A strange solitary coral: The effects of heat stress on *Astrangia solitaria*

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Abstract

Global climate change has caused a significant increase in sea surface temperatures (SST) in the last century. Thermal stress due to the rise of sea surface temperatures and an increase in marine heat waves is a significant factor in the decline of coral reefs. While symbiotic coral stress responses, such as bleaching, are widely studied, less is known about aposymbiotic corals and how their physiology is affected by warming waters. This study explores the heat tolerance of *Astrangia solitaria*, a severely under-researched coral species, retrieved from Port Everglades, Florida. They were found living on steel pilings in 29°C waters, a temperature at the limit of typical optimal thermal ranges. Investigation of their biology revealed the absence of symbiotic photosynthetic algae, indicating their aposymbiotic nature. Through a common heat ramp experiment, we exposed corals to incremental temperature increases from 29°C to 35°C, exploring the effects on feeding behavior, mortality rate, and protein fluorescence. We used polyp extension states and feeding behavior as an indicator of coral health. Despite fluctuations in polyp activity and an increase in mortality with rising temperatures, the corals did not exhibit total mortality in 35°C waters. These findings reveal that *A. solitaria* may exhibit species-specific resilience strategies as an edge population that allows it to withstand significant heat stress, providing novel insights into coral species adaptations. *A. solitaria's* aposymbiotic nature provides opportunities for future investigation of how heat stress impacts coral host physiology. In the face of climate change and rising global ocean temperatures, a greater understanding of resilient species will be crucial as more ecosystems continue to face unprecedented environmental stress.

Keywords: aposymbiotic, coral, edge-population, resilience, heat-stress, *Astrangia solitaria,* thermotolerance

1. Introduction

Anthropogenic factors such as deforestation, burning of fossil fuels, and agriculture have led to a rapid increase in greenhouse gasses emissions and global temperatures (Cheng *et al.*, 2019; Cheng *et al.,* 2022). The Earth's climate system is under threat, with about 90% of the extra heat accumulating in the oceans (Cheng *et al.*, 2024). 2023 experienced the highest sea surface temperatures (SST) on record (Cheng *et al.*, 2024) and SSTs have been increasing globally at a rate of 0.13°C per decade from 1982 to 2023 (von Schuckmann, 2024). Regardless of action, the thermal inertia of water will lead to irreversible continued warming for the foreseeable future (Cheng *et al.*, 2019; Cheng *et al.*, 2024). Furthermore, temperatures are rising at a faster rate in the Atlantic (Cheng *et al.*, 2019; Cheng et al., 2024; Lee *et al.*, 2011), making it crucial to understand the acclimation and adaptation of species in this region.

Rising temperatures have pushed organisms to the edge of their thermal limits (Lindgren et al., 2016) and marine heatwaves are increasing in both frequency and intensity (von Schuckmann *et al.*, 2024). Coral reef ecosystems are distinctly vulnerable to thermal stress — the driving cause of their mortality (Hughes *et al.*, 2017). In the majority of corals the symbiotic relationship with the photosynthetic algae Symbiodiniaceae provides the coral with the bulk of its energetic needs through carbon sugars (Cziesielski *et al.*, 2019). This symbiotic relationship is vulnerable to thermal shifts and even a 1°C temperature increase can push corals beyond their thermal limits and disrupt the symbiosis between hosts and their algal symbionts (Rivera *et al.,* 2020). These temperature shifts can lead to a loss of symbiosis in a process called coral bleaching (Rivera *et al.,* 2020). As the photosynthetic byproducts produced by Symbiodiniaceae are the main source of energy production in obligate symbiotic corals, bleaching severely increases their mortality (Dimond & Carrington, 2007).

The majority of research on coral stress responses to increased temperatures has focused on tropical reef building symbiotic corals (Putnam, 2021). However, in recent years, the facultatively symbiotic *Astrangia poculata* has gained interest due to its ability to survive in both symbiotic and aposymbiotic states (Wuitchik *et al.*, 2021). Previous work has found that stress responses of aposymbiotic *A. poculata* are rooted in metabolic decline, protein degradation, reduced immunity, inability to fend off settling organisms, and tissue deterioration (Dimond & Carrington, 2007). In addition, when *A. poculata* is placed under thermal extremes, it can lead to polyp retraction — a potential defense response wherein they enter periods of quiescence (Wuitchik *et al.*, 2021). Interestingly, *A. poculata's* congeric aposymbiotic counterpart, *Astrangia solitaria*, is unstudied. *Astrangia solitaria* (Dwarf Cup Coral) is a shallow water scleractinian coral that has been recorded throughout the western Atlantic Ocean and the Caribbean Sea. It is an encrusting coral with a reptoid structure, small calicles (Serra *et al.*, 2023), and clear/white, green, or brown polyps (Cairns, 2000; Serra *et al.*, 2023). Though its morphology is not well documented, it is assumed that the coenosarc does not connect polyps to each other. Although *A. solitaria* is widespread, there is insufficient research documenting the physiological responses of *A. solitaria* under stress.

Coral communities living at the edge of their environmental limits have potential to serve as resilience hotspots and can provide insights into how coral reefs might function in future ocean conditions (Schoepf *et al.*, 2023). These extreme reefs facilitate acclimation/adaptation to climate change-induced stressors (Schoepf *et al.*, 2023). *Astrangia solitaria* exhibits wide habitat ranges, as they are generally found growing attached to coral rubble and undersides of platy corals across all reef zones (Cairns, 2000; Goreau, 1959). The creation of novel settlement habitat by anthropogenic activity (Aguilera *et al., 2023*) has resulted in *A. solitaria* colonizing anthropogenic artificial substrates such as

polypropylene fishing rope (Alves-Júnior *et al.*, 2024). This wide range across extreme and edge habitats may lead to *A. solitaria* having high thermal resilience; however, few studies have explored the physiological response of *A. solitaria* to thermal stress.

In this study, we investigated specimens of *A. solitaria* collected from steel pilings in Port Everglades, Florida. (Figure 1) Port Everglades is a major metropolitan area and cruise ship port in Fort Lauderdale, Florida. This urban setting has likely pushed coral species like *A. solitaria* to adapt to their modified environment. According to the NOAA Florida Keys National Marine Sanctuary, the optimal temperature range for reef-building corals is 23°C to 29°C (NOAA, 2024). Given that our *A. solitaria* samples were collected from waters with a mean temperature of 29°C, it is assumed that these corals are already sitting at the edge of their optimal thermal range. This high acclimation temperature, combined with its urban habitat, characterizes *A. solitaria* as an edge population. In light of its unique life history, we investigated how increasing water temperatures affect feeding behavior, mortality, and protein fluorescence in *A. solitaria.*

We hypothesized that warmer waters would increase mortality rate and lead to the gradual health decline of *A. solitaria.* A secondary hypothesis is that *A. solitaria* will be able to withstand temperatures above what is considered the thermal limit for Floridian corals. This study will help develop our understanding of *A. solitaria's* responses to heat stress in order to expand our knowledge of coral resilience as they approach thermal limits and provide understanding of *A. solitaria* biology, ecological niche, and adaptive strategies in an era of continued global change.

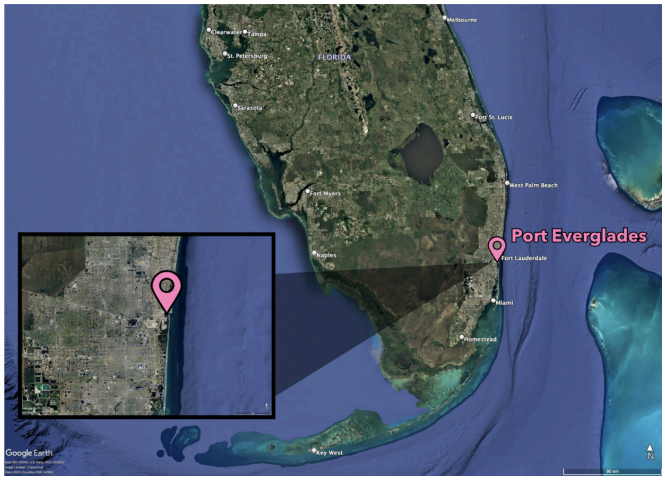

Figure 1. Map of Port Everglades, Florida. This map shows the collection site for the *Astrangia solitaria* samples used in this study. The inset map highlights the high-traffic, urban port of the collection site.

2. Methods

2.1 Experimental Design

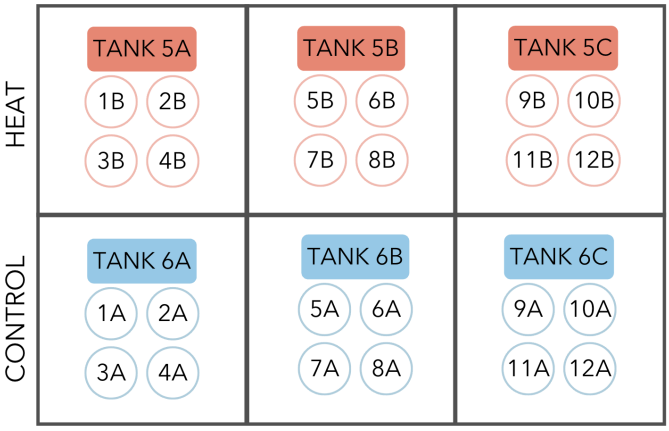

Figure 2. *Astrangia solitaria* thermal stress experimental design. Diagram of fragment distribution between three heated (Red) and three control (Blue) tanks. Genets are divided into "A" and "B" ramets, with "A"s being placed in the controlled treatment and "B"s in the heated treatments. Fragments with the same number represent the same genet.

Twelve *Astrangia solitaria* fragments were collected via scuba from steel pilings in Port Everglades, Florida (26.0816° N, 80.1206° W) and shipped to Boston University (BU). Upon arrival, corals were acclimated at 29°C for 101 days in the Davies Marine

Invertebrate Research Facility. Each coral fragment was randomly divided into an "A" and "B" ramet, resulting in a total of 24 fragments. These fragments were divided evenly between control and heated tanks, with 12 nubbins per treatment and 4 nubbins per tank. All "A" ramets were placed in the control tanks and "B" ramets were heated to ensure equal genetic variability in each treatment (Figure 2). To test the response of *A. solitaria* to heat stress, we had 3 heat treatment tanks and 3 control tanks, creating a total of 6 tanks.

All tanks were maintained at the same light cycle of 12 L:12 D (12:30 am - 12:30 pm), with PAR ranging from 30-40 during light cycles. Flow conditions were kept consistent via powerheads. Nubbins were evenly distributed in the center of the tank to ensure equal light exposure. The heat treatment tanks underwent a thermal ramp of 1°C every morning until signs of mortality were observed when set to 36°C, where temperature was maintained for the remainder of the experiment (Figure 3).

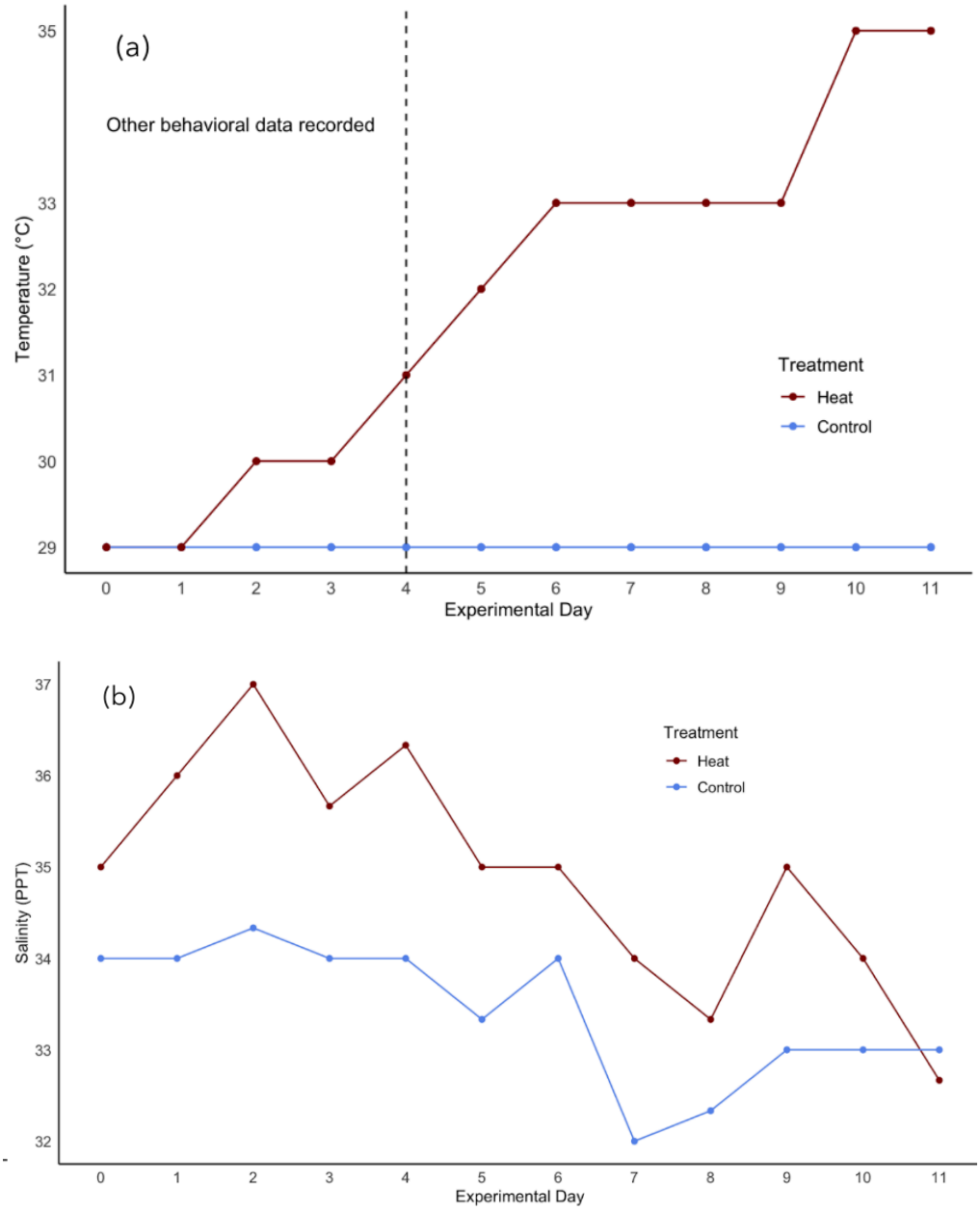

Figure 3. Water temperature and salinity measurements for heated and controlled tanks during the heat stress experiment duration. **(a)** Afternoon temperature (˚C) recordings. The dotted line represents the day feeding time and behavior measurement procedures changed. **(b)** Salinity recording in parts per thousand (PPT), which were taken in the morning.

2.2 Water Quality

Water quality was measured three times a day. Temperature was measured three times daily (morning, midday, evening) using a Durac glass thermometer. Salinity was measured once a day (morning) using a *The Aquarium Solution* 'H_2Ocean' refractometer. Auto top-off systems were refilled with distilled water daily to maintain salinity and account for evaporation. Filter socks and protein skimmers were cleaned every other day to ensure filtration remains consistent between treatment tanks and sumps.

2.3 Fluorometry

Initially, Pulse Amplitude Modulation (PAM) fluorometry measurements were planned to be taken every three days to measure the photosynthetic efficiency of Photosystem II in the coral polyps (Xia *et al.*, 2023). PAM measurements were conducted twice at the beginning of the experiment after dark acclimation. However, after day 4 corals were placed under a fluorescent microscope and it was determined that they were aposymbiotic so any F_V/F_m value that was measured was likely from endolithic algae or nearby encrusting algae on the fragments. Therefore, PAM data are not included in this study.

2.4 Health Assessment

For days 1 through 5 of our experiment, feeding was conducted daily in the morning between 9:00 am and 11:00 am. Upon the observation of higher activity during the dark cycle, from day 5 onwards, feeding occurred between 1:00 pm and 3:00 pm. Food for each treatment was a mixture of 0.3 cc Reef Chili and 0.3 cc PolypLab Reef Roids diluted with about 100mL of seawater taken directly from either the heated or controlled tanks. The 100mL mixture was target-fed to polyps and distributed as evenly as possible between the three tanks for each treatment. Polyp expansion after feeding was used as a proxy for holobiont health. The behavior and activity of the coral polyps were noted as the level of tentacle extension 30 minutes after feeding. The assessment methods were adapted from Rastelli et al. (2020), where each fragment is assigned an expansion state using the following metrics: Value 0: totally retracted polyps, Value 1: tentacles or polyps emerging from the gastric cavity, and Value 2: polyps with extended tentacles. The percentage of polyps on a single fragment displaying each expansion category was recorded. The percentage of total open polyps per fragment was later calculated by summing emerging and totally expanded polyps. The same individual conducted feeding assessments to avoid differences in judgment on extension and minimize bias.

2.5 Photo Analysis

Standardized top-down photos of each fragment were taken at the beginning and end of the experiment. The initial purpose was to conduct Red Channel analysis of the images. After discovery of *A. solitaria*'s aposymbiotic nature, these photos served the purpose of documenting general visual changes to the coral fragments following heat challenge. Photos were taken in a studio set-up using a Sony A7 IV camera. Coral fragments were taken out of water and placed on a Coral Watch Coral Health Chart (Figure 4).

Figure 4. Photo of *A. solitaria* coral fragment 2B (heat treatment) at day 0 of experiment.

Figure 5. Three states of polyp extension. Three states of polyp extension observed in *Astrangia solitaria*. **(a)** Value 0 - fully retracted polyp, where tentacles are completely withdrawn into the corallite, indicating minimal activity. **(b)** Value 1 - partially extended polyp, with tentacles emerging from the gastric cavity, suggesting moderate activity or responsiveness. **(c)** Value 2 - fully extended polyp, where tentacles are maximally extended, indicating a high level of activity and feeding readiness.

To determine whether *A. solitaria* was symbiotic or aposymbiotic, close-up photos of non-experimental samples were taken using a Leica Olympus SZX16 microscope. Photos were captured through microscope software as well as by positioning an iPhone 14 camera through the microscope eyepiece to observe the presence of symbiotic algae. This set-up was also used to visualize the different categories of polyp extension (Figure 5). Finally, our Leica microscope was used for fluorescent microscopy to document Green Fluorescent Protein expression in the control versus heated fragments at the end of the experiment.

2.6 Statistical Analysis

ANOVAs were run on linear regression models to test the effects of treatment on the different polyp extension states at day 5 (first day of updated behavior measurements) and day 11 (last day of the experiment). Additionally, a linear regression model (lm) was performed on feeding data (Polyp Expansion State vs Temperature/Day) split by temperature treatment to determine if feeding behavior was modulated by temperature. Temperature, salinity and feeding data were visualized in boxplots, bar graphs and line graphs. A general map of the site was made using Google Earth.

3. Results

3.1 Water Quality

The control tanks were maintained at a constant 29±1℃ over the course of the experiment. Temperature in the heat treatment ramped from 29 to 35℃, raised by 1℃ everyday up to day 8 of the experiment. As the water in the heat treatment tanks took time to actually see an increase in water temperature, fluctuations in the actual water temperature were recorded as seen in Figure 3a. Salinity was kept relatively constant throughout the experiment ranging from 32 to 34 ppt with (average of 33.4 ppt) in the control and 32 to 37 ppt (average of 34.9 ppt) the heated tanks (Figure 3b).

3.2 Qualitative Observations

Our observations reveal that *Astrangia solitaria* is an aposymbiotic coral. It was found living on steel, an unnatural substrate (Figure 14). The coral consists of individual polyps not connected by a clear coenosarc. The polyps ranged in proximity to each other, some

appearing joined at the base. The corallites are a brown red color with the corallites having distinctly raised septa (Figure 4). Under blue light, polyp tentacles appear clear with fluorescent spots (Fig. 14). We observed higher polyp activity during the dark cycle, indicating the nocturnal feeding behavior of these heterotrophic animals. As the thermal ramp progressed, polyp activity visibly decreased and the base of some polyps appeared a lighter pink color. In both treatments, other organisms were found living on the corals, such as coralline algae, which bleached following heat treatment (Figure 6).

3.3 Feeding Behavior

Polyp extension data was visualized in a histogram and found not normal even after log and square root transformations. A regression was run on different variables against the percent of retracted polyps. With the data from the heat treatment separated, we found a positive correlation between the amount of retracted polyps and temperature with an R-squared value of 0.35 and a significant p-value of 0.001 (Figure 7). There was a significant correlation between the amount of retracted polyps and experimental day with an R-squared value of 0.38 and a significant p-value of 0.001 (Figure 8). The control treatment showed a comparatively weaker correlation between the amount of polyps retracted and the experimental day with an R-squared value of 0.08 and a p-value of 0.001 (Fig. 8).

We analyzed the average polyp extension level between treatments across days 5 to 11. The overall trend was that full polyp extension in the heat treatment decreased and the proportion of retracted polyps increased (Figure 9). The control displayed small fluctuations throughout the course of the experiment but no major trends emerged (Fig. 9). Overall, there was a significant decrease in feeding behavior for the heat compared to a slight overall increase for the control treatment (Fig. 9).

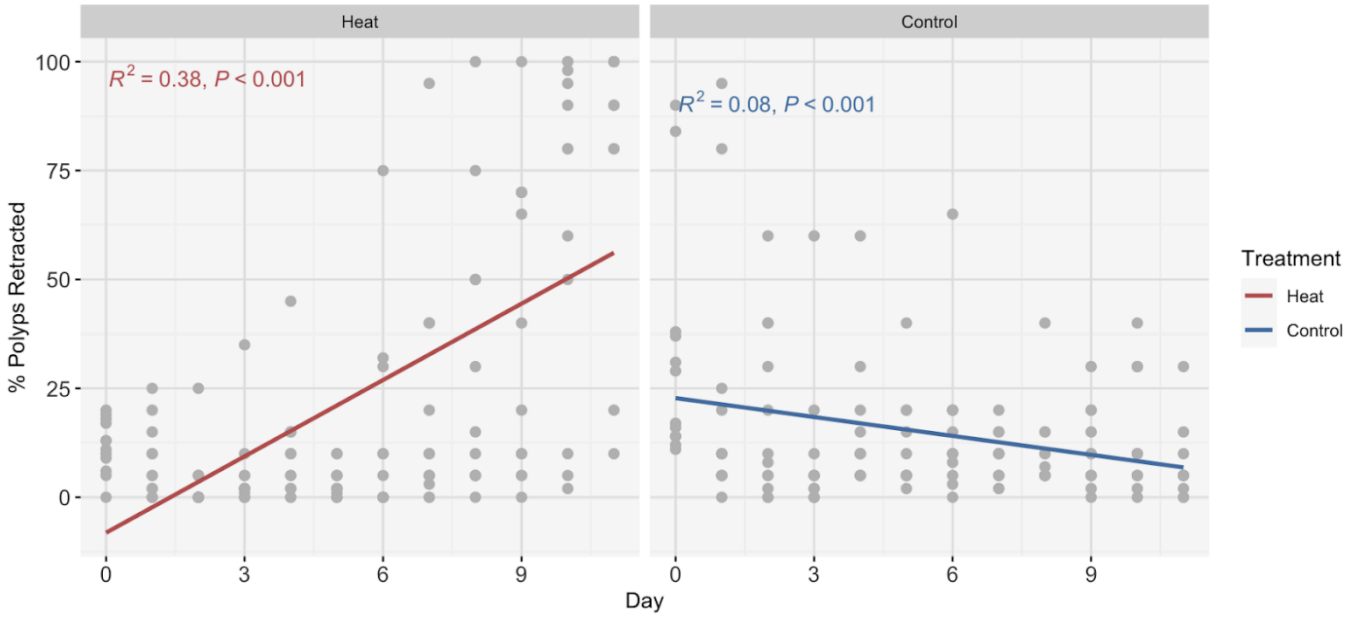

Figure 8. Retracted polyps by treatment. Linear regression of % polyps retracted by experimental day for *Astrangia solitaria* under heat and control treatments. In the heat treatment, % polyp retraction shows a positive trend over time (R2=0.38, P-value<0.001), while the control group shows a slight negative trend (R2=0.08, P-value<0.001). This suggests that elevated temperatures increase polyp retraction over time.

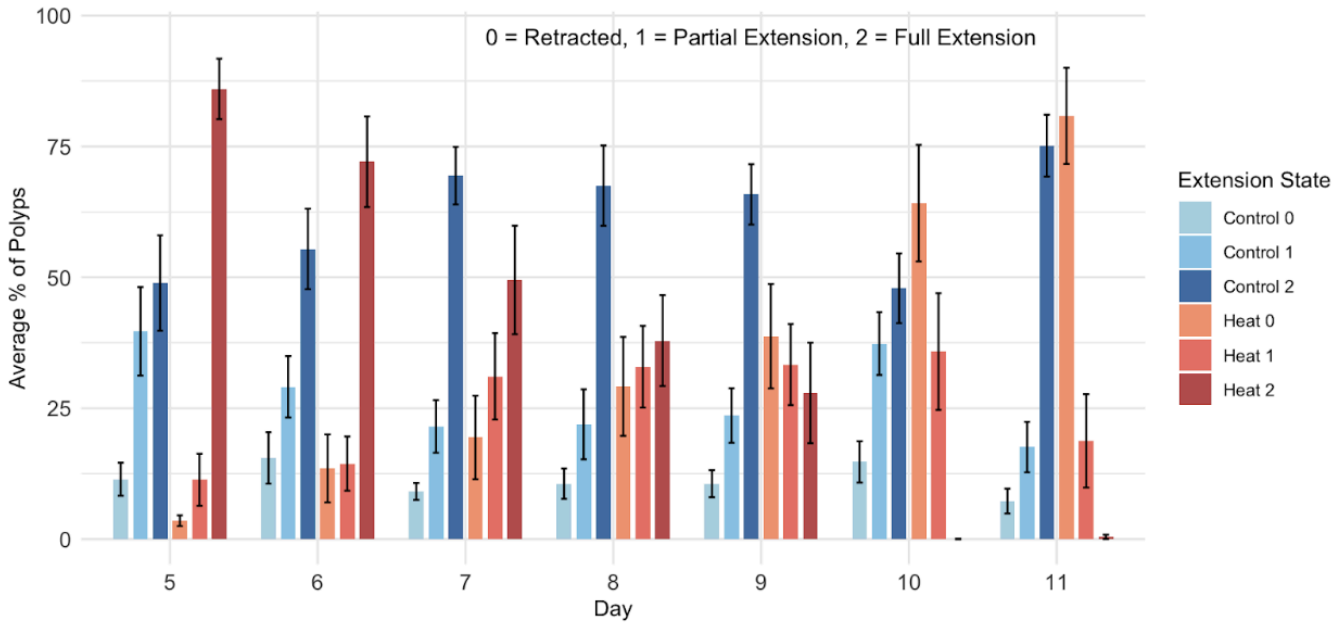

Figure 9. Average polyp extension level between treatments. Average percentage of *Astrangia solitaria* polyps in each extension state (0 = retracted, 1 = partial extension, 2 = full extension) between heat and control treatments over the course of the experiment. Control polyps generally displayed higher levels of full extension (blue bars), while polyps in the heat treatment showed a greater proportion of retraction (red bars) over time. Bars represent standard error. This indicates that elevated temperatures influence polyp extension behavior, reducing full extension and increasing retraction as a stress response to heat.

We ran a comparison of polyp activity levels on day 5 versus day 11 of the experiment and found significant differences. The average amount of polyps retracted in the heated treatment increased from less than 5% on day 5 to around 75% on the final day. Inversely, the amount of fully extended polyps decreased from 80% on day 5 to nearly 0% on the final day (Figure 10). The control treatment showed minimal change between the two days with the majority of the polyps being fully extended on the final day. We ran six one-way analysis of variances (ANOVA) to compare polyp extension levels on day 5 to day 11. An ANOVA was run for each of the three extension states in the two treatments (Table 1 & 2).

For the heat treatment, the ANOVAs yielded a significant p-value for retracted and fully extended polyps (Table 3). For the control treatment, the ANOVA revealed that there was no significant difference between the amount of retracted polyps on day 5 and day 11 (Table 4).

Lastly, the data was divided by genet and the extensions states were visually displayed in stacked bar plots. This revealed that at day 10 the majority of fragments in heat appeared to have a major drop off in feeding activity (Figure 11). There was no significant change in polyp extension in heated tanks between days 8 and 9 but a significant decrease in full extension from around 25% to 0% from day 9 to 10 ($p < 0.01$; Fig. 9; Table 5 & 6).

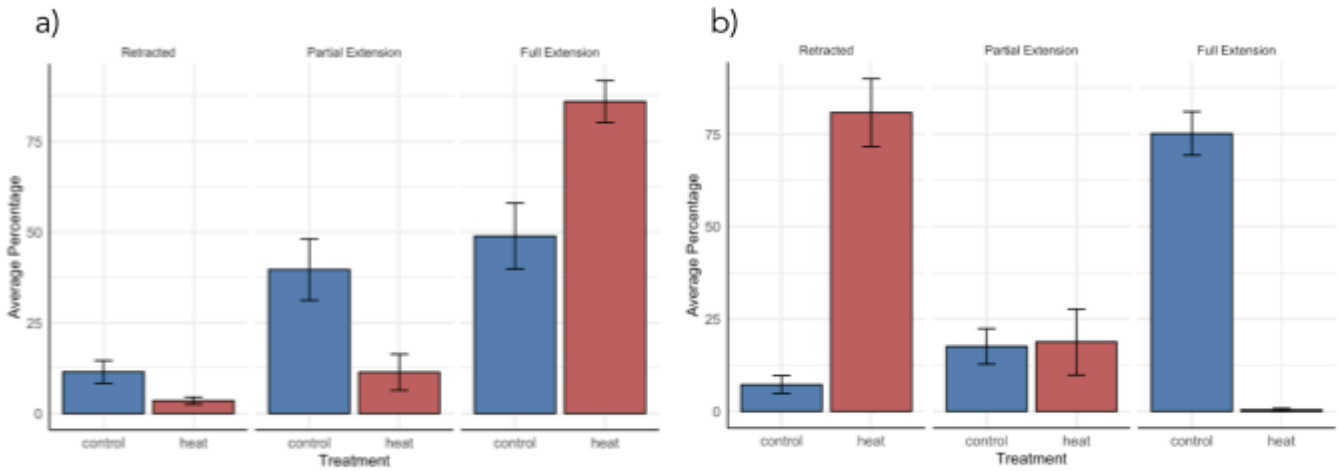

Figure 10. Comparison of *Astrangia solitaria* polyp activity between control and heat treatments on Day 5 and Day 11. Bars represent the average percentage of polyps in each extension state: retracted, partial extension, and full extension. Error bars represent standard error. **(a)** Day 5 polyps in the heat treatment exhibited higher full extension compared to controls **(b)** Day 11, the heat-treated polyps showed a significant increase in retraction and a marked reduction in full extension.

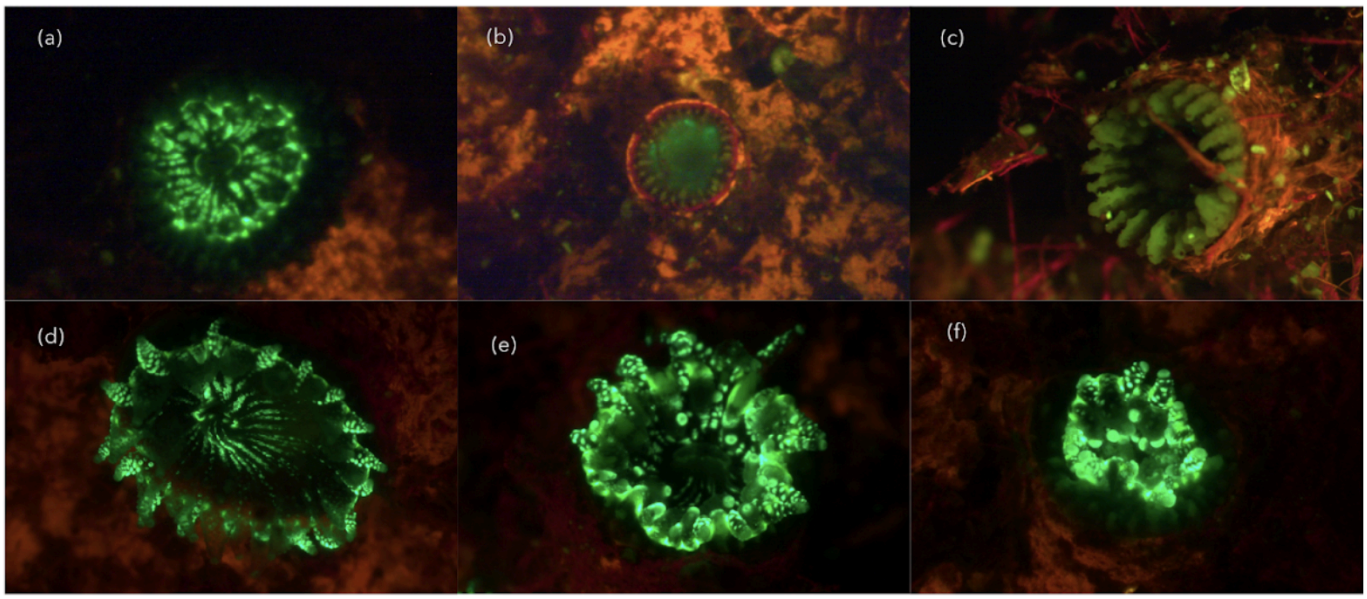

Figure 12. Green Fluorescent Protein (GFP) concentrations of experimental corals. (a) Heated, alive; **(b)** Heated, dying; **(c)** Heated, dead; **(d)** Controlled, alive; **(e)** Controlled, alive; **(f)** Controlled, alive. This is in heat and control treatments, illustrating varying health states. GFP intensity and distribution vary by health status, with live polyps displaying more defined GFP expression, while dying or dead polyps in the heat treatment show reduced or fragmented fluorescence.

3.4 Green Fluorescent Protein

At the end of the experiment, the majority of both experimental and control nubbins exhibited levels of Green Fluorescent Protein (GFP), indicating the presence of live polyps (Figure 12). All fragments showed some level of survival, indicated by punctuated fluorescence in corallite structures. Although a few dead polyps were observed, the majority of randomly selected polyps appeared alive. In control samples, GFP was clearly visible in the tentacles when exposed to blue light (Fig. 12E). In contrast, polyps in a retracted state under the microscope showed GFP concentrated around the mouth area (Fig. 12A). However, polyps from the heated tanks displayed less defined fluorescence, particularly among those dead or in poor health (Figure 12B, C).

4. Discussion

The objective of this study was to simulate the impacts of rising temperatures in order to look at the physiological responses of heat-stressed *A. solitaria.* Our results showcase several important findings but emphasize the need for further research.

4.1 Thermal Stress Responses

4.1.1 Feeding Responses

Ocean heat content reached an increase of 464 ± 55 ZJ in 2023, the highest level on record (Cheng *et al.*, 2024). The irreversible nature of this warming highlights the urgent need to understand the widespread impacts of climate change (Cheng *et al.*, 2024). This includes learning more about underrepresented species and how their biology is impacted by thermal stress. Through our research on *A. solitaria*, we aimed to understand a unique aposymbiotic coral and its specific stress responses, behavior, and biology. We hypothesized that the health of *A. solitaria* polyps would decline as temperatures increased. This hypothesis was supported through the observation of decreased polyp extension as the thermal ramp progressed (Fig. 9). Over the 11-day experiment, we observed a significant positive correlation between the proportion of retracted polyps and temperature (Fig. 7). In contrast, control corals experienced a slight increase in feeding as the experiment went on, likely due to acclimation after handling during experimental setup (Fig. 8). Decreased feeding at higher temperatures is consistent with data on other aposymbiotic corals such as *Astrangia poculata* (Wuitchik *et al.,* 2021).

We observed polyps continuing to feed even at 35°C, although the percentage was significantly reduced (Fig. 9). This is well beyond the 21.7 to 29.6°C global average thermal tolerance limit of coral reefs (Guan et al., 2015). The corals were living in Florida waters at the upper limit of 29°C, however as the region continues to experience rising temperatures corals have been documented surviving in conditions up to 40℃ (NOAA, 2024). In 2023, Florida experienced the worst bleaching on record with almost 100% of coral bleaching and water temperatures up to 38℃ (Neely et al., 2024). The significant decline of polyp activity on day 10, when temperatures reached 35℃ and 36℃, could reveal an upper thermal limit of *A. solitaria* (Fig. 9). Future research could explore smaller thermal changes to define this limit.

As metabolic demand increases with heat stress corals must cope nutritionally (Dobson et al., 2021; Rädecker et al., 2021). Therefore, we predicted an initial increase in feeding behavior of thermally stressed *A. solitaria*. However, this was not observed in our experiment (Fig. 13). In addition to increasing heterotrophy, symbiotic corals may catabolize energy reserves, decrease respiration, and decrease calcification rates in response to high temperatures (Dobson et al., 2021). A potential explanation for the lack of increased feeding in *A. solitaria* is the utilization of these other strategies.

Coral bleaching occurs at high temperatures due to a breakdown of the relationship between Symbiodiniaceae algae and the coral host causing an ejection of the colored algae symbionts (Cziesielski et al., 2019). One perspective on coral bleaching is the oxidative theory which states that the excess production of reactive oxygen species (ROS) by damaged symbionts leak into host cells, resulting in tissue damage (Nielsen et al., 2018). This causes the coral to expel its symbionts in an attempt to reduce its physiological stress (Nielsen et al., 2018). A potential explanation for the survival of *A. solitaria* at 35℃ may be the absence of the negative byproducts of ROS being produced intracellularly by the algal symbionts. Future studies should investigate the role of ROS in determining thermal tolerance limits in the coral host.

4.1.2 Green Fluorescent Protein

The presence of GFP observed in all nubbins supports the theory that *A. solitaria* is able to survive temperatures up to 35℃, however this is clearly at a cost as noted by the significant drop in full extension from an average of 86% on day 5 to 0.42% on day 11 (Fig. 10 & 12). More information is needed on the physiology behind these changes to fully understand the resilience and recovery of these corals. Future research should look at long term studies that encompass recovery periods after stress.

When stressed, anthozoans have been seen to upregulate fluorescent protein (FP) concentrations (Palmer et al., 2009). This is a potential response to changes in ROS exposure (Palmer et al., 2009). Oxygen radicals are produced through metabolic pathways to manage tissue redox states especially by symbiotic corals (Palmer et al., 2009). During thermal and light stress events, ROS expression is elevated and oxidative stress can occur in the symbiont and the coral host (Palmer et al., 2009). Given *A. solitaria*'s aposymbiosis, we can specifically focus on ROS stress responses in the coral host and predict a higher thermal tolerance. However, previous studies have shown variability in the response of fluorescent proteins to thermal stress with their exact function remaining unknown (Dizon et al., 2021; Roth and Deheyn, 2013). Our observations of decreased GFP in thermally stressed *A. solitaria* supports the decrease in GFP concentrations alongside declining coral health found by Roth and Deheyn (2013) in a symbiotic coral species (Figure 12 a-c).

GFP presence was observed in the majority of polyps on our experimental ramets, even those that we assumed had experienced total mortality (Fig. 12). This finding provides us with many opportunities for further research on *A. solitaria*. Given that we did not measure GFP throughout the course of the experiment and only conducted fluorescent microscopy on the final day, we may have missed certain points of upregulation of FP transcripts in response to initial thermal stress. Establishing checkpoints for polyp photography and fluorescence measurement would be beneficial to the analysis of this coral's stress response. Dizon et al. (2021), outline temporal changes in gene expression in response to heat stress in different coral species, noting that some demonstrated significant upregulation 24-48 hours after exposure and then downregulation back to baseline values by 72 hours. This suggests that *A. solitaria* could have exhibited different levels of transcript expression throughout the experiment depending on where they were in the heat ramp. More frequent fluorescent microscopy could provide more detail on their biological responses. Looking at fluorescence after a cool-down period where we decrease the temperature back down to their ambient state could also provide valuable insight on coral recovery. Additionally, tissue analysis of GFP concentrations and fluorescent protein structures in experimental corals would broaden our understanding of the coral's stress response mechanisms. Finally, given the knowledge gap on the genetic variation between *A. solitaria* individuals, gene sequencing of the experimental polyps will allow for analysis of GFP concentrations

and potential intraspecific variation in FP across the individuals on a fragment (Dizon et al., 2021).

4.1.3 Analysis of Qualitative Results

The reddish color of the polyps may result from crustose coralline algae (CCA) encrusting over the skeletal base of polyps as the source of pigment in *A. solitaria* remains unknown. While coral coloration typically arises in coral from their symbiotic algae, this is not the case in aposymbiotic species (Rivera & Davies, 2021). We observed large amounts of CCA present throughout the nubbins, with noticeable bleaching in the heated tanks (Fig. 6B). Although CCA was not explicitly measured in this study, it is known to play a key role in coral ecosystems by facilitating recruitment, colonization, and the survival of coral larvae (Littler & Littler, 2013). Investigating the extent of this relationship in *A. solitaria* could provide novel insights into their established ability to improve coral recovery and restoration as it appears to have a very close association in this species (Littler & Littler, 2013).

During our feedings with the blue light of the tanks we observed specks of color visible in the clear tentacles (Fig. 14). We believe this could be the result of fluorescent protein as they contribute to various coloration in marine organisms including coral (Dizon et al., 2021). This was also present in the microscope when assessing extension states (Fig. 5).

4.2 Edge population

The corals used in this investigation were found on steel pilings in Port Everglades (Fig. 14). Their use of an urban structure near a metropolitan center suggests that they could be synanthropic, meaning they live in close proximity to humans and benefit from their activities and expansion. They have also been found growing on fishing rope, further supporting this idea (Alves-Júnior et al., 2024). With increased urbanization and increased population, *A. solitaria* may be able to find new substrates to use as habitats and survive as urban sprawl continues to intensify. The major shipping lane at Port Everglades has had a significant impact on marine systems in this coastal environment, leading to an increase in stress for many species (Sabdono et al., 2024; Fig. 1). Additionally, a port widening and deepening project has been proposed to make room for larger, more modern vessels, which would in turn risk the loss of many coral species in the surrounding waters (Aguirre & Brenman, 2024). *Astrangia solitaria's* proximity and survival in rapidly expanding urbanization highlights the urgent need for understanding this unique species.

Corals in the Florida Reef Tract, which includes Port Everglades, experience multiple interacting stressors in addition to thermal stress including disease, coastal construction, pollution, increased sedimentation and suspension, and runoff (Sena, 2024). Substrate area is a known limiting factor in marine habitats (Fisher, 2023) and hence the ability of coral species to colonize non-traditional substrates, displayed by *A. solitaria*, is a crucial adaptive strategy. Future experiments should explore the colonization of various substrates by the larvae.

4.3 Experimental Limitations

Due to the relatively understudied nature of *A. solitaria* and limited literature available, a central focus of our experiment was actively observing, documenting, and uncovering its biological and phenotypic characteristics. The unknown genetic structure and population dynamics of *A. solitaria* posed a challenge in determining whether to evaluate individual polyps or treat entire fragments as colonies.. To address this uncertainty, we measured the percentage of extended polyps per nubbin, ensuring our data could be applied to either scenario. We used fluorescent microscopy to confirm their aposymbiotic state on day 4 of the experiment, which resulted in a modification of our experimental procedure for the remaining 7 days. On day 4, we also observed more polyp activity and extension in

dark conditions, resulting in the switch to a nocturnal feeding schedule as opposed to daytime feeding. Our behavioral observations were consistent with previous findings on nocturnal coral behaviors (Sweeney, 1976).

In addition, due to the biology of this coral species, mortality could not be estimated visually. Feeding behavior was originally intended to be a proxy for mortality, but this was found to be inaccurate as many polyps that were inactive during feeding times were still alive when seen under the fluorescent light of the microscope on the last day (Fig. 12). The GFP results of *A. solitaria* following heat treatment were compared to those of the control group on day 12 of the experiment. However, GFP levels were not measured at the start of the experiment, which limits our ability to detect changes over time and may mask the full extent of heat-induced changes in GFP expression. Additionally, without a standardized method to quantify GFP, such as using MATLAB or similar software (Roth et al., 2010), the results remain qualitative and subjective. A possible source of error is adjusting the light levels to visualize the coral. This could have had an effect on GFP measurements, as different light intensities might alter the appearance of fluorescence. Future studies should address these limitations by incorporating baseline GFP measurements, using quantitative tools for fluorescence analysis, and standardizing light conditions to ensure more accuracy.

Another potential source of error in the experiment was variable salinity. Although it was maintained within the acceptable broad range for coral survival. It may have fluctuated enough to influence physiological responses and confound results. Corals are sensitive to environmental changes, where salinity variations could impact their stress levels and overall health (Suryono et al., 2021). In addition, the short duration of the experiment limited the strength of the data, as it may not have captured the long-term or cumulative effects of the stressors. Lastly, using aposymbiotic corals allowed us to focus solely on coral host responses without the influence of symbiotic algae, simplifying the separation of physiological changes (Wuitchik et. al. 2021). However, their inability to bleach made it more challenging to assess phenotypic stress responses, further complicating efforts to evaluate mortality visually and relying instead on indirect measures like feeding behavior and fluorescence.

4.4 Future Projects

The results and observations found in this experiment gave rise to many potential future projects regarding *A. solitaria*. At the end of the experiment, the coral samples were prepared for two new projects: firstly, RNA responses to heat stress, and secondly, the relation between individual polyp DNA and distances from each other.

For RNA testing, all fragments were observed under a Leica Olympus SZX16 microscope and blue light. Polyps were randomly selected and verified as alive through their GFP intensity and the presence of live tissue. Heated fragments were photographed in the photo booth set-up. Polyps were removed from both the heated and controlled samples using shears and placed in microcentrifuge tubes filled with 1 milliliter of ethanol (C2H5OH). Tubes were labeled with the ID of each fragment. These samples will be used for RNA analysis by the Davies Marine Invertebrate Lab at Boston University to test whether RNA sequences differ between heat stressed and controlled *A. solitaria* polyps.

In the second experiment, polyps were only removed from the controlled fragments. An iPad was used to take photos of each nubbin in the photo booth. Five polyps were selected for removal based on their proximity to each other and labeled on the image. One polyp was selected as the primary polyp; two neighboring polyps and two polyps at the far edge were chosen (Figure 15). These polyp samples were stored in ethanol-filled microcentrifuge tubes. Each tube was labeled with the fragment ID and the associated polyp ID as was annotated on the

fragment photo (ie. 2A-1). These corals will be used for DNA sequencing by the Davies Marine Invertebrate Lab to analyze the genetic differences between polyps based on their spatial distribution on a substrate.

5. Conclusion

Our results support the detrimental effects that rising ocean temperatures have on the health of coral species. We identified key biological characteristics on *Astrangia solitaria*, a relatively unstudied coral, such as its aposymbiotic nature. Understanding the critical characteristics of this coral will contribute significantly to further studies that can investigate its resilience as an edge population species and its ability to live in strained environments above the global average thermal tolerance limit of coral reefs. Results indicated that *A. solitaria* experiences reduced polyp extension and feeding activity under heat stress. Their responses show they are capable of surviving in 35°C but at significantly reduced activity. Controls showed minimal change, maintaining higher GFP fluorescence and feeding activity throughout the experiment. Further studies should investigate the RNA responses of *A. solitaria* to heat stress and explore the genetic identity of polyps to improve our understanding of its biology.

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We would like to express our gratitude to Dr. Sarah Davies and Jeric Da-Anoy for their guidance and leadership throughout this research. We also extend our thanks to Kian Thompson for his assistance with husbandry and lab work guidance.

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Appendix

ANOVA Tables

Treatment	Extension State	P-value	Significance
Control	value_0	0.0262	*
Control	value_1	0.0085	*
Control	value_2	0.0024	*
Heat	value_0	0.0262	*
Heat	value_1	0.0085	*
Heat	value_2	0.0024	*

Table 1: Results of ANOVAs for Day 5 of extension states between Heat and Control Treatments. Indicating significant differences between extension states and treatment values.

Treatment	Extension State	P-value	Significance
Control	value_0	9.558e-08	*
Control	value_1	9.094e-01	
Control	value_2	1.519e-11	*
Heat	value_0	9.558e-08	*
Heat	value_1	9.094e-01	
Heat	value_2	1.519e-11	*

Table 2: Results of ANOVAs for Day 11 of extension states between Heat and Control Treatments. Indicating significant differences between extension states and treatment values. It shows no significant difference in partial extension (value_1) states between control and heat.

	value_0	value_1	value_2
Degrees of Freedom	1	1	1
Residuals	22	22	22
F-value	70.28	0.527	218.5
p-value	2.69e-8***	0.476	6.6e-13***

Table 3: Results of One-Way ANOVA for polyp extension in heat treated nubbins on day 5 and 11. Indicating significant differences for value_0 and value_2

	value_0	value_1	value_2
Degrees of Freedom	1	1	1
Residuals	22	22	22
F-value	1.111	5.15	5.839
p-value	0.303	0.0334*	0.0244*

Table 4: Results of One-Way ANOVA for polyp extension in control nubbins on day 5 and 11. Indicating significant differences for value_2 and value_3 but non for retracted polyps (value_0)

	value_0	value_1	value_2
Degrees of Freedom	1	1	1
Residuals	22	22	22
F-value	0.487	0.001	0.597
p-value	0.493	0.97	0.448

Table 5: Results of One-Way ANOVA for polyp extension in heat nubbins on day 8 and 9. Showing no significant difference between those days.

	value_0	value_1	value_2
Degrees of Freedom	1	1	1
Residuals	22	22	22
F-value	2.89	0.034	8.451
p-value	0.103	0.855	0.00817**

Table 6: Results of One-Way ANOVA for polyp extension in heat nubbins on day 9 and 10. Showing a significant difference between those days for fully extended polyps.

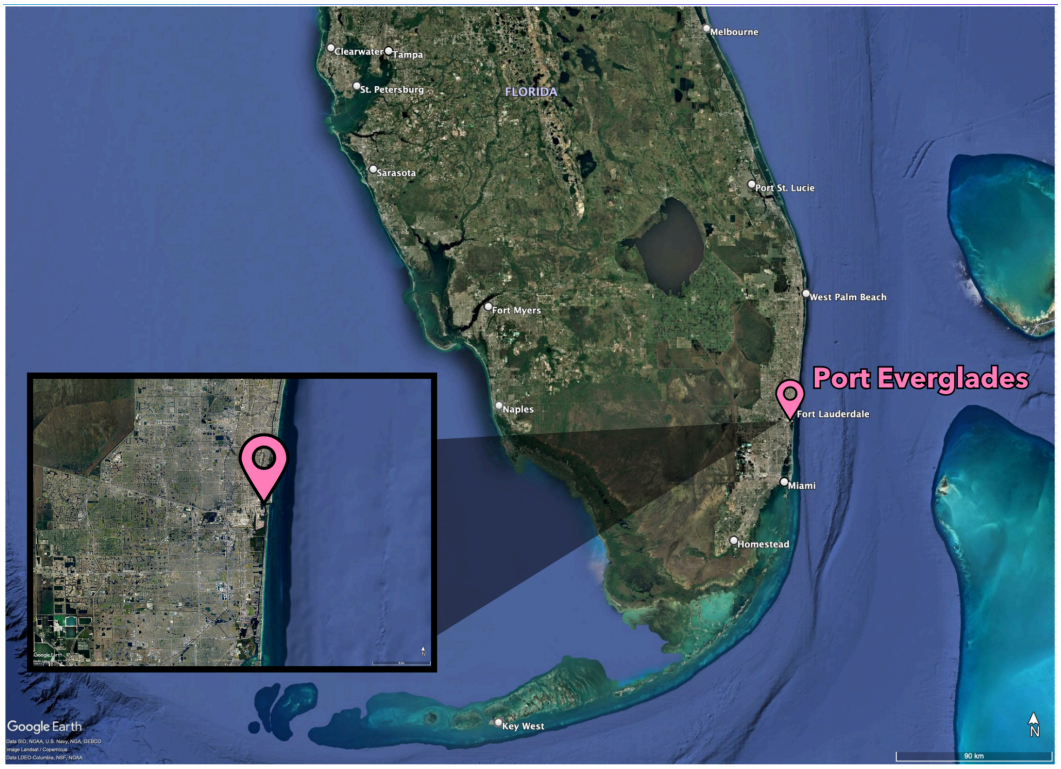


Figure 1: Map of Port Everglades, Florida. The map shows the collection site for the *Astrangia solitaria* samples used in this study. The inset map highlights the high-traffic, urban port of the collection site.

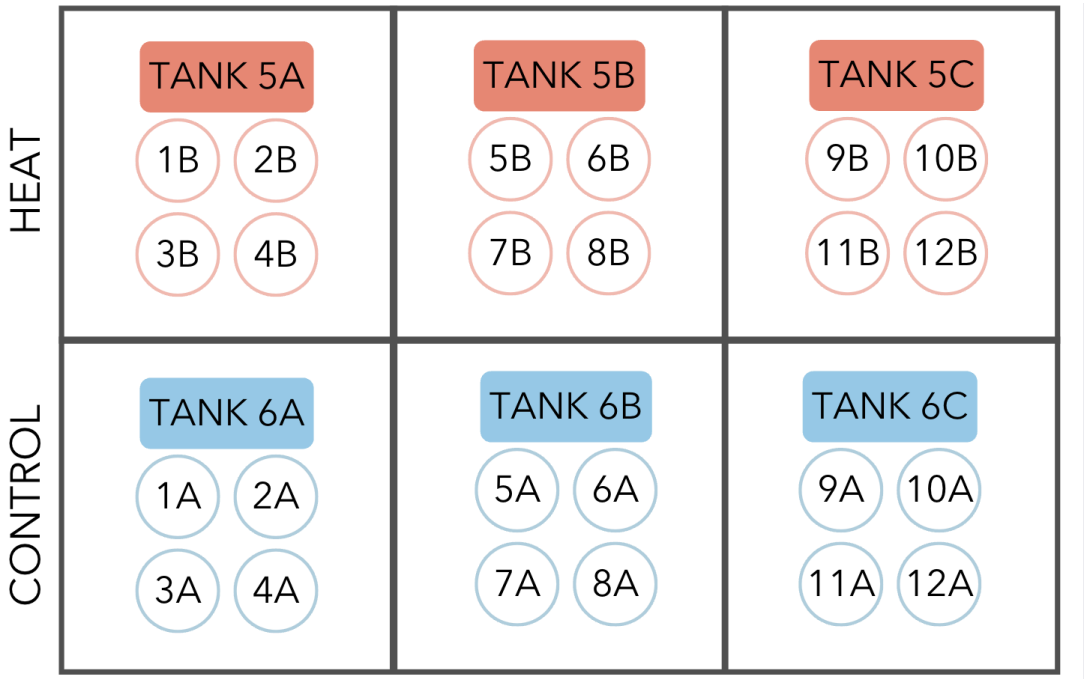


Figure 2. *Astrangia solitaria* thermal stress experimental design. Diagram of fragment distribution between three heated (Peach) and three control (Lavender) tanks. Genets are divided into "A" and "B" ramets, with "A"s being placed in the controlled treatment and "B"s in the heated treatments. Fragments with the same number represent the same genet.

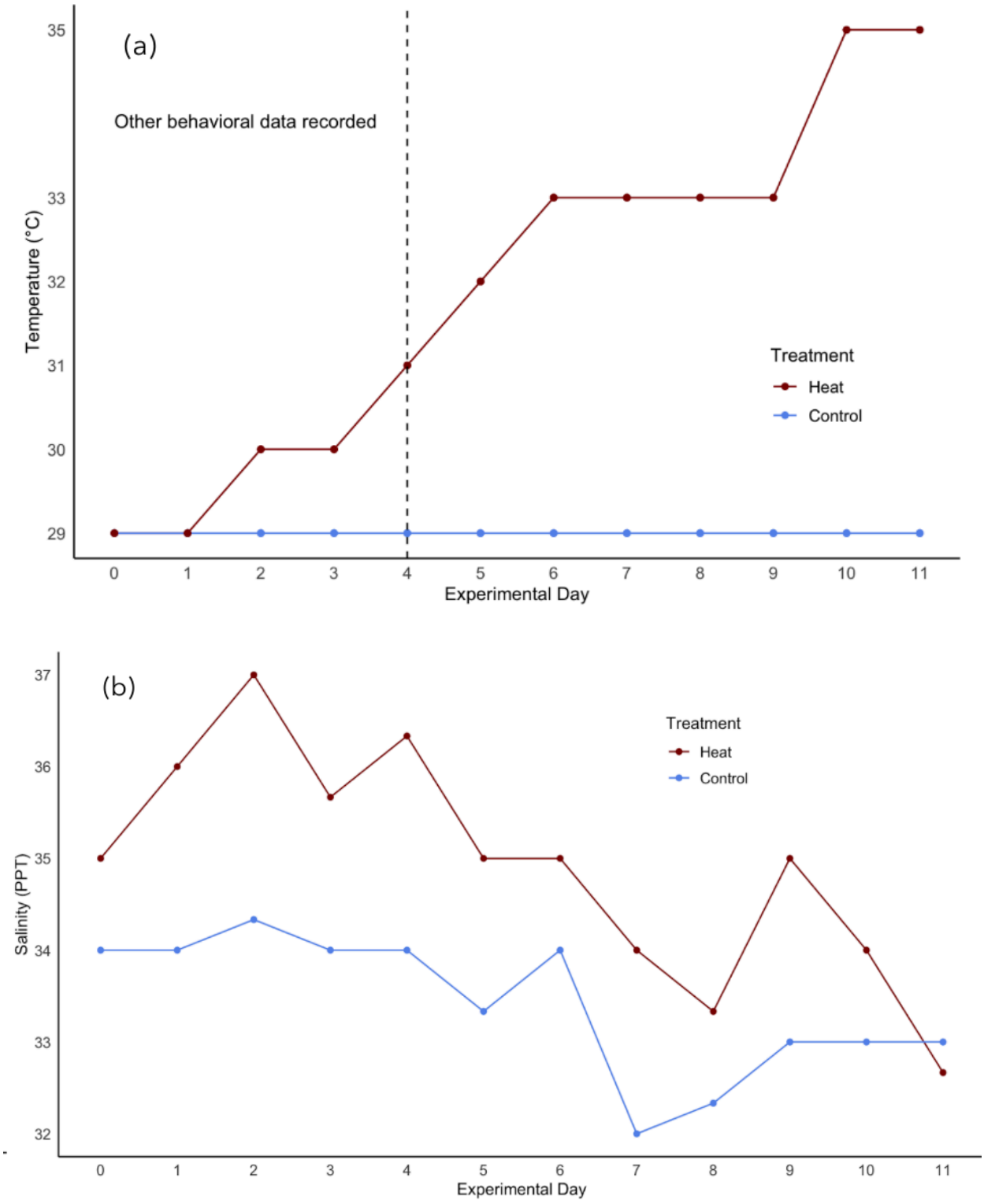


Figure 3. Daily water temperature and salinity readings for heat treatment and control tanks during the experiment duration. a) Afternoon temperature (˚C) recordings. The dotted line represents the day feeding time and behavior measurement procedures changed. b) Salinity recording in parts per thousand (PPT), which were taken in the morning.

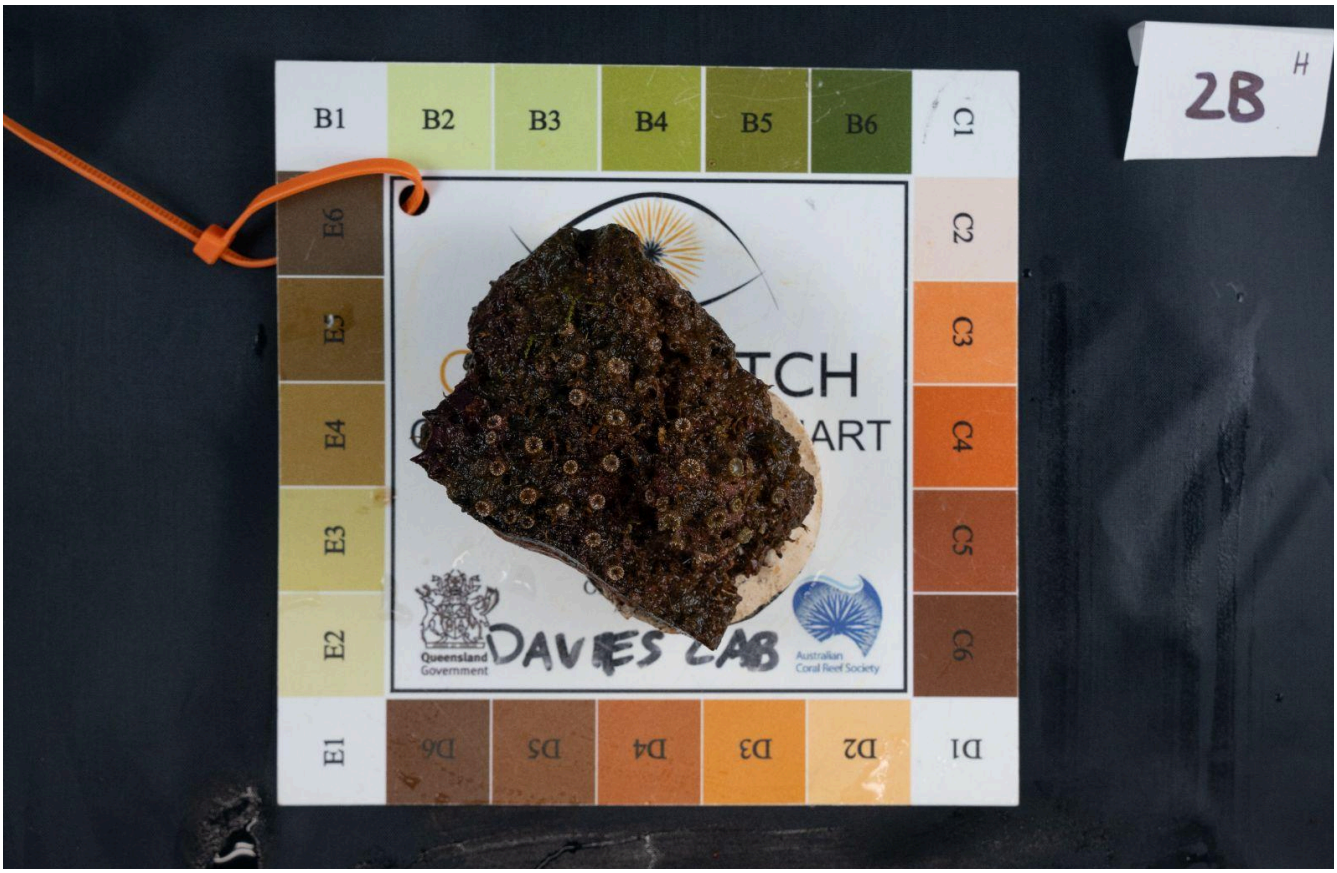


Figure 4. Photo of *A. solitaria* coral fragment 2B (heat treatment) at day 0 of experiment. Photos were taken for qualitative and comparative assessments of health and pigmentation changes in response to heat treatment.

Figure 5. Three states of polyp extension. Three states of polyp extension observed in *Astrangia solitaria*. **(a)** Value 0 - fully retracted polyp, where tentacles are completely withdrawn into the corallite, indicating minimal activity. **(b)** Value 1 - partially extended polyp, with tentacles emerging from the gastric cavity, suggesting moderate activity or responsiveness. **(c)** Value 2 - fully extended polyp, where tentacles are maximally extended, indicating a high level of activity and feeding readiness.

Figure 6. Example photographs from before and after heat treatment. a) *Astrangia solitaria* sample 5B on day 0 of the experiment, before heat treatment**. b)** sample 5B on day 11 of the experiment, after heat treatment. The red box on both pictures highlights the portion of the coral where coralline algae bleached after heat treatment.

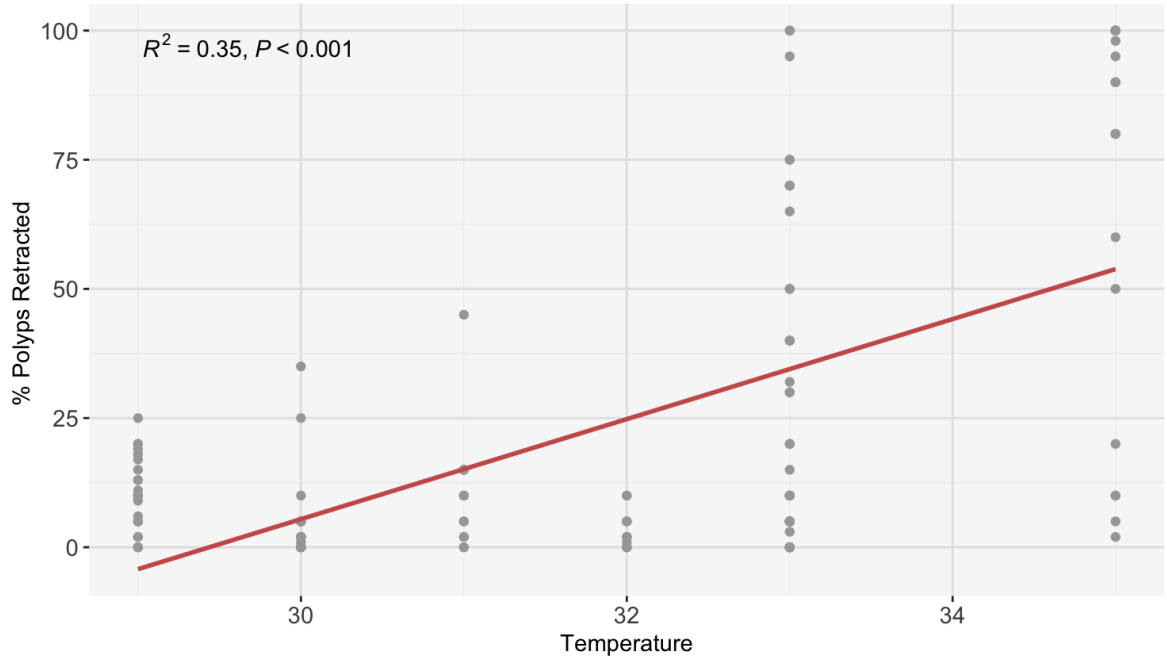


Figure 7. Heat treatment regression with temperature. Linear regression of temperature versus % polyps retracted in *Astrangia solitaria* under heat treatment. The regression shows a positive relationship, with R2=0.35 and P-value<0.001, indicating increased polyp retraction at higher temperatures.

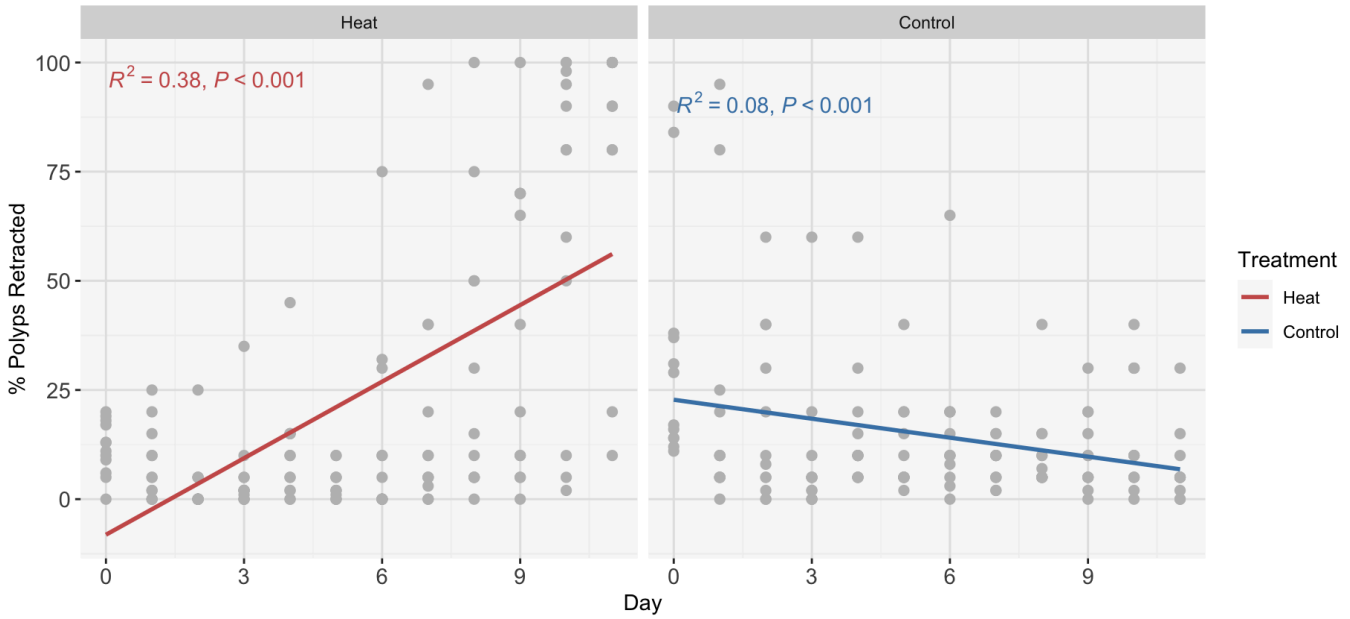


Figure 8. Retracted polyps by treatment. Linear regression of % polyps retracted by experimental day for *Astrangia solitaria* under heat and control treatments. In the heat treatment, % polyp retraction shows a positive trend over time (R2=0.38, P-value<0.001), while the control group shows a slight negative trend (R2=0.08, P-value<0.001). This suggests that elevated temperatures increase polyp retraction over time.

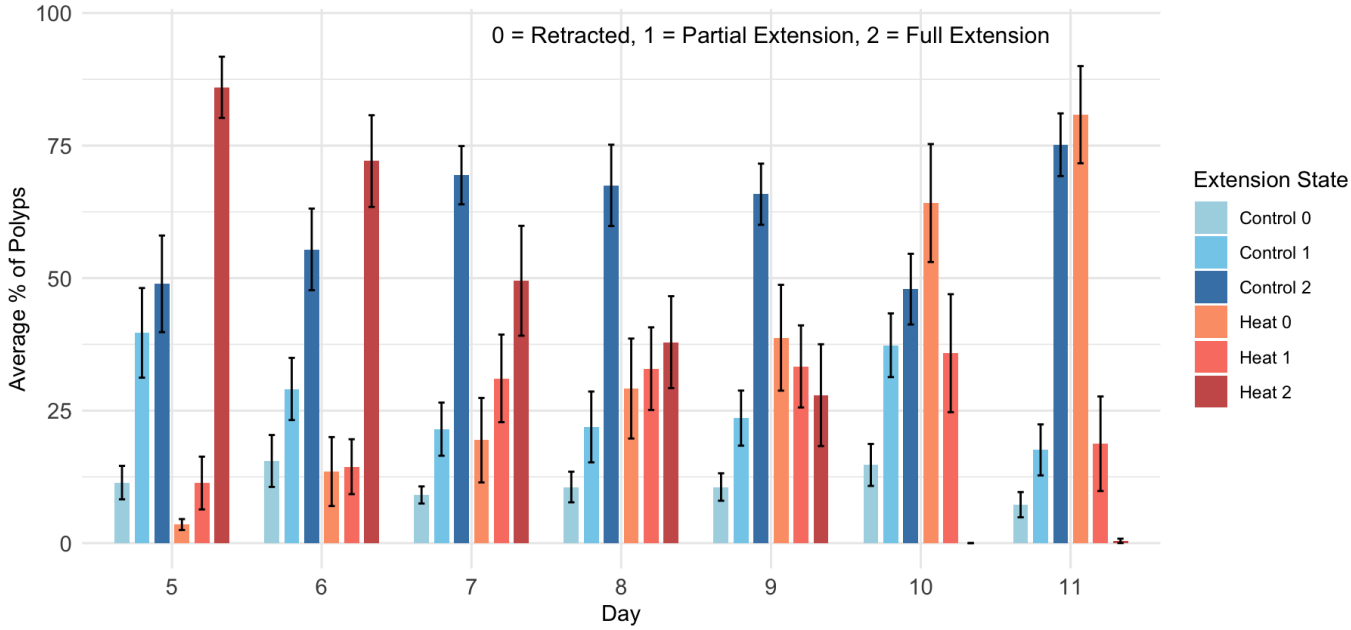
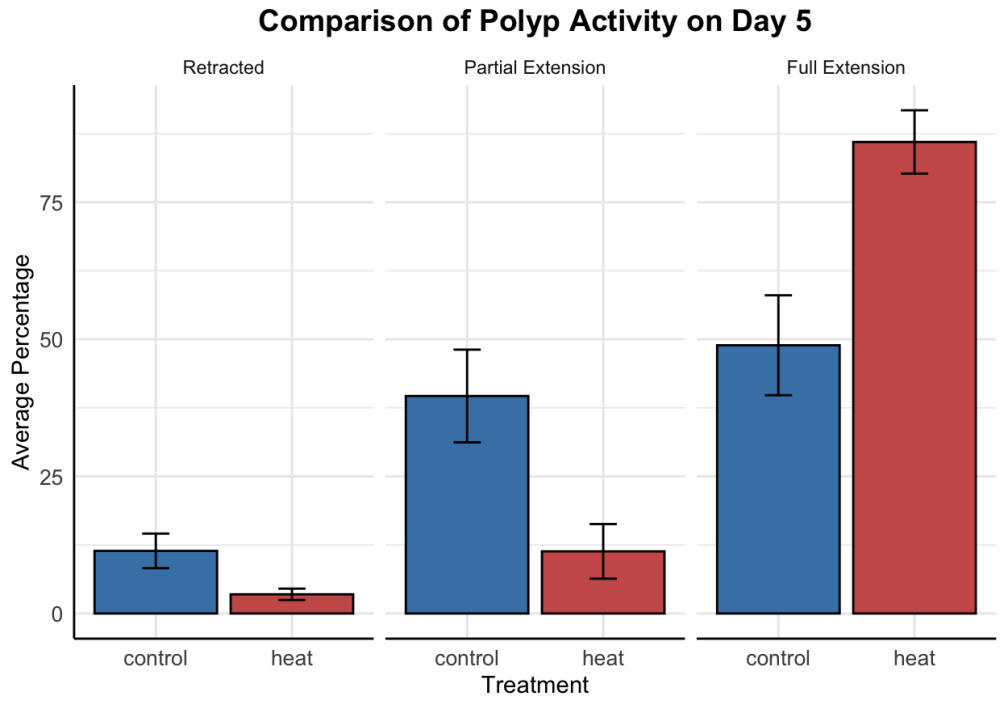


Figure 9. Average polyp extension level between treatments. Average percentage of *Astrangia solitaria* polyps in each extension state (0 = retracted, 1 = partial extension, 2 = full extension) between heat and control treatments over the course of the experiment. Control polyps generally displayed higher levels of full extension (blue bars), while polyps in the heat treatment showed a greater proportion of retraction (red bars) over time. Bars represent standard error. This indicates that elevated temperatures influence polyp extension behavior, reducing full extension and increasing retraction as a stress response to heat.

a)



b)

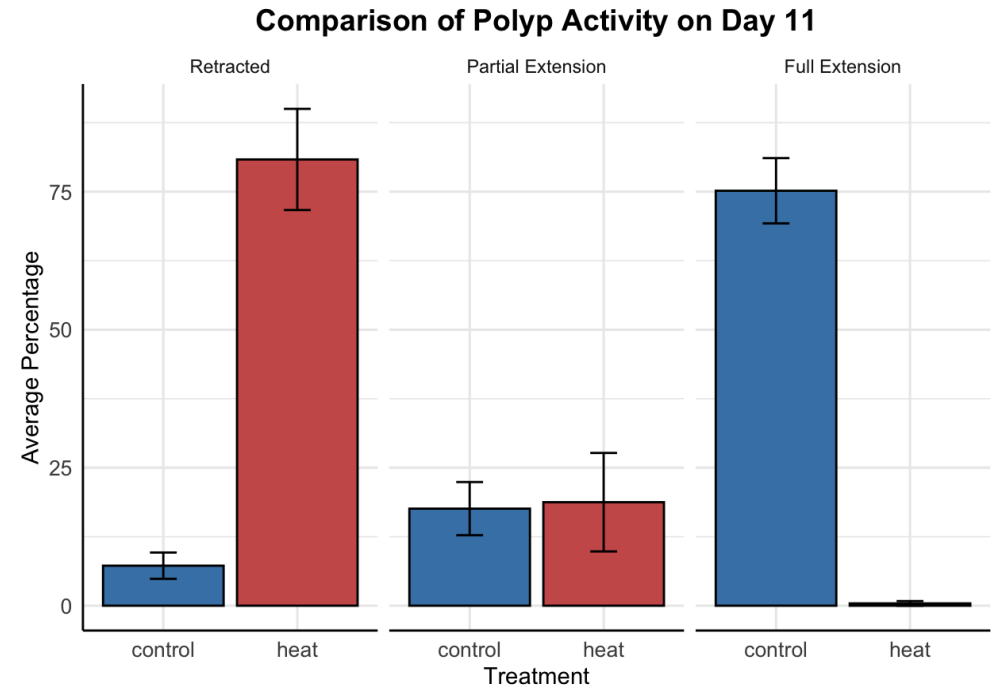


Figure 10. Comparison of *Astrangia solitaria* polyp activity between control and heat treatments on Day 5 and Day 11. Bars represent the average percentage of polyps in each extension state: retracted, partial extension, and full extension. Error bars represent standard error. **(a)** Day 5 polyps in the heat treatment exhibited higher full extension compared to controls. **(b)** Day 11, the heat-treated polyps showed a significant increase in retraction and a marked reduction in full extension.

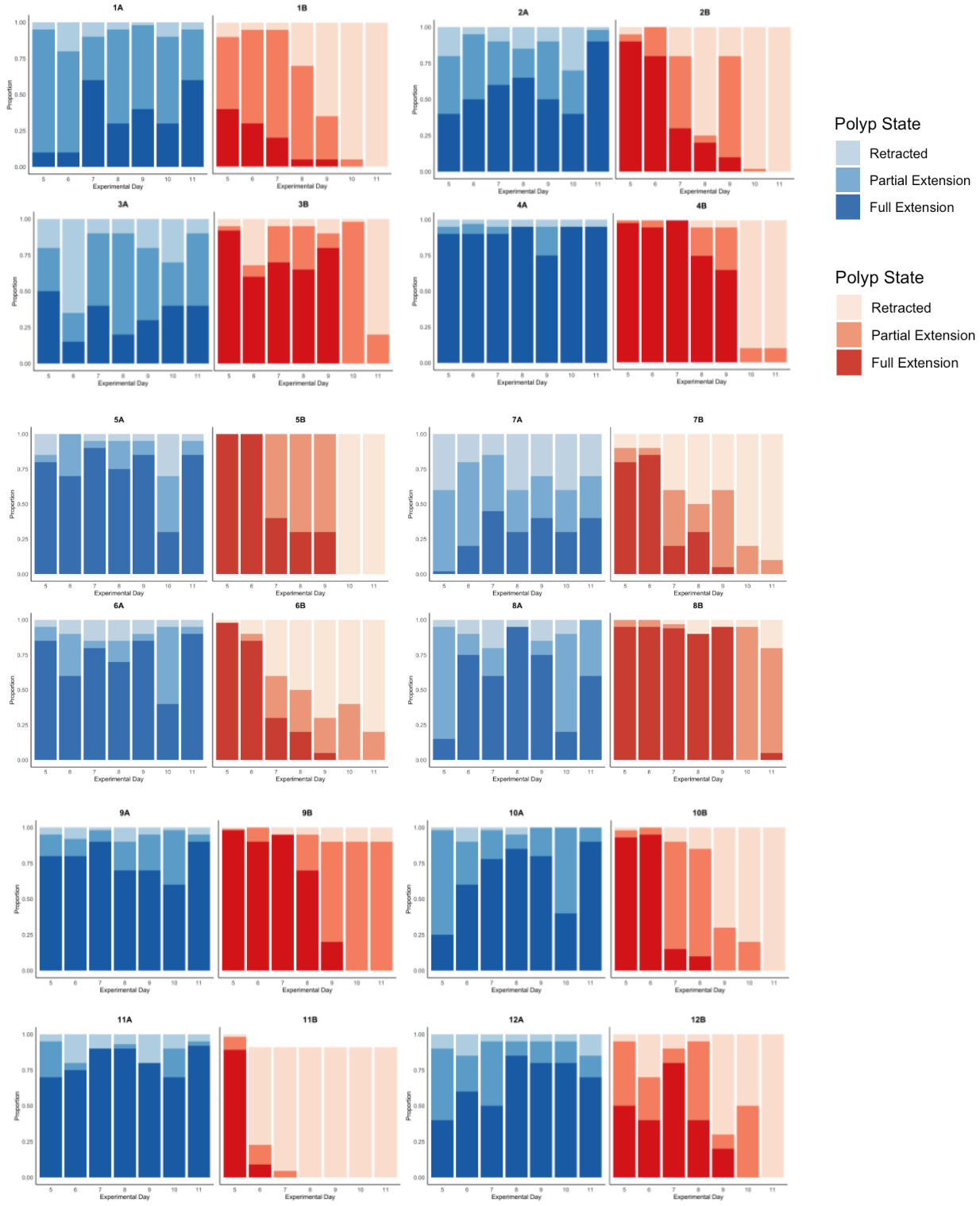


Figure 11. Daily proportion of polyp extension states (retracted, partial extension, and full extension) across all 24 *Astrangia solitaria* coral fragments over the course of the experiment. Each panel represents individual coral fragments (1A, 1B, 2A, 2B, etc.), with blue shades indicating the control treatment and red shades representing the heat treatment. This illustrates variation in polyp activity across fragments and highlights the impact of heat treatment, with fragments under heat showing a gradual shift towards increased polyp retraction over time compared to controls.

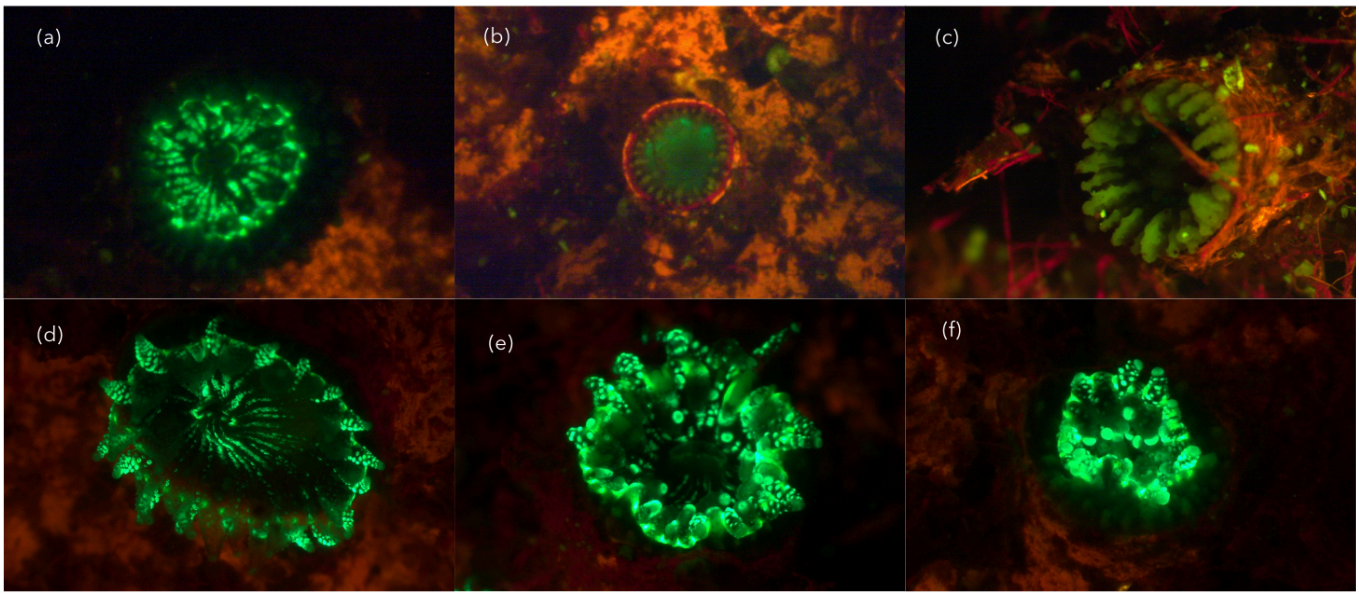


Figure 12. Green Fluorescent Protein (GFP) concentrations of experimental corals. (a) Heated, alive; **(b)** Heated, dying; **(c)** Heated, dead; **(d)** Controlled, alive; **(e)** Controlled, alive; **(f)** Controlled, alive. This is in heat and control treatments, illustrating varying health states. GFP intensity and distribution vary by health status, with live polyps displaying more defined GFP expression, while dying or dead polyps in the heat treatment show reduced or fragmented fluorescence.

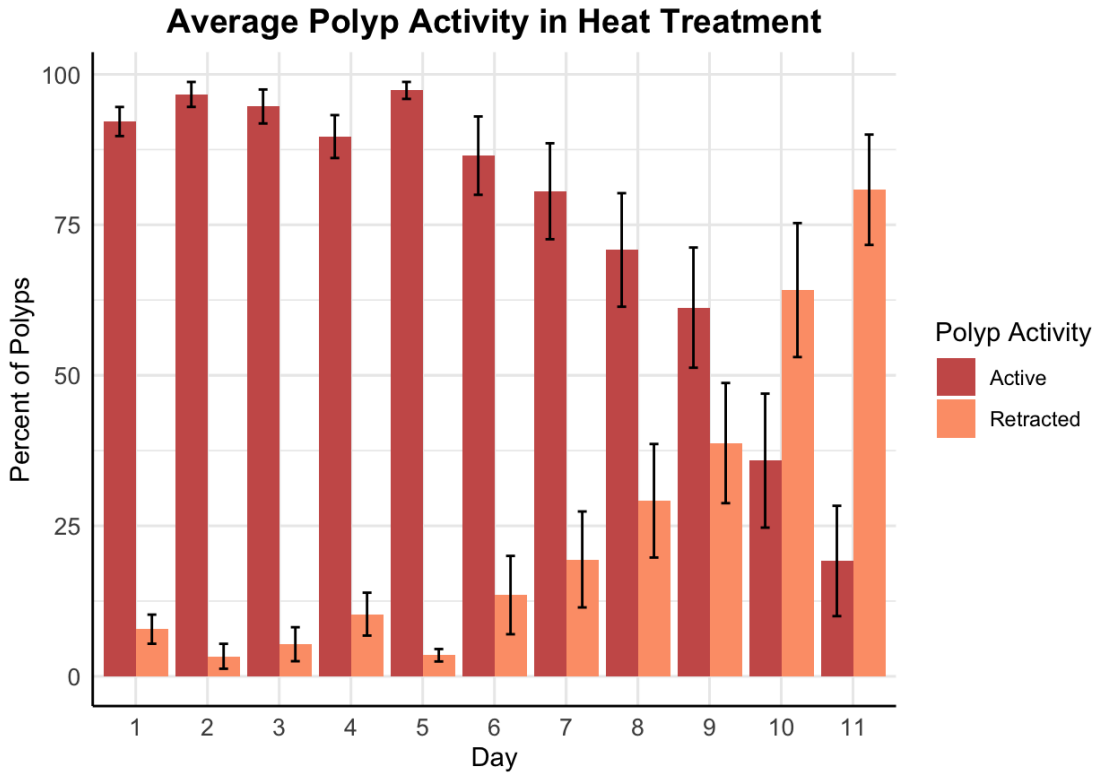


Figure 13. Average polyp activity (active vs. retracted) of *Astrangia solitaria* under heat treatment over 11 experimental days. The proportion of active polyps decreases significantly over time, while retracted polyps increase, particularly after Day 5. Error bars represent standard error.

Figure 14. Image of *Astrangia solitaria* coral fragment under blue light, highlighting the polyp tentacles fully extended and their fluorescence. The image also illustrates the steel and metal material that the corals are growing on. The individual nature of the corallites is visible.

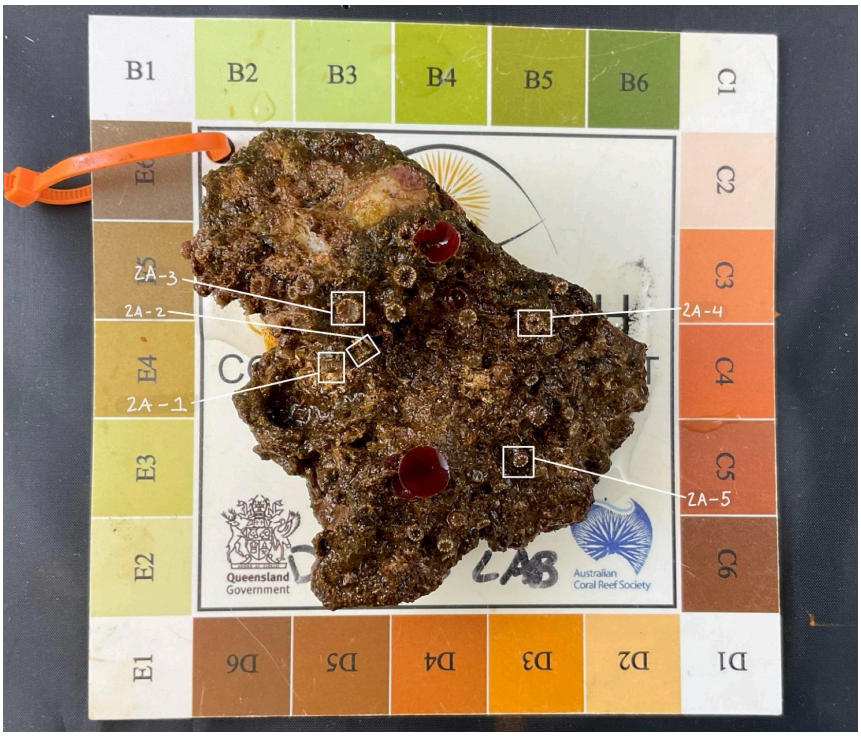


Figure 15. Image of polyps selected for the DNA extraction experiment for *A. solitaria* sample 2A. Polyp 2A-1 is the primary polyp, 2A-2 and 2A-3 are the neighboring polyps, and 2A-4 and 2A-5 are farther polyps. All control fragments were labeled similarly.