**The impact of climate change on marine symbiosis**

**in *Anthopleura xanthogrammica***

by

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# Abstract

Anthropogenic climate change is affecting costal 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 heat on temperate intertidal ecosystems. This paper investigates how short-term heatwaves effect the temperate sea anemone *Anthopleura xanthogrammica* and its symbiotic relationship with two marine algae, zoochlorellae and Symbiodinaceae. 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. *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 marine symbiosis in temperate ecosystems can be resistant to short-term heat exposure, but this is not seen in all cnidarians. This study can be used as a foundation for future research on the effect of heat and climate-change related impacts on temperate intertidal ecosystems.

# 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 the extreme surface and air temperatures. A 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 by 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 & Wyttenback, 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 proxy for symbiont fitness; a decrease in photosynthesis indicates a decline in algal health (Dykens & Shick, 1984; Fujise, 2014). Contrarily, 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 are intertidal organisms sharing the same phylum and class as corals, who 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. McCloseky et al. (1996)found that anemones control agal 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).

# Brandt 1835

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 and is an exceptional example of relationships where one host has 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 *(*Dykens & 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; McCloseky 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 of 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 (Dykens, & 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 photosynthetic partner, but the host fitness would not be affected. As such, we predict that not only symbiont density will decrease but also that measures of algal stress, such as photosynthetic capacity 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* = 12 per treatment). 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.

## Statistical Analysis

The Shapiro-Wilk test (Shapiro and Wilk, 1965) and Bartlett test (Arsham, 2011) 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 for 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. All of the above analyses were performed in R (V 4.1.1, R Core Team, 2021)

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 (Levine & Muller-Parker, 2012). Aposymbiotic anemones with symbiont density lower than 1000 cells per mg of tissue were removed from statistical analysis [CITE]. Both the Kruskal-Wallis rank sum (Kruskal & Wallace, 1952) and Dunn Kruskal-Wallis (Dinno, 2015) tests were used to assess the relationship between heat treatments and photosynthetic capacity of the symbionts.

### Symbiont Density

### To assess the symbiont fitness, 1-4 tentacles were clipped per anemone at the following timepoints: end of acclimation period, after 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 (10-12µm), while zoochlorellae are green and smaller (6-8µm) (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. Statistical analysis was performed using both Kruskal-Wallis rank sum (Kruskal & Wallace, 1952) and Dunn Kruskal-Wallis (Dinno, 2015) tests.

### 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). Statistical differences between treatments were assessed using an ordinal regression model (Christense, 2015).Since less healthy anemones 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.

# Results

## Symbiont Fitness

## *Photosynthetic Efficiency*

Photosynthetic efficiency increased after the heatwave and decreased after the recovery period (Fig. 2). These trends were similar among treatments and the control. The difference between the 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-adj = 0.025). There was no significant difference between the control and the extreme heat treatment (Dunn Kruskal-Wallis, Z = -0.67, p-adj = 0.065) or between the control and the mid heat treatment (Dunn Kruskal-Wallis, Z = 2.14, p-adj = 0.50).

## *Symbiont Density*

Zoochlorellae density in *Anthopleura xanthogrammica* tissue of both heat treatments showed a slightly decreasing trend after the heatwave and recovery periods, compared to the control (Fig. 4A). Across the experiment, the extreme heatwave treatment had significantly higher zoochlorellae density then the control and the mid heatwave treatment (p = 1.5 x 10-5). We observed no consistent patterns for the change in Symbiodiniaceae density (Fig. 4B). There was statistical difference in the Symbiodiniaceae density between the control and the mid heatwave treatment (p = 0.0029) and between the control and the extreme heatwave treatment (p = 0.031). The heat treatments did not significantly affect density of zoochlorellae and Symbiodiniaceae in *A. xanthogrammica* as there were no statistical differences between events within each treatment (p > 0.05 for all, Table S1).

## *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. 3A). No clear patterns in the proportion of dividing Symbiodiniaceae cells can be observed (Fig. 3B). 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-adj= 0.15), or control and extreme heatwave treatment (Dunn Kruskal-Wallis, Z = 1.73, p-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-adj = 1.00) or the control and the extreme heatwave treatment (Dunn Kruskal-Wallis, Z = -0.62, p-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). At the end of the recovery period, both the extreme heatwave treatment (p = 0.15) and the control (p = 0.00088), showed a significant increased base diameter while the mid heatwave treatment showed a significant decrease (p = 0.017) (Fig. 5).

## *Anemone Behaviour*

Behavioural responses of *A.* *xanthogrammica* showed a consistent pattern among treatments over the 3-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).

**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 as host fitness remained constant, we found that *A. xanthogrammica* showed significant behavioural responses to heat. Additionally, we observed no biologically significant changes, 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; McCloseky 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 capacity, algal symbionts followed a similar pattern in all three groups (Fig. 2), independently from heat exposure, suggesting no correlation between heat treatments and variations in photosynthesis. These findings correspond with Pryor et al. (2021) who found that the photosynthetic capacity 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 capacity 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 capacity 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 algae fitness such as mitotic index and symbiont density were analyzed to provide insight into the symbiotic relationship.

Possibly add another sentence here. Measures of mitotic index were not biologically different between heat treatments and the control after the heatwave, indicating that the symbionts’ ability to asexually reproduce was not impeded by heat. However, in zoochlorellae 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 zoochlorellae 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 independently of seasonal heat exposure in temperate anemones. As such, we may not conclude that variations in mitotic index is caused by heat exposure, as hypothesized earlier. 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 capacity, 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 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 was 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 at summer low tides by closing. 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. The mid and extreme treatments also 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 3-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 the sea anemones. One possibility is that by retracting their tentacles and closing at high temperatures, *A. xanthogrammica* individuals can regulate their internal temperature, which in turns benefits their algal symbiont as observed by Secord and Augustine (2000). Not only do the temperate anemones protect themselves, but this mechanism also protects their main nutrient source 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 (Golden, 2021) during a heatwave, 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 3-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 the proper nutrition, affecting not the just 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.

Conclusions

Our study set out to investigate the effects of increasing temperatures on marine symbiosis due to anthropogenic climate change. We found that *Anthopleura xanthogrammica* had negative physical reactions to increasing temperature, 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. Anthropogenic 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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# References

Aichelman, H. E., Townsend, J. E., Courtney, T. A., Baumann, J. H., Davies, S. W., & Castillo, K. D. (2016). Heterotrophy mitigates the response of the temperate coral *Oculina arbuscula* to temperature stress. *Ecology and Evolution*, *6*(18), 6758-6769. https://doi.org/10.1002/ece3.2399

Aichelman, H. E., & Barshis, D. J. (2020). Adaptive divergence, neutral panmixia, and algal symbiont population structure in the temperate coral *Astrangia poculata* along the Mid-Atlantic United States. *PeerJ*, *8*, 1-27.  https://doi.org/10.7717/peerj.10201

Ainsworth, T. D., & Brown, B. E. (2021). Coral bleaching. *Current Biology*, *31*(1), R5-R6. https://doi.org/10.1016/j.cub.2020.10.048

Ainsworth, T. D., Leggat, W., Silliman, B. R., Lantz, C. A., Bergman, J. L., Fordyce, A. J., Page, C. E., Renzi, J. J., Morton, J., Eakin, C. M., & Heron, S. F. (2021). Rebuilding relationships on coral reefs: Coral bleaching knowledge‐sharing to aid adaptation planning for reef users. *BioEssays*, *43*(9), 1-9. https://doi.org/10.1002/bies.202100048

Angeli, A., Zara, F. J., Turra, A., & Gorman, D. (2016). Towards a standard measure of sea anemone size: assessing the accuracy and precision of morphological measures for cantilever-like animals. *Marine Ecology*, *37*(5), 1019-1026. https://doi.org/10.1111/maec.12315

Aslam, S. (2021, September 17). *June’s heatwave was deadliest weather event in Canadian history*. CityNews. Retrieved November 29, 2021, from https://vancouver.citynews.ca/2021/09/17/deadliest-heatwave-bc/

Arsham, H., Lovric M. (2011) Bartlett’s Test. *International Encyclopedia of Statistical Science*. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-04898-2\_132

Baghdasarian, G., & Muscatine, L. (2000). Preferential expulsion of dividing algal cells as a mechanism for regulating algal-cnidarian symbiosis. *The Biological Bulletin*, *199*(3), 278-286. <https://doi.org/10.2307/1543184>

Bates, A. (2000). The intertidal distribution of two algal symbionts hosted by *Anthopleura xanthogrammica* (Brandt 1835). *Journal of Experimental Marine Biology and Ecology*, *249*, 249-262. https://doi.org/10.1016/s0022-0981(00)00203-3

Bates, A. E., Mclean, L., Laing, P., Raeburn, L. A., & Hare, C. (2010). Distribution patterns of zoochlorellae and zooxanthellae hosted by two Pacific northeast anemones, *Anthopleura elegantissima* and *A. xanthogrammica*. *The Biological Bulletin*, *218*(3), 237-247. https://doi.org/10.1086/bblv218n3p237

Bedgood, S. A., Mastroni, S. E., & Bracken, M. E. S. (2020). Flexibility of nutritional strategies within a mutualism: food availability affects algal symbiont productivity in two congeneric sea anemone species. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1940), 1-10. https://doi.org/10.1098/rspb.2020.1860

Christense, R.H.B. (2019). *Regression Models for Ordinal Data* [R]. https://cran.r-project.org/web/packages/ordinal/.

Dimond, J. L., Bingham, B. L., Muller-Parker, G. l., Wuesthoff, K., & Francis, L. (2011). Seasonal stability of a flexible algal–cnidarian symbiosis in a highly variable temperate environment. *Limnology and Oceanography*, *56*(6), 2233-2242. https://doi.org/10.4319/lo.2011.56.6.2233

Dinno, A. (2015). Nonparametric Pairwise Multiple Comparisons in Independent Groups using Dunn’s Test. *The Stata Journal: Promoting Communications on Statistics and Stata*, *15*(1), 292-300. https://doi.org/10.1177/1536867x1501500117

Dunn, S. R., Thomason, J. C., le Tissier, M. D. A., & Bythell, J. C. (2004). Heat stress induces different forms of cell death in sea anemones and their endosymbiotic algae depending on temperature and duration. *Cell Death & Differentiation*, *11*(11), 1213-1222. https://doi.org/10.1038/sj.cdd.4401484

Dykens, J. A., & Shick, J. M. (1984). Photobiology of the symbiotic sea anemone, *Anthopleura* *elegantissima*: defenses against photodynamic effects, and seasonal photoacclimatization. *The Biological Bulletin*, *167*(3), 683-697. https://doi.org/10.2307/1541419

Fernandes De Barros Marangoni, L., Rottier, C. & Ferrier-Pagès, C. (2021). Symbiont regulation in *Stylophora* *pistillata* during cold stress: An acclimation mechanism against oxidative stress and severe bleaching. *J. Exp. Biol.* *224*(3), 1-10. https://doi.org/10.1242/jeb.235275

Fujise, L., Yamashita, H., Suzuki, G., Sasaki, K., Liao, L. M., & Koike, K. (2014). Moderate thermal stress causes active and immediate expulsion of photosynthetically damaged zooxanthellae (symbiodinium) from corals. P*loS One*, *9*(12), 1-18. https://doi.org/10.1371/journal.pone.0114321

Gates, R. D., Baghdasarian, G., and Muscatine, L. (1992). Temperature stress causes host cell detachment in symbiotic cnidarians: Implications for coral bleaching. *The Biological Bulletin, 182*(3), 324-332. https://doi.org/10.2307/1542252

Golden, H. (2021, August 25). *Video shows salmon injured by unlivable water temperatures after heatwave*. The Guardian. Retrieved December 14, 2021, from https://www.theguardian.com/us-news/2021/jul/27/salmon-boiled-alive-pacific-north-west-heatwave-video

Harley, C. D. G., Randall Hughes, A., Hultgren, K. M., Miner, B. G., Sorte, C. J. B., Thornber, C. S., Rodriguez, L. F., Tomanek, L., & Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters*, *9*(2), 228-241. https://doi.org/10.1111/j.1461-0248.2005.00871.x

Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world’s coral reefs. *Marine and Freshwater Research*, *50*(8), 839-866. https://doi.org/10.1071/mf99078

Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Dietzel, A., Eakin, C. M., Heron, S. F., Hoey, A. S., Hoogenboom, M. O., Liu, G., McWilliam, M. J., Pears, R. J., Pratchett, M. S., Skirving, W. J., Stella, J. S., & Torda, G. (2018). Global warming transforms coral reef assemblages. *Nature*, *556*(7702), 492-496. https://doi.org/10.1038/s41586-018-0041-2

Kinzie III, R. A., Takayama, M., Santos, S. R., & Coffroth, M. A. (2001). The adaptive bleaching hypothesis: experimental tests of critical assumptions. *The Biological Bulletin*, *200*(1), 51-58. https://doi.org/10.2307/1543084

Kruskal, W. H., & Wallis, W. A. (1952). Use of Ranks in One-Criterion Variance Analysis. *Journal of the American Statistical Association*, *47*(260), 583-621. https://doi.org/10.1080/01621459.1952.10483441

LaJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R., & Santos, S. R. (2018). Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. *Current Biology*, *28*(16), 2570-2580. https://doi.org/10.1016/j.cub.2018.07.008

Levine, M. R., & Muller-Parker, G. (2012). Distribution patterns and nutritional contributions of algal symbionts in the sea anemone *Anthopleura* *xanthogrammica*. *Marine Ecology Progress Series*, *453*, 79-94. https://doi.org/10.3354/meps09602

Lukacs, E. (1955). A Characterization of the Gamma Distribution. *The Annals of Mathematical Statistics*, *26*(2), 319-324.  https://doi.org/10.1214/aoms/1177728549

Lüskow, F., Kløve-Mogensen, K., Tophøj, J., Pedersen, L. H., Riisgård, H. U., & Eriksen, N. T. (2019). Seasonality in Lipid Content of the Demosponges *Halichondria panicea* and *H. bowerbanki* at Two Study Sites in Temperate Danish Waters. *Frontiers in Marine Science*, *6*, 1-7. https://doi.org/10.3389/fmars.2019.00328

Margulis, L. (1971). Symbiosis and Evolution. *Scientific American*, *225*(2), 48-61. https://doi.org/10.1038/scientificamerican0871-48

McCloskey, L., Cove, T. G., & Verde, E. A. (1996). Symbiont expulsion from the anemone *Anthopleura* *elegantissima* (Brandt) (Cnidaria; Anthozoa). *Journal of Experimental Marine Biology and Ecology*, *195*(2), 173-186. https://doi.org/10.1016/0022-0981(95)00079-8

Migdal, A. (2021a, June 30). *Western Canada’s heat wave is “unprecedented.” Scientists say it will become more common with climate change*. CBC. Retrieved October 24, 2021, from <https://www.cbc.ca/news/canada/british-columbia/bc-heat-wave-climate-change-1.6085275>

Migdal, A. (2021b, July 5). *More than a billion seashore animals may have cooked to death in B.C. heat wave, says UBC researcher*. CBC. Retrieved October 24, 2021, from <https://www.cbc.ca/news/canada/british-columbia/intertidal-animals-ubc-research-1.6090774>

Moore, J. A. Y., Bellchambers, L. M., Depczynski, M. R., Evans, R. D., Evans, S. N., Field, S. N., Friedman, K. J., Gilmour, J. P., Holmes, T. H., Middlebrook, R., Radford, B. T., Ridgway, T., Shedrawi, G., Taylor, H., Thomson, D. P., & Wilson, S. K. (2012). Unprecedented Mass Bleaching and Loss of Coral across 12° of Latitude in Western Australia in 2010-11. *PLoS ONE*, *7*(12), 1-11. https://doi.org/10.1371/journal.pone.0051807

Murdoch, P. S., Baron, J. S., & Miller, T. L. (2000). Potential effects of climate change on surface-water quality in North America. *JAWRA Journal of the American Water Resources Association*, *36*(2), 347-366. https://doi.org/10.1111/j.1752-1688.2000.tb04273.x

O'Brien, T. L., & Wyttenbach, C. R. (1980). Some effects of temperature on the symbiotic association between zoochlorellae (Chlorophyceae) and the sea anemone *Anthopleura* *xanthogrammica*. *Transactions of the American Microscopical Society*, ­*99*(2), 221-225. https://doi.org/10.2307/3225711

Oladi, M., Shokri, M. R., & Rajabi-Maham, H. (2017). Application of the coral health chart to determine bleaching status of *Acropora* *downingi* in a subtropical coral reef. *Ocean Science Journal, 52*(2), 267-275. <https://doi.org/10.1007/s12601-017-0025-4>

Oliver, E. C. J., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., Benthuysen, J. A., Feng, M., Sen Gupta, A., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Straub, S. C., & Wernberg, T. (2018). Longer and more frequent marine heatwaves over the past century. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-03732-9

Ottaway, J. (1973). Some effects of temperature, desiccation, and light on the intertidal anemone, *Actinia tenebrosa* Farquhar (Cnidaria: Anthozoa). *Marine and Freshwater Research*, *24*(1), 103-126.  https://doi.org/10.1071/mf9730103

Padgett, W. J. (2011). Weibull Distribution. *International Encyclopedia of Statistical Science*, 1651-1653. https://doi.org/[10.1007/978-3-642-04898-2\_611](https://doi.org/10.1007/978-3-642-04898-2_611)

Pryor, S. H., Andrews, L., Kelaher, B. P., Tagliafico, A., & Scott, A. (2021). Ocean temperature, but not acidification, causes sea anemone bleaching under a near-future climate scenario. *Coral Reefs*, *40*(2), 355-364. https://doi.org/10.1007/s00338-021-02050-9

Purcell, J. E. (2008). Extension of methods for jellyfish and ctenophore trophic ecology to large-scale research. *Hydrobiologia*, *616*(1), 23-50.

Oladi, M., Shokri, M. R., & Rajabi-Maham, H. (2017). Application of the coral health chart to determine bleaching status of *Acropora* *downingi* in a subtropical coral reef. *Ocean Science Journal, 52*(2), 267-275. <https://doi.org/10.1007/s12601-017-0025-4>

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Richier, S., Rodriguez-Lanetty, M., Schnitzler, C. E., & Weis, V. M. (2008). Response of the symbiotic cnidarian *Anthopleura* *elegantissima* transcriptome to temperature and UV increase. *Comparative Biochemistry and Physiology*,*3*(4), 283-289. https://doi.org/10.1016/j.cbd.2008.08.001

Rigby, R. A., & Stasinopoulos, D. M. (2005). Generalized additive models for location, scale and shape (with discussion). *Journal of the Royal Statistical Society: Series C (Applied Statistics)*, *54*(3), 507-554.  https://doi.org/10.1111/j.1467-9876.2005.00510.x

Rossi, S., Ribes, M., Coma, R., & Gili, J. M. (2004). Temporal variability in zooplankton prey capture rate of the passive suspension feeder *Leptogorgia* *sarmentosa* (Cnidaria: Octocorallia), a case study. *Marine Biology*, *144*(1), 89-99. https://doi.org/10.1007/s00227-003-1168-7

Ruggiero, P., Komar, P. D., & Allan, J. C. (2010). Increasing wave heights and extreme value projections: The wave climate of the U.S. Pacific Northwest. *Coastal Engineering*, *57*(5), 539-552. https://doi.org/10.1016/j.coastaleng.2009.12.005

Russo, S., Dosio, A., Graversen, R. G., Sillmann, J., Carrao, H., Dunbar, M. B., Singleton, A., Montagna, P., Barbola, P., and Vogt, J. V. (2014). Magnitude of extreme heat waves in present climate and their projection in a warming world. *JGR Atmospheres.* *119*, 12,500-12,512.  https://doi.org/10.1002/2014jd022098

Sawyer, S. J., & Muscatine, L. (2001). Cellular mechanisms underlying temperature-induced bleaching in the tropical sea anemone *Aiptasia pulchella*. *Journal of Experimental Biology*, *204*(20), 3443-3456. https://doi.org/10.1242/jeb.204.20.3443

Secord, D., & Augustine, L. (2000). Biogeography and microhabitat variation in temperate algal‐invertebrate symbioses: zooxanthellae and zoochlorellae in two Pacific intertidal sea anemones, *Anthopleura* *elegantissima* and *A. xanthogrammica*. *Invertebrate Biology*, *119*(2), 139-146. https://doi.org/10.1111/j.1744-7410.2000.tb00002.x

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, *52*(3-4), 591-611. https://doi.org/10.1093/biomet/52.3-4.591

Stillman, J. H. (2019). Heat Waves, the New Normal: Summertime Temperature Extremes Will Impact Animals, Ecosystems, and Human Communities. *Physiology*, *34*(2), 86-100. https://doi.org/10.1152/physiol.00040.2018

Suharsono & Brown, B.E. (1992). Comparative measurements of mitotic index in zooxathellae from a symbiotic cnidarian subject to temperature increase. *Journal of Experimental Marine Biology and Ecology*, *158*(2), 179-188. https://doi.org/10.1016/0022-0981(92)90225-y

Verde, E. A., & McCloskey, L. (2007). A comparative analysis of the photobiology of zooxanthellae and zoochlorellae symbiotic with the temperate clonal anemone *Anthopleura* *elegantissima* (Brandt). III. Seasonal effects of natural light and temperature on photosynthesis and respiration. *Marine Biology*, *152*(4), 775-792. https://doi.org/10.1007/s00227-007-0737-6

*WinControl-3* (3.30). (2020). [Pulse Amplified Modulation (PAM)]. Microsoft. https://www.walz.com/products/chl\_p700/junior-pam/downloads.html

Wuitchik, D. M., Almanzar, A., Benson, B. E., Brennan, S., Chavez, J. D., Liesegang, M. B., Reavis, J. L., Reyes, C. L., Schniedewind, M. K., Trumble, I. F., & Davies, S. W. (2021). Characterizing environmental stress responses of aposymbiotic *Astrangia* *poculata* to divergent thermal challenges. *Molecular Ecology*, *30*(20), 5064-5079. https://doi.org/10.1111/mec.16108

Wyllie de Echeverria, V. R., & Thornton, T. F. (2019). Using traditional ecological knowledge to understand and adapt to climate and biodiversity change on the Pacific coast of North America. *Ambio*, *48*(12), 1447-1469. https://doi.org/10.1007/s13280-019-01218-6

# Figures

Chart, line chart

Description automatically generated

**Figure 1.** Average temperature increases in each treatment over the 6 hours of heatwave.

Chart, radar chart

Description automatically generated

**Figure 2.** The average photosynthetic efficiency (Fv/Fm) of algal symbionts 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 capacity.

**Chart, box and whisker chart

Description automatically generated**

**Figure 3.** The mitotic index (dividing cells/total cells) of both algal symbionts in *Anthopleura* *xanthogrammica*, A. zoochlorellae (ZC) and B. Symbiodiniaceae (S), 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. Letters above box indicate the results of a Dunn Kruskal-Wallistest; boxes that do not share the same letter are significantly different (p < 0.05).

Chart, box and whisker chart

Description automatically generated

**Figure 4.** The symbiont density (cells/mL) in *Anthopleura xanthogrammica* of A. zoochlorellae (ZC) and B. Symbiodiniaceae (S) 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. Letters above box indicate the results of a Dunn Kruskal-Wallistest; boxes that do not share the same letter are significantly different (p < 0.05).

Chart, line chart

Description automatically generated

**Figure 5.** The 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.

Chart, bar chart

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**Figure 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).

Chart, box and whisker chart

Description automatically generated

**Figure 7.** The 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.**

Symbiont density zoochlorellae, using gamlss with Weibull distribution. Significance compared to pre-heat of control.