

The impact of climate change on marine symbiosis in
Anthopleura xanthogrammica

by

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Abstract

Anthropogenic climate change is affecting coastal ecosystems in the North Pacific due to large temperature fluctuations and increased storm activity throughout the year. Many studies have looked at the effects of increasing temperature on tropical marine species, but there remains a gap in knowledge on the effects of heatwaves on temperate intertidal ecosystems. This paper investigates how short-term heatwaves affect the temperate sea anemone *Anthopleura xanthogrammica* and its symbiotic relationship with two marine algae, zoochlorellae and Symbiodiniaceae. We hypothesized that increasing temperature would cause algal symbionts to be expelled from the anemone due to a decrease in algae health, following the oxidative bleaching hypothesis. We simulated the conditions of a tidepool during mid-day low tide over a three-day summer heatwave, including both a mid and an extreme heatwave treatment. We found *A. xanthogrammica* had negative behavioural responses, but there was no evidence of a decrease in algae fitness. We propose that by retracting their tentacles, the anemones regulate their internal temperature, thereby protecting the symbionts from heat. This research suggests that anemone-algal symbiosis can be resistant to short-term heat exposure, which is not seen in all cnidarians. Our study can be used as a foundation for future research on the effect of heat and climate-change related impacts on temperate intertidal ecosystems.

Keywords

Mutualism, Sea anemone, Symbiodiniaceae, Temperate symbiosis, Temperature Stress, Zoochlorellae

Introduction

Northern Pacific coastal ecosystems are one of the most biodiverse temperate marine systems in the world (Wyllie de Echeverria & Thornton, 2019). These ecosystems are threatened by increased heatwave frequency and intensity as well as elevated summer temperatures caused by climate change (Russo et al., 2014; Stillman, 2019). The intertidal zone is particularly vulnerable to these changes as intertidal organisms not only suffer from the rapid warming of shallow waters but also from extreme surface and air temperatures. The recent heatwave event that occurred over the North Pacific in June 2021 caused a mass mortality event among intertidal invertebrate populations (Aslam, 2021; Migdal, 2021a; Migdal, 2021b). As a result, questions remain about the interactions between intertidal species following heat stress, especially when looking at symbiotic relationships.

Mutualistic symbiosis is a beneficial relationship between two or more unrelated species living together in close proximity (Margulis, 1971). Symbiotic relationships between photosynthesizing organisms and invertebrates have evolved repeatedly and independently, an example being the sponge *Halichondria bowerbanki* and cyanobacteria (Lüskow et al., 2019). Climate change and increasing ocean temperatures have proven to hinder the interactions between symbiotic species, as seen in the well-documented case of coral bleaching (Hoegh-Guldberg, 1999; Hughes et al., 2018; Ainsworth et al., 2021). The process of bleaching occurs when all symbionts are expelled from the host (Pryor et al., 2021). The well-known phenomenon in coral has generated a lot of speculation about the underlying mechanisms behind bleaching.

The oxidative bleaching hypothesis predicts that the algal symbionts react to temperature changes before the host by overproducing reactive oxygen species (ROS) (Fernandes De Barros Marangoni et al., 2021). The excess ROS, causing cellular damage, is sensed by the host, thereby causing it to expel the faulty symbiotic partners. To test this hypothesis, previous research used both symbiont and host fitness as metrics to assess the relationship. Asexual reproduction, indicated by mitotic cell

division in unicellular symbionts, can reflect algal symbiont stress; a decrease in mitotic index indicates a decrease in algae health (O'Brien & Wyttenbach, 1980; McCloskey et al., 1996; Bates et al., 2010; Dimond et al., 2011). Similarly, photosynthetic efficiency, a measure of photosynthesis output, can also be used as a proxy for symbiont fitness; a decrease in photosynthesis indicates a decline in algal health (Dyken & Shick, 1984; Fujise, 2014). Alternatively, host fitness can be assessed through behavioural activity (Wuitchik et al., 2021). The oxidative bleaching hypothesis is difficult to determine in obligately symbiotic corals as the algae-host relationship is perfectly intertwined; the symbionts represent the only source of food for the host (Aichleman et al., 2016; Wuitchik et al., 2021). As a result, research must turn to facultative symbiotic relationship between algae and marine invertebrates to further test the oxidative bleaching hypothesis.

Sea anemones share the same phylum and class as corals and sometimes partake in facultative symbiotic relationships with algae. This means they can survive without the algal symbiont(s), making it easier to test the oxidative bleaching hypothesis.

McCloskey et al. (1996) found that anemones control algal symbiont populations within their tissues by expelling them in a bolus of mucus and undigested material if they are no longer wanted or needed. While this is a form of normal algal control, extremely stressed anemones can undergo bleaching. The process of bleaching can happen with changes in temperature, salinity, and UV exposure (Pryor et al., 2021). Studies have researched the effects of increased temperature and UV stress on anemones in the *Anthopleura* genus (Richier et al., 2008).

The giant green anemone (*Anthopleura xanthogrammica*, Brandt 1835) is a common host of two algal symbionts: symbiotic dinoflagellates in the family Symbiodiniaceae (previously known as zooxanthellae, LaJeunesse et al., 2018), and symbiotic green algae, zoochlorellae (O'Brien & Wyttenbach, 1980; Secord & Augustine, 2000; Bates et al., 2010; Levine & Muller-Parker, 2012). The giant green anemone is abundant along the West coast of Vancouver Island (Dimond et al., 2011). *A. xanthogrammica* is an exceptional example of mutualism between one host and multiple symbionts, making it

a great organism to portray symbiotic relationships (Bates, 2000). The abundance of Symbiodiniaceae and zoochlorellae varies within the temperate anemone *A. xanthogrammica* depending on food availability (McCloseky et al., 1996), temperature, and irradiance (Dyken & Shick, 1984; Bates et al., 2010). Previous research has shown that an increased presence of Symbiodiniaceae and decreased presence of zoochlorellae is more common in anemones at lower latitudes, or in more sun-exposed intertidal zones (O'Brien & Wyttenbach, 1980; McCloskey et al., 1996; Verde & McCloskey, 2007; Levine & Muller-Parker, 2012). This indicates that Symbiodiniaceae are more adapted to warmer environments than zoochlorellae, but it is unclear whether the abundance of the dinoflagellate within anemone tissues is a preference of the symbiont or the host. This is one of the many gaps in literature concerning the impacts of temperature on anemone-algal symbiosis.

Previous studies looked at the long-term effects of increased water temperature on *A. xanthogrammica* and found a decrease in density and mitotic activity in its algal symbionts (O'Brien & Wyttenbach, 1980). However, there remains a gap in knowledge on the effects of short-term heatwaves on anemones. In addition to a scarcity of knowledge on the effects of acute heat stress, most research on temperature impacts on anemone-algae symbiosis focus on tropical rather than temperate species (Gates et al., 1992; Sawyer & Muscatine, 2001; Pryor et al., 2021). Pryor et al. (2021) found that the tropical species *Entacmaea quadricolor* bleached in response to high temperature treatments but not to increased CO₂ concentration in the water. Tropical environments provide a more stable ecosystem with less variation in temperature across seasons compared to temperate intertidal zones that suffer from changing seasonality (Dyken & Shick, 1984; Verde & McCloskey, 2007). Further investigation into the effects of short-term heatwaves is needed in order to fill this gap in knowledge.

This study aims to quantify the effects of short-term heatwaves on temperate anemone-algae symbiosis by measuring the differences in fitness between host and symbionts. The symbiotic relationship is investigated to determine whether the expulsion of algal symbionts is due to a decline in anemone or algae health. Following the oxidative

bleaching hypothesis (Fernandes De Barros Marangoni et al., 2021), we hypothesize that increased water temperature causes a decrease in symbiont density in *A. xanthogrammica*, as the stressed symbionts will produce an excess of ROS which will be detected by the host. The anemone would thereby expel its faulty photosynthetic partner, but the host fitness would not be affected. As such, we predict that measures of algal stress, including symbiont density, photosynthetic efficiency, and mitotic index will decrease as a result of the heatwave event. Alternatively, we hypothesize that a decrease in symbiont density could also be caused by a decline in anemone fitness. In this case, we would predict a decrease in symbiont density and a decline in anemone size, feeding behaviour, and tentacle activity. Finally, the host could decide to retain its symbiont as they provide a source of food during this stress period. Thereby, we might see no change, or an increase in symbiont density in *A. xanthogrammica*.

Methods

Collection and Husbandry

Anthopleura xanthogrammica were collected from three highly biodiverse intertidal sites: Scott's Bay (48°50'07.4"N, 125°08'38.9"W) (n = 12), Bamfield Marine Science Center (BMSC) Foreshore (48°49'59.8"N, 125°08'11.8"W) (n = 14), and Bluestone Point (48°48'55.5"N, 125°10'27.9"W) (n = 10). Individuals were collected from the mid-to-low intertidal zone (approximately 1.5m elevation) at each site in October 2021. Anemones were transported in coolers with seawater to the aquatic facilities of BMSC. *A. xanthogrammica* individuals were kept in individual containers in flow through sea tables for an acclimation period ranging from 6-15 days. Anemones experienced a 12L:12D photoperiod. Individuals were fed twice a week with pieces of fish during the acclimation and recovery periods but were not fed during the three-day heatwave event to avoid regurgitation due to stress.

Experimental Design

A. xanthogrammica were randomly placed into three treatments: control (approximately 12°C), mid heatwave (25°C), and extreme heatwave (30°C) ($n_{\text{treatment}} = 12$). Each treatment consisted of four buckets with three anemones in each bucket. During the three-day treatment, tide pool conditions were simulated by stopping the flow of continuously supplied seawater to the anemones, allowing for the temperature to be increased slowly over six hours (Fig. 1). After the six-hour low-tide conditions in the control and heatwave treatments, seawater flow (at approximately 12°C) was restored to simulate a rising tide.

Symbiont Fitness

Photosynthetic Efficiency

Photosynthetic efficiency was measured at the end of the acclimation period, then twice a day during the heatwave period, with a final measurement taken 5 days after. Measurements were taken with a pulse amplitude modulation (PAM) fluorometer, using the PC software *WinControl-3* (2020). PAM measurements of *A. xanthogrammica* specimens were taken after a period of dark acclimation ($n = 30$). Before and after the three-day heat stress event, anemones were dark acclimated overnight and PAM measurements were taken in the morning. During the heatwave simulation, after a 20-minute dark acclimation period, PAM measurements were taken twice daily. The probe was applied at three random places around the top of the anemone column and averaged to give a single measure (Levine & Muller-Parker, 2012). Aposymbiotic anemones with symbiont density lower than 1000 cells per mg of tissue were removed from the statistical analysis of photosynthetic efficiency.

Symbiont Density

To assess the symbiont fitness, 1-4 tentacles were clipped per anemone at the following timepoints: end of the acclimation period, after the three-day heating event, and after five days of recovery. Tentacles were stored at -20°C before being homogenized in 0.5

mL of filtered seawater using a mortar and pestle. A hemocytometer was used to count the number of symbiont cells under the microscope (40x magnification, $n = 30$). Algal symbionts were easy to differentiate as Symbiodiniaceae are golden brown and larger in size (10-12 μ m), while zoochlorellae are green and smaller (6-8 μ m) (Secord and Augustine, 2000; Bates et al., 2010; Dimond et al., 2011).

Mitotic Index (MI)

Using a hemocytometer, the number of dividing cells were manually counted under the microscope (40x magnification, $n = 30$). MI was calculated by dividing the number of cells undergoing mitosis by the total number of cells counted. MIs of zoochlorellae and Symbiodiniaceae were counted separately.

Host Fitness

Anemone Health

To quantify anemone stress response, three base measurements were taken with a calliper to track changes in body size: width, length, and diagonal ($n = 36$). An average base diameter was calculated using the mean of these measurements.

Anemone Behaviour

A. xanthogrammica stress was measured through behavioural responses. Tentacle activity was tracked every hour during the heatwave ($n = 36$), recording whether the anemone was open (all tentacles visible), partially open (some tentacles visible), or closed (no tentacles visible). Since less healthy cnidarians usually have decreased foraging behaviour (Wuitchik et al., 2021), we recorded the time it took each anemone to ingest food as a proxy ($n = 9$).

We also recorded the time it took for each anemone to ingest the food they were given at every feeding event ($n = 9$). Feeding time was measured from the point at which food was administered to the point at which food was no longer visible, either by ingestion or

by covering completely with tentacles. Measurements longer than one hour were declared extreme outliers and discarded.

Statistical Analysis

The Shapiro-Wilk test (Shapiro and Wilk, 1965) and

Bartlett test (Arsham and Lovric, 2014) were conducted on all datasets to check for assumptions of normality and equal variance. When assumptions were not met, a fit of univariate distributions to non-censored data (*fitDist*) function from the package *gamlss* (Rigby & Stasinopoulos, 2005) was run to determine the appropriate distribution of data. The distributions were then used to run a Generalized Additive Model for Location, Scale, and Shape (GAMLSS, Rigby & Stasinopoulos, 2005). The Rigby and Stasinopoulos (2005) algorithm was utilized in all models. A variety of distributions were used in our models for testing our treatments and control against different response variables: symbiont density of zoochlorellae (Weibull, Padgett, 2011), symbiont density of Symbiodiniaceae (Gamma, Lukacs, 1955), body size (log-normal), and feeding time (log-normal). Three random factors were considered in all GAMLSS models including the individual anemone, field site, and acclimation period. Additionally, both the Kruskal-Wallis rank sum (Kruskal & Wallace, 1952) and Dunn Kruskal-Wallis (Dinno, 2015) tests were used to assess statistical significance of photosynthetic efficiency and mitotic index of symbionts. Finally, an ordinal regression model was used to analyze tentacle activity (Christense, 2015). All the above analyses were performed in R (V 4.1.1, R Core Team, 2021).

Results

Symbiont Fitness

Photosynthetic Efficiency

Photosynthetic efficiency (F_v/F_m) increased by an average of 0.07 after the heatwave and decreased by an average of 0.09 after the recovery period (Fig. 2). These trends

were similar among treatments and the control. The difference between experimental groups was significant (Kruskal-Wallis rank sum test, chi-squared = 8.04, df = 2, $p = 0.018$). This was primarily driven by the difference between extreme and mid heatwave treatments (Dunn Kruskal-Wallis, $Z = -2.64$, $p\text{-adj} = 0.025$). There was no significant difference between the control and the extreme heat treatment (Dunn Kruskal-Wallis, $Z = -0.67$, $p\text{-adj} = 0.065$) or between the control and the mid heat treatment (Dunn Kruskal-Wallis, $Z = 2.14$, $p\text{-adj} = 0.50$).

Symbiont Density

Zoochlorellae density in *Anthopleura xanthogrammica* tissue in both heat treatments showed a slightly decreasing trend after the heatwave and recovery periods, compared to the control (Fig. 3A). Across the experiment, the extreme heatwave treatment had significantly higher zoochlorellae density than the control and the mid heatwave treatment ($p = 1.5 \times 10^{-5}$). The control and the mid heatwave treatments showed no statistical differences ($p = 0.71$). We observed no consistent patterns for the change in Symbiodiniaceae density (Fig. 3B). Without taking the interaction of multiple events into account, there was a statistical difference in Symbiodiniaceae density between the control and mid heatwave treatment ($p = 0.0029$) and between the control and extreme heatwave treatment ($p = 0.031$). However, the interaction between events and heat treatments did not significantly affect density of zoochlorellae (Table S1) and Symbiodiniaceae (Table S2) in *A. xanthogrammica* ($p > 0.05$ for all).

Mitotic Index

We observed a slight decreasing trend in the proportion of cell division (cells undergoing mitosis) in zoochlorellae within the mid heatwave treatment and a slight increasing trend in dividing zoochlorellae within the extreme heatwave treatment, but these trends remain insignificant (Fig. 4A). No clear patterns in the proportion of dividing Symbiodiniaceae cells can be observed (Fig. 4B). The difference between the two heat treatments was significant for zoochlorellae (Kruskal-Wallis rank sum test, chi-squared = 9.49, df = 2, $p = 0.0087$), but not significant for Symbiodiniaceae (Kruskal-Wallis rank

sum test, chi-squared = 0.51, df = 2, $p = 0.77$). However, there was no statistical significance in the proportion of dividing zoochlorellae between the control and mid heatwave treatment (Dunn Kruskal-Wallis, $Z = -1.44$, $p\text{-adj} = 0.15$), or control and extreme heatwave treatment (Dunn Kruskal-Wallis, $Z = 1.73$, $p\text{-adj} = 0.17$). There was also no statistical difference in the proportion of dividing Symbiodiniaceae between the control and the mid heatwave treatment (Dunn Kruskal-Wallis, $Z = -0.61$, $p\text{-adj} = 1.00$) or the control and the extreme heatwave treatment (Dunn Kruskal-Wallis, $Z = -0.62$, $p\text{-adj} = 1.00$).

Host Fitness

Anemone Health

Base diameter showed inconsistent patterns through the events of the experiment (Fig. 5). The base diameter of *A. xanthogrammica* slightly increased across both the mid treatment ($p = 0.31$), extreme treatment ($p = 0.91$) and the control ($p = 0.21$) after the simulated heatwave, showing insignificant growth in the anemones (Fig. 5, Table S3). At the end of the recovery period, the control ($p = 0.00088$) showed a significant increase in base diameter while the mid heatwave treatment showed a significant decrease ($p = 0.017$) (Fig. 5, Table S3). The extreme heatwave treatment showed no significant changes post-recovery ($p = 0.15$).

Anemone Behaviour

Behavioural responses of *A. xanthogrammica* showed a consistent pattern among treatments over the three-day heatwave; as the temperature increased, the anemones retracted their tentacles and remained closed (Fig. 6). When temperatures reached above 25°C, most individuals became partially or fully closed (Fig. 6). At the end of each heatwave, all individuals in the extreme heat treatment showed strong behavioural responses. There was a statistically significant difference between both the mid heatwave ($p = 0.00018$) and extreme heatwave ($p = 0.0035$) when compared to the control.

Feeding time of *A. xanthogrammica* showed an increase after heat exposure in both the mid ($p = 0.12$) and extreme ($p = 0.043$) heatwave treatments but remained relatively constant in the control ($p = 0.33$) (Fig. 7, Table S4).

Discussion

The objective of this experiment was to investigate the effects of short-term marine heatwaves on anemone-algal symbiosis by looking at the differences in fitness of the host, *Anthopleura xanthogrammica*, and its symbionts. Although we hypothesized that symbiont fitness would decrease and host fitness would remain constant, we found that *A. xanthogrammica* showed significant behavioural responses to heat. Additionally, we observed no biologically significant changes in symbiont fitness, showing some level of resistance by the algae. However, some variations in algal health remain unresolved.

While we predicted that Symbiodiniaceae would be more resistant to heat stress than zoochlorellae (O'Brien & Wyttenbach, 1980; McCloskey et al., 1996; Verde & McCloskey, 2007; Levine & Muller-Parker, 2012), we saw inconsistent trends in symbiont fitness and found no biological significance supporting the oxidative bleaching hypothesis. With respect to photosynthetic efficiency, algal symbionts followed a similar pattern in all three groups (Fig. 2), independently from heat exposure. This suggests that there is no correlation between heat treatments and variations in photosynthesis. These findings correspond with Pryor et al. (2021) who found that the photosynthetic efficiency of tropical anemones did not vary significantly in response to heat treatments, despite showing evidence of bleaching. It is possible that unhealthy symbionts are expelled, while healthy ones are retained, so measuring the photosynthetic efficiency of algae within anemone tissues may not reflect the overall symbiont fitness. A study looking at expelled Symbiodiniaceae following coral bleaching, found a decrease in photosynthetic efficiency of damaged symbionts (Fujise et al., 2014). However, as we found no significant variation in symbiont density, this methodology of measuring photosynthetic efficiency outside of the host may not apply to our experiment. As measures of photosynthesis were inconclusive, other indicators of algal fitness such as

mitotic index and symbiont density were analyzed to provide insight into the symbiotic relationship.

We found that there was no biological difference in measures of mitotic index between heat treatments and the control after the heatwave, indicating that the symbionts' ability to asexually reproduce was not impeded by heat. However, in zoochloellae we did observe a significant difference between the mid and extreme heatwave treatments, but the interpretation of the significance remains unclear. This difference could potentially be explained by a higher proportion of dividing symbionts from the start of the experiment through the random distribution of individuals. The inconclusive patterns of cell division do not align with O'Brien & Wyttenbach (1980), who found a decrease in mitotic index in the sea anemone *A. xanthogrammica* at increased temperatures, pointing to some unknown physiological response in our experiment. Additionally, the proportion of dividing zoochloellae was much higher than dividing Symbiodiniaceae, which is likely due to species-specific differences in reproduction rates. This is supported by Verde and McCloskey (2007) who found similar patterns in temperate anemones independently of seasonal heat exposure. Nevertheless, our data contradicts our prediction that variations in mitotic index is caused by heat exposure. While some researchers propose that differences in mitotic index can be explained by variations in symbionts density (Baghdasarian & Muscatine, 2000; Oladi et al., 2017), we found no evidence of such occurrence.

No biologically relevant patterns were seen in symbiont density, as there was no significant increase or decrease after the heat treatments. While Pryor et al. (2021) found that tropical sea anemones bleached as a response to increased temperature, we found no evidence that this phenomenon occurs in *A. xanthogrammica*, at least in the short-term. With no clear differences in symbiont density, photosynthetic efficiency, or mitotic index between heatwave treatments, our evidence does not support the oxidative bleaching hypothesis which predicts a decrease in symbiont fitness. Perhaps the symbiont resistance to heat exposure can be explained by the behavioral mechanisms of the anemones.

A. xanthogrammica displayed a strong negative behavioural response to the short-term heatwave treatments by exhibiting changes in base size, tentacle exposure, and feeding time. Anemones showed varying base diameters (Fig. 5), but this data remains unresolved. Although Angeli et al. (2016) suggests that the pedal disc diameter is the best estimator of the true size of an anemone, *A. xanthogrammica* individuals were visibly moving within their containers throughout the experiment, which could have affected the accuracy of our measurements. While variability in changing base size could be attributed to movement, this should be further investigated to look at possibly interesting physical reactions to heat. Although base diameter measurements were inconclusive, behavioural responses provided better insight regarding anemone fitness.

Increased tentacle retraction and decreased feeding time were prevalent in the two heatwave treatments after the rise in temperature. When temperatures reached 25°C and above, most individuals completely retracted their tentacles (Fig. 6). We believe that tentacle retraction could be a mechanism for regulating internal body temperature, as discussed by Secord and Augustine (2000) who found that *A. xanthogrammica* was able to maintain temperatures closer to ambient seawater by closing at summer low tides. This is also supported by the findings of Dykens and Shick (1984), showing that *Anthopleura elegantissima* retracted its tentacles and attached debris to the body column for protection from sun exposure. Furthermore, the mid and extreme treatments experienced slower feeding time after heat exposure. As we collected feeding data for a subset of our sample, we cannot assess this correlation with certainty, but we believe these trends should be further investigated with a larger sample size. Warmer water temperatures have been shown to decrease digestion time in two closely related cnidarians, including jellyfish (Purcell, 2009) and corals (Rossi et al., 2004). Arguably, feeding behaviour in anemones could also be affected by heatwaves. Tentacle activity and feeding behaviour informs us that the anemones were generally negatively affected by increased temperatures over a short simulated three-day heatwave, while base measurements do not clearly inform us about host fitness. This supports our alternative hypothesis stating that anemone fitness, not algae fitness, would be more affected by acute heat exposure.

Despite not seeing the expected bleaching patterns, our experiment highlights an important protection mechanism from *A. xanthogrammica*. One possibility is that by retracting their tentacles and closing at high temperatures, sea anemones can regulate their internal temperature, which in turn benefits their algal symbionts as observed by Secord and Augustine (2000). Not only do the temperate anemones protect themselves, but this mechanism also protects their main nutrient source (the symbionts) while they remain closed (Bedgood et al., 2020). With regards to marine symbiosis, this suggests that the symbiotic relationship between *A. xanthogrammica*, zoochlorellae, and Symbiodiniaceae is more adapted to resist marine heatwaves than other cnidarian-algal partnerships. Studies have found that some temperate corals will completely bleach at similar temperatures to our extreme heatwave treatment (Moore et al., 2012; Aichelman et al., 2016). While coral bleaching remains a concerning issue exacerbated by climate change (Ainsworth & Brown, 2021), our study shows that not all mutualistic symbiosis will breakdown in short-term heatwave conditions. However, to encompass the multifaceted impacts of climate change, future research must incorporate a more holistic approach.

Our laboratory experiment did not entirely replicate natural heatwave conditions. Anemones collected in the fall were acclimated to colder conditions than what they would normally experience in the summer, when heatwaves are most prevalent, and therefore may respond differently to heat exposure. By running on an open system, the anemones faced a greater cold shock when water flow was restored at the end of each heatwave. It is also possible that this difference in water temperature led to a quicker recovery from *A. xanthogrammica* individuals. With rising tide, we expect temperatures of 21 degrees during a heatwave (Golden, 2021), while our anemones experienced approximately 12-degree waters during their recovery. Future experiments should be run in similar conditions to natural summer heatwaves to reduce excessive temperature shocks. Examining the effects of longer and more intense heat stress is also a valuable direction for future research, as heat waves are increasing in frequency and intensity with the onset of climate change (Russo et al, 2014).

Running a three-day heatwave will show short term results on anemone-algae symbiosis but cannot address how temperature fluctuations throughout an entire summer would affect the mutualistic relationship. As seen in our experiment, if *A. xanthogrammica* individuals remain closed during extreme heat stress events, and heatwaves increase in duration and intensity, anemones would be unable to catch prey, requiring more algal symbionts to obtain nutrients (Bedgood et al., 2020). Furthermore, Dunn et al. (2004) showed that Symbiodiniaceae performs apoptosis-like cell death within anemone tissues at high temperatures. If anemones cannot feed with their tentacles, and symbionts cannot survive in their tissues, anemones would lack proper nutrition. This could affect not only the anemone but the integrity of the symbiotic relationship. An avenue for future studies could explore the increasing frequency of short-term heatwaves and observe the cumulative impacts on marine symbiosis.

Conclusion

Our study set out to investigate the effects of climate-change induced increases in temperature on marine symbiosis. We found that *A. xanthogrammica* had negative physical reactions to heatwave conditions, but its algal symbionts were not affected. Our findings suggest that this symbiotic relationship is somewhat resistant to increases in temperature within tidepools. While we provide evidence showing that *A. xanthogrammica* has strong behavioural responses to heat, it is unclear how the symbiotic relationship with zoochlorellae and Symbiodiniaceae is affected. Further exploration is required to understand both the short and long-term effects of climate change on marine symbiosis within temperate anemones, but this study provides multiple avenues for future studies. Climate change will continue to affect marine symbiosis, and many of the consequences on temperate intertidal species are unknown and understudied (Harley et al., 2006). With increasing frequencies in heatwaves (Oliver et al. 2018), storms (Ruggiero, 2010) and worsening water quality (Murdoch et al., 2000), researchers must investigate how these changes will challenge the intertidal ecosystem.

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Figures

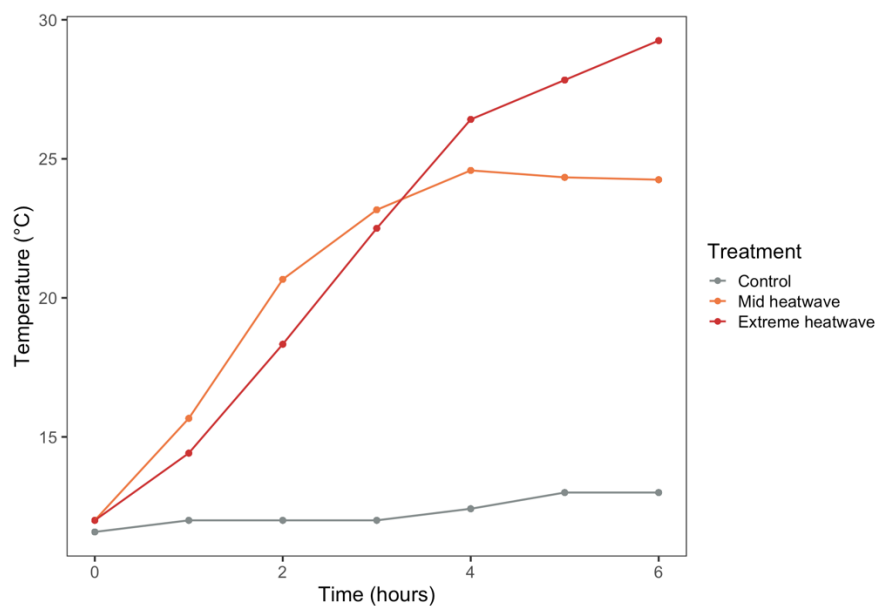


Fig. 1. Temperature increases in each treatment over the 6-hour heatwave, averaged over the three heatwaves.

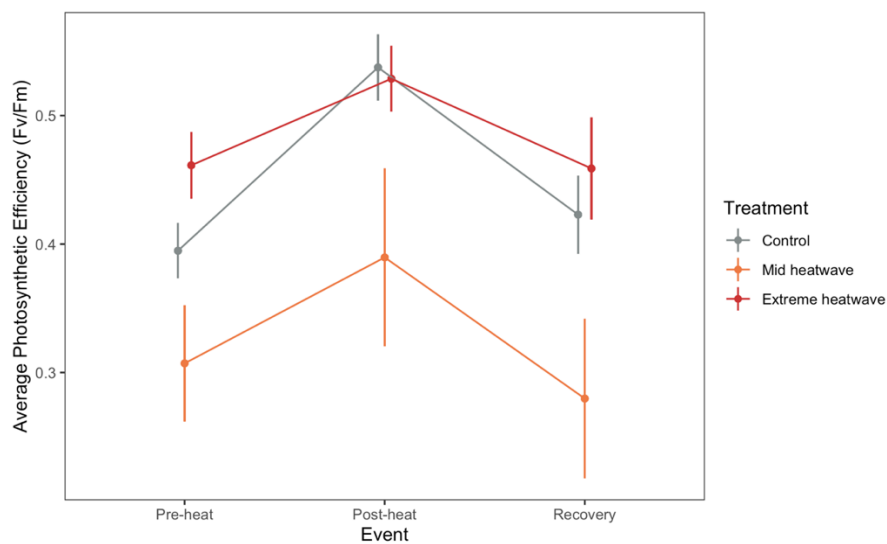


Fig. 2. Average photosynthetic efficiency (Fv/Fm) of zoochlorellae and Symbiodiniaceae in *Anthopleura xanthogrammica* in the control (12°C), mid heatwave treatment (25°C), and extreme heatwave treatment (30°C) over three events: pre-heat, post-heat, and recovery. Error bars indicate standard error of the mean photosynthetic efficiency.

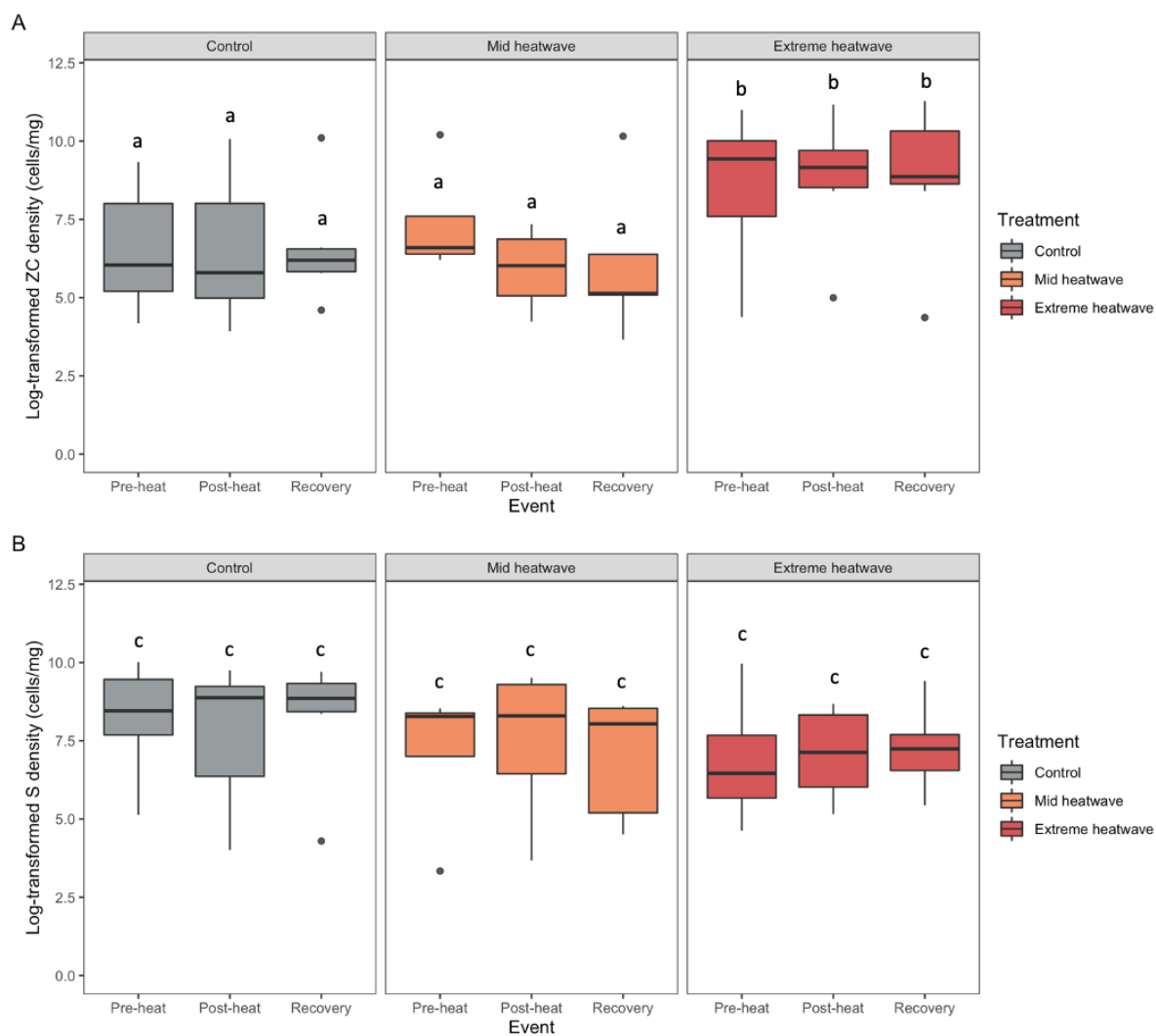


Fig. 3. Log-transformed symbiont density (cells/mL) of *A. zoochlorellae* (ZC) and *B.* Symbiodiniaceae (S) in *Anthopleura xanthogrammica* for the control (12°C), mid heatwave treatment (25°C), and extreme heatwave treatment (30°C) over three events: pre-heat, post-heat, and recovery. Letters above box indicate the results of a Dunn Kruskal-Wallis test; boxes that do not share the same letter are significantly different ($p < 0.05$).

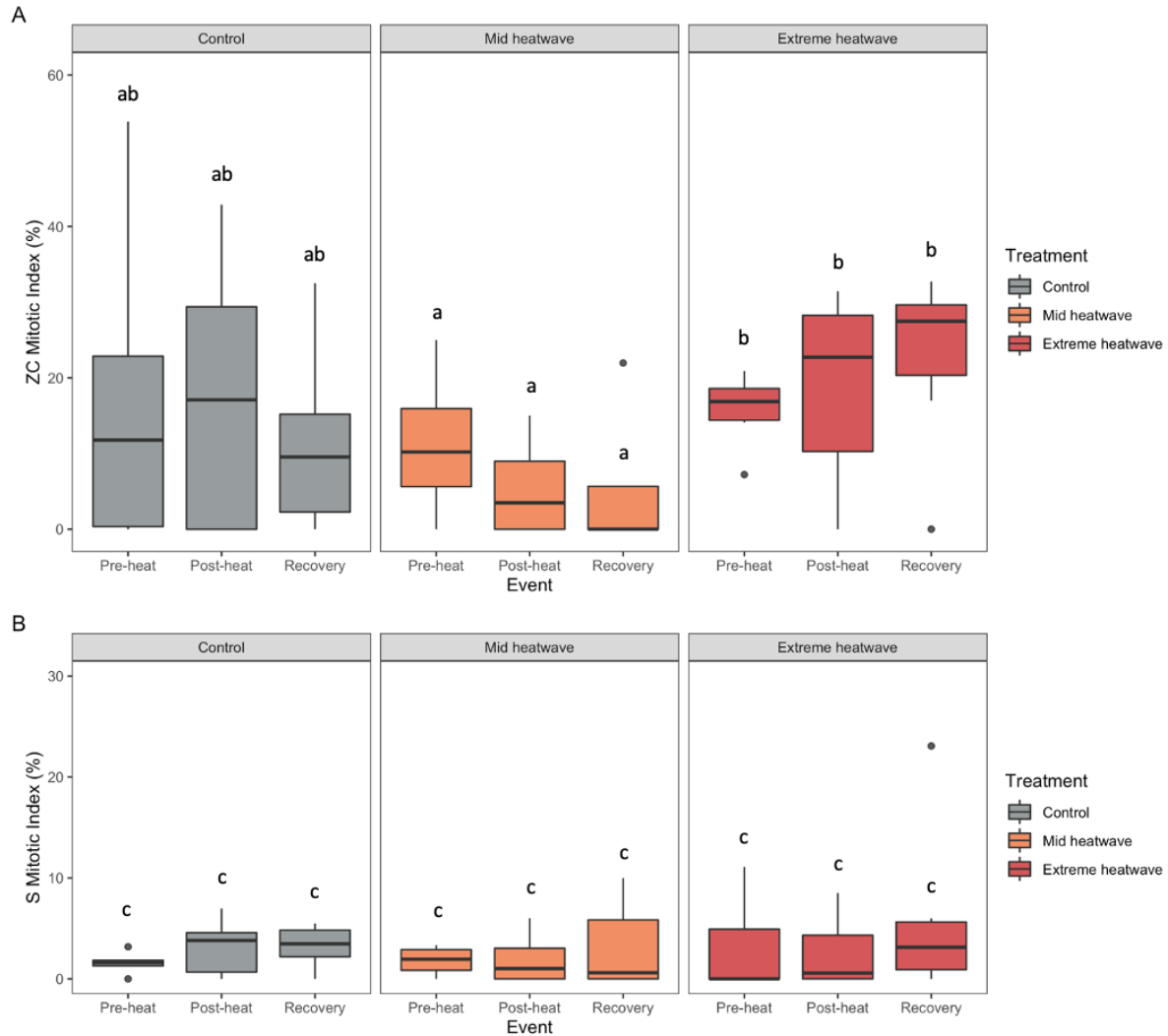


Fig. 4. The mitotic index (dividing cells/total cells) of both algal symbionts, A. zoochlorellae (ZC) and B. Symbiodiniaceae (S), in *Anthopleura xanthogrammica* for the control (12°C), mid heatwave treatment (25°C), and extreme heatwave treatment (30°C) over three events: pre-heat, post-heat, and recovery. Letters above box indicate the results of a Dunn Kruskal-Wallis test; boxes that do not share the same letter are significantly different ($p < 0.05$).

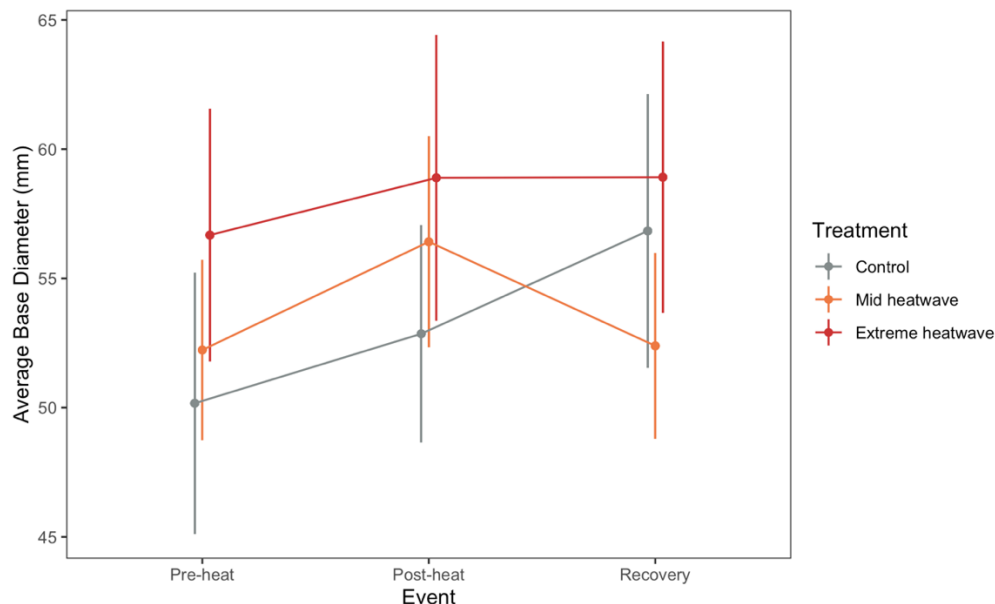


Fig. 5. Average base diameter of *Anthopleura xanthogrammica* in the control (12°C), mid heatwave (25°C), and extreme heatwave treatment (30°C) over three events: pre-heat, post-heat, recovery. Error bars indicate standard error of the mean base diameter.

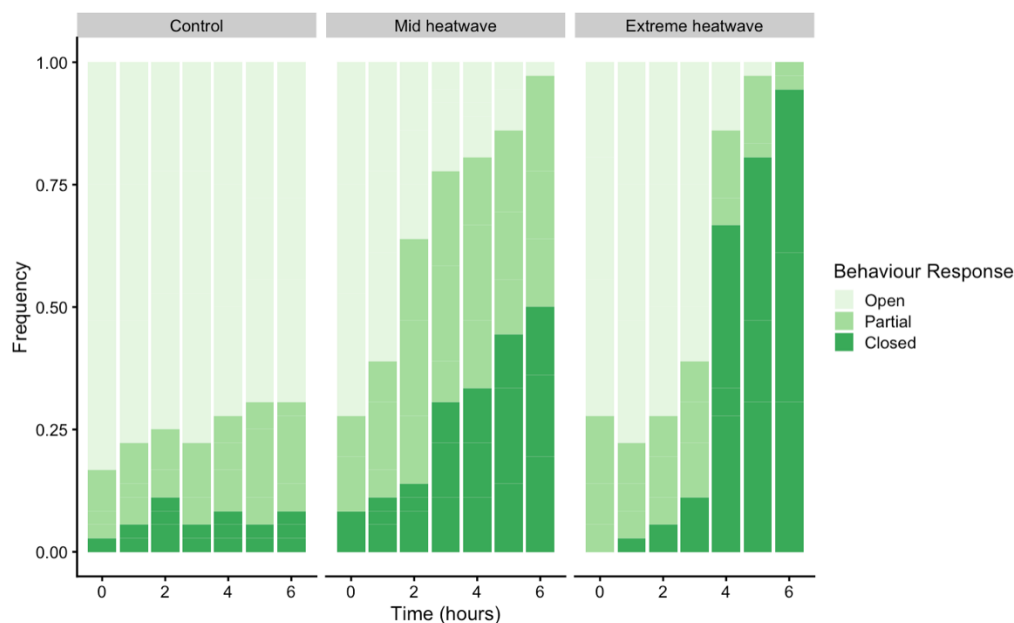


Fig. 6. Frequency of observed behavioural response (open, partially open, or closed) of *Anthopleura xanthogrammica* at three temperature treatment levels: control (12°C), mid heatwave (25°C), extreme heatwave (30°C).

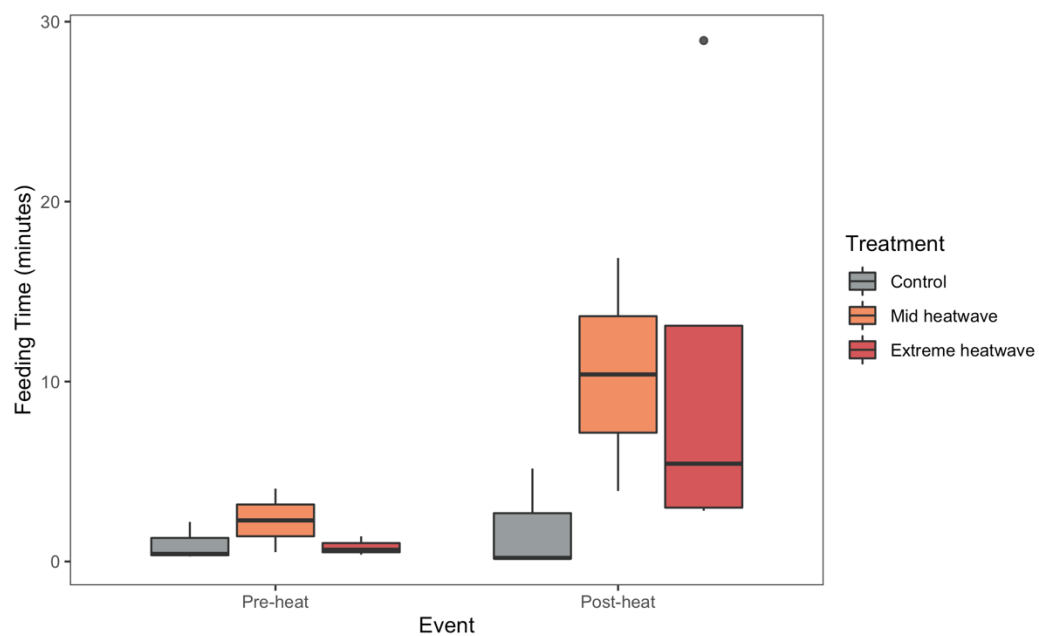


Fig. 7. Feeding time in minutes of *Anthopleura xanthogrammica* before and after heatwaves at three treatment levels: control (12°C), mid heatwave (25°C), and high heatwave (30°C).

Supplemental Information

Table S1. Statistical output of a Generalized Additive Model for Location, Scale, and Shape, using a Weibull distribution, to assess the significant differences in zoochlorellae density between treatments and events. Difference is assessed using the pre-heat control as a baseline. Treatments are control (12°C), mid heatwave (25°C), and extreme heatwave (30°C). Events are pre-heat, post-heat, and recovery.

Model Terms	Estimate	Standard Error	t-value	p-value
[Intercept]	7.02648	0.20520	34.242	< 0.01
Post-heat	-0.17523	0.27804	-0.630	0.5354
Recovery	0.28886	0.28856	1.001	0.3283
Mid heatwave	-0.12147	0.32291	-0.376	0.7106
Extreme heatwave	1.47524	0.26344	5.600	< 0.01
Post-heat : Mid heatwave	0.05102	0.45057	0.113	0.9109
Post-heat : Extreme heatwave	-0.83396	0.44295	-1.883	0.0738
Recovery : Mid heatwave	0.13530	0.38302	0.353	0.7275
Recovery : Extreme heatwave	0.32660	0.38314	0.852	0.4037

Table S2. Statistical output of a Generalized Additive Model for Location, Scale, and Shape, using a Gamma distribution, to assess the significant differences in Symbiodiniaceae density between treatments and events. Difference is assessed using the pre-heat control as a baseline. Treatments are control (12°C), mid heatwave (25°C), and extreme heatwave (30°C). Events are pre-heat, post-heat, and recovery.

Model Terms	Estimate	Standard Error	t-value	p-value
[Intercept]	8.06079	0.25027	32.208	< 0.01
Post-heat	-0.16934	0.34106	-0.496	0.6245
Recovery	-0.23477	0.35394	-0.663	0.5141
Mid heatwave	-1.32556	0.39574	-3.350	0.0029
Extreme heatwave	-0.74465	0.32308	-2.305	0.0310
Post-heat : Mid heatwave	0.43494	0.55161	0.788	0.4389
Post-heat : Extreme heatwave	1.03874	0.54261	1.914	0.0687
Recovery : Mid heatwave	-0.23333	0.46976	-0.497	0.6244
Recovery : Extreme heatwave	0.03936	0.46977	0.084	0.9340

Table S3. Statistical output of a Generalized Additive Model for Location, Scale, and Shape, using a Log-Normal distribution, to assess the significant differences in average base size measurements between treatments and events. Difference is assessed using the pre-heat control as a baseline. Treatments are control (12°C), mid heatwave (25°C), and extreme heatwave (30°C). Events are pre-heat, post-heat, and recovery.

Model Terms	Estimate	Standard Error	t-value	p-value
[Intercept]	3.89112	0.01940	200.618	< 0.01
Post-heat	0.03437	0.02685	1.280	0.2053
Recovery	0.09388	0.02685	3.496	< 0.01
Mid heatwave	0.03913	0.02685	1.457	0.1501
Extreme heatwave	0.10920	0.02685	4.067	< 0.01
Post-heat : Mid heatwave	0.03822	0.03756	1.018	0.3129
Post-heat : Extreme heatwave	-0.09243	0.03756	-2.461	0.0167
Recovery : Mid heatwave	-0.00411	0.03756	-0.109	0.9133
Recovery : Extreme heatwave	-0.05528	0.03756	-1.472	0.1462

Table S4. Statistical output of a Generalized Additive Model for Location, Scale, and Shape, using a Log-Normal distribution, to assess the significant differences in average feeding time between treatments and events. Difference is assessed using the pre-heat control as a baseline. Treatments are control (12°C), mid heatwave (25°C), and extreme heatwave (30°C). Events are pre-heat, post-heat, and recovery.

Model Terms	Estimate	Standard Error	t-value	p-value
[Intercept]	-0.5298	0.5149	-1.029	0.3330
Post-heat	0.2449	0.7281	-0.336	0.7451
Mid heatwave	0.8233	0.8141	1.011	0.3409
Extreme heatwave	0.3369	0.7281	0.463	0.6557
Post-heat : Mid heatwave	1.9684	1.1513	1.710	0.1249
Post-heat : Extreme heatwave	2.3532	0.9970	2.360	0.0453