

2022

Stressed out in a changed world: investigating the strength of the temperate coral response to acute and chronic anthropogenic stress

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BOSTON UNIVERSITY
GRADUATE SCHOOL OF ARTS AND SCIENCES

Thesis

**STRESSED OUT IN A CHANGED WORLD:
INVESTIGATING THE STRENGTH OF THE TEMPERATE CORAL
RESPONSE TO ACUTE AND CHRONIC ANTHROPOGENIC STRESS**

by

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B.A., Kenyon College, 2018

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science

2022

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ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. Randi Rotjan for her support, guidance, optimism, and endless energy for “wall-science”, without whom this thesis would not have been possible. I would also like to extend my utmost gratitude to Cassandra Swartz, who shall now be revered as the coral-fragmentation expert, for her insurmountable enthusiasm and dedication to this project. Additionally, I owe many thanks to Mikhayla Osborne, Aby Yoon, Anna Lapadula, and Marius Siscar for their hard work and diligence during this experiment. And to Isabela Trumble, who’s role in designing, fabricating, and building the experimental aquarium system was invaluable, and for her constant support and encouragement throughout this process.

I would also like to thank the following members of the Rotjan Lab for their support and instrumental contributions to this project: Caroline Fleming, Brian Kennedy, Anna Gauthier, Stephanie Kennedy, and Jacob Jaskiel. Additionally, from the Davies’ Lab: Hannah Aichelman, Dr. Colleen Bove, and Alexa Huzar, for their help with methodology, statistical analysis, and modeling.

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ABSTRACT

Both global and local chronic environmental stressors associated with climate change and anthropogenic sources are increasing in severity, and can compromise the resilience of key marine ecosystems such as foundational coral reefs. Despite the impacts, however, there are major knowledge gaps in our understanding of direct interactive and compounding effects of multiple chronic environmental stressors on coral animals. Further, chronic stress may have sublethal, downstream impacts; for example, inhibiting the recognition and response to sudden acute stressors in the marine environment. The goal of this study was to determine the direct impacts of multiple chronic environmental stressors - elevated temperature (global), microplastic pollution, light availability, and food availability (local) - on survival and physiological performance of the emerging temperate coral model *Astrangia poculata*, and to determine how exposure to different chronic stressors affects their ability to deal with sudden acute stress. To achieve this, we exposed individual coral polyps to different combinations of stressors, and quantified the

response of the coral host (growth) and symbiont (photosynthetic efficiency, chlorophyll a density). Coral polyps were then challenged with an acute stress near the onset, midpoint, and end of the experiment to quantify the impact of chronic environmental stress on the ability to mount a response to an acute stress. We found that the local stress of microplastic exposure had no impact on lethal or sublethal measures of the coral holobiont, while light was beneficial in maintaining coral mass. In contrast, elevated temperatures (representing global stress) reduced survival, diminished host and symbiont performance, and repressed the coral metabolic response under acute stress challenge. Feeding, however, was beneficial in preserving symbiont function, but has consequences for fitness and coral growth when presented with thermal stress, lending support to the growing hypothesis that this established mutualism shifts towards parasitism in stressful environmental conditions. Despite the magnitude of these combined stressors, over 80% of coral polyps survived, highlighting the overall resilience of *A. poculata* to diverse environmental challenges. These findings underline the complexity with which anthropogenic stressors interact to affect coral survivorship and resilience to future global change.

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INTRODUCTION

Organismal response to immediate and acute stress has been the topic of rigorous scientific investigation because intense short-term vulnerability can have long-term implications for downstream fitness (Crespi and Warne 2013). Downstream effects can induce immediate mortality or impose sub-lethal permanent effects that, in some cases, can be conferred to new generations (Coslovsky and Richner 2011; Pravosudova et al. 2001). In the Anthropocene, acute stress responses are compounded by the persistent and underlying chronic stressors of global change, which increase organismal exposure to elevated temperatures, changes in climate patterns, and increasing storm frequency and intensity (Letcher 2021). For marine organisms, these are further exacerbated by the addition of oceanic chronic stressors including changes in sea level rise (Bellard, Leclerc, and Courchamp 2014), ocean temperature (Abraham et al. 2013), and ocean pH (Hönisch et al. 2012).

Environmental context determines the degree to which an organism can respond to acute stressors. Organisms that are exposed to prolonged environmental stressors (aka chronic stress), respond differently to acute stressors such as the threat of predation or an acute novel pathogen (Hughes et al. 2003), compared to organisms that have not experienced chronic stress. Regardless of which chronic stressor most strongly impacts any given organism, it has been previously shown that exposure to chronic stress can influence organismal tolerance and resilience to acute stress in multiple taxa including

birds (Dickens et al. 2010), amphibians (Hall et al. 2020), plants (Boscaiu et al. 2013) and fish (Brunel and Boucher 2007). Populations of the freshwater frog *Lithobates sylvaticus*, for example, have been subjected to persistent salinization of freshwater habitats due to chronic salt runoff which, as an energetically costly stressor, has amplified the susceptibility of these frogs to the common ranavirus, an acute stressor associated with mass-mortality events (Hall et al. 2020). Organismal response to an acute stressor is mediated by the state of the environment and determines the degree to which an organism is capable of responding. However, the known consequences of chronic stress in the era of global change may need to be re-examined.

Rising global ocean temperatures remain one the largest threats to ocean ecosystems worldwide. Studies show that warming ocean temperatures can lead to increased levels of cortisol hormones - key indicators of stress - in both larvae and juveniles of European sea bass (Goikoetxea et al. 2021). Additionally, decreases in fish recruitment and stock productivity have been associated with increased temperatures (Brunel and Boucher 2007). In foundational coral reef ecosystems, rising temperatures have led to a decrease in calcification and growth (Kornder, Riegl, and Figueiredo 2018), a reduction in biomass of the coral host and algal symbiont (ie. holobiont) (Thornhill et al. 2011), declining coral recruitment and survivorship (Hughes et al. 2007), and induced mass coral bleaching events (Heron et al. 2016; Wilkinson and Souter 2008; Hughes et al. 2003; Hoegh-Goldberg 1999) leading to the decline of coral reef ecosystems and their associated communities.

Further, rising ocean temperatures have altered key processes in the marine environment. Increased temperatures have been shown to alter seasonal phytoplankton blooms as well as decrease overall phytoplankton abundance (Gittings et al. 2018). As ocean warming spreads, it exacerbates thermal stratification and prevents cold, nutrient-dense deep water from entering the relatively nutrient-void surface layer, thereby limiting essential primary production of phytoplankton and other photosynthetic organisms (Polovina, Howell, and Abecassis 2008; Van de Waal and Litchman 2020). The lack of mixing concurrent with elevated temperatures can limit essential nutrients and reduce prey availability thereby adding additional chronic stress to the marine ecosystem at the very base of the food web (Edwards and Richardson 2004).

Chronic stressors, however, can also be more local in nature. For example, the increasing and ubiquitous presence of marine microplastic pollution in the ocean has put additional pressures on marine organisms. Microplastic pollution originates from both land and ocean sources (Schwarz et al. 2019; Li et al. 2016) and threatens reef ecosystems because of their proximity to land-based inputs, high fishing activity, and susceptibility to wind- and wave-mediated transport and deposition (Lartaud et al. 2021; de Oliveira Soares et al. 2020). For benthic organisms like corals, passive exposure to and adhesion of microplastics in the ocean can lead to reduced skeletal growth (Chapron et al. 2018; Mouchi et al. 2019; Reichert et al. 2019), reduced photosynthetic efficiency of endosymbionts (Lanctôt et al. 2020; Reichert et al. 2019; Syakti et al. 2019), tissue necrosis (Reichert et al. 2018; Syakti et al. 2019), and ultimately coral bleaching (Reichert et al. 2019; Syakti et al. 2019). Reef-building scleractinian corals have been

shown to ingest microplastics (Axworthy and Padilla-Gamiño 2019; Rotjan et al. 2019) and have been observed in-situ with microplastics embedded in internal tissues (Allen, Seymour, and Rittschof 2017; Huang et al. 2019; Montano et al. 2020; Rotjan et al. 2019). The direct consequences of ingesting microplastics include the consumption of potentially toxic plasticizer additives (Huang et al. 2020), nutrient loss and energy depletion, (Rotjan et al. 2019) and blockage of digestion and subsequent nutrient uptake (Hall et al. 2015).

These chronic stressors can be categorized in one of two ways based on the scale in which they operate. First, chronic stressors that impact a range of organisms simultaneously and across diverse ecosystems and ecological niches can be considered global stressors. Negative impacts of global chronic stress, like rising ocean temperatures, affect numerous taxa across marine ecosystems and can be seen in marine mussels from temperate estuaries (Marigomez et al. 2017), kelp forests in tropical, temperate, and polar regions (Smale 2020), and subtropical epipelagic fish in the Mediterranean Sea (Moltó et al. 2021). These global stressors are wide-reaching but can impact species and ecosystems differently depending on individual environmental contexts.

Second, more centralized chronic stressors are considered to be local, where impact is more limited to the immediate surroundings within a particular environment. Examples of local stressors are sedimentation from coastal development (Stubler, Duckworth, and Peterson 2015), sewage effluent and pollution runoff from urbanized areas (Kaczmarzsky, Draud, and Williams 2005), and nutrient (Zaneveld et al. 2016) and microplastic pollution (Patterson et al. 2020). While local stressors can be present in

multiple regions and affect a multitude of ecosystems, they are not present universally. As such, the interaction between local and global stressors is likely to be spatially and temporally dynamic, creating a shifting landscape of organismal exposure and impact. Chronic stressors take on multiple forms, and are increasing globally due to climate change and amplifying locally due to human population rise and urbanization; as such, global and local stressors can and often do interact. Pacific oysters, for example, are vulnerable to interactions between ocean acidification, rising ocean temperatures, and changing salinities in the Pacific Northwest region of the United States (Ko et al. 2014). Meanwhile, phytoplankton are impacted by the interaction between changes in carbon dioxide concentrations ($p\text{CO}_2$), elevated temperatures, nutrient limitation, and changes in light intensity, whereby adaptive responses may be size-dependent (Van de Waal and Litchman 2020).

These types of global and local chronic stress interactions have been observed in coral reef ecosystems, which are particularly vulnerable to environmental changes (Wilkinson 1999; Ban, Graham, and Connolly 2014). The coral holobiont, which is comprised of an animal host, algal endosymbiont, and other interacting taxa, are susceptible to global stressors that impact the holobiont as a whole (rising ocean temperatures), and local stressors that target the host (limited food abundance) and symbiont (light stress, sedimentation) independently (Mieog et al. 2009). Studies have investigated the impact of interactive chronic stressors such as rising temperatures and increasing ocean acidification on the dynamics of this symbiotic relationship (Abrego et

al. 2008; Ban et al. 2014; Grottoli et al. 2018; Reichert et al. 2021) in an attempt to unravel the ways in which corals may survive in a shifting climate.

In the oceans, coral reefs are experiencing dramatic declines globally as a result of chronic stress followed by pulses of acute stress such as coral bleaching (Berkelmans et al. 2004) and disease outbreaks (Muller et al. 2020; Bruno et al. 2007). In addition to chronic stressors, corals can experience a variety of acute stressors following storms (sedimentation) (Goatley and Bellwood 2013) or following short-term human inputs (pollution, various forms) (Lee and Mohamad 2009; van Dam et al. 2011; Patterson et al. 2020) that can all interact with each other. The ability of corals to tolerate these multitude of acute stressors has been the main focus of numerous studies to date.

To manage chronic and acute stressors, corals can increase their energetic resources to combat stress by leveraging heterotrophic feeding. Known as the heterotrophic rescue effect, heterotrophy has been shown to mitigate thermally-induced coral bleaching when autotrophic nutrient acquisition is eliminated or compromised (Rodrigues and Grottoli 2007; Aichelman et al. 2016). In one study, *Montipora capita* coral were able to maintain their daily metabolic energy requirements, usually obtained through endosymbiont photosynthesis, by increasing active consumption following bleaching (Grottoli, Rodrigues, and Palardy 2006). Others have shown that at chronic elevated temperatures, experimentally fed scleractinian corals maintain higher photosynthetic activity (Hoogenboom et al. 2012) and chlorophyll pigmentation (Borell et al. 2008) compared to starved corals, despite the significant disruption to photosynthesis within photosystem II.

To address how the holobiont may compensate for and respond to stress, understanding the specific role of endosymbionts in response to climate change stress and conferred resilience to the coral holobiont is an active area of investigation. One study found a high abundance of the symbiont clade *D* (proposed reclassification to genus *Durusdinium sp.*, LaJeunesse et al. 2018) present within scleractinian corals located in areas that were either chronically exposed to higher relative sea surface temperatures, or high frequencies of local sedimentation stress from coastal development and have demonstrated a history of coral bleaching (Stat and Gates 2011). This particular symbiont clade has been associated with higher thermotolerance and increased bleaching resistance in numerous other studies (Jones et al. 2008; Berkelmans and Van Oppen 2006), though the universality of this resistance is challenged (Abrego et al. 2008). One study suggests that flexibility to shift symbiont clade communities within the holobiont, rather than association with a single clade, may facilitate acclimatization and adaptation to climate change stress (Silverstein, Correa, and Baker 2012).

In most cases, shallow water tropical corals respond to acute stress via the loss of symbiosis (aka dysbiosis), or coral bleaching (Lough and van Oppen 2009). As such, it is difficult to discern the origin of holobiont failure (host or symbiont) when faced with stress. Coral symbionts are particularly sensitive to changes in light availability and irradiance (Anthony and Fabricius 2000; Hoogenboom et al. 2012), which can originate from increasing turbidity and sedimentation in shallow coastal waters (Anthony et al. 2004; Mi et al. 2019). In addition, temporal seasonality is a main driver of changes in light availability, leading to symbiont stress, loss of pigmentation and potentially coral

bleaching (Brown et al. 1999). As climate change intensifies and alters seasonal environments and atmospheric oscillation, algal symbionts within the coral holobiont may become increasingly stressed from light limitation.

Unlike the many obligate coral species that live in most tropical ecosystems, the temperate coral, *Astrangia poculata*, exhibit facultative symbiosis whereby these corals exist both in symbiosis with endosymbiotic algae, *Breviolum psygmophilum*, (symbiotic) and without (aposymbiotic). This facultatively symbiotic relationship enables the isolation of physiological responses from the host and symbiont independently under chronic and acute stress regimes, making this organism an essential and emerging model system (Neff 2020).

Importantly, the facultative nature of this symbiotic relationship confers a greater degree of resilience to chronic stressors. In regions where nutrient availability is highly variable this coral employs a mixotrophic strategy in order to meet their metabolic demands (Burmester et al. 2018; DiRoberts et al. 2021). Symbiotic corals rely on acquisition of photosynthates derived from their endosymbionts via autotrophy (Houlbrèque and Ferrier-Pagè 2009) while aposymbiotic corals primarily rely on heterotrophy through active predation (Muscatine et al. 1984). Food availability and light conditions are essential components for healthy corals, and therefore, we can determine the impact of food stress conditions on the host (aposymbiotic) and light stress conditions on the endosymbiont (symbiotic) in isolation.

Additionally, *A. poculata* has an extensive ecological range along the Eastern coast of North America and experience a high degree of seasonal thermal variation that

can range from 27-0°C between summer and winter months (Aichelman, Zimmerman, and Barshis 2019; Dimond and Carrington 2007). This facultative symbiosis and flexibility in feeding strategies have been shown to mitigate detrimental effects of thermally-induced coral bleaching (Rodríguez and Grottoli 2007; Aichelman et al. 2016), where heterotrophic, aposymbiotic corals are more resilient. This allows us to investigate the impact of environmental stressors on “bleached” corals (i.e., aposymbiotic) without inducing bleaching stress. However, while facultative symbiosis and flexibility in feeding strategies are advantageous for coping with some anthropogenic stressors, it also makes *A. poculata* more vulnerable to chronic local pollution, e.g. microplastics (Axworthy and Padilla-Gamiño 2019; Rotjan et al. 2019). Cities and other urbanized coastal areas located near or within the range of *A. poculata* contribute to the input of oceanic microplastic pollution (Mabry 2021), which has been shown to vector microbes among other consequences (Lamb et al. 2018; Rotjan et al. 2019; Feng 2020). There is also a relatively large body of literature on *A. poculata* demonstrating its response to thermal stress, food stress, wound healing, pollution, etc., making this an ideal system to test how symbiotic state, interacting with multiple stressors, can contribute to coral resilience.

In this study, we examined the influence of multiple chronic stressors on an emerging temperate model coral, *Astrangia poculata*. These multiple chronic stressors included a mix of globally-relevant stressors (elevated temperature) and locally-relevant stressors (food availability and microplastic pollution). Because of the facultatively symbiotic nature of this model coral, we were also able to test the relative contributions of symbiosis to chronic stress tolerance by manipulating light in the context of symbiotic

state (symbiotic or aposymbiotic). To determine which chronic stressor, or combination thereof, played the most substantial role on coral fitness, we tested coral polyp response to acute stress near the beginning, midpoint, and end of a 22-day chronic stress exposure in a randomized block design. Using this approach, we asked the key question: what stressor or combination of stressors most influences coral resilience, and how is that modulated by symbiosis? Under elevated temperatures, we manipulated light availability, food availability, and plastic exposure for both symbiotic and aposymbiotic colonies, and hypothesized that symbiotic colonies would be better able to tolerate chronic and acute stress whenever experimental conditions were favorable for the symbiont (no plastic pollution, light, fed).

METHODS

Collection, fragmentation, and husbandry

On July 13, 2021, 1 mixed, 25 aposymbiotic, and 27 symbiotic *Astrangia poculata* colonies were collected by SCUBA divers in water less than 10 meters at Fort Wetherill State Park in Jamestown RI (41° 28'40" N, 71° 21'34" W). All colonies were removed of surficial algae before transportation to Boston University where they were maintained in aquaria at 19°C with recirculating seawater (Instant Ocean ®) under 54W fluorescent aquarium lights (ATI, North Rhine-Westphalia, Germany) in 12h light:12h dark cycles. Colonies were fed frozen *Calanus fimarchius* copepods (Piscine Energetics, Vernon, BC) three times per week and left to acclimate to aquaria conditions for three weeks.

After acclimation, colonies were fragmented into individual coral polyps over five days using a handheld rotary saw (Dremel Manufacturing, Racine, WI, USA). Polyp fragments ranged from 1-37 per colony (16.3 ± 6.5 , mean \pm SD). All fragments ($n = 480$) were weighed to the nearest thousandth gram using a microscale (Mettler Toledo, USA) to determine initial dry mass for growth analysis. Fragments were adhered to custom-fabricated acrylic disks (57 mm diameter, 20 mm height) in groups of 5 polyps. Mounted experimental fragments were allowed to recover in the same ambient aquaria for at least 4 weeks before experimental treatments began to allow adequate recovery from

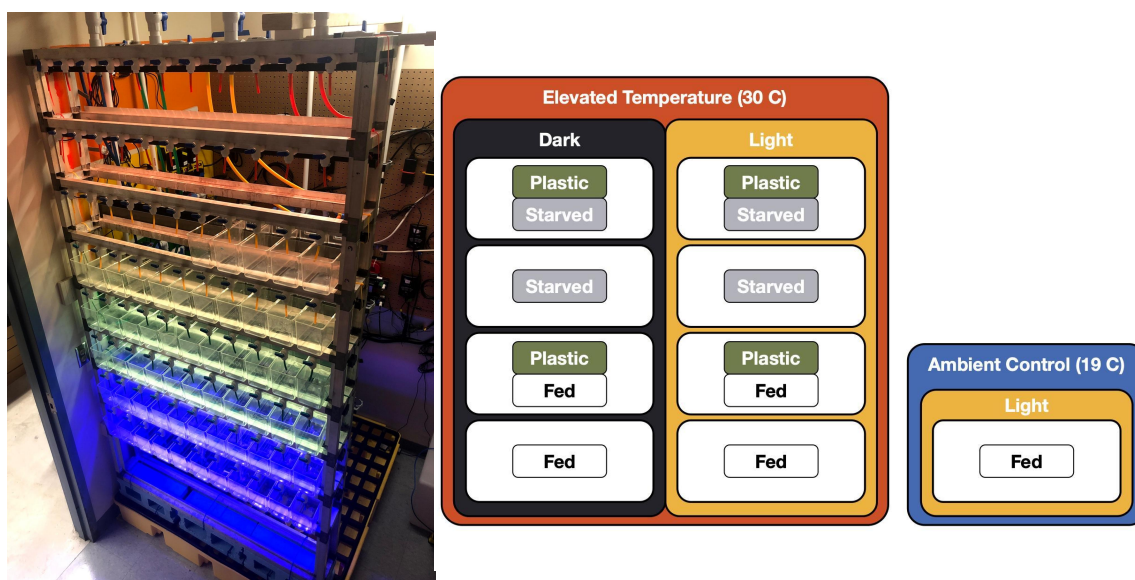


Figure 1. Experimental aquarium system (left) and conceptual design (right). The aquarium rack is equipped with four independent water sumps that each supply two rows of 1.6L tanks (11 tanks per row), so that each row is an independent treatment. Within the elevated temperature conditions (30°C) each treatment represents one of the eight possible combinations of exposure to chronic stress; exposure to plastic pollution, light availability, and food availability.

fragmentation. Coral microfragmentation has been recently utilized as an ecosystem restoration technique to promote rapid coral growth for outplanting in degraded reefs (Page, Muller, and Vaughan 2018). This novel technique enables quantification of stress response within individual polyps, which may be capable of resisting anthropogenic stress to the same degree as whole colonies (Schlecker et al. 2022; Page, Muller, and Vaughn 2018).

Experimental setup and manipulations

After recovery, 320 polyp fragments (n = 160 aposymbiotic, 160 symbiotic) were assigned to one of eight stress treatments in the experimental aquarium rack (Figure 1), while 160 fragments remained in the ambient control conditions (n = 80 aposymbiotic, 80 symbiotic).

The experimental treatments were combinations of three stressors; +/- plastics, +/- light, and +/- food and were all maintained at 30°C (Figure 1). Experimental treatments are designated by a three-string acronym associated with each stressor: P or N for plastic or no plastic, L or D for light or dark, and F or S for fed or starved. Polyp fragments from the same parental colony (genet) were divided across experimental treatments to account for genotype influence (assuming all polyps from the same colony are of a single genotype; Figure 2).

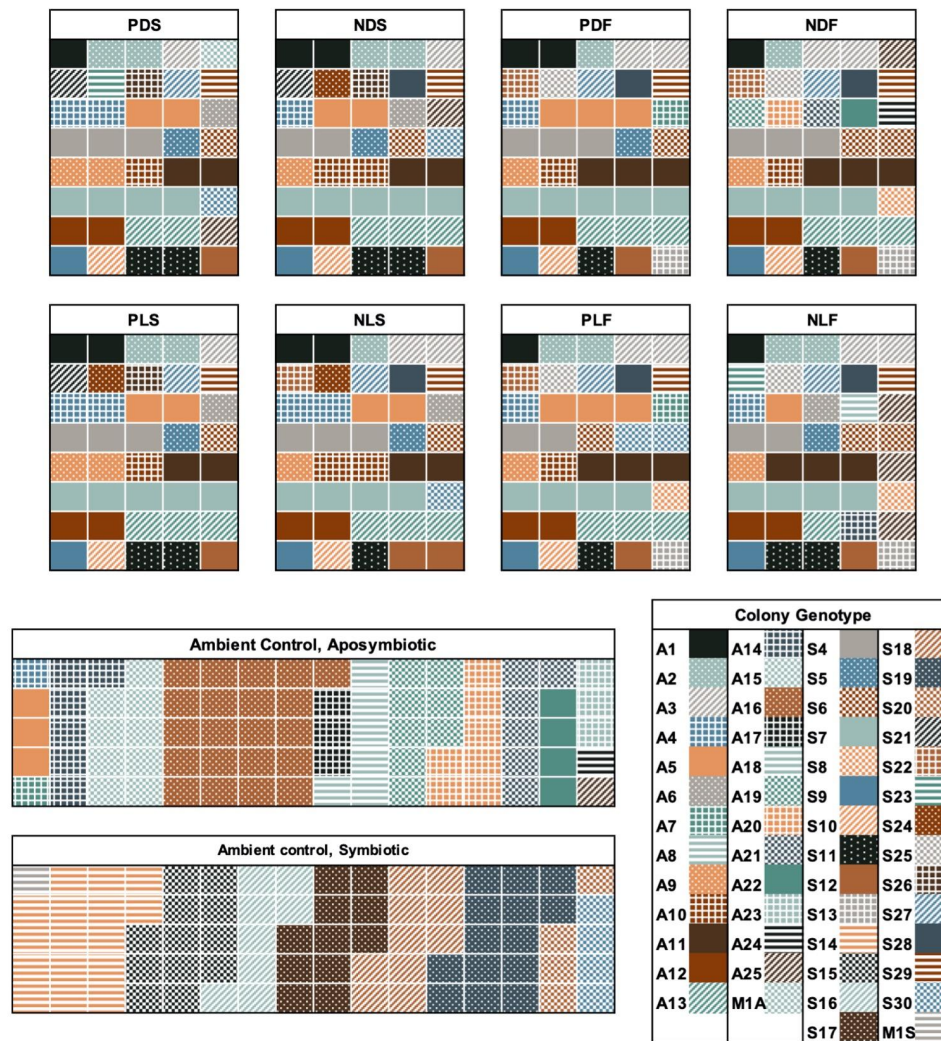


Figure 2. Coral colony genotype distribution across experimental treatments and ambient control. In total 25 aposymbiotic, 30 symbiotic, and 1 mixed colony were fragmented into 480 individual coral polyps.

Temperature:

Coral fragments were assigned to one of two thermal conditions; ambient control (19°C) or experimentally elevated temperatures (30°C). In Rhode Island, *A. poculata* experiences thermal variation that reaches as high as 27°C in the

summer, and as low as 0°C in the winter (Dimond and Carrington 2007; Dimond et al. 2012). Therefore, 30°C was used in this study to thermally challenge *A. poculata* past their *in-situ* environmental threshold. Ambient control conditions were maintained at 19°C as Rhode Island populations are frequently subjected to temperatures between 18-20°C (Aichelman, Zimmerman, and Barshis 2019).

Light:

To test the hypothesis that absence of light would disproportionately influence symbiotic colonies, and to examine the impact of light (or lack thereof) as a stressor, aposymbiotic and symbiotic polyps were placed into dark or light treatments. Custom-built experimental tanks were equipped with Aquasky LED aquarium lights (Fluval ®) and kept on a 12h light:12h dark cycle for light treatments and 0h light: 24h dark cycle for dark treatments. Photosynthetically active radiation (PAR) was maintained between 80-90 $\mu\text{mol photos m}^{-1}\text{s}^{-1}$ during light hours for the light treatment. Lights remained off and tanks were covered with blackout cover to minimize PAR (0-4 $\mu\text{mol photos m}^{-1}\text{s}^{-1}$) for the dark treatments.

Microplastics and food:

To test the impact of starvation stress on corals, corals were either starved or fed. At the same time, some corals were stimulated with a non-nutritive particle (plastics) versus not. While plastics were primarily used as a non-nutritive food

proxy, plastics themselves have been shown to have negative impacts in corals (de Oliveira Soares et al. 2020) and in *A. poculata* (Rotjan et al. 2019; Allen et al. 2017). To test the influence of plastics, UV-fluorescent green polyethylene spheres ranging from 355-425 μm in diameter were used in this study (1.035 g cc^{-1} , Cospheric LLC, Santa Barbara, CA, USA). In preparation, the plastic spheres were pre-acclimated in circulating, aerated seawater for at least 5 days prior to experimental dosing to cultivate a microbial biofilm. Correspondingly-sized food particles were given to corals in the fed treatment: decapsulated brine shrimp eggs (BSE) were used as a food source because they are comparable in size and shape to microplastic beads (Rotjan et al. 2019).

Plastics were dosed at a concentration of 10 mg L^{-1} , while food was dosed at twice the amount of microplastics (20 mg L^{-1}) to ensure that corals had sufficient opportunity of feeding and interacting with both materials. This concentration was chosen in order to observe the interactive effects between exposure to microplastics and other environmental stressors. Studies have demonstrated that lower environmentally relevant concentrations of microplastics ($\sim 0.01\text{-}0.2 \text{ mg L}^{-1}$) alone impact marine organisms in myriad ways (Revel et al. 2019; Zhang et al. 2019; Naidoo and Glassom 2019). Therefore, a concentration greater than environmentally observed was used in order to ensure that the interactive effects between these stressors are truly represented in the data collected.

For treatments that received plastic (PDS, PLS), food (NDF, NLF), or plastic and food (PDF, PLF), feeding occurred 3 times per week for a total of 9 feedings. During feeding, flows to individual tanks were turned off to isolate the tanks from the larger system. Oxygen stones remained on to aid in circulation. Polyp fragments were target-fed to allow ample opportunity to ingest either or both materials and left to feed with water flow off for 30 minutes. After this duration flow was restored and any rafting food or plastic was filtered out of circulation through a series of sock and mechanical filters. Any food or plastic remaining in the tanks were siphoned out and a partial water change was completed.

Survival

To assess the fitness consequences of each treatment, survival and mortality were scored for each polyp on a daily basis for the duration of the experiment. Fragments were considered dead only when coral tissues were absent from the skeletal septa. Fragments displaying tissue regression or detachment were considered living and remained in experimental treatments.

Survival rate was analyzed with a Cox Proportional Hazards model (CPH) using the ‘survival’ package in RStudio (RStudio team 2020, version 4.0.2). Coral polyps that were removed from the experiment for non-mortality related reasons were right-censored, shown as ‘+’ in the Kaplan-Meier survival curves, to indicate their removal from the

experiment. This accounts for the decrease in sample size which is excluded from statistical analysis to determine the CPH Hazard Ratio (HR).

For all treatments, excluding no-plastic/light/fed (NLF) and no-plastic/dark/fed (NDF), fragment survival was analyzed from days 1-22. In these two treatments, there was an aquarium malfunction where temperature and salinity dropped significantly overnight. Therefore, survival was analyzed up until days 16 and 17 for NLF and NDF respectively since mortalities after this point could not be attributed to the experimental treatments alone. All fragments that were living up until days 16 and 17 were right-censored to indicate the termination of survival scoring for these experimental treatments. Since the ambient control treatment experienced zero mortalities during the experiment, 2 mortalities (1 aposymbiotic, 1 symbiotic) were added to each treatment to allow for statistical comparisons between groups.

Quantifying chlorophyll density

Symbiotic state (symbiotic or aposymbiotic), determined by association with *Breviolum psygmophilum* algal symbionts, can be non-destructively assessed via visual inspection and color quantification. Color is regularly used as a proxy for quantifying chlorophyll density as previously described (Burmester et al. 2018; Sharp et al. 2017; Winters et al. 2009; Dimond and Carrington 2007). Fragments were photographed prior to the onset of the experiment on day 1 (pre-treatment) and on day 22 after the experimental treatment (post-treatment). Photographs were taken with a Tough TG-6 digital camera at 11x zoom (Olympus, Tokyo, JP) under a custom LED light ring

apparatus. Photographs were analyzed using open-source code published by Burmester et al. (2017) in MATLAB R2007b (The MathWorks, Natick, MA, USA). Red, green, and blue values were determined for each polyp fragment against a red-green-blue (RGB) color standard that was present in every photo to account for any differences in light intensity. Red (R) color values are used to estimate the chlorophyll-a density on a dark-light gradient where an increasing R intensity correlates to a whiter value, and subsequently a less chlorophyll dense polyp as noted in Winters et al. (2009). Coral R color intensity was analyzed using mixed effects linear models in the ‘lmer’ package with polyp ID as a random effect to account for repeat measurements. Post hoc pairwise comparisons were performed using the ‘emmeans’ package and model comparisons were performed using the ‘anova’ function.

Photosynthetic efficiency

To measure symbiont activity, photosynthetic efficiency (F_v/F_m) of photosystem II was measured in triplicate for each polyp fragment on days 8, 15, and 22 for the experimental treatments, and day 22 for the ambient control group, using a Walz Junior-PAM pulse-amplitude modulated fluorescence meter as previously described (Dimond and Carrington 2007; Burmester et al. 2018; DiRoberts et al. 2021; Aichelman, Zimmerman, and Barshis 2019). Polyp fragments were dark-acclimated for at least 30 minutes before determining minimal (F_0) and maximal fluorescence (F_m). Minimal fluorescence was measured by exposing polyps to 6 seconds of far-red illumination, while maximal fluorescence was determined by exposing polyps to a 0.6 second

saturating pulse of 10,000 μmol . The photosynthetic efficiency of polyps is calculated as the difference between maximal and minimal fluorescence over the maximal fluorescence (F_v/F_m). To prevent saturation of the reaction centers in photosystem II for individual polyps, F_v/F_m was measured once for every polyp before circling back to measure a second and third time. The three measurements were then averaged to determine a single representative F_v/F_m for each polyp. For experimental treatments, F_v/F_m was analyzed using mixed effects linear models in the 'lmer' package with polyp ID as a random effect to account for repeat measurements. Post hoc pairwise comparisons were performed using the 'emmeans' package and model comparisons were performed using the 'anova' function. For the ambient control polyps, linear regression models and Tukey HSD post hoc analyses were performed since each polyp is represented once in the data.

Change in polyp size

To assess whether polyps gained or lost tissue over the course of the experiment, polyp growth/loss was determined by the change in dry mass over time. Dry mass was measured to the nearest thousandth gram at four time points (after fragmentation, and on days 8, 15, and 22) for fragments in the experimental treatments and two points (after fragmentation and on day 22) for fragments in the ambient control condition using a microscale. Fragments were dried using a kimwipe to remove external moisture before mass was determined. For fragments that died during the treatment period, their final mass was considered the last weight measured and normalized to the number of days in treatment on the day that it was weighed. For example, if a fragment was determined to

be dead on day 18 of the experiment but was last weighed on day 15, that weight was recorded as the final mass and normalized to 15 days. All fragments that were alive up until the end of the experiment were normalized to 22 days. Growth rate was converted to mg day^{-1} and log-transformed to improve normality distribution and reduce directional skew for statistical modeling and analysis. Since growth rate accounts for all measurements of mass over time, the parental colony genotype was included in all linear mixed effects models using the ‘lmer’ package to account for repeat measurements. All post hoc pairwise analyses were performed using the ‘emmeans’ package, while model comparisons were performed using the ‘anova’ function.

Respiration

Bacterial ligands, such as Toll-like receptors (TLR), have been shown to induce immune responses in vertebrates such as *Drosophila* (Lemaitre et al. 1996) and mice (Poltorak et al. 1998; Tabeta et al. 2004) while similar receptors have been categorized in invertebrates like *Caenorhabditis elegans* (Pujol et al. 2001), *Nematostella vectensis* (Margolis et al. 2021), and *Hydra magnipapillata* (Franzenburg et al. 2012). For *cnidarians*, which demonstrate innate immunity against pathogens (Miller et al. 2007), these ligand receptors are essential components for bacterial recognition (Underhill et al. 1999; Hayashi et al. 2001; Zhang et al. 2004). In this study, we used lysed *Escherichia coli* cells as immunostimulants to mimic the acute threat of a pathogenic disease in order to quantify the degree of bacterial recognition and the impact on metabolic respiration under chronic stress conditions.

Two 24-channel closed system respirometers (one for challenge assays, and one for basal respiration assays) were used for non-invasive oxygen sensing using 2 mL glass vials with pre-calibrated internal sensors (SDR SensorDish Reader, PreSens, Germany). Experiments were performed on days 8, 15, and 22 for all living polyps in the experimental treatments and on day 22 for a subset of fragments in the ambient control condition (n = 80). Prior to every respirometry experiment, fragments were carefully removed from their acrylic mounts, weighed, and displaced in saltwater to determine mass and volume-specific respiration rates. Fragments were divided into two respirometry conditions: saltwater (SW, 35 ppt) or saltwater + lysed *E. coli* bacterial cells (EC) as basal and acute stress challenge groups. Fragments were exposed to the same respirometry condition in subsequent experiments in order to allow for pairwise comparisons in metabolic respiration rates (MR) over time.

During each experiment all living polyps from a given treatment were placed in their own vial with sterilized SW or SW+EC (water temperature ~20°C), air bubbles were removed, and vials were placed on the sensor dish. Four additional vials were used as controls to measure the background rate of respiration. Oxygen measurements (pO₂ in percent air saturation) were recorded every 15s using the SDR version 4.0.0 software (PreSens, Germany). Fragments were removed from the experiment when oxygen pO₂ dropped below 65%, or after 4 hours to prevent coral exposure to hypoxic conditions.

Challenge assays:

To test how corals exposed to chronic stressors respond to a potential immune stimulus, coral polyps were exposed to “challenge treatments”.

Escherichia coli cells were cultured with chloramphenicol resistance in Luria Broth (LB) on a shaking incubator at 32°C for 5 days. Optical density was determined for the liquid culture (OD₆₀₀ = 0.868) and aliquoted into 1.5 mL vials. Cultures were then frozen at -18°C for a minimum of 24 hours to lyse the cells and kill all living *E. coli* bacteria. Pilot studies showed no difference in the rate of respiration for fragments exposed to living versus lysed *E. coli* indicating that exposure to lysed bacterial cells was sufficient to elicit a metabolic response while eliminating bacteria-driven mortality and bacterial respiration. During acute stress experiments, polyp fragments were challenged with lysed *E. coli* in room temperature saltwater at a concentration of 50 million cells μL^{-1} .

Basal respiration assays:

To examine the basal oxygen consumption rates of polyps in different chronic stress treatments, respiration rate was measured for polyps in sterile room temperature saltwater without the presence of an acute bacterial challenge.

After each experiment, challenged fragments were rinsed thoroughly with fresh saltwater to remove lingering bacterial cells. All fragments were then re-adhered to the acrylic mounts and placed back into the experimental aquarium tanks.

To account for sensor drift, which occurs naturally over time due to use, oxygen concentration for the negative oxygen controls (Pyroscience Technologies, Germany) were averaged and subtracted from all treatment values. Re-scaled values were further calibrated to internal respirometry condition controls per plate, per experimental run. For corals challenged with acute bacterial stress, respiration rates were adjusted to the background respiration of internal plate control vials containing sterile saltwater and the equivalent concentration of bacterial cells. Similarly, basal respiration rates were adjusted for polyps that were not acutely challenged using internal plate controls containing sterile saltwater only.

Rate of oxygen consumption for all internal plate controls were calculated over time, and these background rates were then subtracted from the experimental rates. Thus, all experimental rates have been corrected for sensor drift and background respiration prior to analysis and visualization. Finally, individual polyp respiration rates were adjusted by the mass of the polyp and volume of saltwater within the respiration vial to calculate mass-specific rates of respiration. Mass-specific metabolic rates were calculated and background adjusted using the ‘RespR’ package (version 1.1.0) in Rstudio (Harianto, Carey, and Byrne 2019). Calculated rates ($\mu\text{mol O}_2 \text{ min}^{-1}\text{g}^{-1}$) were then log-transformed and analyzed with mixed effects linear modeling using the ‘lmer’ package. Coral polyp ID was included as a random effect to account for repeat measures of individual polyps over time. All post hoc pairwise analyses were performed using the ‘emmeans’ package, while model comparisons were performed using the ‘anova’ function.

Logistical experimental design compromises

Respirometry experiments plastic/dark/starved (PDS) 1 and plastic/light/starved (PLS) 1 used lysed *E. coli* from August 10, while all following experiments used more recent cultures from September 9. Respirometry data for no-plastic/dark/fed (NDF) day 8, acute stress challenge was lost due to technical computer issues. Acrylic tile 52 (from no-plastic/light/starved (NLS) treatment) and tile 47 (from no-plastic/dark/fed (NDF) treatment) were accidentally swapped for 2 days. No feeding occurred during those two days, nor did any data collection. All fragments from tile 52 were alive and in good health. On tile 47, four of the five fragments were still alive. The following day one fragment adhered to tile 52 was determined to be dead.

In the experimental aquarium rack system, the sump that supplied water to treatments no-plastic/dark/fed (NDF) and no-plastic/light/fed (NLF) malfunctioned overnight on days 17 and 16 respectively. Water drained from the sump and auto-top-offs and stopped circulating to the experimental tanks, isolating them from the system. The salinity in the sump dropped to 15 ppt, while temperature remained between 28-31°C through the course of the night. The salinity and temperature of the individual tanks remains unknown since they were isolated. The following morning water was added to the system to re-stabilize water quality and return water circulation. Since any fragment mortality observed after this period could be attributed to the system failure rather than the stress of the treatments themselves, these treatments were right-censored at days 17 and 16 for survival analysis. All mortality after this period was recorded but not used for analysis.

RESULTS

Survival

We determined the risk of survival (and corresponding mortality) for each polyp within all experimental and control treatments. During the 22-day experiment, there were no observed mortalities (0) in the ambient temperature control group (0%), compared to the 54 total polyps (16.9%) that died in elevated temperatures across all experimental treatments (Figure 3A). The plastic/light/fed (PLF) treatment experienced the greatest loss of 12 individuals where polyps were 34.2 times more likely to die than those in the control conditions (Cox Proportional Hazards likelihood ratio test: $X^2 = 79.43$, $df = 8$, $p = 2.96 \times 10^{-6}$). However, coral polyps had the highest risk of mortality in the no-plastic/dark/fed (NDF) treatment. With 11 mortalities, NDF polyps experienced a risk of mortality 53.8 times greater than control polyps (LR test: $X^2 = 79.43$, $df = 8$, $p = 2.37 \times 10^{-7}$). Both the no-plastic/dark/starved (NDS) and no-plastic/light/starved (NLS) treatments lost one polyp, and were ranked as 6.3 and 6.1 times as likely to die compared to the ambient control corals respectively (LR test: $X^2 = 79.43$, $df = 8$, $p = 0.044$ (NDS), $p = 0.048$ (NLS)).

To examine which stressor had the largest contribution to mortality risk (hazard ratio), we combined all stressors in a single CPH model and found that elevated temperatures significantly increased the risk of mortality (Hazard Ratio = 33.36) while starvation significantly reduced the risk (Figure 3B; HR = 0.41, LR test: $X^2 = 64.73$, $df = 4$, global $p = 2.9 \times 10^{-13}$).

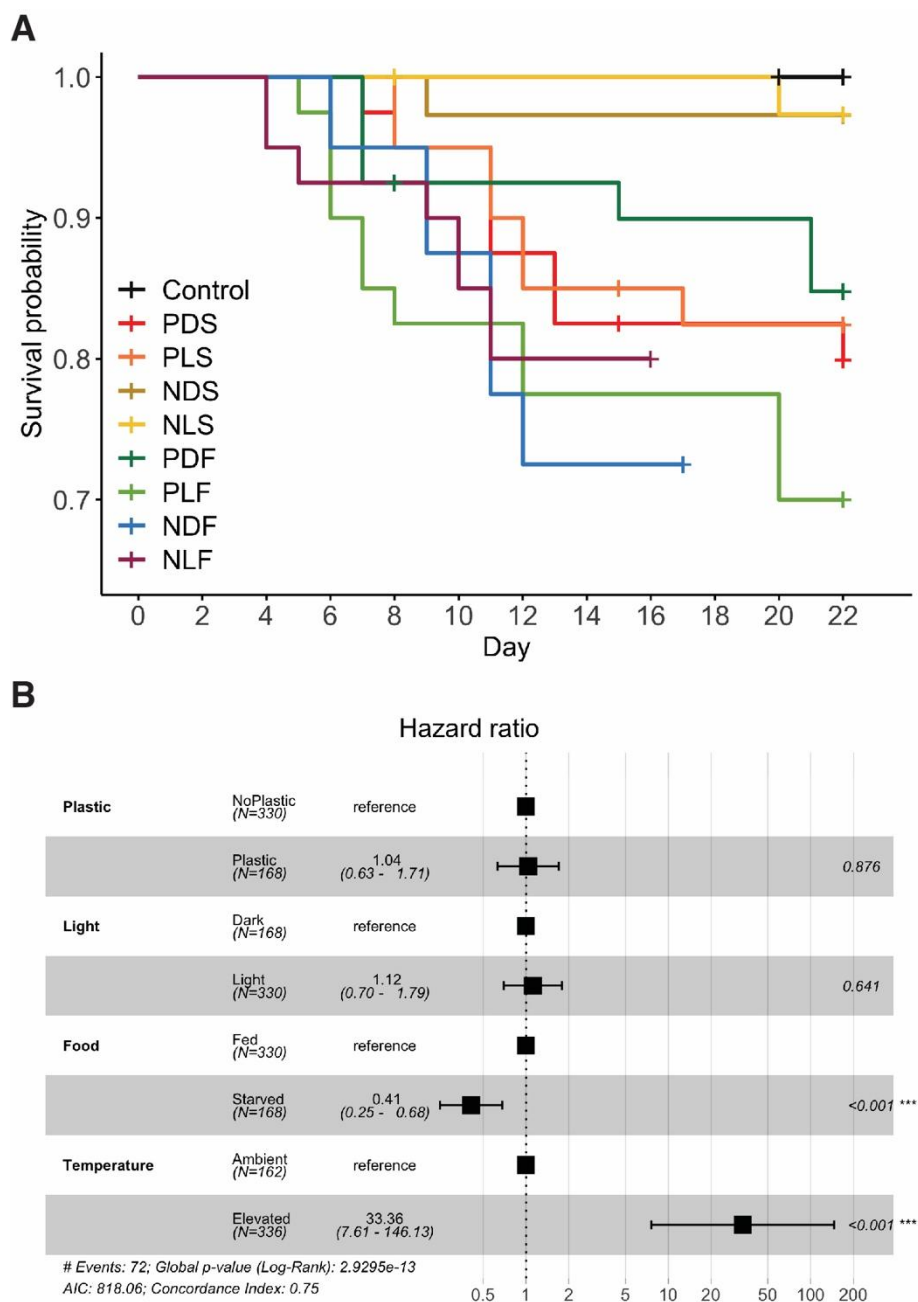


Figure 3. Kaplan-Meier survival curves by treatment (A). ‘+’ indicates where a coral polyp was removed, or censored, from the experiment. The removal of the individual does not impact statistical analysis in the Cox Proportional Hazards model. Temperature and food availability influenced mortality, assessed via the CPH Model (B) where a Hazard Ratio (HR) > 1 indicates an increase in the risk of mortality associated with a given condition, and HR <1 indicates a reduction of the risk.

To examine the contextualized impact of each stressor (without considering multiple stressors), we looked at the influence of each stressor independently on survival and mortality. At elevated thermal conditions (30°C), coral polyps were at risk of mortality 21.1 times greater than those living at ambient temperatures (19°C) (Figure 4A; LR test: $X^2 = 51.39$, $df = 1$, $p = 8 \times 10^{-13}$). Within elevated temperatures (not including ambient controls), neither plastic nor light treatments impacted the rate of survival. Although coral fragments exposed to plastics had an increased risk of mortality (HR = 1.5), the observed mortalities were indistinguishable from coral fragments that were not exposed to plastics (Figure 4B; LR test: $X^2 = 1.88$, $df = 1$, $p = 0.2$).

Interestingly, corals exposed to the dark stress conditions experienced marginally increased survivorship (HR = 0.91), though not significantly greater than corals in the light conditions (Figure 4C; LR test: $X^2 = 0.12$, $df = 1$, $p = 0.7$). In contrast to expectations, starvation significantly improved the survival probability of coral polyps within elevated temperatures (Figure 4D). Fed coral fragments experienced mortality 2.6 times more than starved corals (LR test: $X^2 = 11.36$, $df = 1$, $p = 7 \times 10^{-4}$).

Although aposymbiotic polyps experienced greater mortality during the experiment than their symbiotic counterparts (Figure S1; HR = 2.4), the risk of dying associated with symbiotic phenotype was influenced by time and therefore did not pass the assumptions test of the Cox Proportional Hazards model (Goodness-of-fit test for Schoenfeld's residuals; $X^2 = 9.92$, $df = 1$, $p = 0.0044$).

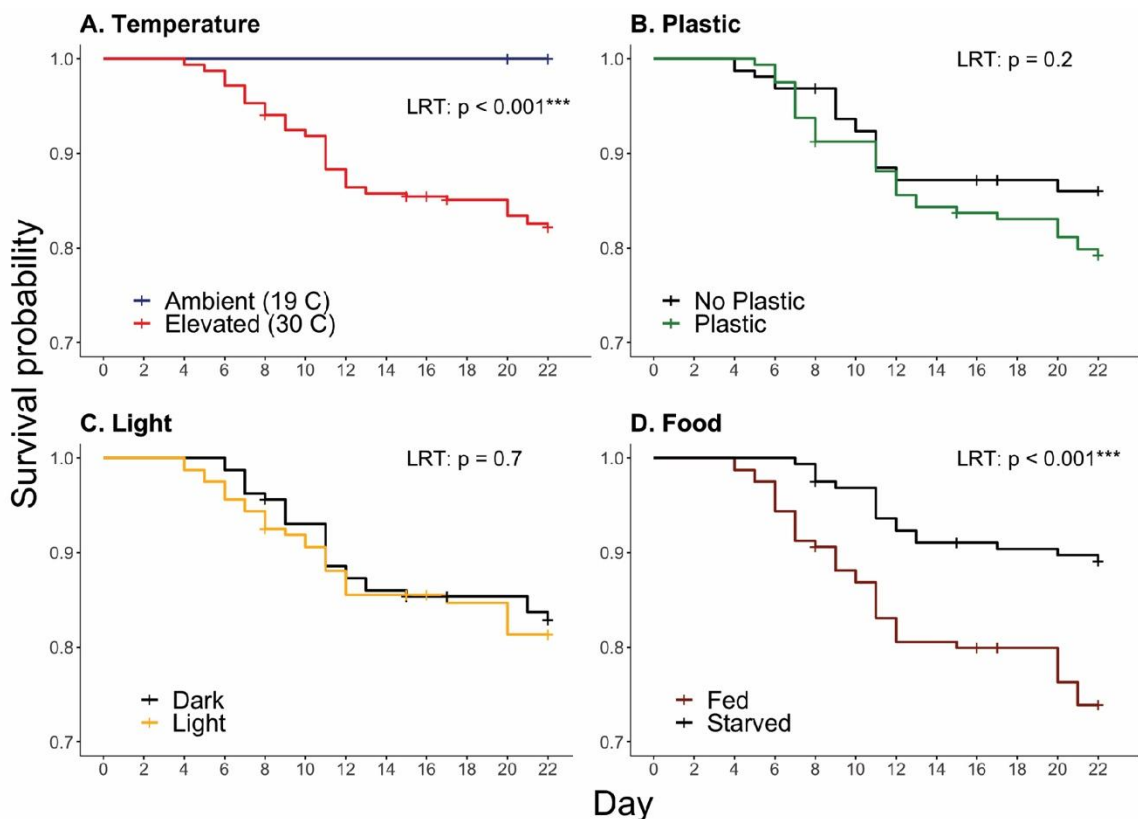


Figure 4. Kaplan-Meier Survival Curves for individual stressors; (A) temperature, (B) plastic, (C) light and (D) food. Plastic, light, and food conditions are contextualized within elevated temperatures, and do not include individuals within the ambient controls. '+' indicates censorship of a coral polyp from the experiment and is not included in the Cox Proportional Hazard analysis to determine relative hazard. Significance is shown based on the Likelihood Ratio test (LRT) and indicates where $p < 0.001^{***}$, $p < 0.01^{**}$.

Photosynthetic Efficiency of *Breviolum psygmophilum* symbionts

Photosynthetic efficiency (F_v/F_m) varied significantly over time in all experimental treatments except in the no-plastic/dark/starved (NDS) (ANOVA; $X^2 = 2.23$, $df = 1$, $p = 0.14$) and plastic/light/fed (PLF) treatments (ANOVA; $X^2 = 2.29$, $df = 1$, $p = 0.13$), in which F_v/F_m remained constant (Figure 5A). Additionally, association with *B. psygmophilum* symbionts influenced F_v/F_m in three experimental conditions (Figure

5B: ANOVA; PDS, $X^2 = 4.29$, $df = 1$, $p = 0.034$; NLS, $X^2 = 10.75$, $df = 1$, $p = 0.001$; PLF, $X^2 = 8.31$, $df = 1$, $p = 0.004$) and in the ambient control group (linear regression; $F = 507.8$, $df = 155$, Tukey HSD; $p < 2 \times 10^{-16}$). Within all elevated temperature treatments, phenotype significantly impacted photosynthetic efficiency over time (Figure 5C: ANOVA: $X^2 = 44$, $df = 2$, $p = 2.73 \times 10^{-10}$) where a notable shift occurred between the two phenotypes between days 1 and 8 of chronic stress exposure.

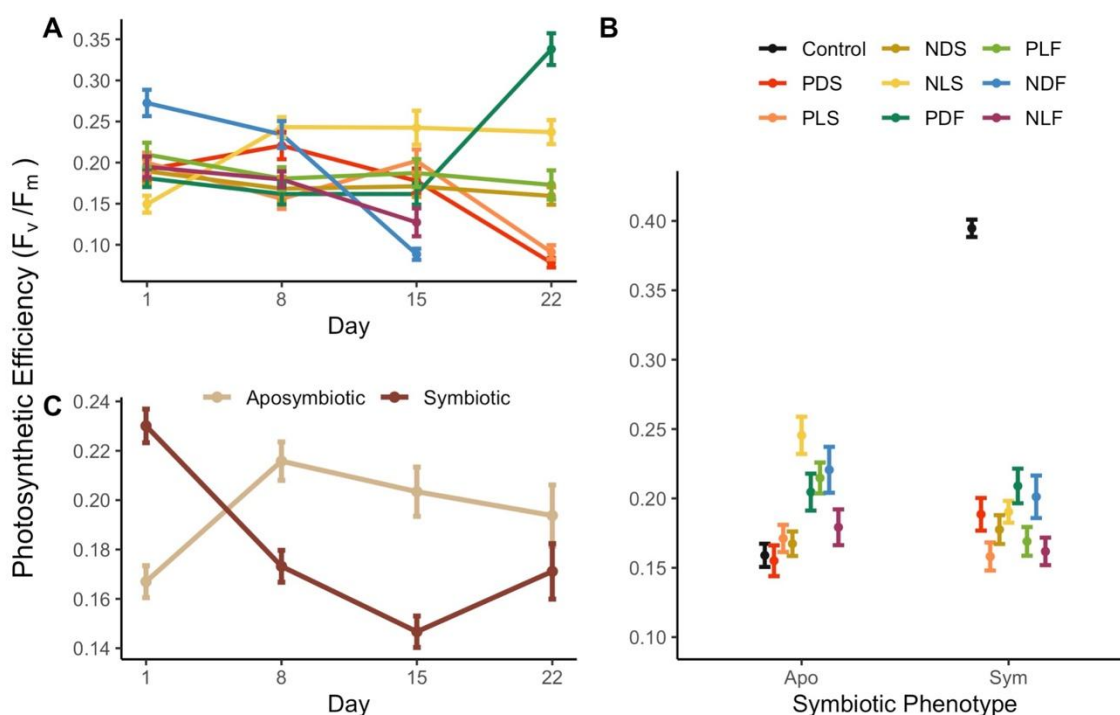


Figure 5. Photosynthetic efficiency (F_v/F_m) of *Breviolum psymophilum* endosymbionts. (A) F_v/F_m changed significantly across time in all experimental treatments except NDS and PLF (excludes ambient control), (B) F_v/F_m between aposymbiotic and symbiotic phenotypes for all treatments, (C) symbiotic phenotypes significantly affected F_v/F_m over time in elevated temperatures. Data represents mean and standard error for all panels.

Symbiotic phenotype, time, and the interaction between symbiotic phenotype and time significantly impacted F_v/F_m in the experimental treatments plastic/dark/starved (PDS) and no-plastic/dark/starved (NDS) (Figure S2, Table 1; LMER all predictors; $p < 0.05$). In contrast, F_v/F_m was not significantly affected by symbiotic phenotype, time, or their interaction in the plastic/light/fed (PLF) treatment (Figure S2, Table 1; LMER; all predictors; $p < 0.05$).

Elevated thermal stress significantly decreased F_v/F_m in *B. psymophilum* symbionts compared to those in the ambient control conditions on day 22 (Figure 6A; LM; $f = 49.17$, $df = 352$, $p = 1.21 \times 10^{-11}$). Symbionts in brown coral polyps in the ambient conditions averaged $0.395 \pm 0.006 F_v/F_m$ (mean \pm SEM), more than twice as efficient than brown coral symbionts at elevated temperatures across all time points (0.182 ± 0.004 , mean \pm SEM). In contrast, photosynthetic efficiency in aposymbiotic white corals did not differ between ambient and elevated temperatures, averaging 0.159 ± 0.008 at 19°C and 0.193 ± 0.005 at 30°C (mean \pm SEM). At elevated temperatures, time significantly impacted F_v/F_m (Figure 6A; ANOVA; $X^2 = 8.0$, $df = 1$, $p = 4.8 \times 10^{-3}$).

Within the experimental conditions at elevated temperatures, feeding significantly maintained F_v/F_m (Figure 6D; LMER; $p = 0.041$, ANOVA; $X^2 = 4.22$, $df = 1$, $p = 0.04$) while plastic exposure and light availability had no effect (Figure 6B Plastic; LMER; $p = 0.115$, ANOVA; $X^2 = 2.51$, $df = 1$, $p = 0.11$; Figure 6C Light; LMER: $p = 0.752$, ANOVA; $X^2 = 0.101$, $df = 1$, $p = 0.75$).

When considering the change in F_v/F_m over time, the interaction between the respective stressor and time did not impact F_v/F_m for plastic exposure (Figure 6B; LMER:

t = 0.21, p = 0.84;), food availability (Figure 6D; LMER; t = -1.3, p = 0.2), or light availability (Figure 6C; LMER; t = 1.2, p = 0.22) despite time playing a significant factor within the light vs dark treatment (LMER; t = -2.9, p = 4.1x10⁻³).

Table 1. Mixed effects model outputs for the effect of phenotype and time on photosynthetic efficiency (F_v/F_m) of coral polyp fragments within each treatment group. Coral polyp ID was included as a random effect to account for repeat measures.

		Liner effects mixed model			
		estimate	df	t	Pr(> t)
PDS	Phenotype	0.0727	139	2.930	0.004 **
	Time	-0.0033	139	-2.494	0.014 *
	Phenotype X Time	-0.0038	139	-2.020	0.045 *
PLS	Phenotype	0.0346	140	1.564	0.120
	Time	-0.0017	140	-1.408	0.161
	Phenotype X Time	-0.0042	140	-2.544	0.012 *
NDS	Phenotype	0.0921	129	4.074	8.04e-05 ***
	Time	0.0024	129	2.177	0.032*
	Phenotype X Time	-0.0073	129	-4.724	6.65e-06 ***
NLS	Phenotype	0.0209	135	0.835	0.405
	Time	0.0074	135	5.973	2.53e-08 ***
	Phenotype X Time	-0.0068	135	-3.947	1.36e-4 ***
PDF	Phenotype	0.0472	142	1.697	0.092
	Time	0.0085	142	5.691	6.97e-08 ***
	Phenotype X Time	-0.0042	142	-2.013	0.046 *
PLF	Phenotype	-0.0308	129	-1.217	0.226
	Time	-0.0007	129	-0.480	0.632
	Phenotype X Time	-0.0013	129	-0.681	0.497
NDF	Phenotype	0.0004	99	0.014	0.989
	Time	-0.0118	78	-5.145	1.95e-06 ***
	Phenotype X Time	-0.0013	75	-0.416	0.678
NLF	Phenotype	0.0546	101	2.272	0.025 *
	Time	0.0006	75	0.335	0.738
	Phenotype X Time	-0.0094	71	-3.871	2.39e-4 ***

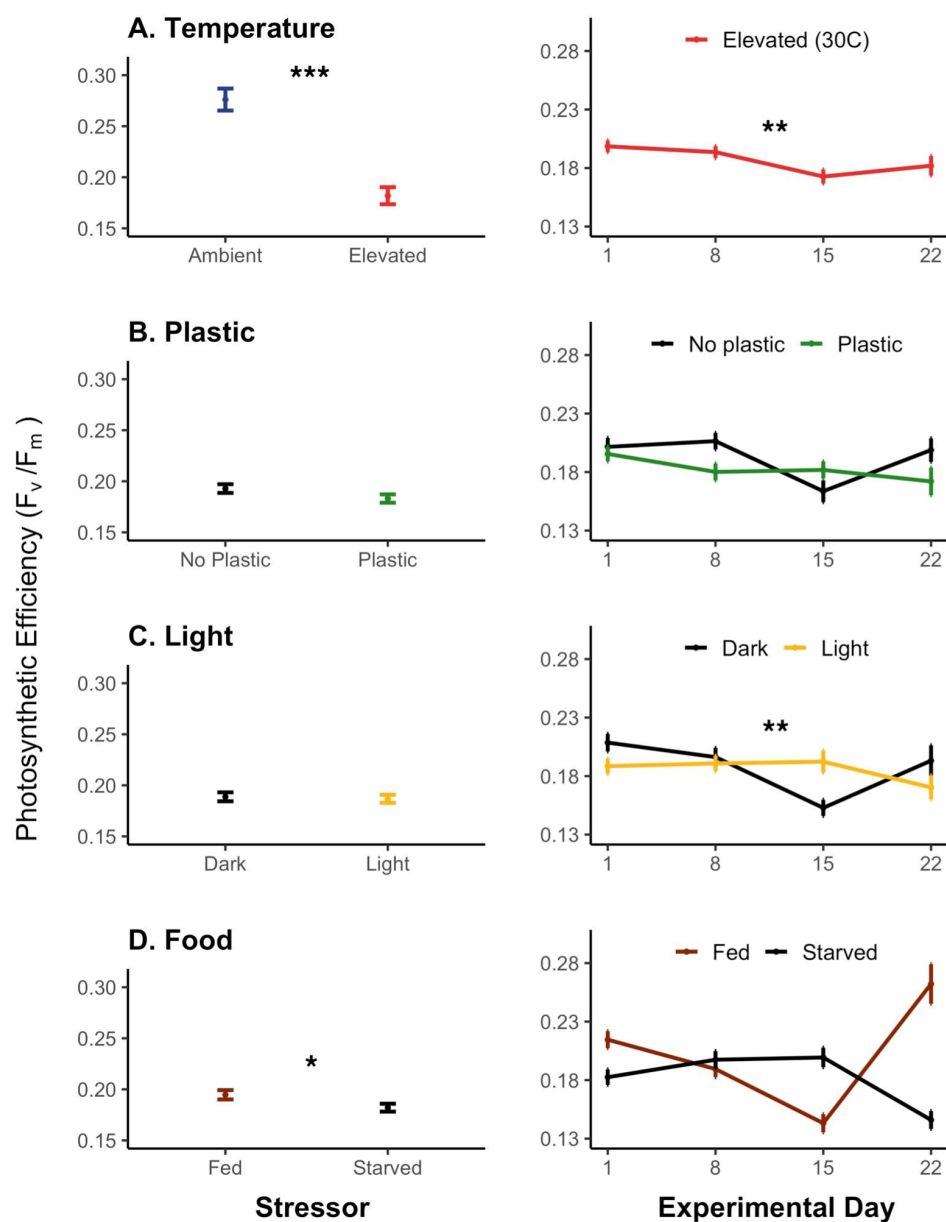


Figure 6. Photosynthetic efficiency by stressor (left), and by stressor over time (right) for (A) temperature, (B) microplastic exposure, (C) light availability, and (D) food availability. Plastic, light, and food conditions are contextualized within elevated temperatures, and do not include individuals within the ambient controls. Data represent mean and standard error. Significance is denoted where treatment (left) and time (right) impacted F_v/F_m . The interaction between stressor and time was not significant for any stressor (LMER). Asterisks indicate where $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***.

Chlorophyll density of *B. psygmophilum*

To approximate chlorophyll density over time, we used coral color, which is a non-destructive, non-invasive method. Intensity in the red (R) color channel has been shown to inversely correlate with coral color and chlorophyll density, where higher R channel values implicate a whiter and more aposymbiotic coral (Winters et al. 2009), and is demonstrated here (Figure 7). In this experiment, five out of the eight treatments experienced an overall increase in the intensity in the red color channel, while the remaining three experienced an overall decrease in intensity over the duration of the experiment (Figure 8). Simultaneous post hoc pairwise comparisons using Tukey's HSD test indicated that the changes in red channel intensity increase significantly over time

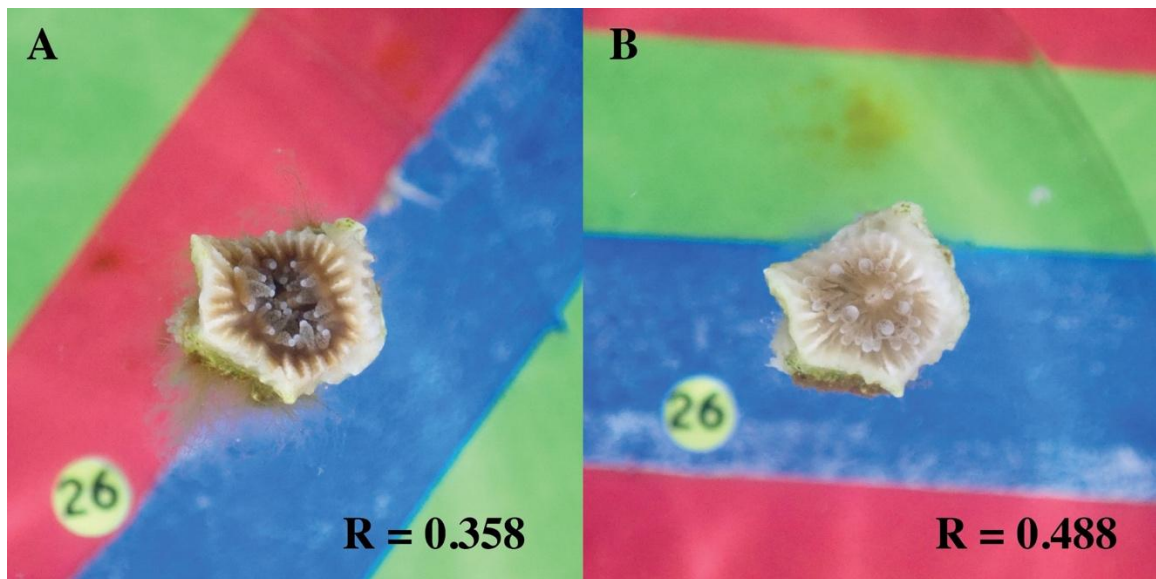


Figure 7. Color photographs of a single *A. poculata* coral polyp (ID: Y26) before (A) and after (B) the experiment. R values represent the color in the red channel intensity as determined in Matlab (Burmester et al. 2018) against RGB color standards, where increasing R values correspond with decreasing chlorophyll density.

within the PDS ($t = -3.2$, $df = 34.5$, $p = 0.003$) and NDF ($t = -2.3$, $df = 23.1$, $p = 0.031$) treatments, and significantly decrease in the NLF treatment ($t = 2.92$, $df = 35.7$, $p = 0.006$).

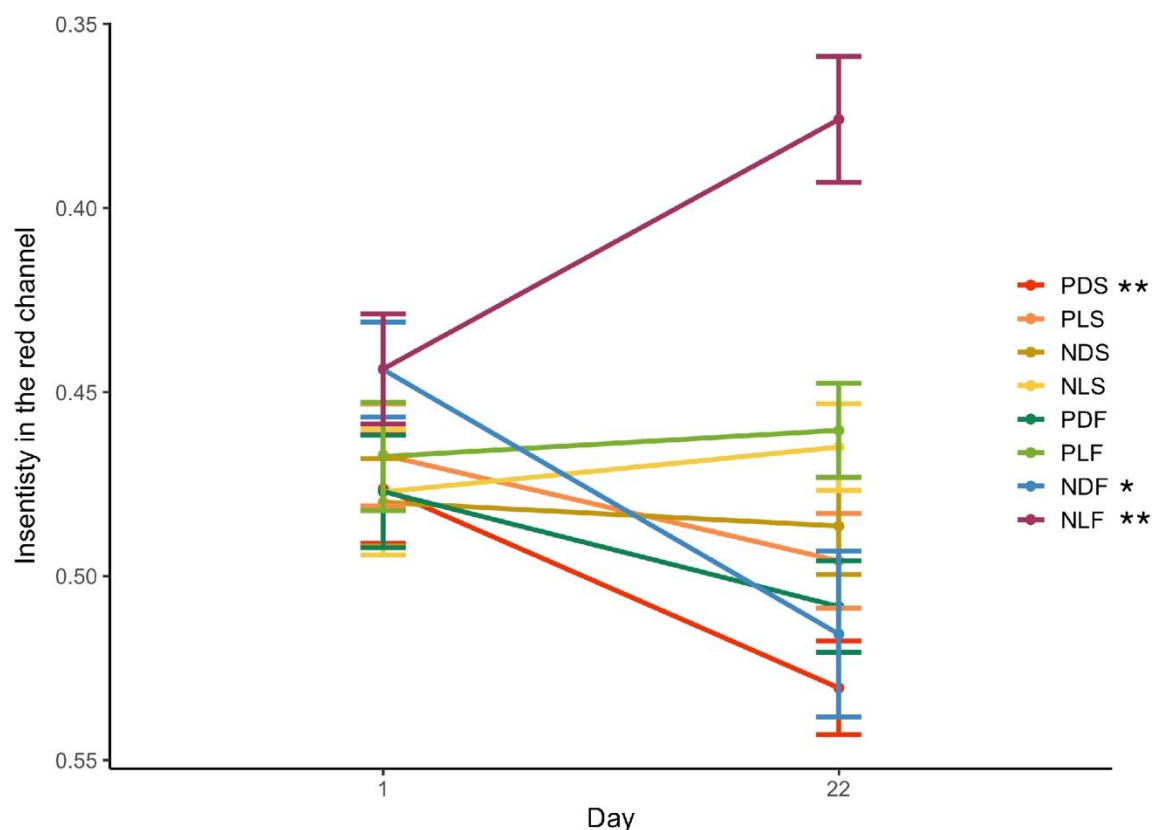


Figure 8. Change in the intensity of the red channel as a proxy for chlorophyll density across treatments. Data represent mean and standard error. An increase in the red color correlated with a loss of chlorophyll density and coral bleaching. Asterisks indicate Tukey's HSD post-hoc comparisons where $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***.

Further, changes in the red channel intensity vary by symbiotic phenotype within each treatment (Figure 9). In five treatments, symbiotic coral polyps experienced a significant increase in red channel intensity (Table 2; Tukey's HSD: NLS and NDF, $p <$

0.05; PDF, $p < 0.01$; PDS and PLS, $p < 0.001$), suggesting a decline in approximated chlorophyll density. For aposymbiotic fragments, changes in the red channel intensity were inconsequential in all treatments except for the no-plastic/light/starved (NLS) (TukeyHSD; $t = 4.39$, $df = 36.7$, $p < 0.001$) and no-plastic/light/fed (NLF) treatments (TukeyHSD; $t = 3.89$, $df = 39.4$, $p < 0.01$; Table 2), where approximated chlorophyll densities increased.

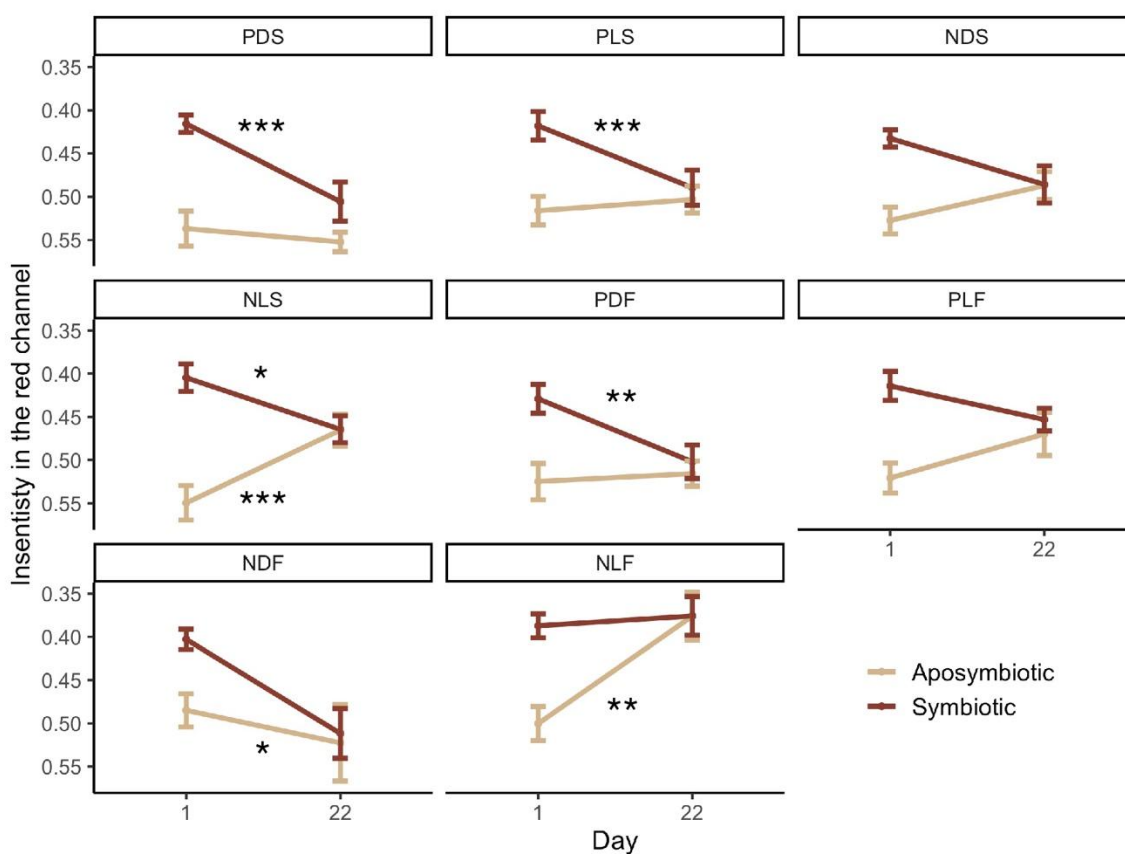


Figure 9. Change in the intensity of the red channel as a proxy for chlorophyll density by symbiotic phenotype within treatments. An increase in the red color indicates a loss of chlorophyll density and coral bleaching. Data represent mean and standard error, significance denoted where symbiotic (brown) or aposymbiotic (tan) coral polyps experienced a significant change in R color over the experimental period based on Tukey's HSD, where $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***.

Table 2. Mixed effects linear model for the effect of phenotype and time on changes in the red channel intensity (R) as a proxy of chlorophyll density. Tukey's Honest Significant Difference post-hoc comparisons represent the difference in R between day 1 and 22 of the experiment within each phenotype. Coral polyp ID was included as a random effect to account for repeat measures.

Model: R Color Intensity ~ Phenotype * Time + (1 Polyp ID)									
		Liner effects mixed model				Tukeys HSD pairwise: Phenotype ~ Day			
		estimate	df	t	Pr(> t)	estimate	df	t	p
PDS	Phenotype * Time	0.083	30.70	3.09	0.00426**				
	Apo					-0.01	32.90	-0.47	0.9660
	Sym					-0.09	34.60	-4.69	0.0002**
PLS	Phenotype * Time	0.085	33.49	3.84	0.00052***				
	Apo					0.02	32.90	1.19	0.6358
	Sym					-0.07	31.80	-4.31	0.0008***
NDS	Phenotype * Time	0.094	37.14	3.34	0.00191**				
	Apo					0.04	36.40	2.09	0.1769
	Sym					-0.05	36.40	-2.63	0.0574
NLS	Phenotype * Time	0.140	36.10	5.32	0.000001***				
	Apo					0.08	36.70	4.39	0.0005***
	Sym					-0.06	36.00	-3.11	0.0183*
PDF	Phenotype * Time	0.085	30.10	2.98	0.00561**				
	Apo					0.02	35.10	0.81	0.8497
	Sym					-0.07	32.80	-3.50	0.0072**
PLF	Phenotype * Time	0.096	30.62	3.28	0.00257**				
	Apo					0.06	33.10	2.62	0.0610
	Sym					-0.04	29.60	-1.96	0.2252
NDF	Phenotype * Time	0.068	25.15	1.23	0.23194				
	Apo					-0.04	29.60	-0.82	0.8431
	Sym					-0.11	23.40	-2.83	0.0437*
NLF	Phenotype * Time	0.113	62.00	2.71	0.00873**				
	Apo					0.12	39.40	3.89	0.002**
	Sym					0.03	30.70	0.42	0.9751

For individual stressors within experimental conditions (excluding ambient controls), linear mixed effects models indicated that differential stress conditions did not significantly impact the red channel intensity over time when considering symbiotic phenotype in plastic (Figure 10; LMER: $t = 0.74$, $df = 487.7$, $p = 0.46$) or light treatments (LMER: $t = 0.26$, $df = 486.4$, $p = 0.80$). In contrast, feeding significantly maintained red intensity over time and indicates greater chlorophyll retention (LMER: $t = 2.0$, $df = 498.3$, $p = 0.046$). In all stress conditions within the same model framework, symbiotic phenotype significantly predicted the change in red intensity over the duration of the

experiment (LMER Plastic; $t = -8.83$, $df = 487.8$, $p < 2 \times 10^{-16}$, Light; $t = -8.06$, $df = 486.4$, $p = 6.14 \times 10^{-15}$, Food; $t = -8.03$, $df = 489.3$, $p = 7.4 \times 10^{-15}$).

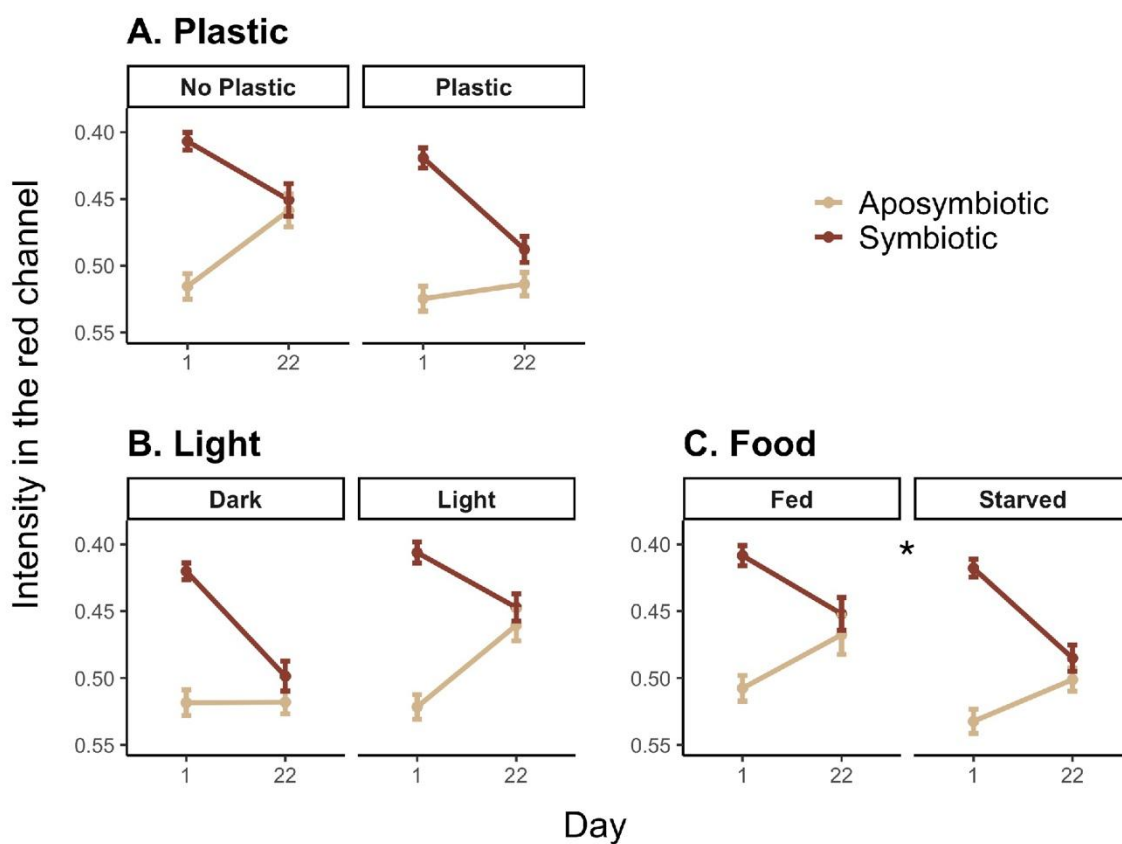


Figure 10. Change in intensity in the red color channel (R) for symbiotic (brown) and aposymbiotic (tan) coral fragments in plastic (A), light (B), and food (C) treatments within elevated temperature experimental conditions. Data represent mean and standard error, significance denoted where $p < 0.05$ *.

Change in polyp size

Over the course of the experiment, polyps in the ambient control conditions lost an average of 0.8 ± 0.12 (mean \pm SEM) mg of dry mass per day while polyps in all elevated temperature experimental conditions lost 1.2 ± 0.1 mg per day. Simultaneous post hoc pairwise comparisons show that no-plastic/dark/fed (NDF) is the only treatment that lost significantly more mass over time compared to the control group (Figure 11;

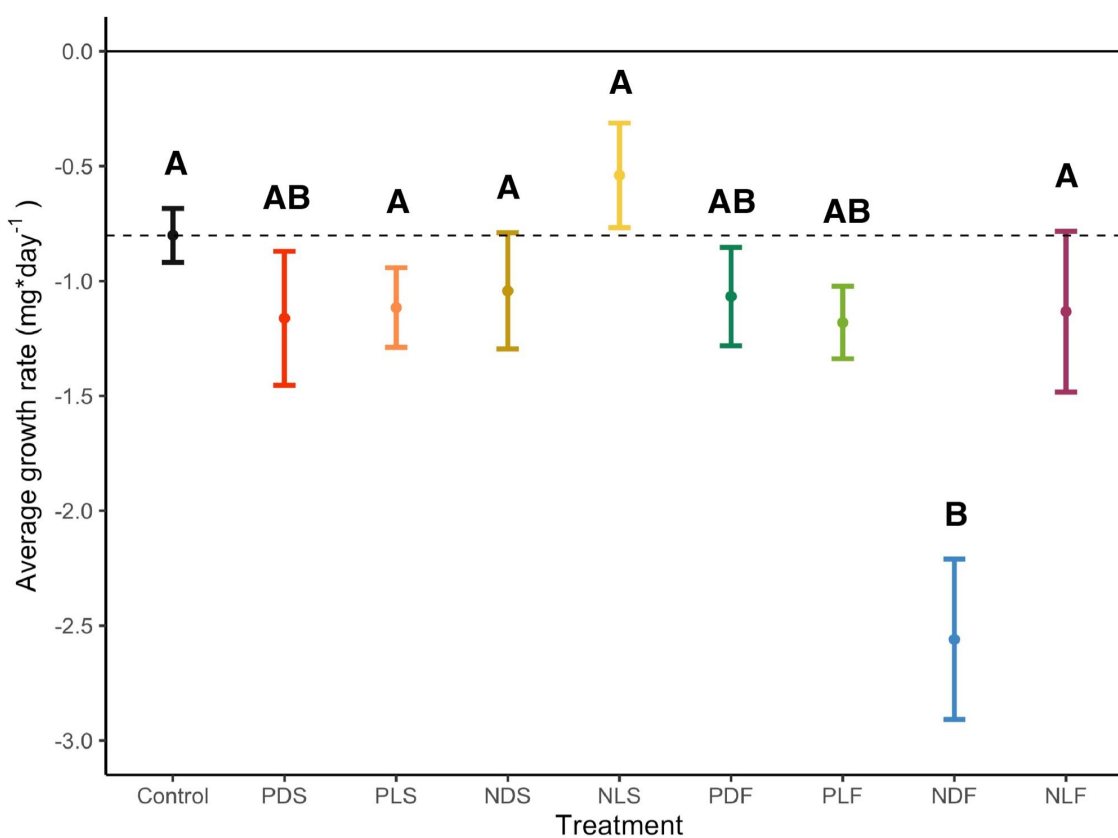


Figure 11. Average growth rate of coral polyps across treatments. Data represent mean and standard error. Dashed line indicates the mean growth rate of polyps in the control group (-0.8 ± 0.12 mg per day). Letters indicate significant differences between treatment groups as determined by Tukey's Honest Significant Difference post-hoc test.

Tukey HSD; $t = 5.9$, $df = 310$, $p < 0.001$). The rate of decrease in mass in the remaining treatments was not statistically different from the controls.

When considering individual stressors, elevated temperature significantly increased the rate of loss of mass of coral polyps compared to ambient temperatures (Figure 12A; LMER: $p = 0.0013$, ANOVA: $X^2 = 11.16$, $df = 1$, $p = 8.3 \times 10^{-4}$).

Within the elevated temperature experimental treatments, light and food treatments significantly impacted the growth rate of coral polyps, while plastic did not.

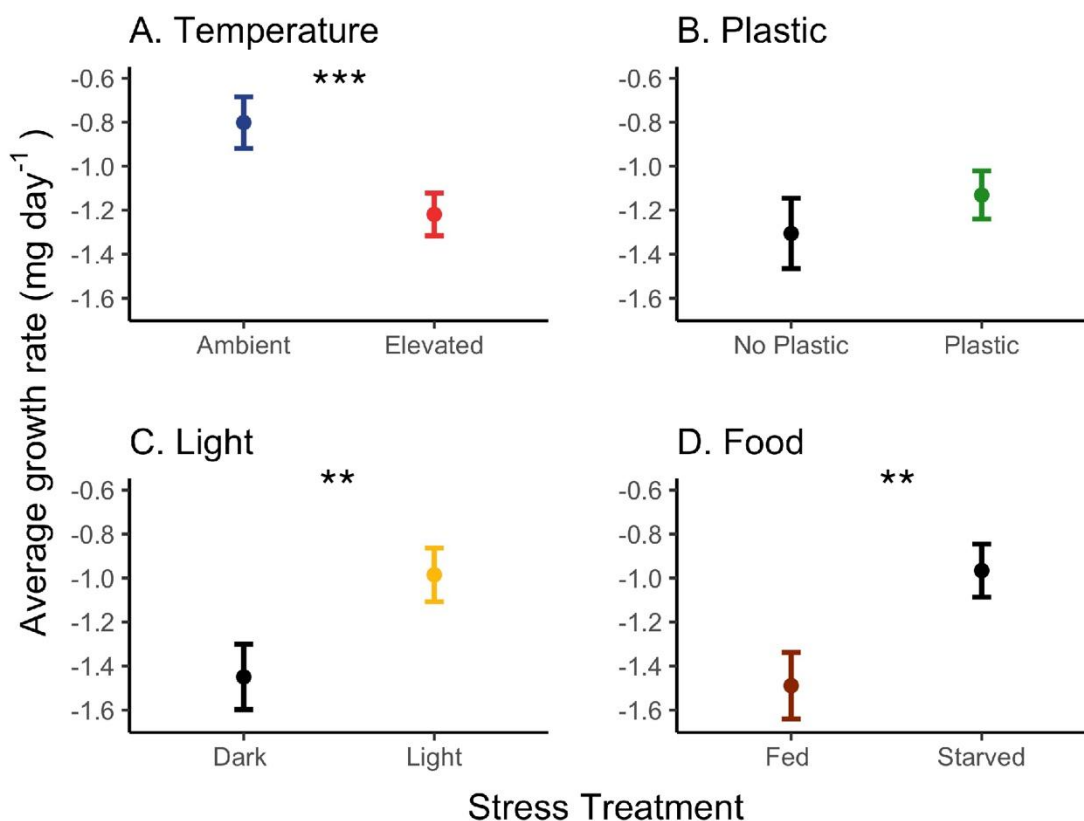


Figure 12. Average growth rate of coral polyps exposed to individual stressor treatments (A) temperature, (B) plastic, (C) light, and food (D). Plastic, light, and food conditions are contextualized within elevated temperatures, and do not include individuals within the ambient controls. Data represent mean and standard error. Significance is indicated where ANOVA $p < 0.01$ **, $p < 0.001$ ***.

Coral fragments in the light treatments sustained a slower loss of mass compared to fragments in the dark conditions (Figure 12C; LMER: $p = 0.0071$, ANOVA: $X^2 = 7.34$, $df = 1$, $p = 0.007$). Similarly, although contrary to our predictions, coral fragments in the starvation stress treatments maintained a slower loss of mass compared to fed corals (Figure 12D; LMER; $p = 0.01$, ANOVA: $X^2 = 6.76$, $df = 1$, $p = 0.009$). Finally, exposure to microplastics had no effect on the rate of change in mass (LMER: $p = 0.501$, ANOVA: $X^2 = 0.45$, $df = 1$, $p = 0.51$).

Within each stress regime, Tukey's HSD pairwise comparisons showed that elevated temperature, dark conditions, and feeding corresponded to a faster rate of decrease in mass of polyps (Table 3; Tukey HSD $p < 0.05$).

When considering all stressors together in a mixed model framework, plastic was insignificant, while temperature, light, and food significantly impacted growth rate (LMER Temperature; $t = -2.33$, $df = 148$, $p = 0.021$, Plastic; $t = -0.67$, $df = 418$, $p = 0.5$, Light; $t = 2.88$, $df = 414$, $p = 0.004$, Food; $t = 2.83$, $df = 441$, $p = 0.0049$; Table 4). Symbiotic phenotype did not impact growth rate alone or in the mixed effects models.

Table 3. Mixed effects model output for the effect of individual stressors on coral growth. Tukey's Honest Significant Difference post-hoc test shows pairwise comparisons within each stress treatment. Colony is included as random effect to account for multiple measures of coral genets.

Model: Log(Growth Rate) ~ "Stressor" + (1 Colony ID)								
	Liner effects mixed model				Tukeys HSD pairwise: ~ "Stressor"			
	estimate	df	t	Pr(> t)	estimate	df	t	p
Ambient Temperature (intercept)	-0.0037	47.21	-4.65	2.69e-5***				
Elevated Temperature Amb ~ Elev.	-0.0034	48.69	-3.41	0.0013**	0.0034	52.1	3.36	0.0014**
No Plastic (intercept)	-0.0067	51.41	-7.55	7.13e-10 ***				
Plastic NP ~ P	-0.0007	262.90	-0.67	0.501	0.0007	272.0	0.67	0.5016
Dark (intercept)	-0.0085	54.33	-9.75	1.55e-13 ***				
Light D ~ L	0.0029	261.40	2.72	0.00707 **	-0.0029	270.0	-2.71	0.0071 **
Fed (intercept)	-0.0085	56.78	-9.69	1.25e-13 ***				
Starved F ~ S	0.0028	286.40	2.60	0.00972 **	-0.0028	288.0	-2.60	0.0099

Table 4. Mixed effects linear model output for the effect of individual stress treatments on polyp growth rate. The full model includes all stress parameters while the reduced excludes plastic as it had an insignificant effect in the full model. Colony is included as random effect to account for multiple measures of coral genets.

Full Model: Log(Growth Rate) ~ Temperature + Plastic + Light + Food + (1 Colony ID)						
	Full			Reduced		
	Estimates	t statistic	p	Estimates	t statistic	p
(Intercept)	-0.0067	-5.189	<0.001***	-0.0067	-5.2078	<0.001***
Temperature [Elevated]	-0.003	-2.3118	0.022*	-0.0034	-2.7546	0.007**
Plastic [Plastic]	-0.0007	-0.6703	0.503			
Light [Light]	0.0029	2.882	0.004**	0.003	2.8988	0.004**
Food [Starved]	0.0029	2.8275	0.005**	0.0029	2.8182	0.005**
Observations		451			451	
AIC		-2917.75			-2931.23	
log-Likelihood		1465.87			1471.62	

Respiration of the coral holobiont

Metabolic respiration (MR) rate of coral polyps from the ambient temperature control conditions (n = 80) were measured on day 22 of the experiment in order to establish the baseline metabolic rates of healthy *A. poculata* corals in resting conditions (saltwater) compared to acute stress exposure (*E. coli* bacterial ligands). Acute stress significantly increased metabolic rate of polyps in the ambient control conditions (Figure 13A; LM; $t = -4.96$, $df = 77$, $p = 4.29 \times 10^{-6}$). The basal metabolic rate (SW) of corals in

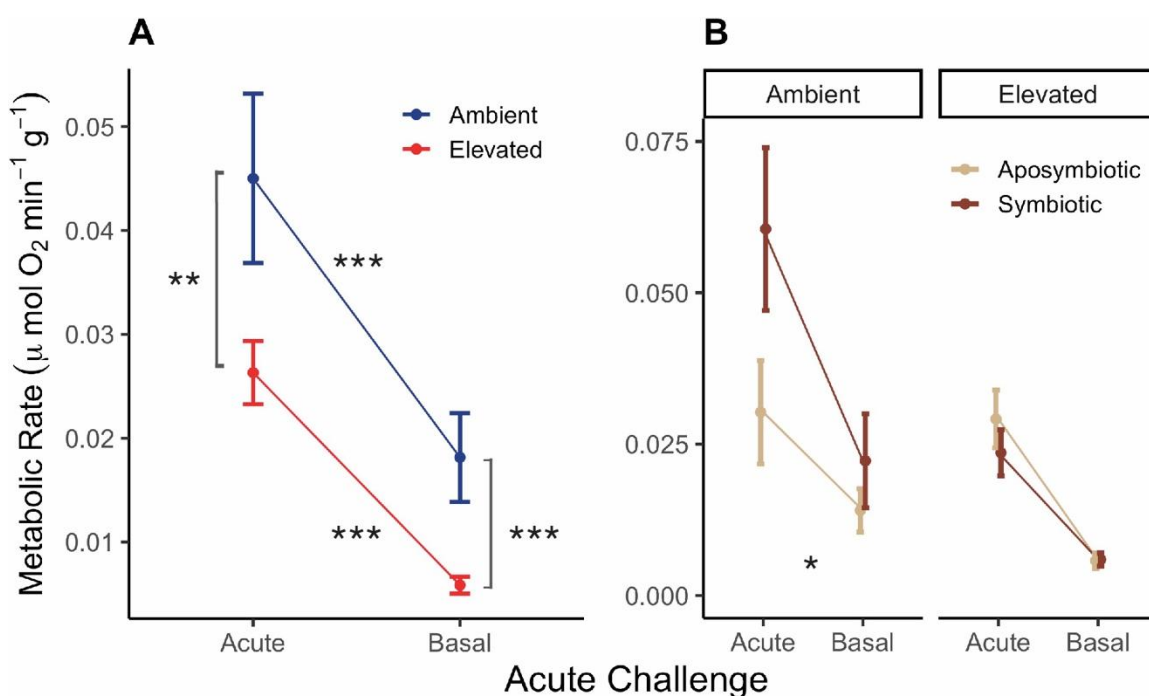


Figure 13. The influence of temperature regime on basal metabolic rate and in response to acute bacterial challenge (A), mediated by symbiosis (B) on day 22. Data represent mean and standard error. Significance is indicated for Tukey's HSD pairwise comparisons both within and between thermal regimes (A), and linear models for the influence of phenotype on metabolic response within respective temperature regimes (B). Significance is denoted where $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***.

the ambient temperature treatment averaged $0.018 \pm 0.004 \mu\text{mol O}_2 \text{min}^{-1}\text{g}^{-1}$ (mean \pm SEM), 2.5 times slower than the metabolic rate of corals facing acute stress which averaged $0.045 \pm 0.008 \mu\text{mol O}_2 \text{min}^{-1}\text{g}^{-1}$ (Figure 13A; TukeyHSD; $p = 4.3 \times 10^{-6}$).

Additionally, symbiotic phenotype significantly impacted MR of coral polyps in the ambient temperature conditions (Figure 13B; LM; $t = 2.57$, $df = 75$, $p = 0.012$). MR was significantly faster in coral polyps exposed to the acute challenge for both phenotypes (TukeyHSD Apo; $p = 0.033$, Sym; $p < 0.001$).

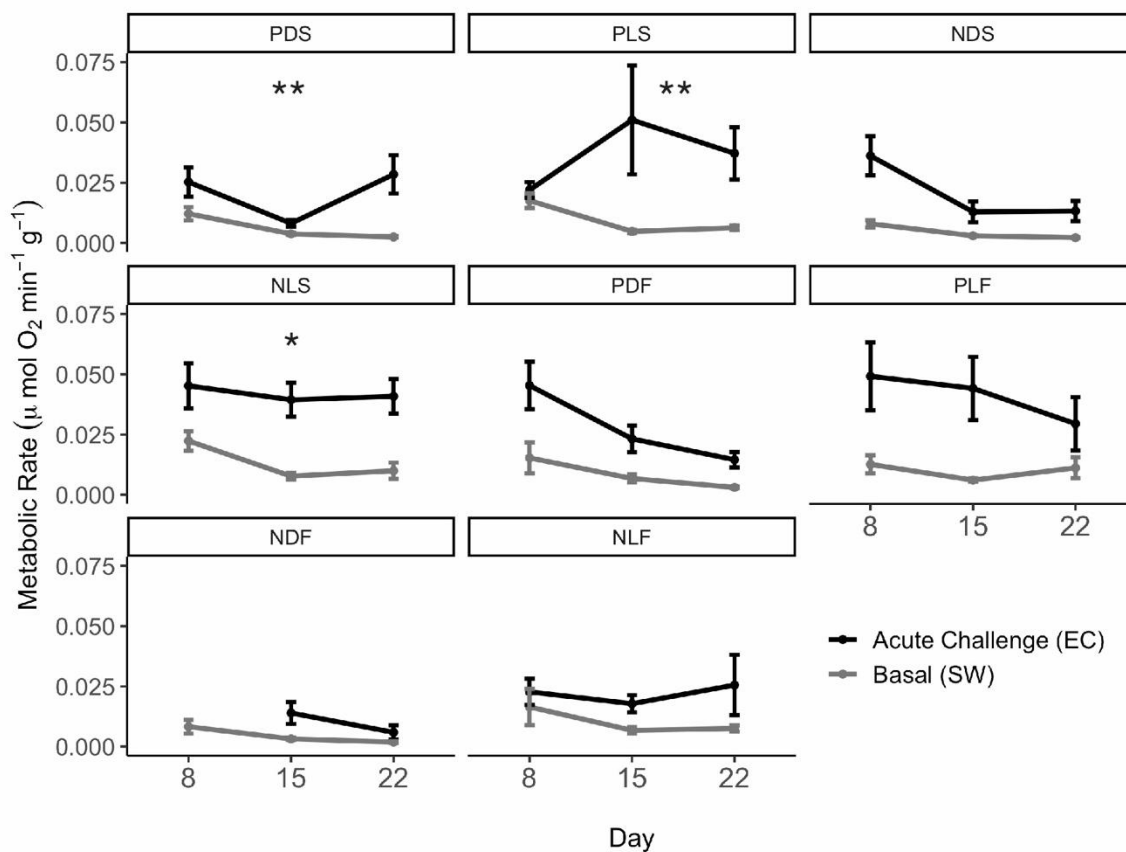


Figure 14. Metabolic rate over time by acute stress challenge for all experimental treatments. Data represent mean and standard error. Significance is denoted for treatments in which the interaction between acute stress challenge and time was significant where $p < 0.05$ *; $p < 0.01$ **.

In line with our hypothesis, elevated temperature significantly reduced coral fragments' basal metabolic rate and metabolic rate in response to acute stress compared to ambient temperatures (Figure 13A; LM; $t = -3.76$, $df = 305$, $p = 2.1 \times 10^{-4}$). Elevated temperature significantly dampened basal MR to an average of $0.009 \pm 0.0008 \mu\text{mol O}_2 \text{ min}^{-1} \text{ g}^{-1}$, 3.1 times slower than in ambient conditions (TukeyHSD; $p = 2.7 \times 10^{-6}$). Similarly, coral fragments in thermal stress responded 1.7 times slower ($0.026 \pm 0.031 \mu\text{mol O}_2 \text{ min}^{-1} \text{ g}^{-1}$) when acutely challenged (TukeyHSD; $p = 1.2 \times 10^{-3}$). However, within elevated temperature treatments, symbiotic phenotype had no influence on MR in either acute stress condition (Figure 13B; LM; $t = -0.816$, $df = 765$, $p = 0.415$)

Exposure to acute stress (bacterial *E. coli* ligands) initiated a metabolic response and resulted in faster metabolic respiration (MR) compared to basal metabolic rate across all experimental treatments (LMER; $p < 0.05$; Table 5). Metabolic rate was significantly impacted by time and the acute stress challenge independently, but not the interaction between time and acute stress, for the no-plastic/dark/starved (NDS), plastic/dark/fed (PDF), and plastic/light/fed (PLF) treatments (Table 6; LMER; $p_{\text{Time} \times \text{Acute Stress}} < 0.05$). MR for corals in the no-plastic/light/fed (NLF) treatment was affected the by acute stress challenge (Table 6; LMER; $t = -2.02$, $df = 91$, $p = 0.047$) but not day (Table 6; LMER; $t = -0.44$, $df = 91$, $p = 0.66$). Finally, MR in the no-plastic/dark/fed (NDF) treatment was not influenced by any term (Table 6; LMER; $p > 0.05$).

Table 5. Mixed effects linear model output for the effect of bacterial stress treatment on the metabolic rate of coral polyps within each experimental treatment. Tukey's HSD post hoc test is shown for the comparison between MR in basal and acute challenge treatments. Coral polyp ID was included as a random effect to account for repeat measures.

Model: Log (MR) ~ 'Acute Stress' + (1 Polyp ID)									
Liner effects mixed model					Tukey's HSD				
		estimate	df	t	Pr(> t)	estimate	df	t	p
PDS	Acute	-4.36	100	-34.33	< 2e-16 ***				
	Basal	-1.10	100	-6.30	7.93e-09 ***	1.1	34	6.284	<.0001***
PLS	Acute	-3.89	36	-27.31	< 2e-16 ***				
	Basal	-1.03	36	-5.17	9.12e-06 ***	1.03	34	5.163	<.0001***
NDS	Acute	-4.43	32	-34.27	< 2e-16 ***				
	Basal	-1.31	31	-7.34	2.71e-08 ***	1.31	35	7.337	<.0001***
NLS	Acute	-3.47	111	-33.07	< 2e-16 ***				
	Basal	-1.23	111	-8.11	7.35e-13 ***	1.23	36	8.111	<.0001***
PDF	Acute	-3.98	103	-33.74	< 2e-16 ***				
	Basal	-1.31	103	-7.67	1.01e-11 ***	1.31	34	7.664	<.0001***
PLF	Acute	-3.73	30	-23.59	< 2e-16 ***				
	Basal	-1.17	30	-5.34	8.83e-06 ***	1.17	31	5.337	<.0001***
NDF	Acute	-4.91	39	-22.45	<2e-16 ***				
	Basal	-0.63	34	-2.32	0.0264 *	0.629	31	2.292	0.0289*
NLF	Acute	-4.16	93	-38.36	< 2e-16 ***				
	Basal	-0.84	93	-5.61	2.04e-07 ***	0.84	32	5.594	<.0001***

Symbiotic phenotype did not impact metabolic response to the acute stress challenge for any experimental treatment except for no-plastic/light/starved (NLS) (Figure 15; LMER; $t = -2.6$, $df = 109$, $p = 0.011$). Additionally, both aposymbiotic and symbiotic polyps in the no-plastic/light/starved (NLS) treatment consumed oxygen significantly faster in the acute stress challenge compared to the basal MR of the same

phenotype (TukeyHSD Apo; $t = 6.55$, $df = 33.5$, $p < 0.0001$, Sym; $t = 4.99$, $df = 34.3$, $p = 0.0001$).

Table 6. Mixed effects linear model output for the effect of time and acute bacterial stress treatment on coral polyps in each experimental treatment. Coral polyp ID is included as a random effect to account for repeated measures.

Model: Log (MR) ~ Time * 'Acute Stress' + (1 Polyp ID)					
Liner effects mixed model					
		estimate	df	t	Pr(> t)
PDS	Time	-0.08	98	-0.58	0.567
	Challenge	0.12	98	0.31	0.759
	Time X Challenge	-0.62	98	-3.29	0.002 **
PLS	Time	0.13	70	1.00	0.319
	Challenge	0.13	93	0.32	0.748
	Time X Challenge	-0.61	69	-3.30	0.002 **
NDS	Time	-0.55	69	-4.79	9.23e-06 ***
	Challenge	-1.37	101	-3.80	2.49e-4 ***
	Time X Challenge	0.03	68	0.19	0.847
NLS	Time	-0.08	74	-0.70	0.488
	Challenge	-0.44	97	-1.18	0.242
	Time X Challenge	-0.39	74	-2.29	0.029 *
PDF	Time	-0.52	70	-4.55	2.16e-05 ***
	Challenge	-1.10	94	-3.00	0.003 **
	Time X Challenge	-0.10	70	-0.60	0.550
PLF	Time	-0.28	59	-2.18	0.033258 *
	Challenge	-1.50	87	-3.69	3.87e-4 ***
	Time X Challenge	0.17	59	0.99	0.325
NDF	Time	-0.88	36	-2.02	0.051
	Challenge	-1.74	35	-1.68	0.102
	Time X Challenge	0.33	36	0.69	0.495
NLF	Time	-0.06	91	-0.44	0.660
	Challenge	-0.77	91	-2.02	0.047 *
	Time X Challenge	-0.03	91	-0.17	0.865

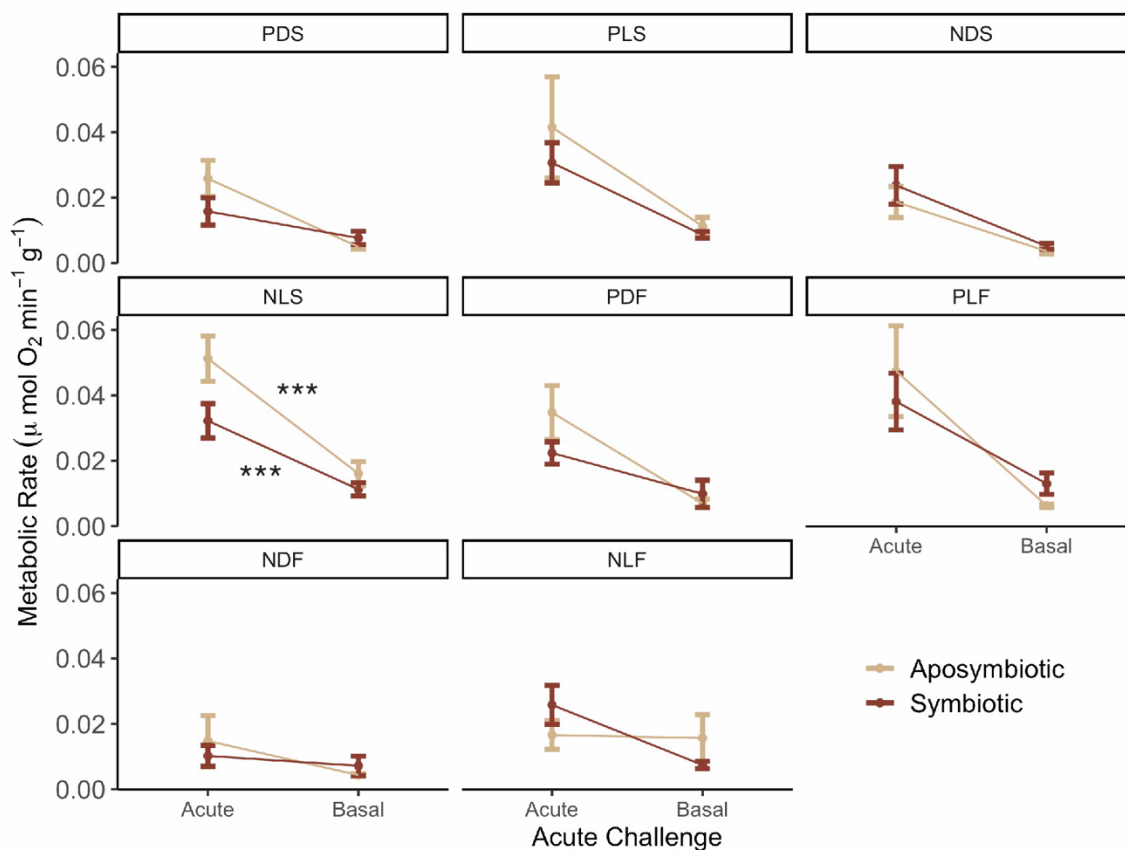


Figure 15. Metabolic rate of aposymbiotic (tan) and symbiotic (brown) coral polyps across experimental treatments. Data represent mean and standard error. Significance of Tukey's HSD post hoc is shown for the difference in response between phenotypes within experimental treatments where $p < 0.001$ ***.

Within the elevated temperature experimental conditions, neither plastic nor food influenced metabolic response to the acute stress challenge (Figure 16A, LMER plastic; $t = 0.932$, $df = 277.4$, $p = 0.352$; Figure 16C, food; $t = 0.591$, $df = 279.8$, $p = 0.555$). In contrast, light exposure significantly maintained MR in both acute stress conditions (Figure 16B, LMER; $t = 5.4$, $df = 237.4$, $p = 1.47 \times 10^{-7}$). Post-hoc pairwise comparisons show that MR is significantly faster for coral fragments exposed to light in the basal

conditions (TukeyHSD; $t = -6.64$, $df = 266$, $p < 0.001$) and in the acute stress challenge (TukeyHSD, $t = -5.4$, $df = 279$, $p < 0.001$) compared to dark-exposed corals.

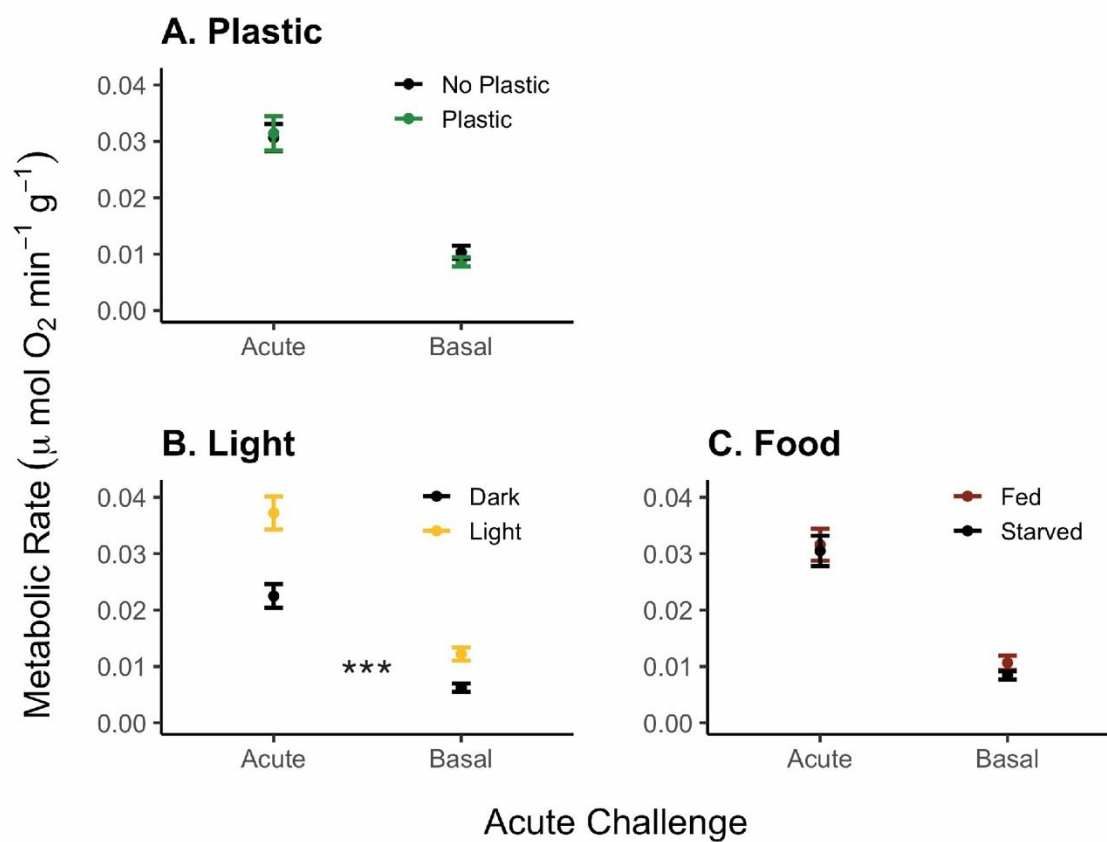


Figure 16. Metabolic rate of coral polyps within individual chronic stress treatments (A) plastic, (B) light, and (C) food. Data represent mean and standard error. Significance is shown where a given stressor influenced metabolic response (LMER) where $p < 0.001$ ***.

DISCUSSION

In this study, we investigated the impacts of multiple climate change and anthropogenic stressors on the coral holobiont to determine which stressor, or combination thereof, most influenced coral resilience. Additionally, we leveraged the facultative symbiotic coral *Astrangia poculata* to further investigate how symbiotic state influences responses to acute and chronic stressors. To the best of our knowledge, this is the first study to assess this particular suite of stressors together, and further, to investigate the impact of multiple chronic stressors on sublethal response to acute stressors in corals. Coupled with our survivorship results, this study disentangles how local and global chronic stressors can impact acute stress response and mortality. We found that across all metrics of coral host and symbiont performance, elevated temperature was the strongest driver of reduced coral physiology, while local stressors played a more variable role.

Direct impacts of stress on coral mortality

Thermal stress affected the survival of coral polyps over the duration of the experiment, where only 83% survived in elevated temperatures compared to 100% survival under ambient temperatures. The reduction of survival under elevated temperature conditions is expected, as there is a large body of literature demonstrating the negative impact of increasing temperature on coral survivorship (Carballo-Bolaños, Soto, and Chen 2019; Drury 2020; Mayfield et al. 2013; McClanahan et al. 2007; Jokiel and

Coles 1990). Given this broad scale trend, it is surprising that even with elevated temperatures (30°C) coupled with a variety of chronic stressors, 83% of polyps survived, demonstrating the overall resilience of this facultatively symbiotic coral. *A. poculata* has previously been shown to be resilient to thermal stress (Wuitchik et al. 2021, Burmester et al. 2017; Dimond et al. 2013; Dimond and Carrington 2007), which is thought to contribute to the large latitudinal range of this species (Thornhill et al. 2008; Dimond et al. 2013), also enabling this coral to live through seasonal variation. Some tropical corals have also been shown to be resilient to thermal stress (Jury and Toonen 2019; Barshis et al. 2013), but the high survivorship of *A. poculata* is much greater than what is typically shown elsewhere (Anderson et al. 2019), especially given the interacting stressors.

Interestingly, even with elevated risk of mortality in high temperatures, some factors that were *a priori* assumed to be chronically challenging actually appeared to improve survivorship. In particular, starvation reduced the risk of mortality in high temperature conditions, lending further support to an emerging hypothesis that lack of food input can lead to a metabolic quiescence that may help to buffer animal survivorship in periods of low food availability (e.g. winter) (Burmester et al. 2018; Breef-Pilz et al, in prep). Metabolic quiescence has also been demonstrated in the field, but has primarily been attributed to colder temperatures (Grace et al. 2017).

Survival is a powerful fitness metric (Darwin 1859), however given the overall high survival rates of these polyps, it is important to instead examine sublethal influences of each of the chronic stressors in this experiment. As such, sublethal indicators of holobiont stress, such as change in polyp mass, may provide important insight into

survival trajectory before a mortality effect is observed (Rosic et al. 2014; Anthony et al. 2009).

Influence of chronic stress on coral growth

Coral growth rate is a useful predictor of coral health as it requires substantial energy surplus to extend the coral skeleton and continue to grow (Shoepf et al. 2013). Here, we saw that elevated temperatures significantly accelerated the rate at which coral polyps lost mass over time. This finding is consistent with previous studies in scleractinian corals (Lough and Cantin 2014; Cantin et al. 2010; Crabbe 2008). However, reduced growth rate has been noted in this species at lower temperatures near their Northern range in Woods Hole, MA (Dellaert et al. 2022; Grace 2017; Dimond et al. 2013) and has been attributed to reduction of coral behaviors such as polyp extension and response to physical stimuli (Wuitchik et al. 2021). In one experiment, *A. poculata* colonies exhibited similar reductions in polyp behavior when they were unfed at ambient water temperatures (18°C) (Burmester et al. 2018).

Interestingly, contrary to our hypothesis, in addition to the survival advantage granted to starved polyps, we saw that feeding significantly increased the loss of polyp mass. This suggests that food consumption is detrimental at elevated temperatures, and reduces energy reserves needed for growth, even though heterotrophic feeding has been shown to help maintain growth during heat challenge in other scleractinians (Rodrigues and Grottoli 2007; Aichelman et al. 2016). The mitigating effect of heterotrophy may therefore not be universal, and instead may be dependent on the context of other chronic

stress factors. Burmester et al. (2018) proposed a nutritionally-cued metabolic dormancy in response to stress, in which corals will begin to shut down energetically taxing processes when prey is not readily available. Our findings support this hypothesis given that starvation slowed down the rate of loss of mass, and therefore may lead to metabolic quiescence in *A. poculata* in an attempt to buffer stressful environmental conditions.

Additionally, dark conditions negatively impacted growth while plastic exposure did not, even though corals were exposed to microplastics at a concentration 50 times greater than used in previous studies (de Soares et al. 2020). This can be further observed in the no-plastic / dark / fed (NDF) treatment which lost the most mass over time and was the single treatment that differed from the controls. This finding is particularly interesting because, although it is not the treatment with the most compounding stressors (plastic / dark / starved, PDS) it ranked highest in mortality risk, whereby corals in this treatment were over 50 times more likely to die at any point during this experiment compared to ideal conditions. Since both microplastic pollution (Mouchi et al. 2019; Reichert et al. 2019; Chapron et al. 2018) and lack of light (Houlbrèque and Ferrier-Pagès 2009; Osinga et al. 2011) inhibit skeletal growth by altering energy reserves, we predicted that the Plastic / Dark / Starved (PDS) treatment would exhibit the most exaggerated growth response due to the compounding presence of local chronic stressors. Given that the only supposed local stressor present in the NDF treatment is lack of light, it is possible that the increased loss of mass observed is due to reduced function in the symbionts. Although polyps in dark conditions lost mass significantly faster than those in light conditions, other treatment combinations exposed to darkness (PDF, PDS, NDS) were not impacted

to the same degree as polyps in NDF. Therefore, to further examine the role of symbiosis in holobiont maintenance during chronic stress, we can look to indicators of symbiont function such as photosynthetic activity and chlorophyll densities.

The impact of chronic stress on symbiont performance

Elevated temperature stress reduced photosynthetic efficiency (F_v/F_m) in brown (symbiotic) but not white (aposymbiotic) *A. poculata* coral polyps. In fact, aposymbiotic polyps exposed to elevated temperatures averaged F_v/F_m values 1.2 times higher than polyps in ambient control conditions. Further, it is interesting that, in looking at the change in photosynthetic activity over time, we observed a reversal in activity by phenotype, where aposymbiotic coral increased efficiency while symbiotic coral efficiency decreased at the onset of this experiment. Similarly, estimations of approximated chlorophyll density mirrored the observations for photosynthetic efficiency, where under elevated stress, symbiotic coral lost chlorophyll density and became whiter while aposymbiotic colonies increased pigmentation over time. In essence, regardless of symbiotic state at the origin of the experiment, all corals converged on a similar F_v/F_m by the end of the experiment. It is no surprise that symbiotic corals experienced reduced photosynthetic capacity and algal paling, as the impact of thermal stress on symbiont performance has previously been explored in both facultative and obligate coral species (Hoogenboom et al. 2012; Abrego et al. 2008; Warner et al. 2002; Heron et al. 2016; Wilkinson and Souter 2008; Hughes et al, 2003; Hoegh-Goldberg

1999). A recent study showed that *Breviolum psygmophilum* symbionts downregulate more genes involved in photosynthesis and oxidative stress response when exposed to extended thermal stress (Chan et al. 2021, preprint), and might suggest that *B. psygmophilum* are more thermally sensitive than their coral hosts. The initial increase in F_v/F_m coupled with the increase in chlorophyll density within aposymbiotic polyps observed here may be a short-term compensatory mechanism to increase nutrient acquisition under sudden stress since symbionts play a large role in autotrophic energy transfer (Tremblay et al. 2012; Ferrier-Pagès et al. 2011). Further investigation to determine whether this observation is consistent across variable feeding regimes is warranted to assess the impact of nutrient availability on symbiont acquisition in aposymbiotic corals.

Within a week of experimental treatment, aposymbiotic coral efficiency increased but then demonstrated a gradual reduction of F_v/F_m after 8 days of chronic stress. This pattern may indicate a shift from initial mutualism (increased F_v/F_m) to parasitism (decreased F_v/F_m) as the endosymbionts become increasingly compromised from chronic stress and can no longer contribute to the holobiont, but may still be sequestering resources from the host. Studies have hypothesized that when coral organisms are not in optimal conditions, maintenance of the symbiotic relationship becomes untenable when symbionts impose a significant metabolic burden on their host (Dellart et al. 2022; Baker et al. 2018; LaJeunesse et al. 2015; Lesser, Stat, and Gates 2013; Yakovleva et al. 2009; Stat, Morris, and Gates 2008). The mutualism-parasitism gradient hypothesis (Bronstein

2009), might explain why symbiotic polyps experienced a decline in photosynthetic efficiency within the first 15 days of this experiment.

As expected, in fed treatments, photosynthetic efficiency and chlorophyll density were maintained in corals exposed to elevated temperatures, lending support to the heterotrophic rescue hypothesis (Grottoli, Rodrigues, and Palardy 2006). Heterotrophy has been shown to offset the impacts of thermal stress by helping to maintain sufficient photosynthetic activity (Hoogenboom et al. 2012; Ferrier-Pagés et al. 2010; Borell et al. 2008) and mitigating coral bleaching (Aichelman et al. 2016; Borell et al. 2008; Rodrigues and Grottoli 2007; Grottoli, Rodrigues, and Palardy 2006). However, it is interesting to note that food availability appears to mitigate external chronic stressors of the symbiont (F_v/F_m , Chl-a), whereas food was inconsequential in helping to maintain coral mass (as a proxy for host tissue biomass). One study looked at the allocation of dissolved inorganic nitrogen (DIN) to the holobiont in different nutrient enriched environments and found that, when conditions were favorable and nutrients were plentiful, *B. psymophillum* symbionts benefited the host through DIN translocation to the host tissue, while scarcity of nutrients led symbionts to selfishly retain DIN and force the host to rely on internal lipid stores for energy (DiRoberts et al. 2021). This putatively parasitic mechanism of symbiont energy hoarding might shed light on the differential impact of heterotrophy as a mitigating factor to stress in this experiment and highlight that symbionts can be detrimental to hosts across a broad suite of both local and global environmental stressors.

Finally, we observed that neither plastic nor light treatments impacted F_v/F_m or chlorophyll density. Studies have assessed the impact of microplastic exposure on photosynthetic performance and chlorophyll densities with varied results. In some corals, exposure to microplastics has increased photosynthetic efficiency (Lanctôt et al. 2020; Rocha et al. 2020), while it has decreased in others (Reichert et al. 2021). Similarly, microplastics have been shown to decrease (Reichert et al. 2021), increase (Tang et al. 2018) or do not impact coral tissue brightness (Reichert et al. 2021) as a proxy for chlorophyll density and coral bleaching. Here, we saw that chronic exposure to microplastics did not result in significant changes in F_v/F_m or coral color (representing chlorophyll density). Tang et al (2018) proposes that increased chlorophyll content is a response to microplastic adhesion to the coral tissue, which reduces light availability, and subsequently increases algal chlorophyll production in an attempt to adapt to darker conditions (Falkowski 1980). Some plastics were observed to adhere to coral tissues, although the degree of adhesion was not quantified here.

Lack of light is proposed to lead to increased chlorophyll content (Falkowski 1980), but we observed a decrease in chlorophyll for symbiotic polyps and no change in aposymbiotic polyps in elevated thermal stress. In contrast, light exposure pushed the phenotypes to converge towards a single color where aposymbiotic colonies gained density and symbiotic polyps lost density, mirroring photosynthetic efficiency as described above. Previously, studies have observed a negative correlation between irradiance and symbiont density where increase in light intensity, or irradiance, results in a decrease in symbiont density (Cunning et al. 2017; Titlyanov et al. 2001), similar to

what we observed in our experiment. Downs et al. (2013) determined that, for the tropical coral *Pocillopora damicornis*, high light conditions (at 25° and 31°C) induced a higher degree of photo-oxidative stress compared to those in prolonged darkness in both temperatures. For *B. psygophilum* symbionts of *A. poculata*, saturating irradiance at which the maximum photosynthesis occurs has been identified (400 photos $\mu\text{mol m}^{-1}\text{s}^{-1}$ at 18°C) (Aichelman et al. 2019; Jacques, Marshall, and Pilson. 1983). Although the irradiance used in this experiment (80-90 photos $\mu\text{mol m}^{-1}\text{s}^{-1}$) was well below these levels, it is possible that the threshold for photoinhibition is lowered at elevated temperatures (Takahashi et al. 2004; Bhagooli and Hidaka 2004), and therefore light in this experiment acted as a stressor rather than a benefit to the symbiont as predicted. The relationship between temperature and photosynthetic capacity should be further investigated for this coral species. Regardless, in order to further determine how chronic exposure to sublethal stressors (microplastic exposure, starvation, darkness) impacts *A. poculata*, we examined the differential response to the physiological challenge of an acute stress.

The influence of chronic stress on acute stress response

Metabolic rate (MR) was significantly elevated when coral polyps from ambient control conditions were exposed to an acute stress challenge. This indicates that, when “healthy” corals are exposed to an acute stress, like the mimicked-threat of a harmful bacterial pathogen (lysed *E. coli* ligands), they are able to recognize its presence as a potential immune threat and respond accordingly. Under thermal stress, however, the

metabolic response to the acute stress challenge was subdued across all treatment groups compared to the response of coral polyps at ambient temperature. Research has indicated that some coral species might be mitigating the detrimental effects of chronic thermal stress through differential regulation of select genes associated with immune responses (Louis et al. 2017; Barshis et al. 2013). Barshis et al. (2013) determined that thermally-resistant corals had markedly different, and reduced, transcriptomic responses during heat exposure, and upregulation of genes associated with immune response, compared to more thermally-sensitive groups. They postulate that thermally-resistant corals have an increased threshold at which an immune response is activated which may confer greater resilience when exposed with an acute pathogen stressor. In our study the observed subdued MR responses could potentially indicate the inability of corals to recognize immunostimulatory constituents as a threat, or could indicate their inability to sufficiently respond due to chronic stress exposure.

Additionally, symbiotic association significantly elevated the MR of ambient control polyps that were exposed to the acute stress challenge, though there was no impact of symbiosis on basal respiration rates. While we could not distinguish between mechanisms, the elevated response could have been directly mediated by the symbiont (increased symbiont respiration), or indirectly mediated (host/holobiont has increased responsiveness when partnered with symbionts). For obligate-symbiotic cnidarians, studies have previously relied on analysis of algal cell density (Hoogenboom, Beraud, and Ferrier-Pagés 2010; Hoegh-Guldberg and Smith 1989), quantification of mitochondrial enzyme activity (Hawkins et al. 2016), and analysis of photosynthesis to

respiration ratios (P:R) (Muscatine, McCloskey, and Marian 1981; Coles and Jokiel 1977) as predictors of symbiont respiration. Studies have hypothesized that algal respiration constitutes roughly 10% of the gross photosynthetic capacity, and may increase in response to environmental stress such as fluctuations in salinity (Ferrier-Pagès, Gattuso, and Jaubert 1999), heavy sedimentation (Telesnicki and Goldberg 1995; Riegl and Branch 1995), and increased temperature (Verde and McCloskey 2001; Coles and Jokiel 1977). However, in this study, while there was no significant effect of symbiotic state on metabolic response at elevated temperatures (except in the NLS treatment, which maintained amplified responsiveness to acute stress), we observed a reversal of the role of phenotypes between temperature regimes. Although algal respiration is hypothesized to increase in response to stress, reduced metabolic response has been demonstrated in other obligate scleractinians when exposed to stressors (Nyström, Nordemar, and Tedengren 2001; Porter et al. 1989; Muthiga and Szmant 1987). In this study, chronic stress may impede symbiont performance to the extent that symbionts no longer contribute to metabolic response, either directly or indirectly, when faced with an acute stressor. These findings further support the idea that the relationship between corals and symbionts shifts from initial mutualism towards parasitism when the environment becomes stressful, and the relationship can no longer be equally maintained (Dellart et al. 2022; Baker et al. 2018; LaJeunesse et al. 2015; Lesser, Stat, and Gates 2013; Yakovleva et al. 2009; Stat, Morris, and Gates 2008)

Interestingly, the NLS treatment group was minimally impacted by chronic stressors across all metrics of holobiont performance (high survivorship, maintained

F_v/F_m , minimal changes in chlorophyll density, reduced loss of mass) and maintained elevated metabolic response, like those seen in ambient conditions, during each acute stress challenge. Corals in this treatment were given light to promote healthy symbionts, and were not given microplastics, thus presumably not expending energy to consume and expel microplastics (Reichert et al. 2018; Chapron et al. 2018; Hall et al. 2015). As such, it is possible that even under chronic thermal stress and nutrient limitation, these corals were healthy enough to recognize and respond to acute environmental stress. In contrast, corals in the NDS treatment, which performed similarly across holobiont metrics (97.5% survival, maintained F_v/F_m (0.17 ± 0.007 SEM), no significant change in chlorophyll density), and were exposed to the same plastic and food conditions, demonstrated decreasing metabolic response to acute stress over time. The difference between these two treatment conditions was light availability to *B. psysgophilum* symbionts. When provided with adequate light to promote photosynthesis and general symbiont function, the coral holobiont is able to maintain metabolic homeostasis (Ferrier-Pagés et al. 2011; Grottoli, Rodrigues, and Palardy 2006), while the no-light conditions inhibit the function of symbionts (Anthony and Fabricius 2000; Hoogenboom et al. 2012) and may reduce the metabolic contributions of *B. psysgophilum* to the holobiont. This finding may indicate that the respective “health” of corals in NLS is comparable in some way to corals at ambient temperatures where the algal-host partnership may still be mutually beneficial.

In this study, we demonstrated the interacting effects of chronic stressors, specifically how elevated temperatures (global) interacted with plastic, food, and light (local) availability. We found that elevated temperatures had the strongest negative effect

on coral performance and decreased overall health of the holobiont while the local stressors had more varied impacts. Despite the deleterious effects of thermal stress, corals maintained a high degree of survivorship and demonstrated incredible resilience to chronic stress. Although heterotrophy sustained symbiont-driven functions, it reduced survivorship and holobiont growth, suggesting that algal symbionts may act as parasites when the environment is not favorable. Therefore, starvation-induced metabolic quiescence may be a more advantageous strategy to cope with chronic stress and enable corals to survive more long-term. Importantly, as climate change progresses, many organisms have demonstrated a capacity for genetic and physiological modification to tolerate and survive chronic environmental stress. However, this survival becomes increasingly challenged when organisms are faced with acute stressors while concurrently existing in a chronically stressed state. Our study is among the first to disentangle the multitude of complex factors that contribute to coral survivorship, and affect sublethal fitness by layering the dynamic role of symbiosis with global change, local anthropogenic stress, and acute stress together. Given the IPCC projections of rapid, widespread global change and the projections of exponential human population increase, coupled with the increasingly vanishing global coral populations, this type of study is critical to contextualize and predict coral reef maintenance or loss in a changing world. The UN Decade of Ocean Science calls for “the science we need for the oceans we want”; this type of integrated, nuanced science is the necessary next step in understanding resilience, tolerance, and survival of coral ecosystems.

SUPPLEMENTARY MATERIALS

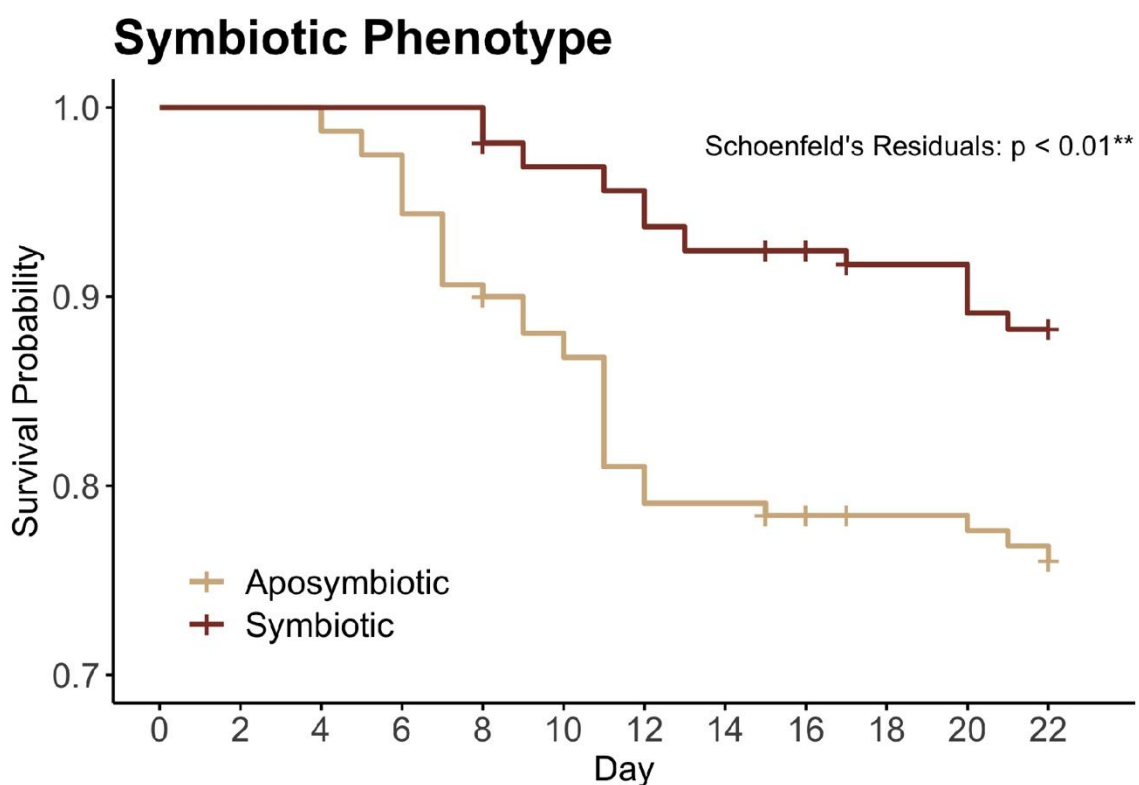


Figure S1. Kaplan-Meier survival curves for symbiotic and aposymbiotic coral polyps in experimental and ambient conditions. Symbiotic corals experienced a higher likelihood of survival compared to aposymbiotic corals (Hazard Ratio = 0.41, Likelihood Ratio Test; $X^2 = 9.92$, $df = 1$, $p = 0.002$). The Cox Proportional Hazards model operates under the assumption that the hazard, or risk of dying, is independent of time and remains proportional. Symbiotic phenotype, as a predictor of coral mortality, violates this assumption as the risk significantly changes with respect to time (Goodness-of-fit test of Schoenfeld's residuals; $X^2 = 8.12$, $df = 1$, $p = 0.0044$).

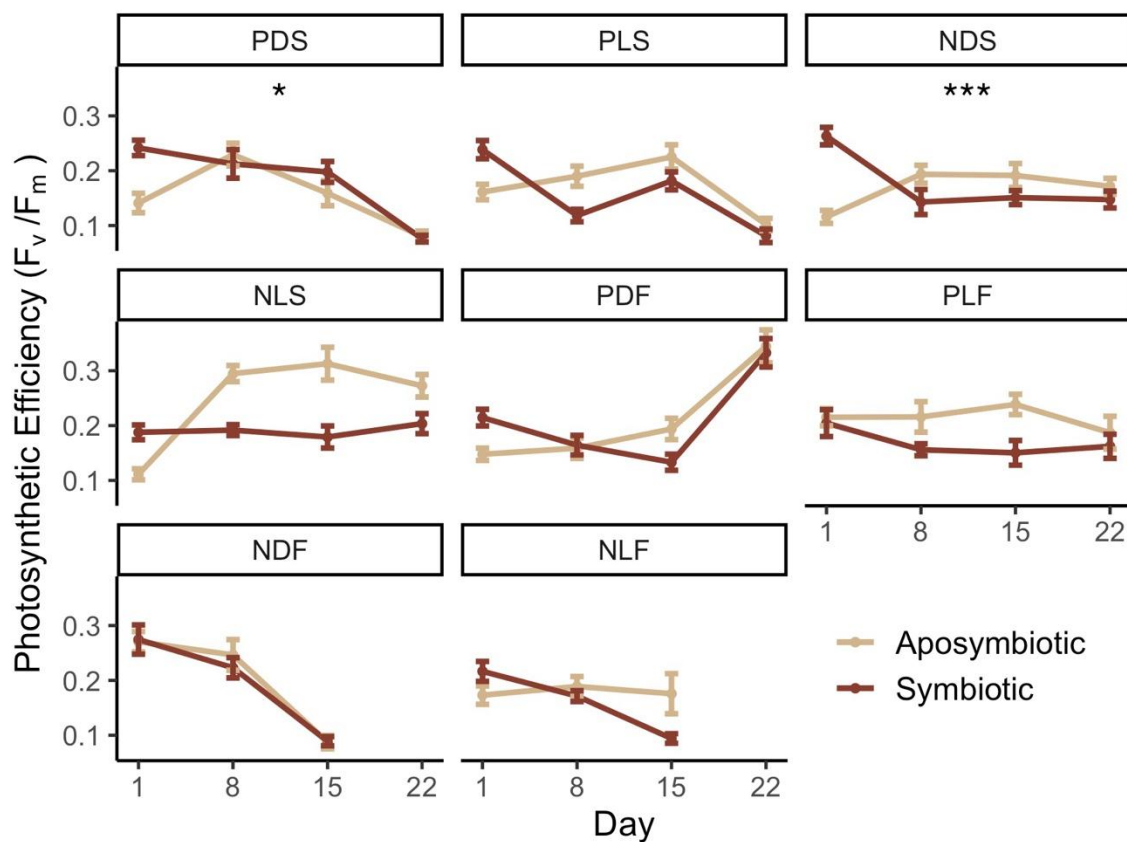


Figure S2. Photosynthetic efficiency (F_v/F_m) over time by symbiotic phenotype in experimental treatments. Data represent mean \pm SEM. For treatments in which phenotype, time, and the interaction between phenotype and time are significant (LMER), asterisks denote where $p_{\text{phenotype} \times \text{time}} < 0.5$ *; < 0.001 ***.

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