple times per day. Consistent with previous care, the corals were not fed for the duration of the experiment.



**Figure 1.** Experimental design: Common garden heat challenge experiment consisting of two experimental systems, each containing three replicate tanks. Four nubbins were placed in every tank, each representing a unique host-symbiont combination.

On the final day of the experiment, two branches of each *P. acuta* nubbin were collected and preserved in liquid nitrogen. One branch will be used for subsequent gene expression analysis, while the other branch will be used for the analysis of physiological traits, including symbiont density, chlorophyll content, holobiont protein content, and carbohydrate content. All samples were stored at  $-80^{\circ}\text{C}$ . The remaining coral nubbins were retained in the experimental tanks, with the temperature gradually reduced to  $26^{\circ}\text{C}$ . The corals were maintained at this temperature until the initiation of the subsequent secondary thermal exposure experiment.

## 2.2 | Host and Symbiont Physiological Parameter Assessments

### (a) Pulse Amplitude Modulation Fluorometry

Pulse amplitude modulation (PAM) fluorometry was performed daily using a Heinz-Walz JUNIOR-PAM fluorometer. Readings of  $F_v/F_m$  indicate the photo efficiency of photosystem II which can be used as a proxy for the health of the algal symbionts. A low  $F_v/F_m$  shows reduced maximum fluorescence of the algal chloroplasts, thus signifying the inefficiency of photosystem II. PAM analysis on a healthy photosystem II will exhibit  $F_v/F_m$  values in the range of 0.5-0.7. PAM was performed on each day of the experiment after the corals had dark-acclimated for at least one hour.  $F_v/F_m$  measurements were taken from three random mid-branch locations on each coral nubbin to avoid bias towards healthy or bleached areas. Following every third day of PAM analysis, each nubbin was relocated in a clockwise fashion within the gridded base to account for lighting and flow differences within each tank.

### **(b) Tissue Sloughing**

Sloughing describes the phenomenon in which coral tissue rapidly peels away from its calcium carbonate skeleton before it has bleached. Once tissue sloughing was observed on nubbins belonging to the X-D host-symbiont pair, we captured daily photographs of the sloughing's progression in this specific pair. We then compared each photograph with the photograph of the same nubbin from the previous day. Based on this comparison, we were able to develop approximate quantitative estimates for the percentage of tissue sloughed across the later duration of the experiment. To ensure consistency, estimates for the percentage of tissue loss were determined by the same team member each day.

### **(c) Photo Analysis**

To avoid glare and ensure maximum clarity in our coral photographs, we utilized a LimoStudio photography box in the lab. This box was designed with three white 'backdrop' walls and a black top to prevent most outside light from entering. Three LED lights were set up on the outside of each of the white walls, creating a luminescent environment inside the box while preventing glare. Lighting conditions were consistent throughout all photograph intervals. All photographs were also taken using the same phone (iPhone 14 Pro Max). Corals were placed on a CoralWatch Coral Health Chart to control for white balance across photographs.

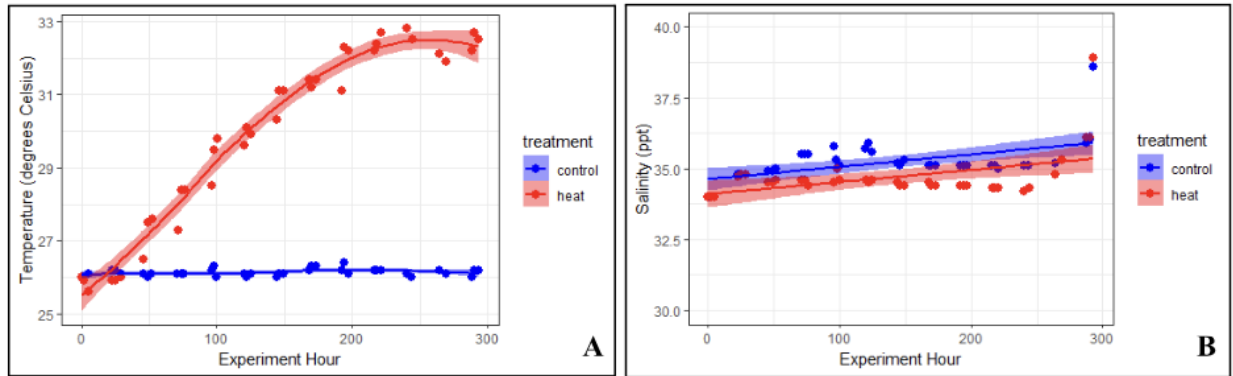
Photos of all fragments were taken on Day 0 and Day 12 to assess coral color changes over the course of the entire experiment. Photos were white-balanced in Photoshop version 25.1. White-balanced photos were then uploaded to MATLAB for color intensity analysis following Winter *et al.* (2009). In brief, ten random points on the coral in each photograph were selected, and the red, green, and blue color intensities were quantified and averaged for each individual.

## **2.3 | Statistical Analyses of Phenotypic Effects**

All statistical analyses used to compare differences in holobiont physiological responses were conducted in R 4.3.1 (R Core Team, 2023). For the photochemical efficiency of symbiont photosystem II, daily Fv/Fm across the duration of the experiment was analyzed within groups of 1) two temperature treatments, 2) two symbiont types, and 3) four host-symbiont pairs. Differences in daily Fv/Fm between two temperature treatments and two symbiont types were analyzed by applying a t-test to the dataset on each day. The analysis of daily Fv/Fm among host-symbiont pairs on each day was performed with ANOVA (analysis of variance). Tukey's HSD tests were used to examine post-hoc pairwise differences. Fv/Fm on the final experimental day (Day 12) was analyzed within groups of 1) each host-symbiont pair, 2) four host-symbiont pairs, and 3) two symbiont types. Differences in Fv/Fm within each host-symbiont pair between control and heat treatment were analyzed using t-tests, while the analysis of Fv/Fm among host-symbiont pairs was performed with ANOVA and post-hoc Tukey's HSD tests. A t-test was used to analyze the variations in Fv/Fm between corals hosting *Cladocopium* and *Durusdinium* symbiont. For coral bleaching, the percentage change in red channel intensity on the final experimental day was analyzed within groups of 1) each host-symbiont pair and 2) four host-symbiont pairs. Within each host-symbiont pair, the percentage change in red channel intensity was analyzed using t-tests, while ANOVA and Tukey's HSD tests were used to analyze the differences across host-symbiont pairs. Daily variations in tissue sloughing of the host-symbiont pair X-D was analyzed with a t-test applied on each day. For all statistical methods, the validity of normality assumptions was verified through Shapiro-Wilk normality tests.

### 3 | RESULTS

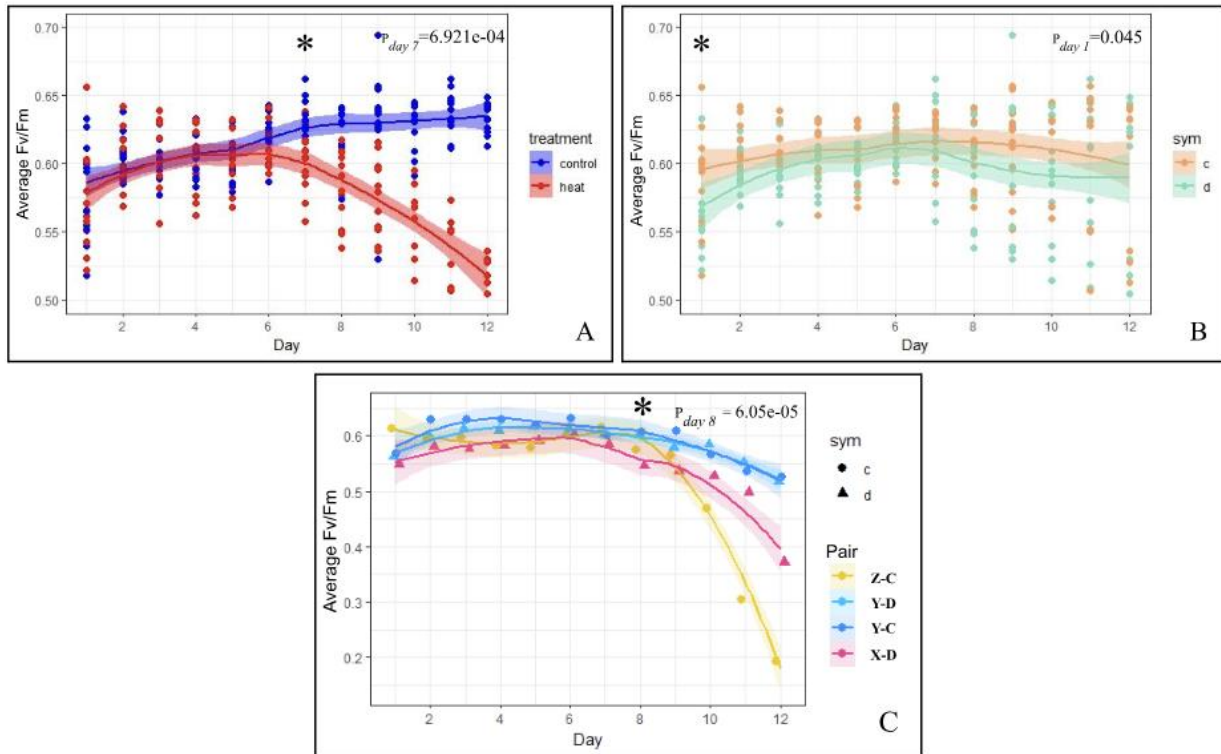
#### 3.1 | Temperature and Salinity



**Figure 2.** Hourly water conditions for the duration of the study. **A)** Temperature ( $^{\circ}\text{C}$ ) between the control system and the experimental heated system. **B)** Salinity (ppt) between the control system and experimental heated system. Each dot represents a salinity reading for one system; salinity measurements were taken 3 times per day for each 3-tank system. Colors indicate tank treatment. Lines indicate a local regression curve relative to proximate points and shaded areas indicate the 95% confidence interval.

The control system was held at a constant temperature of  $26.1 \pm 0.017$   $^{\circ}\text{C}$  for the duration of the experiment (Fig. 2A). The heated system began at the same temperature as the control system and was ramped approximately  $1^{\circ}\text{C}$  each day starting on Day 1 until sloughing and bleaching were observed. This resulted in the temperature plateauing towards the end of the experiment at  $32.4 \pm 0.079$   $^{\circ}\text{C}$  (Fig. 2A). Salinity was kept relatively constant for the duration of the experiment with the control system stabilizing at  $35.2 \pm 0.121$  ppt and the heated system stabilizing at  $34.7 \pm 0.138$  ppt (Fig. 2B). Anomalous salinity readings around hours 100 and 300 reflect issues with the filling of tanks via automatic pumps (Fig. 2B). However, since salinity values did not become concerningly high until the termination of the experiment, the temporary salinity fluctuation most likely had a negligible effect on nubbin physiological stress.

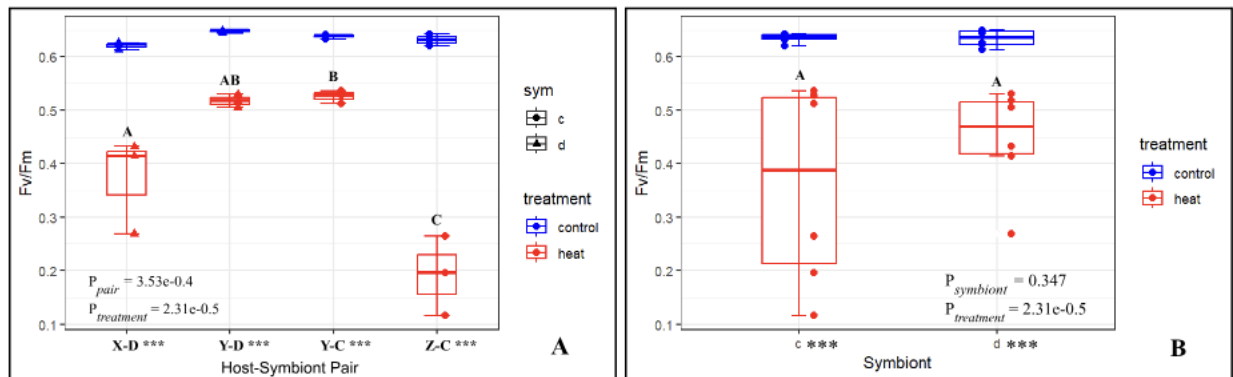
### 3.2 | Photosynthetic Efficiency (Fv/Fm)



**Figure 3.** Average photosynthetic efficiency (Fv/Fm) of *P. acuta* across the duration of the experiment was most impacted by treatment and host-symbiont pair. **A)** Average Fv/Fm of control (blue) and heat-treated (red) corals across the duration of the experiment. N=12 corals per treatment and control. **B)** Average Fv/Fm of corals hosting *Cladocopium* (orange) and *Durusdinium* (teal) across the duration of the experiment. N=12 corals per symbiont type. **C)** Average Fv/Fm of each host-symbiont pair studied across the duration of the experiment in the heated tanks only. Each dot represents the average Fv/Fm for n=3 nubbins of the same host-symbiont pair within the heated treatment. Colors represent host-symbiont pairs and shapes represent symbiont hosted. Lines in all panels indicate a local regression curve relative to proximate points, and shaded areas indicate 95% confidence intervals. Asterisks [\*] indicate the first day during the experiment where a statistically significant difference was observed between variables. Note: y-axis varies for Figure 3C.

An unpaired two-sample t-test run between treatments for each study day proved that average Fv/Fm was similar between control and heated treatments until Day 7 when trends diverged significantly (t-test,  $p=6.92e-4$ ); average Fv/Fm between treatments remained statistically significant from Day 7 until the end of the experiment on Day 12. Average Fv/Fm values from the control treatment demonstrated a positive trend until the end of the experiment while those from the heat treatment decreased consistently (Fig. 3A). An unpaired two-sample t-test run for each day between symbiont types proved that average Fv/Fm between symbiont types (summed from both treatment conditions) was only significant on Day 1 (t-test,  $p=0.045$ ); average Fv/Fm between symbiont types remained statistically insignificant from Day 2 until the end of the experiment on Day 12 (Fig. 3B). A one-way ANOVA run for each study day across all host-symbiont pairs proved that average Fv/Fm was similar until Day 8 when host-symbiont pairs diverged significantly (ANOVA,  $p=6.05e-05$ ); differences between at least two host-symbiont pairs remained statistically significant from Day 8 until the end of the experiment on Day 12 (Fig. 3C). Host-

symbiont pairs Z-C and X-D were the first to show significant declines in Fv/Fm (Tukey's HSD test,  $p < 0.05$ , Fig. 3C).



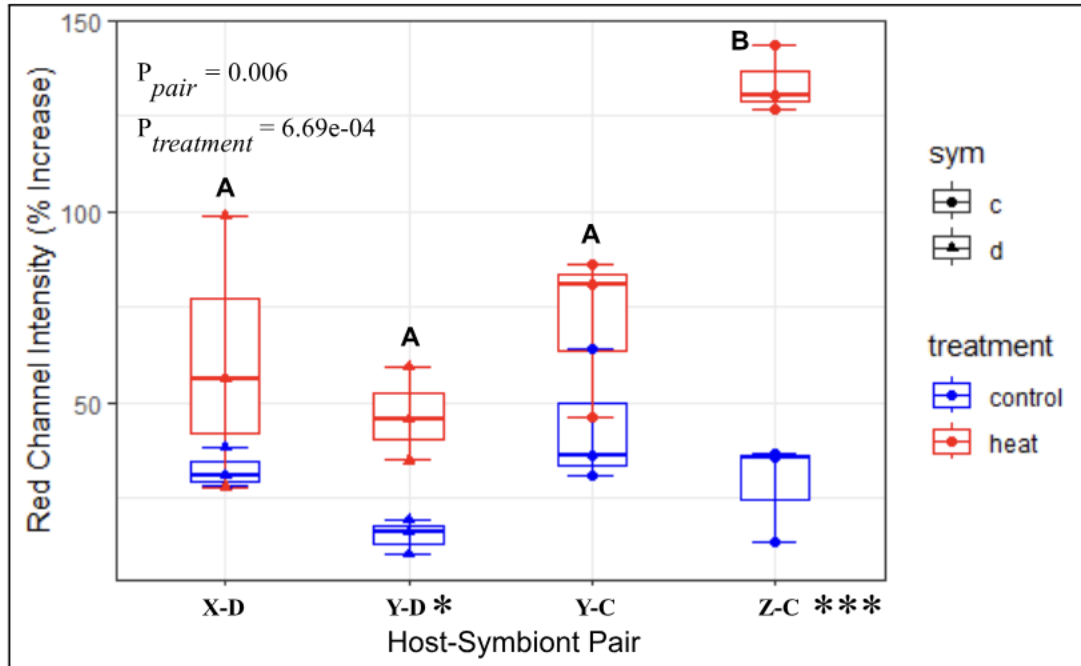
**Figure 4.** Fv/Fm on the final experimental day was most impacted by the host-symbiont pair. **A)** Mean Fv/Fm values across four experimental host-symbiont pairs, X-D, Y-D, Y-C, and Z-C on the final experimental day. Circular points represent corals hosting symbiont *Cladocopium* while triangular points represent corals hosting symbiont *Durusdinium*. **B)** Mean Fv/Fm values between corals hosting *Cladocopium* and *Durusdinium*. Each point represents the mean Fv/Fm value (averaged across 3 PAM measurements) for  $n=1$  nubbin. Labels that contain asterisks (\*) represent a significant difference in Fv/Fm between control and heated corals (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). Differing letters above the heat treatment boxes represent a significant difference in Day 12 Fv/Fm between host-symbiont pairs. Error bars each represent one standard error.

At the end of the 12-day experimental period, significant differences in Fv/Fm were found upon conducting a one-way ANOVA test between different host-symbiont pairs, but no significant difference was found between corals hosting *Durusdinium* and *Cladocopium* (ANOVA,  $p=0.347$ ). While there was no significant difference between the two host-symbiont pairs of genotype Y, host-symbiont pair Z-C exhibited significantly lower Fv/Fm than all other pairs (Tukey's HSD test,  $p < 0.5$ ). All host-symbiont pairs and both symbionts showed significant differences in Fv/Fm values across treatments (t-test,  $p=2.31e-05$ ).

### 3.3 | Red Channel Intensity (Bleaching)

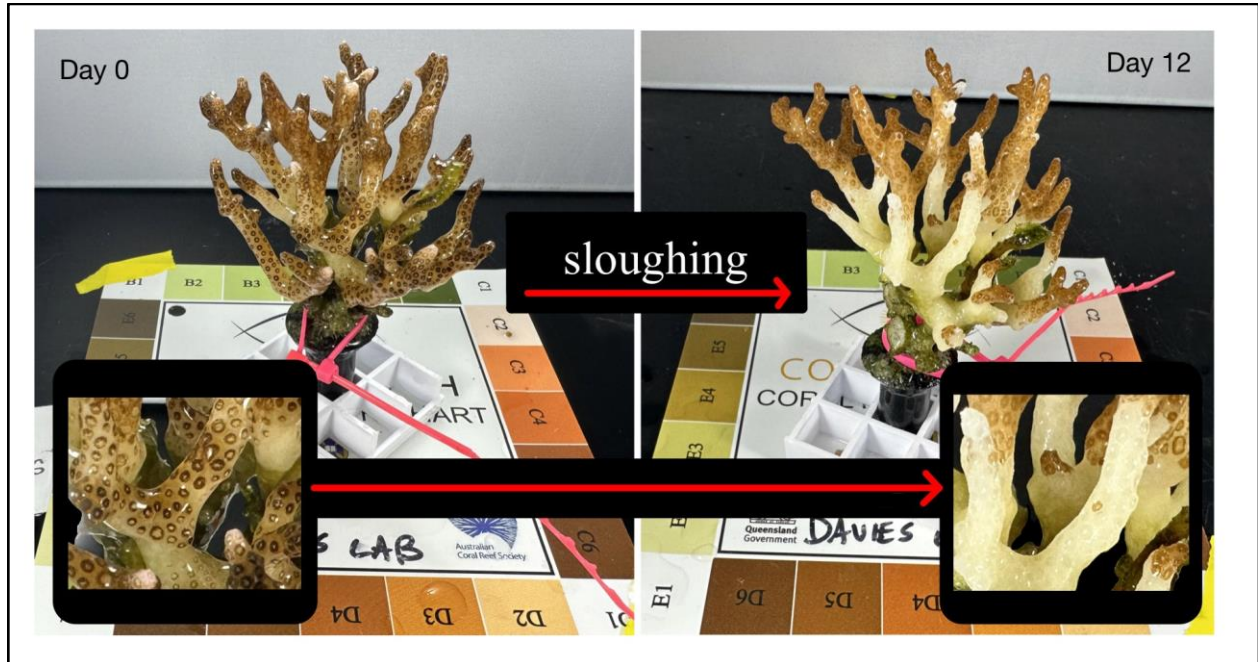
At the end of the 12-day experimental period, we found significant differences in the percentage change in red channel intensity with respect to heat treatment (t-test,  $p=6.69e-04$ ) and host-symbiont pair (ANOVA,  $p = 0.006$ , Fig. 5). Heat treatment resulted in a significant increase in the percentage change in red channel intensity for Y-D (t-test,  $p = 0.014$ ) and Z-C corals (t-test,  $p < 0.001$ ). In addition, Z-C exhibited a  $134 \pm 4.99\%$  increase in red channel intensity under heat treatment which was significantly greater than the red channel intensity increase of X-D, Y-D, and Y-C corals (Tukey's HSD test,  $p < 0.05$ ). Thus, host-symbiont pair Z-C experienced the most severe bleaching of all pairs studied.





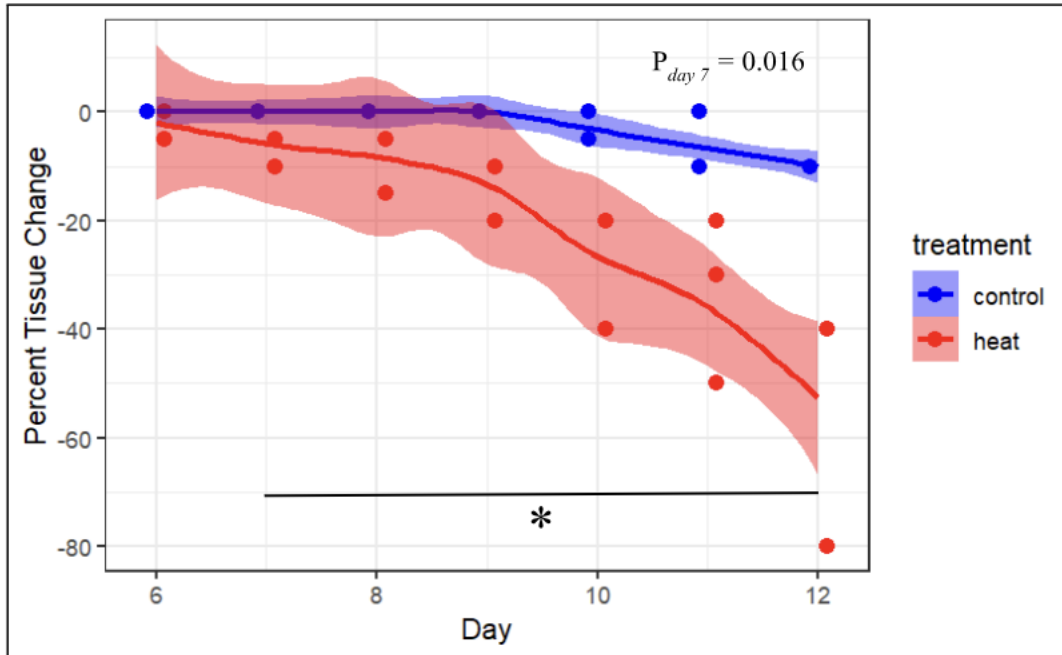
**Figure 5.** Differences in bleaching were identified across host-symbiont pairs on the final experimental day, assessed by the percentage change in the red channel intensity in standardized coral photographs. Circles: corals hosting symbiont *Cladocopium*; triangles: corals hosting symbiont *Durussdinium*. Blue: control (26°C); red: heat treatment (32°C on Day 12). Error bar =  $\pm$  SE. Labels containing asterisks (\*) represent a significant difference in percent change in red channel intensity between control and heated corals (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). Differing letters above the heat treatment boxes represent a significant difference in percent change in red channel intensity among host-symbiont pairs in the heated tanks.

### 3.4 | Tissue Sloughing



**Figure 6.** Notable tissue sloughing was observed from coral host X-D, which hosted the *Durusdinium* symbiont. Photos are a side-by-side comparison of the same nubbin (X-D6) before and after experiencing thermal stress over 12 days; photographs were taken on experimental Day 0 and Day 12 against a standardized coral health color chart.

Throughout the 12-day study, we observed increasingly severe levels of sloughing within corals in the host-symbiont pair X-D. Sloughing was defined as the rapid peeling of coral tissue away from its calcium carbonate skeleton (Fig. 6). Sloughing commenced prior to bleaching in nubbins X-D5 and X-D6, and after bleaching on X-D4. Sloughing was first observed towards the base of each nubbin before it spread towards the tips of each branch (Fig. 6). Though sloughing occurred at different rates on each nubbin, it was observably present on every nubbin on host-symbiont pair X-D.



**Figure 7.** Tissue sloughing on individuals of host-symbiont pair X-D was significantly worse in the heated treatment. Differences in sloughing between treatments remained statistically significant from Day 7 until the end of the experiment on Day 12. Dots represent the percent tissue loss for each X-D nubbin on each given day. Colors represent treatment, lines indicate a local regression curve relative to proximate points, and shaded areas represent 95% confidence intervals. (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ )

Over the first six days, no tissue sloughing was observed. However, on Day 6, tissue sloughing commenced on X-D fragment 5 within the heated treatment (Figure 7). Unpaired two-sample t-tests were run to compare control and heated X-D nubbins for each day and proved that tissue sloughing in each treatment diverged significantly beginning on Day 7 (t-test,  $p = 0.016$ ; Figure 7). Differences in sloughing between treatments remained statistically significant from Day 7 until the end of the experiment on Day 12. Sloughing was observed in nubbins of X-D within the control treatment, but only to an average loss of  $10.0 \pm 0\%$ . Conversely, by Day 12, X-D nubbins within the heat treatment had an average loss of  $53.3 \pm 13.3\%$ . Since tissue that has sloughed is incapable of recovery following experiment termination, the temperature in the heated tanks was subsequently decreased in order to allow for future experiments using the nubbins. Though not to the same extent as X-D, minor sloughing was also observed in heated nubbins of host-symbiont pair Y-D. Therefore, sloughing was solely observed in corals hosting symbiont *Durussdinium*.

#### 4 | DISCUSSION

We found that the coral host and the symbiont type interact to shape the holobiont thermotolerance in *P. acuta* coral. Our results suggest that the coral host exerts a greater influence on thermotolerance than the symbiont partner. The Y-D and Y-C fragments, which are of the same genotype but host different symbionts — *Durussdinium* and *Cladocopium*, respectively — had the least affected photosynthetic efficiencies by the end of the experiment. Fragments X-D and Z-C showed the highest decline in photosynthetic efficiencies, indicating that genotype Y was the most thermotolerant. Although the Symbiodiniaceae type does not necessarily correlate with the

photosynthetic efficiency in the corals that host them, we do see that the host-symbiont pairs affect whether coral bleaching and/or tissue sloughing will occur. Our findings regarding the red channel intensity, a quantitative representation of coral bleaching, show that the Y-D and Z-C fragments had statistically significant increases in this intensity. We were able to physically observe that all fragments containing the symbiont *Durusdinium* showed sloughing during the latter half of the experiment, with the X-D fragments suffering from the highest sloughing rates.

#### **4.1 | The role of the host-symbiont pair in shaping holobiont thermotolerance**

Our results for both photochemical efficiency (Fv/Fm) and bleaching congruently demonstrate that the coral host genotype and symbiont type interact to shape *P. acuta* holobiont thermotolerance rather than the Symbiodiniaceae type alone. This contradicts the findings of Mieong *et al.* (2009), who manipulated coral-Symbiodiniaceae combinations by infecting genetically divergent populations of *Acropora millepora* with *Symbiodinium*, *Cladocopium*, and *Durusdinium* sp. respectively. They identified the symbiont type as the most important predictor for holobiont fitness and thermotolerance, while minimal to no effect was observed from the coral host population. The potential conflicts may be attributed to the use of heterologous (i.e. opportunistic strains hosted following disturbance/algae isolated from a different species of the host) symbiont for infection in Mieong *et al.* (2009). In contrast, our study specifically explored corals naturally associated with their homologous (i.e. symbiont strains hosted under ambient conditions/algae obtained from the same host species) symbionts. The distinct mechanisms involved in detecting homologous and heterologous symbionts, along with the activation of divergent immunity pathways (reviewed by Bove, Ingersoll *et al.* 2022), may contribute to incomparable results between Mieong *et al.* (2009) and our study. In addition, the genetic adaptation of both the coral host and symbiont was demonstrated to collectively enhance the thermal resilience of the holobiont. Howells *et al.* (2016) found that *Platygyra daedalea* corals hosting *Cladocopium* spp. from the hot Persian Gulf exhibited higher thermotolerance compared to their counterparts hosting the presumably thermal-tolerant *Durusdinium* spp. from the milder sea of Oman. The Persian Gulf genotype of *P. daedalea* displayed an enhanced capability to mitigate oxidative stress, with its *Cladocopium* symbiotic partners exhibiting superior retention of photosynthetic performance under elevated temperatures (Howells, Abrego *et al.* 2016). The variations in the holobiont thermotolerance identified in our study may arise from the concurrent genetic adaptation of host-symbiont pairs in *P. acuta*. Subsequent investigation into the transcriptomics of *P. acuta* should elucidate the mechanisms of interaction dynamics between host genotype and symbiont type, revealing how these associations influence holobiont thermotolerance.

#### **4.2 | Evidence of *Durusdinium*-induced tissue sloughing & implications for coral hosts**

An alarming phenomenon observed throughout the duration of this experiment was the sloughing of dead tissue from the coral's skeleton, in which the coral's pigmented tissue peeled off before significant bleaching was observed. One of the study's most unexpected findings was the prevalence of tissue sloughing (coral death) occurring before total bleaching in the two host-symbiont pairings (Y-D and X-D) containing *Durusdinium*. A statistically significant difference in sloughing ( $p=0.01613$ ) was first observed between the X-D nubbins in the control system and the X-D nubbins undergoing heat stress on Day 7. Because sloughing was not expected, our sloughing data from this study is limited and does not reflect the tissue sloughing that was also

observed in Y-D nubbins. Sloughing observations are significant since a key focus of this study was how the symbiont genus *Durusdinium* interacts with its *P. acuta* host coral holobiont when facing thermal stress. *Durusdinium* spp. have become invasive in the Greater Caribbean reef system, establishing new symbioses and potentially shifting the clade composition of corals worldwide (Lawson 2020). Previous research suggests that *Durusdinium* spp. can enhance coral resilience against heat stress (Glynn *et al.* 2001, Berkelmans & Van Oppen 2006, Cunning *et al.* 2015), implying that corals that are biologically invaded by *Durusdinium* spp. may actually fare better under climate change than tropical corals hosting native symbionts, such as *Cladocopium* spp. However, previous literature focuses on bleaching as the primary physiological indicator of coral suffering and often fails to recognize that under rapidly onset heat stress, corals may not get the chance to reach a bleached state before dying.

While the root cause of tissue sloughing in corals is not well understood, research concerning sponges suggests that sloughing may be an organism's attempt to rid itself of a micro-fouling community being established on its surface (Barthel & Wolfrath, 1989). Theories on coral bleaching mechanisms in response to suboptimal environmental conditions could also provide insight into why corals slough their tissue, as both stress responses effectively terminate their symbiotic partnerships. While it has been suggested that the host engages in this detrimental process to flush out damaging reactive oxygen species secreted from endosymbionts, more recent studies fail to establish this causal relationship and suggest that oxidative stress is unlikely to be the driver for symbiont expulsion (Nielsen *et al.*, 2018). However, non-host-associated “free” symbionts have been shown to comprise up to 30% of the total symbiont count within the tissue of naturally bleached corals (Ladriere, 2008). If certain non-symbiotic cells have been demonstrated to be able to live freely within a coral host without being expelled, cell-signaling molecules that increase in corals during heat stress may be going undetected in certain coral-symbiont pairs (Hawkins 2014). Tissue sloughing from corals may potentially be understood as an immune response to hosting a non-native symbiont under heat stress, as corals containing symbiont genus *Durusdinium* in this experiment responded with similar symptoms to those reported for rapid tissue loss syndrome (rapid death, tissue sloughing, full mortality not necessarily associated with bleaching) (Shaver *et al.* 2017). The differential sloughing observed could also be explained by the increased expression of anti-viral transcripts observed in corals hosting *Cladocopium* spp. (Levin *et al.* 2017). The susceptibility of corals hosting *Durusdinium* spp. to tissue loss under heat stress — in contrast to the bleaching response of genotypes containing *Cladocopium* spp. — may suggest that coral hosts are less able to detect and expel non-native symbionts or pathogens, resulting in the stressed organisms ridding themselves of tissue entirely to terminate their symbiosis. It should be noted that this outcome was not an expected finding of this experiment, so further research specifically concerning tissue sloughing induced by heat stress between corals containing native and non-native symbionts should be conducted. Additionally, research concerning the mechanisms of cell signaling between coral hosts and non-native symbionts is needed to prevent future rapid, unrecoverable loss of coral reefs increasingly hosting invasive dinoflagellates.

### **4.3 | Symbiosis & its relationship to coral immunity**

Studies have shown that symbiosis between cnidarian host and symbiont downregulates pathogenicity defense pathways (Mansfield *et al.* 2017) with aposymbiotic individuals demonstrating greater survival rates than symbiotic hosts after exposure to *S. marcescens*

(Mansfield *et al.* 2019). Replication of this study with the integration of aposymbiotic *P. acuta* would not be possible as *P. acuta* is an obligate symbiont, but performing such an experiment with a closely related *Pocillopora* species may help us understand how Symbiodiniaceae impact cnidarian thermotolerance.

A symbiotic state has also been proven to downregulate the nuclear factor kappa light chain (NF- $\kappa$ B) transcription factor pathway, an integral constituent of immunity in many organisms (Mansfield *et al.* 2017). Disruption of the NF- $\kappa$ B pathway may lead to, or increase susceptibility to, disease (Mansfield *et al.* 2017), particularly in symbiotic individuals as proven by Riviera & Davies 2021. According to this study, NF- $\kappa$ B binds more effectively to DNA in aposymbiotic cnidarians than in symbiotic cnidarians. Further research should regard the relationship between stress exposure and the downregulation of the NF- $\kappa$ B pathway to supplement existing knowledge of cnidarian immunity.

However, the NF- $\kappa$ B pathway is but one aspect of cnidarian immunity. Starvation upregulates the NF- $\kappa$ B pathway in the cnidarian species *Exaiptasia pallida* (“*Aiptasia*”), which suggests prioritization of this pathway under energy-limiting conditions (Valadez-Ingersoll *et al.* 2023). Conversely to symbiosis, starvation is more effective at protecting symbiotic *Aiptasia* against pathogen-induced mortality than it is at protecting aposymbiotic *Aiptasia* (Valadez-Ingersoll *et al.* 2023). Current research addressing the combined effects of symbiosis and starvation on the cnidarian NF- $\kappa$ B pathway and subsequent immunity is lacking.

## 5 | CONCLUSIONS

Our results challenge the long-established view that coral resilience to elevated temperatures is exclusively determined by the coral host or symbiont type. Instead, we identify a synergistic effect between coral host genotype and symbiont type in shaping holobiont thermotolerance. We also introduce tissue sloughing as a new and valuable proxy for holobiont health, alongside conventional indicators such as photosynthetic efficiency and bleaching. Future work will target the transcriptomic responses of these coral-symbiont pairs to thermal stress with a specific emphasis on understanding the associated immunity mechanisms.

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