2014

Influence of temperature on the physiological performance of zoochlorellae in two intertidal hosts (Anthopleura elegantissima and A. xanthogrammica)

Gemma S. (Gemma Smith) Woodhouse
Western Washington University

INFLUENCE OF TEMPERATURE ON THE PHYSIOLOGICAL PERFORMANCE OF ZOOCHLORELLAE IN TWO INTERTIDAL HOSTS (*ANTHOPLEURA ELEGANTISSIMA* AND *A. XANTHOGRAMMICA*)

by

Gemma S. Woodhouse

Accepted in Partial Completion

Of the Requirements for the Degree

Master of Science

Kathleen L. Kitto, Dean of the Graduate School

ADVISORY COMMITTEE

Chair, Dr. Brian L. Bingham

Dr. Robin Matthews

Dr. Deborah Donovan

MASTER'S THESIS

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Western Washington University, I grant to Western Washington University the non-exclusive royalty-free right to archive, reproduce, distribute, and display the thesis in any and all forms, including electronic format, via any digital library mechanisms maintained by WWU.

I represent and warrant this is my original work, and does not infringe or violate any rights of others. I warrant that I have obtained written permissions from the owner of any third party copyrighted material included in these files.

I acknowledge that I retain ownership rights to the copyright of this work, including but not limited to the right to use all or part of this work in future works, such as articles or books.

Library users are granted permission for individual, research and non-commercial reproduction of this work for educational purposes only. Any further digital posting of this document requires specific permission from the author.

Any copying or publication of this thesis for commercial purposes, or for financial gain, is not allowed without my written permission.

Gemma S. Woodhouse

July 25, 2014

INFLUENCE OF TEMPERATURE ON THE PHYSIOLOGICAL PERFORMANCE OF ZOOCHLORELLAE IN TWO INTERTIDAL HOSTS (*ANTHOPLEURA ELEGANTISSIMA* AND *A. XANTHOGRAMMICA*)

A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

by

Gemma S. Woodhouse

July 2014

ABSTRACT

The ability of a symbiotic organism to tolerate and respond to stress is dependent on a complex integration of the physiological processes of both host and symbiont. In the intertidal zone, where organisms are exposed to numerous environmental stressors, physiological tolerance limits of algae and animals are often within 1°C - 3°C of the body temperatures they experience there. To understand the association between intertidal sea anemones and their photosymbionts, and how these associations may change with increasing climatic stress, I examined two spatially dominant species in the genus *Anthopleura* (*A. elegantissima* and *A. xanthogrammica*) in symbiotic associations with their relatively sensitive chlorophyte photosymbiont, *Elliptochloris marina*. Anemones hosting *E. marina* were exposed to an increasing thermal regime from 10 - 28°C, under two light treatments, over the course of 10 weeks to establish the upper thermal tolerance limit of *E. marina* in each host, while examining the response of the anemones themselves to the thermal stress. Of the two hosts, *A. xanthogrammica* was less tolerant of high temperatures. A contraction response was triggered for *A. xanthogrammica* at temperatures above 18°C, but *A. elegantissima* showed no contraction until temperatures reached 24°C. To determine how the *E. marina* were responding to the temperature increases, I examined the photochemical efficiency of PS II by measuring photosynthetic efficiency (F_v/F_m) and photosynthetic capacity ($rETR_{max}$) of symbionts within each anemone host at each temperature interval. Photochemical efficiency was strongly affected by temperature; however, there were no apparent host-specific differences. From 10 - 22°C, F_v/F_m remained stable, averaging 0.6 ±

0.1 (SD) for both species. At temperatures above 22°C, photochemical efficiency steadily declined, indicating photoinhibition and the upper thermal tolerance limit of *E. marina*. This relatively low thermal tolerance may influence the competitive balance of symbionts under conditions of increasing global temperatures. Increasing temperatures may cause anemones to adaptively expel symbionts to switch to a more tolerant species. In this study, both *A. elegantissima* and *A. xanthogrammica* expelled symbionts as the temperatures rose. By 28°C, both *Anthopleura* species had expelled the majority of their symbionts; however, *A. xanthogrammica* was able to retain a higher percentage (*A. elegantissima*: 96.7 ± 4.6 % loss; *A. xanthogrammica*: 84.0 ± 18.1 % loss), indicating that they may have an increased ability to buffer temperature changes and maintain algal symbioses during prolonged periods of high temperatures. Field measurements of the internal body temperatures of *A. xanthogrammica* indicated that the anemone has a moderate ability to buffer its symbionts from thermal stress, as the internal body temperatures of lower intertidal anemones remained 6.2 ± 1.1°C cooler than ambient temperatures. This ability to moderate the internal temperature is likely due to host-specific morphological traits, such as a large body size and thick host tissues, which may ultimately provide a more favorable environment for their symbionts under periods of high stress. The relative abilities of *A. elegantissima* and *A. xanthogrammica* to buffer their symbionts, as well as the physiological tolerances of *E. marina*, may have important ecological implications, controlling the range of zoochlorellae at both latitudinal and microhabitat scales.

ACKNOWLEDGEMENTS

I would like to express the deepest appreciation to my advisor, Dr. Brian Bingham, for the mentorship and patient guidance he provided throughout my project. His selfless dedication to both my personal and academic development has had a major impact on my graduate experience, and I am truly fortunate to have had the opportunity to work with such a kind and compassionate advisor. I would also like to thank the other members of my committee, Dr. Robin Matthews and Dr. Deborah Donovan, for their ideas, insightful comments, and constructive feedback throughout the development of my project.

A special thanks goes out to the staff at Shannon Point Marine Center, who provided the resources and support necessary to conduct my research. Gene McKeen and Nate Schwarck provided technical guidance that was essential to my project. I would particularly like to thank Jay Dimond, who provided laboratory and field assistance, insightful comments on my research, and patiently and kindly addressed any of my questions. I would also like to thank Shad Orechovesky for assisting in the collection of specimens, and Amy Oppfelt for her assistance with lab work. In addition, the field portion of my project would not have been possible without feedback from Michael Levine, who was eager to share his extensive knowledge of the Slip Point, WA field site.

I cannot find words to express my gratitude to my husband, Dustin Gleaves, who has been immensely supportive over the last three years. He not only provided the emotional support and encouragement that I needed, but also greatly contributed to my research by lending his time and knowledge. I would like to thank him for all of the nights of late-night editing and thoughtful discussions, as well as his assistance both in the lab and in the field. My research would not have been possible without his help.

Finally, I would like to thank the rest of my family for their unequivocal love and support, including my father, Dr. Clive Woodhouse, who inspired my love of science, and my mother, Elaine Woodhouse, who provided constant emotional support, reminding me during especially taxing times that “this too shall pass.”

This research was supported by grants from the National Science Foundation (NSF IOS 0822179 to Dr. Brian Bingham), Western Washington Fund for the Enhancement of Graduate Research, Western Washington Research and Sponsored Programs, The Charlton Family Endowment, and Huxley College Small Grants.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Coral reefs, some of the most threatened ecosystems on earth, are continuing to decline due to a variety of anthropogenic stressors (Hoegh-Guldberg 1999, Weis 2008). Currently, a third of all coral species are at risk of extinction (Carpenter et al. 2008). The health of corals depends on the obligate association between host and symbiont (referred to in whole as the holobiont). Symbionts produce carbon through photosynthesis and translocate it to the host as simple organic compounds that are essential to the hosts' survival in nutrient-poor tropical waters (Muscatine 1967). In return, the host provides inorganic nutrients in the form of respiratory waste (Yellowlees et al. 2008) and shelter that can reduce the risk of symbiont photoinhibition related to a variety of environmental stressors (Bhagooli and Hidaka 2003). The greatest threat to corals is a malfunction of the symbiotic association, which can lead to expulsion of the algae by the host (referred to as bleaching) (Hoegh-Guldberg 1999, Weis 2008). Bleaching results in decreased growth and reproduction, as well as increased disease and mortality (Brown 1997, Hoegh-Guldberg 1999). Symbiont expulsion is often triggered by environmental stressors, such as increased temperature or light intensity, which compromise the photosynthetic ability of the symbiont. These symbiotic associations are extremely sensitive, and increases in seawater temperature as low as 1°C above seasonal averages can trigger bleaching (Holden 1995).

Recent research has shown that certain corals are more resistant to bleaching than others, likely due to host traits such as tissue thickness, heterotrophic capacity, and presence of heat shock proteins (Baird et al. 2009, Fitt et al. 2009). Symbiont characteristics related to their genetics and physiological response to stressors can also increase bleaching resistance

(Sampayo et al. 2008). Therefore, a holobiont's overall ability to tolerate and respond to stress is dependent on a complex integration of the physiological processes of both host and symbiont (Baird et al. 2009), which makes susceptibility to stress highly variable depending on the specific host-symbiont partnership (Coles and Brown 2003). Due to the complex nature of symbiotic relationships, the relative contributions of host and symbiont to bleaching resistance are poorly understood.

Recent advancements have allowed researchers to study the relationship between specific host and symbionts in relatively non-invasive ways. Previously, most coral research was performed in controlled laboratory environments, which required damaging the host coral by removing it from its natural environment and stripping the tissues to extract the symbionts. In 2007, however, an underwater chlorophyll fluorometer called the Diving-PAM was developed. This instrument allows researchers to examine the photophysiology of symbionts without damaging the hosts, providing information about the *in vivo* efficiency of electron transfer within photosystem II. The PAM fluorometer measures the proportion of absorbed light energy that is actually used for photochemistry, which has become a widely accepted indicator of stress in symbiotic cnidarians (Jones et al. 1999, Warner et al. 1999).

While the majority of PAM fluorometry research has been focused on tropical host-photosymbiont relationships, such as corals, it has equally valuable applications in temperate environments. Unlike their tropical counterparts, temperate associations occur in nutrient-rich waters where the host can obtain many of their nutrients and supplementary carbon heterotrophically (Dubinsky and Jokiel 1994). This makes the majority of temperate host-

photosymbiont relationships facultative rather than obligatory. In addition, temperate associations are subject to dramatic environmental changes, especially when located in the intertidal zone where they are subject to periods of aerial exposure coinciding with high levels of irradiance and temperature. This combination of factors makes the study of temperate symbioses intriguing, providing a very different opportunity to study and understand relationships between symbiont and hosts and the effects of environmental change on the partnership.

There has been substantial research on the thermal tolerance of symbionts within the temperate intertidal anemone *Anthopleura elegantissima* (Brant), an important, spatially dominant member of intertidal communities from Alaska to Baja California (Hand 1955, Dayton 1971, Sebens 1982). These anemones exhibit an unusual symbiotic relationship, hosting two distinct unicellular symbionts within their gastrodermal tissues, allowing for comparison of different algal-symbiont partnerships in a single host. Photosymbionts in *A. elegantissima* can include green chlorophytes (*Elliptochloris marina*, Letsch) referred to as zoochlorellae, and brown dinoflagellates (*Symbiodinium californium*, A. T. Banaszak, R. Iglesias-Prieto & R. K. Trench and *S. muscatinei*, LaJeunesse & R. K. Trench) called zooxanthellae. Both of these endosymbiotic partners benefit the host by providing photosynthate that supplements heterotrophic feeding by the anemones. The additional carbon provided by photosymbionts reduces the weight loss of the anemones hosts during periods of starvation (Muscatine 1961) and may allow the host to extend its range to areas it could not otherwise inhabit due to low food availability (LaJeunesse and Trench 2000). Research suggests that zooxanthellae translocate higher amounts of photosynthate to the host

than do zoochlorellae (Verde and McCloskey 1996, Bergschneider and Muller-Parker 2008). In addition, there may be other ecological consequences of hosting different symbionts, including differential predation on hosts (Seavy and Muller-Parker 2002), and differences in concentrations of UV-absorbing amino acids (Shick and Dunlap 2002), but the advantages of hosting one symbiont over the other are not fully understood.

Like *A. elegantissima*, the anemone *A. xanthogrammica* (Brant) hosts both zoochlorellae and zooxanthellae. However, little is known about *A. xanthogrammica* symbiosis, despite its potential as a second model species. *A. xanthogrammica* and *A. elegantissima* are congeners that live in similar intertidal habitats and are often found together, but there are distinct differences between the two species. While *A. elegantissima* grows to only 6 cm in diameter and forms clonal aggregations though longitudinal fission, *A. xanthogrammica* reaches at least 25 cm diameter, does not reproduce asexually and does not form aggregations with individuals of the same species (Hand 1955, Dayton 1973).

Symbionts within these *Anthopleura* species respond differently to temperature and light, suggesting unique physiological tolerances. These tolerances are reflected in the distribution of the symbionts, which varies across large scale gradients such as latitude, as well as smaller scale light and temperature gradients (LaJeunesse and Trench 2000, Secord and Augustine 2000, Secord and Muller-Parker 2005). In general, *Anthopleura* spp. host zooxanthellae in warmer high-light habitats typical of the upper intertidal zone and lower latitudes, while individuals hosting zoochlorellae are most often found in low intertidal zones and higher latitudes, where they remain cooler with less light exposure (O'Brien and

Wyttenbach 1980). These differential sensitivities to temperature and light control symbiont distribution in the intertidal zone (Hoegh-Guldberg 1999, Weis 2008). Host morphological traits may also affect the distribution of zoochlorellate *Anthopleura*, controlling the range of zoochlorellae at both latitudinal and microhabitat scales (Saunders and Muller-Parker 1997, Bergschneider and Muller-Parker 2008). In the intertidal zone, *A. elegantissima* hosting zoochlorellae are restricted to the cooler low intertidal area, while the larger *A. xanthogrammica* host zoochlorellae much higher in the intertidal zone where temperature and light are generally greater. This pattern is also reflected in latitudinal distributions: zoochlorellate *A. xanthogrammica* occur approximately 6 degrees of latitude farther south (38°N, California) than do zoochlorellate *A. elegantissima* (44°N, Oregon) (Secord and Augustine 2000). This may indicate that the two organisms, when exposed to the same environmental conditions, have different abilities to regulate symbiont stressors. This ability to regulate stressors is poorly understood, but is likely dependent on a combination of individual physiological tolerances, morphological traits, and behaviors. The morphological traits so clearly different in *A. elegantissima* and *A. xanthogrammica* may have a direct effect on the temperatures experienced by the host *in situ*, which has important implications for the distribution of symbionts within the anemones.

In the intertidal zone, a hosts' ability to regulate temperature is extremely important, as the physiological tolerance limits of intertidal organisms are often within 1°C - 3°C of body temperatures experienced *in situ* (Stillman and Somero 1996, Tomanek and Somero 1999). Intertidal anemones have developed a wide range of physiological and behavioral adaptations to cope with the associated stressors of intertidal environments (Dykens and

Shick 1984, Shick and Dykens 1984, Zamer and Shick 1989, Shick 1991). Due to their high exposure to environmental stressors, it is believed that intertidal organisms will be some of the first organisms to be affected by climatic changes (Helmuth et al. 2005, 2006 a, b) and may act as bioindicators for future climate effects. It is critical, therefore, to understand the association between intertidal cnidarians and their photosymbionts, and how these associations may change with increasing climatic stress.

To understand the relationship between *Anthopleura* spp. and their symbionts, as well as the differences in the symbiosis of these two species, I examined the relationship between *A. elegantissima* and *A. xanthogrammica* and their relatively sensitive photosymbiont, *E. marina*, to contrast the physiological tolerances of both the host and symbiont under stressful environmental conditions. Previous studies have documented the body temperatures of *A. elegantissima in situ* during low tide exposures (Dingman 1998, Bingham et al. 2011), and the physiological tolerances of zooxanthellae in both hosts have been well researched (Saunders and Muller-Parker 1997, Muller-Parker et al. 2007). However, limited information exists regarding the temperatures experienced by *A. xanthogrammica in situ* or on the physiological tolerances of *E. marina* hosted within either *A. elegantissima* or *A. xanthogrammica*. My goal was to provide insight into these relationships by: 1) contrasting the physiological tolerances of *A. xanthogrammica* and *A. elegantissima* under increasing temperatures and different light intensities, 2) examining the upper thermal limit for photosynthesis of *E. marina* within *A. elegantissima* and *A. xanthogrammica* under these conditions, and 3) quantifying the internal body temperatures experienced by *A. xanthogrammica in situ* when aerially exposed during a summertime low tide.

MATERIALS AND METHODS

Collection of specimens

Twenty-two *Anthopleura xanthogrammica* were collected from Slip Point, WA on 21 July 2012 (Fig. 1). Since only zoochlorellate individuals were needed, light colored anemones were selected (as darker individuals tend to host *S. muscatinei*). Anemones were collected from a single surge channel spanning tidal heights of -0.60 m to +1.33 m MLLW (Levine 2010), and from a small tidepool at +0.51 m. Anemones were carefully pried from the substrate using a thin metal spatula, taking care not to damage their pedal discs. They were placed in individual zip-top bags, and transported on ice to the Shannon Point Marine Center in Anacortes, Washington. Zoochlorellate *A. elegantisimma* were also collected, but because no zoochlorellate *A. elegantissima* could be found at the Slip Point study site, the necessary zoochlorellate *A. elegantissima* were collected in the same way from Cone Island, Washington (48° 30' 32" N, 122° 41' 02" W) several days later (1 August 2012). At the laboratory, all anemones were cleaned of debris, placed individually in shallow glass dishes (120 x 20 mm for *A. xanthogrammica*, 80 x 20 mm for *A. elegantissima*) and allowed to attach. Anemones in the dishes were submerged in a flow-through seawater table at ambient temperature (11.7 ± 1.1 °C) under natural light from north facing windows, and left to acclimate for approximately six weeks. After six weeks all anemones were responsive and in an expanded state.

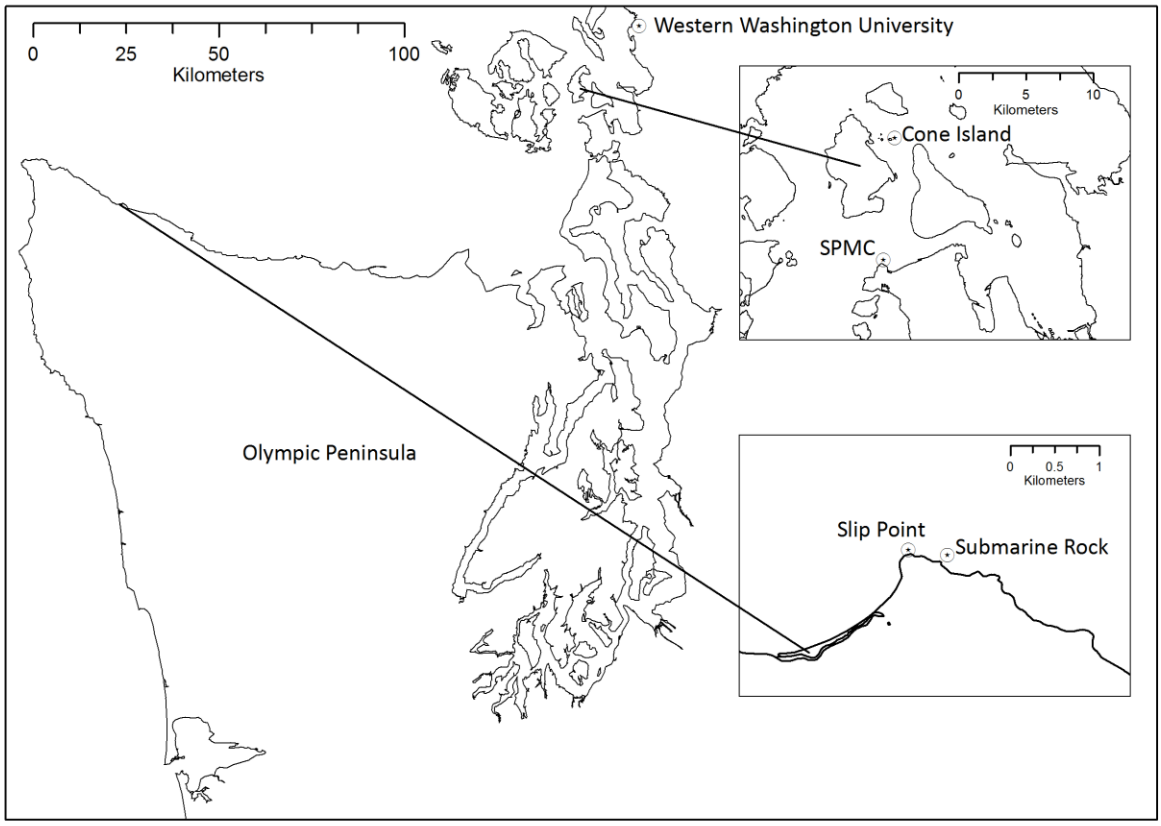

Figure 1. Collection site for *A. xanthogrammica* and *A. elegantissima*. Internal body temperatures of *A. xanthogrammica* were measured at Submarine Rock, near Slip Point, WA and specimens were collected there for use in the laboratory experiments. Zoochlorellate *A. elegantissima* were collected from Cone Island, San Juan Islands, WA.

To ensure all anemones hosted primarily *E. marina*, an initial symbiont cell count was performed and any anemones containing more than 5% zooxanthellae were removed from the experiment. To perform the cell counts, a single tentacle was haphazardly selected and excised from each anemone using small scissors. Symbionts were extracted from the tentacles by gently squeezing the symbionts from the cut end of the tentacle onto a microscope slide. Symbionts, under a compound microscope, were easily distinguishable by color and size (zoochlorellae are green and 6-8 μm in diameter while zooxanthellae are 10-15 μm and brown).

Laboratory measurements of temperature tolerance

To contrast the physiological tolerances of *E. marina* within *A. xanthogrammica* and *A. elegantissima* under conditions of increasing temperature at different light intensities, anemones were placed in an incubator on a 10:14 hour light:dark cycle and exposed to an increasing thermal regime over the course of 10 weeks. Full spectrum fluorescent lights suspended from horizontal shelves were used to create two light treatments: "high light" (mean PAR = 221.8 ± 92.2 μmol quanta m^{-2} s^{-1}, measured at the oral disc of the anemones) and "low light" (mean PAR = 9.3 ± 14.0 μmol quanta m^{-2} s^{-1}). Irradiance levels in the incubator were monitored weekly using a Biospherical Instruments QSL-100 sensor (San Diego, CA, USA). Individual *A. xanthogrammica* for the experiment were distributed into 2L translucent plastic containers (210 x 110 mm), while *A. elegantissima* were placed in 500 ml glass bowls (115 x 65 mm). All containers were filled with 5 μl filtered seawater (salinity =

29.0 ± 1.1 ppt; DO = 7.1 ± 0.8 mg O_2/L ; pH = 7.8 ± 0.5) then distributed into the two light treatments, with six *A. xanthogrammica* and seven *A. elegantissima* per light level. To ensure both species were receiving similar amounts of light, *A. elegantissima* individuals were placed on raised platforms so that their oral discs were the same distance from the lights as the oral discs of the larger *A. xanthogrammica*. To minimize possible differences in light intensity based on placement within the incubator, the anemone positions were rearranged once a week.

After an initial 1-week incubator acclimation to 10°C, the temperature was increased 2°C per week until a maximum of 28°C was reached after 10 weeks. Temperatures inside the incubator were logged every 30 minutes using four Thermochron® iButton temperature loggers (San Jose, CA, USA) submerged in seawater held under each light treatment. To maintain water quality, all anemone bowls were cleaned weekly and refilled using 5 µl filtered sea water held at the appropriate incubator temperatures. Weekly water quality measurements were taken on a subset of anemones in each treatment (n = 3) to monitor dissolved oxygen (DO) and pH levels. Since the light intensity experienced by different areas of the anemone varies based on anemone expansion/contraction, behavior was monitored to examine any differences between species or between treatments. Anemones were visually scored using a ranking system modified from Shick and Dykens (1984), with fully expanded anemones ranked as 2, and fully contracted anemones ranked as 0. After week five, in response to reduced oxygen levels in the treatments and anemone stress, cleanings and water changes were increased to once daily. Throughout the experiment, anemones were fed a single live mussel (*Mytilus* spp.) proportional to their body size immediately after their water

change. The smaller *A. elegantissima* were fed mussels with a shell length ~ 10mm (mean wet weight ~ 0.25 g), while *A. xanthogrammica* were fed mussels with a shell length ~ 40mm (mean wet weight ~ 2.5 g).

Algal cell density, mitotic index and protein analysis

Changes in the density of algal symbionts within an anemone can be used to measure the condition of the holobiont, so *E. marina* density was measured before and after exposure to increasing incubator temperature. To measure density of the symbionts, tentacle samples were taken from anemones immediately after acclimatization in the incubator at 10°C, and again ten weeks later at the end of the 28°C exposure. Tentacles were selected and removed using small scissors. The number of tentacles varied depending on anemone species: five *A. elegantissima* tentacles were generally required to achieve a sufficient number of symbionts for the measurements, while a single *A. xanthogrammica* tentacle was adequate. Due to bleaching, more tentacles had to be sampled at the end of the experiment: ~ 10 tentacles from each *A. elegantissima*, and five from each *A. xanthogrammica*. Once excised, tentacles were frozen at -70°C until they could be processed.

To perform symbiont counts, tentacles were thawed then placed in an Eppendorf tube with 2 or 6 ml of 5µm filtered seawater for *A. elegantissima* and *A. xanthogrammica* respectively. Tissues were homogenized with a Teflon pestle attached to a motorized stirrer (Wheaton Science Products, Millville, NJ, USA). The homogenate was vortexed and divided into two 1.5 ml microfuge tubes. One sample was later used for protein analysis, and the

other for cell counts and mitotic index measurements. Homogenates were frozen at -70°C until processing.

To determine the *E. marina* cell density, counts were performed on a haemacytometer under a compound microscope at 100x. Thawed and vortexed homogenate was loaded into a haemacytometer chamber, and at least 100 zoochlorellae were counted. Six replicates were counted per sample. For samples with low cell densities (fewer than 100 cells per chamber) the number of zoochlorellae within 18 1x1 mm squares was counted. Since anemone body size is highly dependent on water retention, common size measurements such as wet weight and oral disc diameter are generally variable and inaccurate, so protein content was measured and used to standardize the symbiont density measurements. Anemone soluble protein content in the homogenate samples was determined using the Lowry method (Lowry et al. 1951), with bovine serum albumin (BSA) as the standard. Two replicate subsamples were measured. Cell counts were normalized to protein content to determine the cell density per anemone protein biomass.

Growth of a symbiont population can also provide a quantitative estimate of stress in symbiotic cnidarians (Brown and Zamani 1993). To estimate growth rates of *E. marina*, the percentage of dividing cells in each sample was calculated by examining a sample of anemone homogenate under a compound microscope at 400x. One thousand zoochlorellae cells were counted for concentrated samples, and any cells with a well-defined cleavage furrow were scored as dividing. For bleached samples at the end of the experiment, only 500

cells were counted. The mitotic index (MI) was calculated by dividing the number of dividing cells by the total number of cells counted.

Measurements of chlorophyll fluorescence in *E. marina*

To determine how the symbiotic *E. marina* were responding to thermal stress, a pulse-amplitude modulated (PAM) fluorometer (DIVING-PAM, Heina Walz GmbH, Effeltrich, Germany) was used to determine the maximum quantum yield of photosystem II (PS II) in the symbionts within each anemone at each temperature interval. This provided an accurate measure of photochemical efficiency in the symbionts (Warner et al. 1999) and is an accepted indicator of stress in symbiotic cnidarians (Jones et al. 1999).

After an overnight 14-hour dark acclimation in the incubator (to allow PS II reaction centers to reach an open state), anemones were moved in darkness to a dark room to measure the maximum quantum yield of the symbionts. The PAM fluorometer's submersible fiber optic probe was held approximately 5 mm from the surface of the anemone's oral disc, adjacent to the innermost row of tentacles. A weak measuring light (0.15 µmol quanta m^{-2} s^{-1}) was used to assess minimum fluorescence (F_0) of the symbionts while the PS II reaction centers were fully open in their dark-adapted state. This was followed by a short saturation pulse (>10,000 µmol quanta m^{-2} s^{-1} for 8 seconds) to overwhelm all PS II reaction centers causing them to close, producing a measurement of maximum fluorescence (F_m). Maximum quantum yield (F_v/F_m) was then calculated as:

$$\frac{F_v}{F_m} = (F_m - F_o)/F_m$$

where:

F_m = maximum fluorescence

F_o = minimum fluorescence

F_v = variable fluorescence = $F_m - F_o$

Another indication of symbiont health is photosynthetic capacity and how the photosymbionts respond to short-term changes in light intensity. To examine the relationship between irradiance and photosynthetic capacity, the PAM fluorometer was used to perform rapid light curves on extracted *E. marina* to determine the relative (rETR) and maximum relative electron transport rates ($rETR_{max}$) from a subset of experimental anemones every other week, with some resampled. After being exposed to the incubator light for three hours, three *A. xanthogrammica* and three *A. elegantissima* were arbitrarily chosen from each treatment. Tentacles were removed from each anemone using small scissors. The number of tentacles varied depending on anemone size, generally five from *A. elegantissima* and one from *A. xanthogrammica*. Symbionts were squeezed from the cut end of each tentacle onto polycarbonate membrane filters on glass slides. The slides were then placed in a 150 x 30 mm petri dish full of filtered sea water maintained at the corresponding experimental temperature. The fluorometer probe was mounted 5 cm above the petri dish, and rapid light curve analysis was done by increasing the PAR intensity eight times between 0 and 2700

μmol quanta m^{-2} s^{-1} for 10 seconds each, with a measurement of $\frac{F_v}{F_m}$ at each interval. rETR measurements were only performed until 22°C, as tentacles at 26°C were bleached and fluorescence of the remaining symbionts was below the detection limit of the instrument. For the final assessment of rETR at 22°C, measurements were performed on symbionts extracted from tentacles, as well as symbionts freshly expelled by each anemone. This was done to determine whether anemones were selectively expelling damaged symbionts. Pellets containing expelled zoochlorellae were collected from water dishes and an assessment of rETR was immediately performed.

The rETR (relative electron transport rate) was calculated as:

$$rETR = \frac{F_v}{F_m} \times PAR$$

where:

F_m = maximum fluorescence

F_v = variable fluorescence = $F_m - F_o$

PAR = intensity of saturation pulse (μmol quanta m^{-2} s^{-1})

Field temperature measurements

To determine whether *E. marina* hosted by *A. xanthogrammica* naturally experience environmental conditions sufficiently stressful to reduce their photosynthetic capacity, as well as how the thermal environments inside the *A. xanthogrammica* vary during low tide, field measurements were made at Slip Point, WA (Fig. 1). Sampling was performed on Submarine Rock (48° 15' 50" N, 124° 14' 143" W), a rocky outcrop approximately 75 m long x 75 m wide at its widest point and subject to strong tidal exchanges (Levine 2010). At Submarine Rock there is an abundance of *A. xanthogrammica*, occuring in tidepools, surge channels, and along the vertical slopes of the outcrop, with anemones outside the tidepools being regularly immersed at low tide. These anemones were found nestled within thick beds of the California mussel, *Mytilus californianus,* which is one of their primary food sources. Other members of the community included the gooseneck barnacle *Pollicipes polymerus*, the thatched barnacle *Semibalanus cariosus,* and the sea star *Pisaster ochraceus.*

To examine the thermal environment *A. xanthogrammica* and its symbionts experience *in situ,* the internal body temperatures of 14 individuals were measured over a single low tide. A surge channel towards the south end of Submarine Rock was selected for analysis, as all anemones within this channel were aerially exposed at low tide, submerged at high tide, and experienced similar irradiance and exposure. The surge channel spanned a distance of approximately 25 m over tidal heights of -0.60 m to +1.33 m MLLW (Levine 2010) and provided a semi-shaded environment for the anemones. Anemones were selected from within this channel at tidal heights of -0.60 m to +0.36 m (lower intertidal n = 8), and at

+0.36 m to +1.33m (upper intertidal n = 6). A Thermochron® iButton temperature logger (San Jose, CA, USA), set to record temperature at 10-minute intervals, was attached to a monofilament line using plastic zip-ties and inserted into the body cavity of each anemone through the mouth, using a curved needle to "thread" the monofilament through the body wall. The iButton was then pulled snug against the inner wall of the gastrovascular cavity, and secured by a clamp on the outer body wall. Anemones were also individually marked and numbered using plastic tags attached to the pedal disc with monofilament line. To document ambient temperatures, two external iButton temperature loggers were secured with plastic zip ties to a stainless steel eyebolt attached to the substrate with ZSPAR slash zone epoxy (RPM Inc., Medina, OH, USA) at approximately +0.36 m, in a shaded crevice out of direct sunlight. All temperature loggers were left in place for just over 24 hours, from 9:10AM on 5 August 2013 to 10:05AM on 6 August 2013. This allowed for the comparison of internal body temperatures of the anemones to the ambient temperature over an entire tidal cycle.

Statistical analysis

All statistical analyses of data collected from the laboratory experiment were performed using SPSS v.20 (SPSS Inc., Chicago IL). Since each anemone received all temperature treatments, maximum photosynthetic yield data were analyzed with a 3-way repeated measures ANOVA with species and light as the between factors and time as the within factor. The $rETR_{max}$ of the anemones was measured only once at each temperature interval, so those data were analyzed with a 3-way ANOVA using light, temperature, and

species as the factors. A 3-way ANOVA was also used to analyse water pH and dissolved oxygen in the treatments. A 2-way ANOVA was used to compare changes in symbiont density as well as mitotic index as a function of species and light treatment. Data were converted to percent change to account for differences in initial symbiont densities and growth rates. A significance criterion of $\alpha \leq 0.05$ was used for all comparisons. Prior to each analysis, all data were tested to ensure they met the assumptions of ANOVAR or ANOVA as necessary. If the ANOVAR sphericity assumption was violated (as indicated by Mauchley's test of sphericity), a Huynh-Feldt correction was used.

Due to small sample sizes and a lack of replication in one of the treatments, statistical analyses were not used to compare the $rETR_{max}$ between symbionts *in hospite* and expelled. Therefore, only general trends were examined. It was also not possible to statistically compare the internal body temperatures of *A. xanthogrammica in situ* to the ambient external temperature, due to the failure of one of two external iButton loggers. Therefore, only trends between temperatures, not statistical differences, were evaluated. Since expansion behavior data was ranked, it was not possible to perform a standard parametric test on the data. Again, only trends were evaluated. A Pearson's product-moment correlation analysis was done to test for correlations between anemone body size, tidal height, and internal temperature.

RESULTS

Laboratory measurements of temperature tolerance

Temperatures within the incubator remained near the intended temperatures throughout the duration of the experiment. In the low light treatment, average water temperature was within 0.16 ± 0.22°C of the desired temperature. The high light treatment experienced some additional warming due to radiant heat from the overhead lights; however average temperatures were within 0.88 ± 0.25°C of the desired temperature. The pH remained constant throughout the experiment, averaging 7.5 ± 0.1, and did not differ between species or light treatment ($\alpha > 0.05$). The concentration of dissolved oxygen was not as stable, as indicated by a three-way interaction between temperature, species, and light ($p = < 0.001$ with $\varepsilon = 0.29$) (Fig. 2), but averaged 4.2 ± 0.4 mg O_2/L for *A. elegantissima* and 4.0 ± 0.5 mg O_2/L for *A. xanthogrammica*.

All *A. xanthogrammica* and *A. elegantissima* placed in the incubator at 10°C appeared healthy and were fully expanded. At the start of the experiment, *A. xanthogrammica* (n = 10) had a mean wet weight of 133.5 ± 55.7 g, while *A. elegantissima* (n = 14) had a mean wet weight of 13.2 ± 7.6 g. Symbiont cell counts confirmed that *A. elegantissima* were 99.98 ± 0.05% zoochlorellate, while *A. xanthogrammica* were 99.86 ± 0.22%. Appearance and feeding behavior of the anemones stayed consistent as the incubator temperature was gradually increased to 16°C. At 16°C, feeding became irregular in both species, and slowly decreased through the remainder of the experiment. Initial signs of

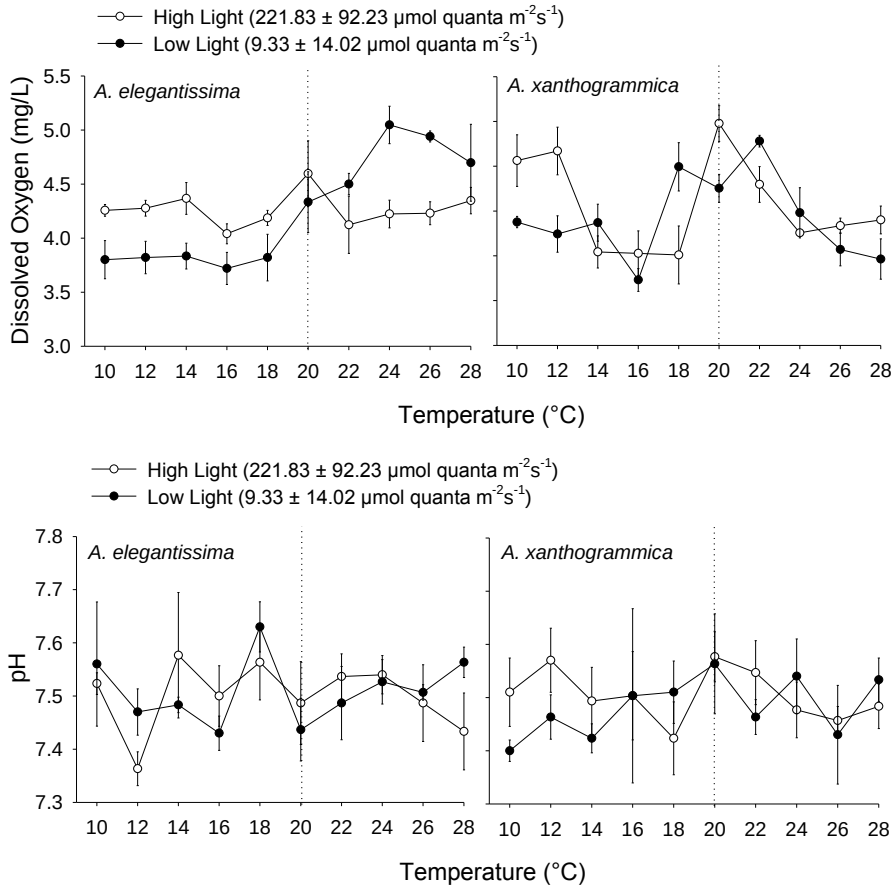

Figure 2. DO and pH (mean ± SE) of anemone holding water. DO and pH were recorded prior to weekly cleanings. At 20°C, in response to reduced oxygen levels and anemone stress, cleanings and water changes (DO = 7.1 ± 0.8 mg O_2/L ; pH = 7.8 ± 0.5) were increased to once daily (represented by dotted line). Subsequent DO and pH readings were taken at the end of each temperature acclimation period.

symbiont expulsion (i.e., reduced pigmentation and presence of symbiont filled boluses in the water dishes) varied between species and light treatment. For *A. elegantissima* in the high light treatment and *A. xanthogrammica* in the low light treatment, signs of expulsion were noted at 16°C. Anemones in these treatments had visibly bleached tentacles by 18°C, and by 22°C *A. elegantissima* in the high light treatment appeared fully bleached (both tentacles and oral disc). Expulsion by *A. elegantissima* in the low light treatment, and by *A. xanthogrammica* in the high light treatment, began to occur at 20°C. By 24°C all anemones were expelling large boluses of symbionts.

In addition to symbiont expulsion, *A. xanthogrammica* started to show other signs of stress at 18°C, evident by high levels of mucus production and slowed reaction times. In contrast, *A. elegantissima* did not exhibit any of these signs of stress during the experiment. At 26°C two non-responsive *A. xanthogrammica* were removed from the experiment; data from these anemones were not used in any analyses. At the conclusion of the experiment, all remaining anemones were returned to ambient temperatures in a flow-through sea water table. Within a few days they resumed feeding and appeared healthy, although largely bleached.

Expansion behavior of the anemones differed between species, temperatures, and light treatments during the experiment. At temperatures below 16°C there was no difference in host response or treatment. Above that threshold, *A. elegantissima* contracted less in response to light and temperature stress than did *A. xanthogrammica*, and showed much less variability (Fig. 3). In the high light treatment, *A. elegantissima* remained fully open for the

duration of the experiment, while *A. xanthogrammica* in the same treatment only remained fully expanded until 22°C, and then partially contracted above that temperature. In the low light treatment, *A. elegantissima* remained expanded until 24°C, and then exhibited sporadic contraction behavior until 28°C when all anemones returned to a fully expanded state. In contrast, *A. xanthogrammica* in the low light treatment showed a contraction response at 18°C, then highly variable individual responses, but largely contracted, for the remainder of the experiment.

Algal cell density and mitotic index

Both anemone species showed a significant loss of symbionts (bleaching) during the experiment (Fig. 4). Prior to exposure, *A. xanthogrammica* had a higher symbiont density than *A. elegantissima*. There was a significant difference between species in the percent loss of symbionts; *A. elegantissima* lost a higher percentage of its symbionts as temperature increased (Fig. 4) (96.7 ± 4.6 % loss for *A. elegantissima*; 84.0 ± 18.1 % loss for *A. xanthogrammica*). Light, however, did not significantly affect symbiont loss. *A. elegantissima* appeared to bleach completely regardless of light treatment, but *A. xanthogrammica* retained some of its symbiont population, especially in the low light treatment.

The percent of dividing zoochlorellae cells, a measure of symbiont growth and an index of overall symbiont health, was significantly influenced by light treatment but not species (Fig. 4, Table 1). Over the course of the experiment, the mitotic index of

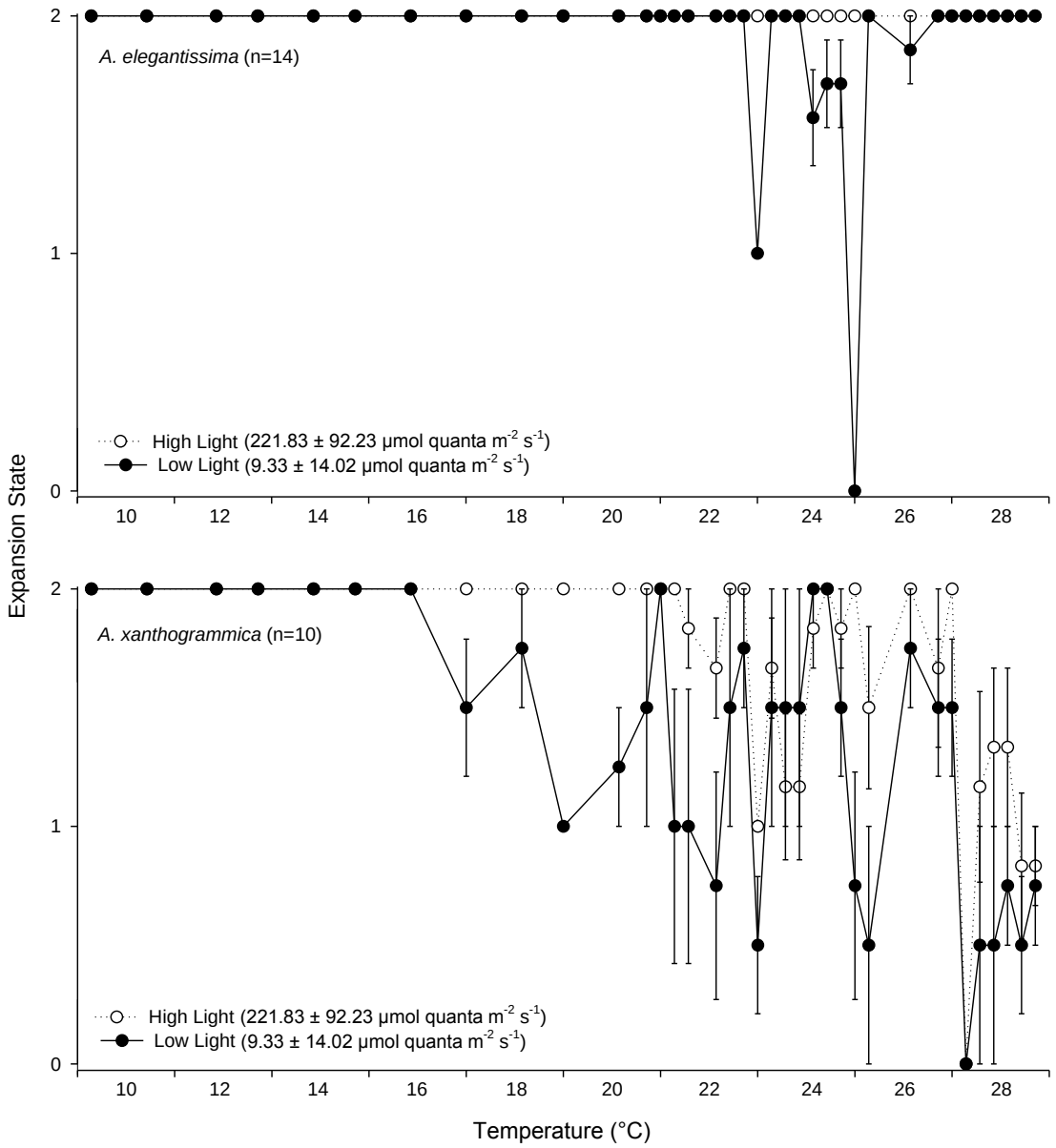

Figure 3. Expansion behavior (mean ± standard error) of *A. elegantissima* and *A. xanthogrammica* from 10 to 28°C. Incubator temperature was increased 2°C per week. The ranking system of expansion/contraction behavior was modified from Shick and Dykens (1984) with fully expanded anemones ranked as 2, and fully contracted anemones as 0.

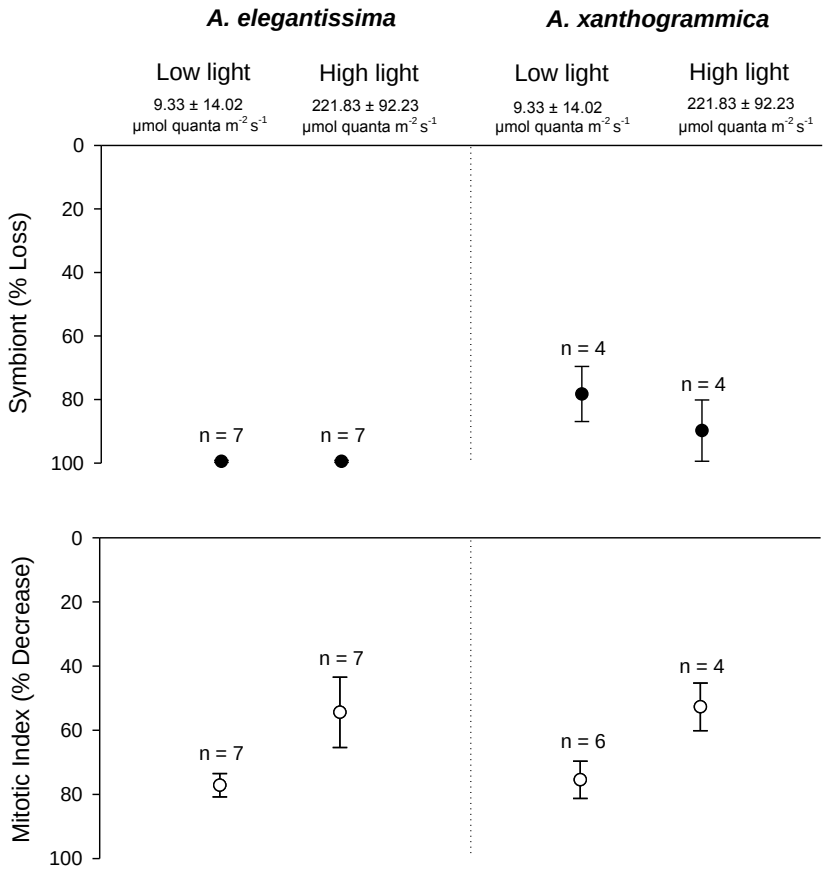

Figure 4. Percent change in density of zoochlorellae per mg tentacle protein and the mitotic index of zoochlorellae (% cells with a visible cleavage furrow) in tentacles removed from *A. elegantissima* and *A. xanthogrammica* (mean ± SE) before exposure at 10°C and after exposure from 10°C - 28°C.

zoochlorellae decreased in both *A. elegantissima* and *A. xanthogrammica* (Fig. 4). Symbionts in the low light treatment were more strongly affected than symbionts in the high light treatment.

Measurements of chlorophyll fluorescence in *E. marina*

Over the course of the experiment, the dark adapted maximum quantum yield of *E. marina* in the oral discs of both species was affected by both temperature and light (Fig. 5). Mauchly's test of sphericity for the maximum quantum yield measurements indicated that the assumption of sphericity was violated ($\chi^2_{44} = 63.3$, $p = 0.04$). Therefore, a Huynh-Feldt correction was used to adjust the degrees of freedom for the tests of significance. There was a statistically significant three-way interaction between species, temperature, and light, indicating that the maximum quantum yield showed different patterns depending on a combination of the 3 factors (Table 3). In general, light appeared to have the greatest effect on the photosynthetic efficiency of the *E. marina* as indicated by an effect size of $\eta^2_p = 0.90$ (Table 3). Symbionts in both *A. elegantissima* and *A. xanthogrammica* had a higher quantum yield in the low light treatment than in the high light treatment regardless of temperature (Figure 4). Maximum quantum yield of *E. marina* remained stable until 22°C, averaging 0.6 ± 0.1 for both species. After 22°C, maximum yield steadily declined in both species and both light treatments except for *A. elegantissima* in the low light treatment, which remained relatively stable.

Table 1. ANOVA table for the effects of temperature and light treatment on the concentration of zoochlorellae per mg tentacle protein in *A. elegantissima* and *A. xanthogrammica*. Significant p-values (lower than $\alpha = 0.05$) are in bold. Partial η^2 values (η^2_p) are effect size measurements that give the proportion of the effects explained by each individual factors or interaction.

Source	SS	df	F	P	η^2_p
Density					
Species	819.95	1	6.74	**0.018**	0.27
Light	369.14	1	3.03	0.099	0.14
Species x Light	46.4	1	0.38	0.545	0.02
Anemone	2190.34	18			
Corrected Total	3382.1	21			
MI					
Species	39.07	1	0.12	0.734	0.01
Light	2713.81	1	8.22	**0.01**	0.29
Species x Light	4.66	1	0.01	0.907	<0.01
Anemone	330.16	20			
Corrected Total	3087.7	23			

Table 2. ANOVAR for the effects of temperature and light treatment on maximum yield of symbionts in *A. elegantissima* and *A. xanthogrammica*. Degrees of freedom were adjusted using a Huynh-Feldt correction to account for violation of the sphericity assumption. Significant p-values (lower than $\alpha = 0.05$) are in bold. Partial η^2 values (η^2_p) are effect size measurements that give the proportion of the maximum yield variation explained by each factor or interaction.

Source	SS	df	F	p	η^2_p
Between-subject factors					
Species	0.18	1	9.57	**0.006**	0.32
Light	3.33	1	181.45	< **0.001**	0.90
Species x Light	0.01	1	0.57	0.46	0.03
Anemone	0.37	20			
Within-subject factors					
Temperature	5.26	7.77	100.83	< **0.001**	0.83
Temperature x Species	0.33	7.77	6.26	< **0.001**	0.24
Temperature x Light	0.45	7.77	8.54	< **0.001**	0.30
Temperature x Species x Light	0.61	7.77	11.64	< **0.001**	0.37
Temperature x Anemone	1.04	155.31			
Total	11.58	209.39			

The maximum electron transport rates ($rETR_{max}$) calculated from rapid light curves, showed different patterns at each temperature depending on the light treatment (Fig. 6). This was verified by a significant temperature x light interaction (Table 4). In general, light treatment appeared to have the greatest effect on the $rETR_{max}$ of the *E. marina* (Fig. 5). Symbionts in both *A. elegantissima* and *A. xanthogrammica* in the high light treatment had a lower $rETR_{max}$ at every temperature interval than anemones in the low light treatment. $rETR_{max}$ showed a general trend of increasing with temperature in the low light treatment, and decreasing with temperature in the high light treatment. Measurements could only be performed until 22°C, as anemones at 26°C were bleached and fluorescence of the remaining symbionts was below the detection limit of the instrument.

In the comparison of symbionts *in hospite* vs. symbionts that had been expelled, only light appeared to affect the $rETR_{max}$ of symbionts. For both anemone species, symbionts in the high light treatment had a similar $rETR_{max}$ both *in hospite* and expelled. In the low light treatment, $rETR_{max}$ was higher *in hospite* than in expelled *E. marina* (Fig. 7).

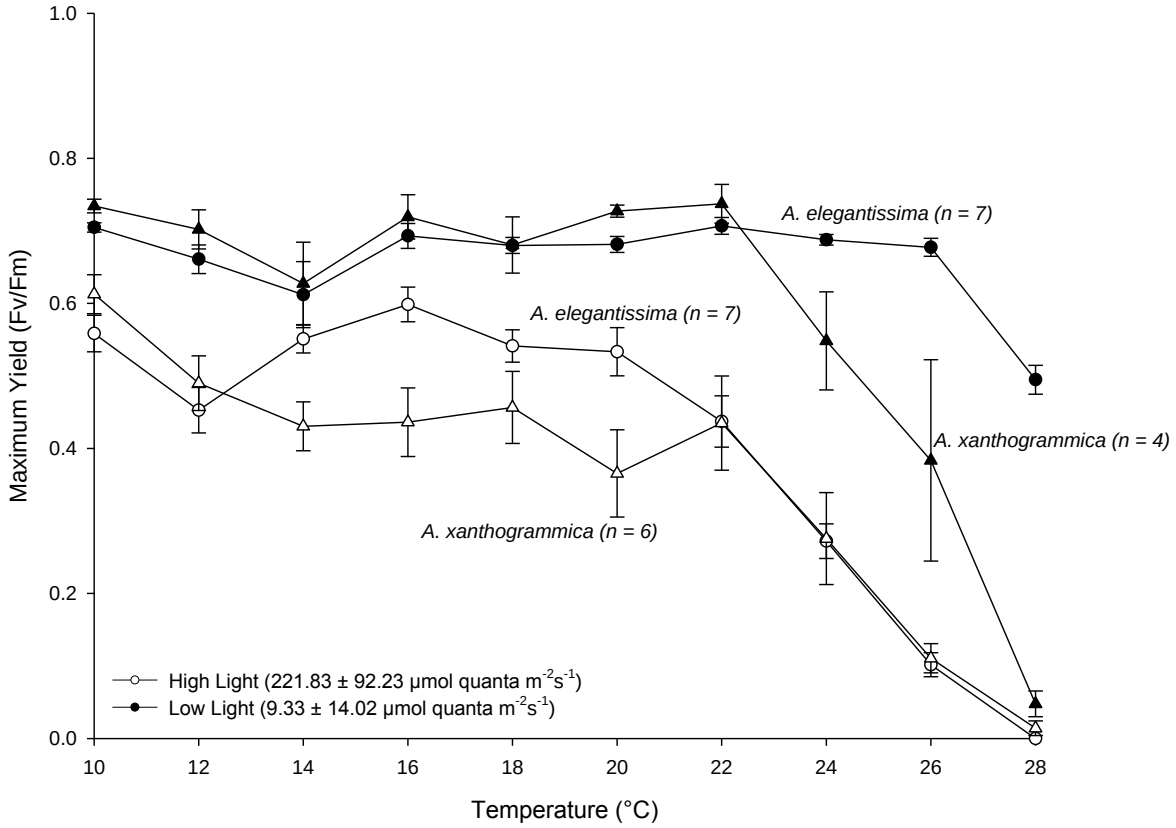

Figure 5. Maximum quantum yield (mean ± standard error) of *E. marina* in the oral discs of *A. elegantissima* and *A. xanthogrammica*. Maximum quantum yield was measured with a PAM fluorometer at each temperature interval after one week of acclimation.

Table 3. ANOVA table for the effects of temperature and light treatment on the $rETR_{max}$ of symbionts in *A. elegantissima* and *A. xanthogrammica*. Significant p-values (lower than $\alpha = 0.05$) are in bold. Partial η^2 values (η^2_p) are effect size measurements that give the proportion of the maximum yield variation explained by each individual factors or interaction.

Source	SS	df	F	P	η^2_p
Temperature	112.16	3	0.997	0.409	0.100
Species	14.65	1	0.391	0.537	0.014
Light	7353.02	1	196.129	**<0.001**	0.879
Temperature x Species	184.37	3	1.639	0.204	0.154
Temperature x Light	869.50	3	7.731	**0.001**	0.462
Species x Light	120.89	1	3.225	0.084	0.107
Temperature x Species x Light	24.97	3	.222	0.880	0.024
Error	1012.25	27			
Corrected Total	11966.02	42			

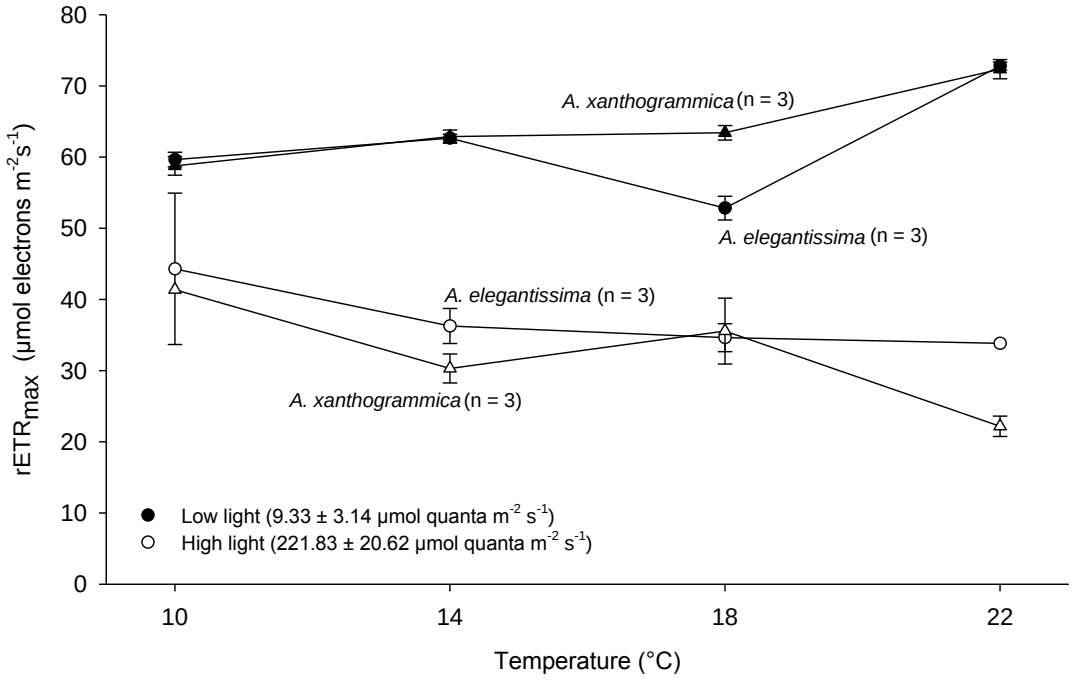

Figure 6. Maximum relative electron transport rate ($rETR_{max}$ mean ± standard error) of *A. elegantissima* and *A. xanthogrammica* at 4 temperature intervals over the course of the experiment. Measurements were only performed until 22°C, as anemones at 26°C were bleached and fluorescence of the remaining symbionts was below the detection limit of the instrument.

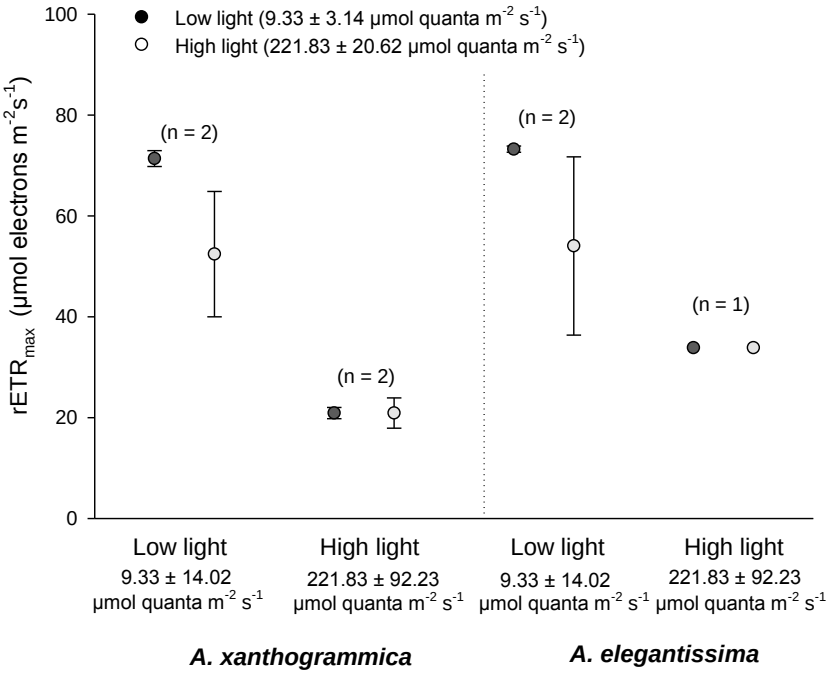

Figure 7. Maximum relative electron transport rate ($rETR_{max}$ mean ± standard error) of *in hospite* and expelled *E. marina* from *A. elegantissima* and *A. xanthogrammica* at 22°C.

Field temperature measurements

Daylight PAR at the Slip Point study site averaged 573.3 ± 495.5 μm m^{-2} sec^{-1}, during the period of 5-6 August with a range of 14.7-1500.0 μm m^{-2} sec^{-1}. The relatively low average irradiance was due to heavy cloud cover in the morning and thick fog throughout the day. Tides ranged from -0.22 m to +2.32 m MLLW. Average body column diameter, measured at the base of the *A. xanthogrammica* sampled during the low tide, was 12.7 ± 2.7 cm when fully contracted, with a range of 9.0 - 17.0 cm. All anemones retained the iButtons that had been inserted into their gastrovascular cavities, and showed no signs of expulsion. The anemones appeared healthy, and many were in an expanded state prior to iButton removal.

The iButton temperature data (Fig. 8) showed that the internal body temperatures of *A. xanthogrammica* (n = 14) were more stable than ambient temperatures (n = 1), and less likely to reach temperature extremes. At its peak, the ambient temperature reached 19.5°C, while the internal body temperatures of the anemones remained 3.8 ± 1.5°C and 6.2 ± 1.1°C cooler on average for upper and lower intertidal individuals respectively. A Pearson's product-moment correlation analysis showed no correlation between anemone body size and tidal height (Fig. 9). However, when measured at the peak ambient temperature, body temperature showed a positive correlation with tidal height. Surprisingly, at the peak ambient temperature, internal anemones body temperature was not correlated with body size (Fig. 9).

Temperatures of lower intertidal anemones showed less fluctuation and more moderate temperatures than those of upper intertidal anemones that were exposed for longer

periods of low tide. The low intertidal anemones also showed lower variability among individuals. Ambient temperatures ranged from 9.0 - 19.5°C, but anemones in the lower intertidal zone remained between 10.0 – 15.5°C. In contrast, anemones in the upper intertidal zone showed a range closer to that of ambient temperatures, ranging from 8.9 – 18.0°C. Body temperatures of all anemones dropped and remained stable once the anemones were submerged at flood tide, then steadily increased as they were exposed by the ebbing tide.

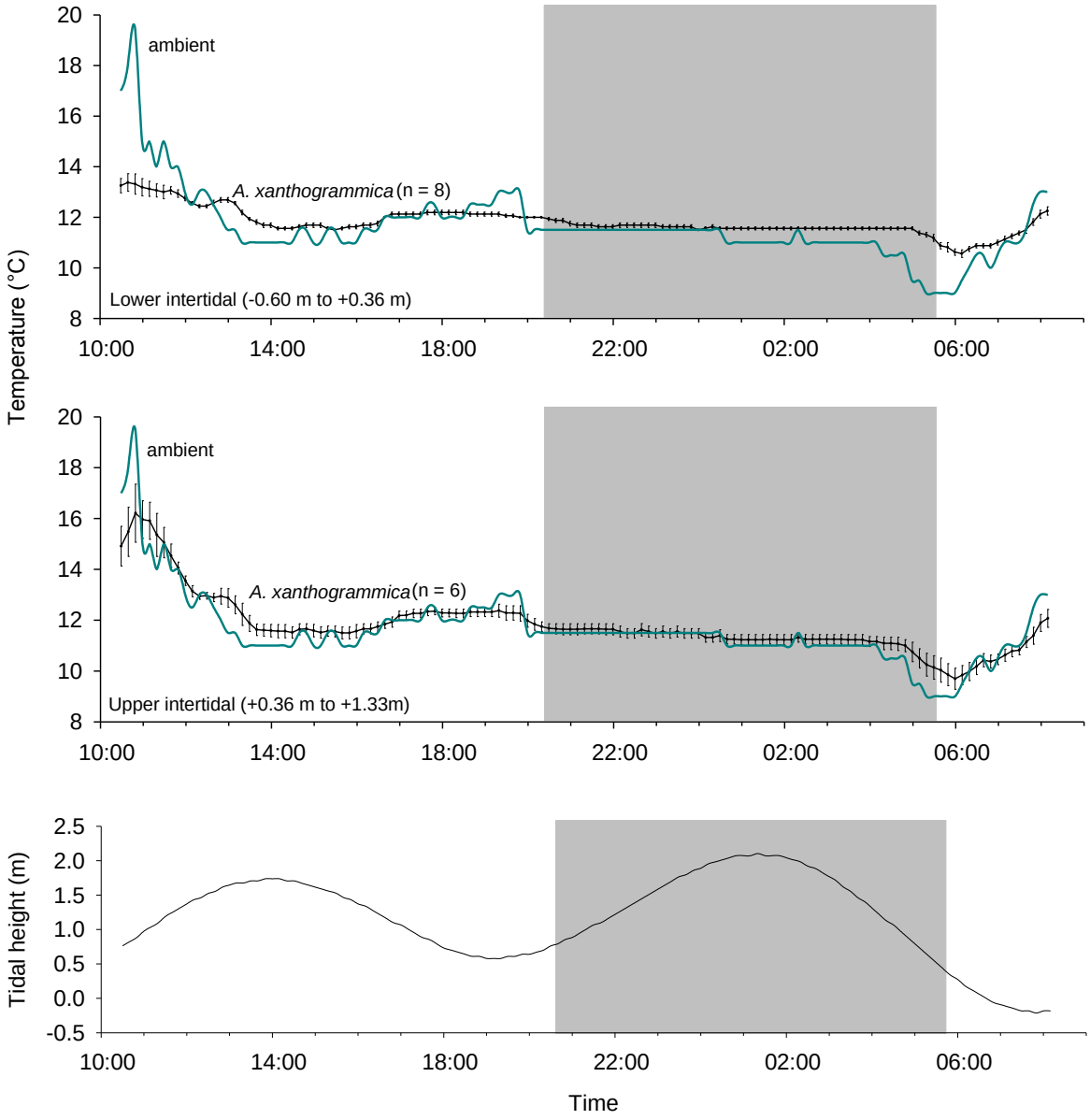

Figure 8. Ambient and internal body temperatures (mean ± standard error) of lower intertidal (-0.60 m to +0.36 m) and upper intertidal (+0.36 m to +1.33m) *A. xanthogrammica* at Slip Point, WA. Grey area highlights the period between sunset and sunrise. Lower plot shows tidal heights (meters relative to MLLW) obtained from the NOAA National Data Buoy Center (Station Id: 9443361 at Sekiu, Clallam Bay, WA).

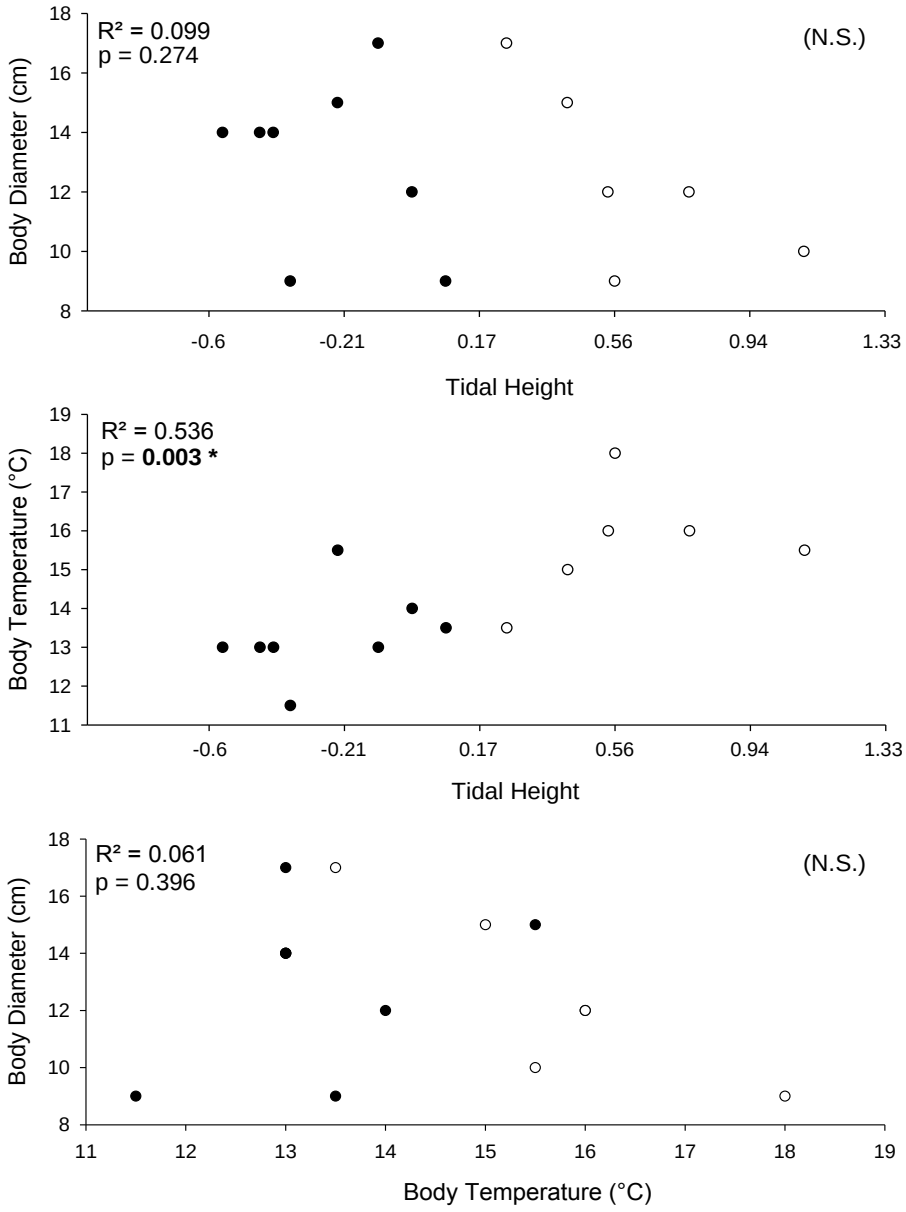

Figure 9. Correlations between: body size (diameter, cm) and tidal height, body temperature and tidal height, and body size and body temperature for *A. xanthogrammica* at Slip Point, WA. Solid points represent lower intertidal anemones (-0.60 m to +0.36 m, n = 8), while open points represent upper intertidal anemones (+0.36 m to +1.33m, n = 6). Internal temperatures were taken at the maximum ambient temperature reached (19.5°C).

DISCUSSION

Overview

To understand what regulates the distribution of *E. marina* in *A. elegantissima* and *A. xanthogrammica,* I examined traits of both the hosts and the symbiont, as each exhibit unique physiological responses that affect the overall response of the holobiont to environmental stressors. A gradual temperature increase of 2°C over the course of 10 weeks was used to establish the upper thermal tolerance limit of *E. marina* in each host, while examining the response of the anemones themselves to the thermal stress. The two light treatments allowed examination of the combined effects of temperature and light stressors.

Host response

Of the two hosts, *A. xanthogrammica* was less tolerant of prolonged high temperatures, exhibiting a stress response indicated by high levels of mucus production and slowed reaction times when temperatures reached 18°C. By 26°C, two individuals were completely non-responsive, had to be removed from the experiment, and did not recover. In contrast, *A. elegantissima* remained responsive throughout the entire experiment, indicating that they can tolerate sustained temperatures of at least 28°C. This high thermal tolerance of *A. elegantissima* is consistent with previous reports. Muller-Parker et al. (2007) found that zooxanthellate *A. elegantissima* exposed to temperatures from 12 - 28°C appeared healthy even at the highest temperature. This differential sensitivity of these two species to temperature may reflect adaptation to their respective habitats. *A. xanthogrammica* were

collected from the Strait of Juan de Fuca, while *A. elegantissima* were collected from the San Juan Islands. Intertidal organisms on the outer coast of Washington and in the Strait of Juan de Fuca generally experience lower levels of heat and irradiance than do anemones in the San Juans (Dayton 1971, Harley and Helmuth 2003).

In addition to the stress responses described above, a contraction response was triggered for *A. xanthogrammica* at temperatures above 18°C, with individuals showing highly variable responses as temperatures continued to increase beyond 18°C. In comparison, *A. elegantissima* only exhibited a brief contraction response between 24 - 26°C, and only in the dark treatment. At 28°C all individuals were fully open and responsive. Contraction is a typical response in anemones to unfavorable stimuli (Pearse 1974) and may have been in direct response to temperature, or to a decrease in available O_2, as *Anthopleura* spp. have been shown to contract when oxygen levels are low (Pearse 1974). Dissolved oxygen varied throughout the experiment depending on a combination of treatment, species, and temperature. These fluctuations likely reflected a combination of anemone and symbiont respiration, as well as symbiont O_2 production. Any microbial populations within the water may have also affected the concentration of dissolved oxygen, as well as any algae, either expelled directly from the anemones, or algae small enough to bypass filtration. There was a higher build-up of algae on the sides of the containers in the high light treatment. These were removed during cleanings, but may have affected O_2 concentrations in that treatment. After 20°C, water changes were increased to reduce anemone stress and algal build up. However, O_2 continued to decrease in the *A. xanthogrammica* water, likely reflecting increased host stress and respiration at higher temperatures. O_2 for *A. elegantissima* remained relatively

stable all the way to 28°C. Although O_2 fluctuated, it is unlikely that they were the primary cause of the contraction response, as the response was elicited before O_2 levels dropped.

Contraction may also be a method to conserve energy under stressful conditions, as contracting reduces metabolic processes in both host and symbiont (Pearse 1974, Shick and Dykens 1984). The contraction responses exhibited by anemones indicate that *A. xanthogrammica* experienced stress at lower temperatures than did *A. elegantissima*. The visible expulsion of symbionts from 16 - 18°C suggests that *A. xanthogrammica* were responding to the stress by attempting to regulate their symbiont density through expulsion (Muscatine and Pool 1979, McCloskey et al. 1996, Baghdasarian and Muscatine 2000, Verde and McCloskey 2002). Expulsion may be an adaptive mechanism, allowing anemones to control the density of symbionts within their tissues. This may be beneficial when symbionts are damaged and are no longer contributing photosynthetic carbon to the host (Muller-Parker et al. 2007). It may also be used as an adaptive measure to reduce productivity of the algae, and the subsequent formation of reactive oxygen species (ROS) which can be highly damaging to both host and symbiont (Pearse 1974, Shick and Dykens 1984).

Symbiont response

To determine how the *E. marina* were responding to thermal stress, the photochemical efficiency of PS II was examined by measuring photosynthetic efficiency (F_v/F_m) and photosynthetic capacity ($rETR_{max}$) of symbionts within *A. elegantissima* and *A. xanthogrammica*. The maximum quantum yield of *E. marina* remained stable until 22°C,

regardless of species or light treatment. After 22°C, however, maximum yield steadily declined in both species and both light treatments except for *A. elegantissima* in the low light treatment, which remained relatively stable. The reduction of photochemical efficiency at 22°C indicates compromised photosynthesis in the symbionts. High light and temperature result in the degradation of D1, a protein essential to the reaction center of PS II (Warner et al. 1999), and damage the thylakoid membrane (Tchernov et al. 2004) and the chloroplast (Weis 2008). Chloroplast damage was apparent after exposure to 28°C, as algae exhibited reduced pigmentation, appearing nearly clear under light microscopy (personal obs.).

From 10 - 22°C, there did not appear to be any host-specific differences in the photophysiology of symbionts, as inhibition occurred at 22°C regardless of species. A similar pattern was reflected in measurements of $rETR_{max}$. After 22°C, only symbionts within *A. elegantissima* in the low light treatment were able to maintain a moderate maximum yield. This indicates that when shaded, symbionts in *A. elegantissima* remain photosynthetically functional until at least 28°C, however, even shaded symbionts in *A. xanthogrammica* experience photoinhibition at 22°C. This suggests different physiological tolerances of symbionts in the two species. Although irradiance was low, the light treatment was sufficient to induce photostress of the symbionts within both species of anemones, indicated by the lower F_v/F_m and $rETR_{max}$ of symbionts in the high light treatment. Symbionts in both species exhibited similar responses to light, even though the tissues in *A. xanthogrammica* attenuate the light more (Dimond et al. 2012). This may suggest production of photoprotective animal pigments in *Anthopleura*, or reduction of photochemical efficiency through non-photochemical quenching (Dykens and Shick 1984, Shick and Dykens 1984).

By 28°C, both *Anthopleura* species had expelled the majority of their symbionts, regardless of light treatment. Overall, *A. elegantissima* lost a higher percentage of its symbionts as temperature increased, while *A. xanthogrammica* retained more symbionts, especially in the low light treatment. This may reflect initial differences in symbionts density, as *A. xanthogrammica* hosts nearly 2.5x more symbionts than *A. elegantissima*. It may also reflect different relative abilities of the hosts to control their symbiont densities. Symbionts are held in symbiosomes within the gastrodermal tissue. Since *A. xanthogrammica* has tissues that are 1.8x thicker than those of *A. elegantissima* (Dimond et al. 2012) they may be less able to actively expel symbionts.

To determine if anemones were preferentially expelling damaged symbionts, the photochemical capacity of *E. marina* in intact anemone tissues was compared to *E. marina* expelled from the same host at the same time. There was no difference in the maximum rETR between expelled and intact *E. marina* from either host species. Since symbionts were still functioning photosynthetically at the time of expulsion (measurements were taken at 22°C), it is likely the expulsion seen in both *Anthopleura* species was a host stress response, or a means of regulating symbionts density.

Experimental treatment

Temperatures within the incubator remained stable and reflected the desired levels, however, some warming did occur in the higher light treatment. While the temperature increase was less than 1°C, it is important to note the temperature discrepancy while

interpreting the results of this experiment. Average light intensity in the high light treatment was 221.8 ± 92.2 µmol quanta m^{-2} s^{-1}, which was below the average daytime irradiance measured at Slip Point, WA (~ 570 µmol quanta m^{-2} s^{-1}) on a foggy day in June. It was also below the average summer daytime irradiance reported for field sites in the San Juan Islands, WA (450 µmol quanta m^{-2} s^{-1}, Muller Parker and Davy 2001). Therefore, anemones in the experiment were subjected to relatively low light and should not have experienced light stress. Exposing the anemones to a moderate light level allowed us to examine the effects of temperature and different light intensities without causing excessive photostress to the anemones or their symbionts.

Field temperature measurements

Internal body temperatures of *A. elegantissima* have been previously measured (Dingman et al. 1998, Bingham et al. 2011). No such measurements had been reported for *A. xanthogrammica*. To examine whether temperatures experienced *in situ* are enough to tax the physiological performance of symbionts within *A. xanthogrammica*, it was necessary to measure the anemones' internal body temperatures. While air and water temperature data alone can provide valuable information, they often do not accurately reflect the temperatures experienced by intertidal organisms *in situ* (Helmuth 1998, 2006b). Body temperatures are also difficult to model, due to multiple synergistic factors including wave height, timing of tides, wind speed, and irradiance (Helmuth et al. 2011). Therefore, measurements of actual body temperatures taken *in situ* are much better indicators of thermal stress (Helmuth 2002).

Internal temperatures of *A. xanthogrammica* in the field remained cooler than ambient temperatures, and remained more stable for the duration of the tidal cycle. Anemones in the lower intertidal zone showed slower rates of warming then anemones in the upper intertidal, and were able to maintain lower body temperatures during periods of increased ambient temperatures. At the peak ambient temperature (19.5°C), the internal body temperatures of lower intertidal anemones remained 6.2 ± 1.1°C cooler than ambient, well below temperatures that impacted *E. marina* in the lab. Anemones in the higher intertidal reached higher temperatures, but were still 3.8 ± 1.5°C below ambient. The maximum temperature experienced by any individual *A. xanthogrammica* was 18°C.

Our measurements of temperatures experienced *in situ* by *A. xanthogrammica* are likely conservative, as ambient temperatures only reached 19.5°C during the sampling period. Temperatures at Slip Point, WA can exceed 30°C during the summer (Levine 2010). Irradiance was also relatively low on the sample dates (5 - 6 August 2013) due to fog and heavy cloud cover. This reduced the impact of solar heating, which has substantial impacts on the body heating of intertidal organisms (Helmuth et al. 2011). Gilman (2006) suggested that the body temperatures of intertidal ectotherms may be closer to the air temperature on days when there is little solar radiation, as was the case during the field study. This indicates that *A. xanthogrammica* could experience much higher body temperatures.

Bingham et al. (2011) measured body temperatures of *A. elegantissima* in the field and found that they frequently exceeded 24°C, but generally remained under the ambient temperature. Dingman (1998) also examined internal body temperatures of anemones in the

San Juans, and found that *A. elegantissima* experience temperatures up to 28°C during aerial exposure at low tide. Dayton (1971) recorded a high of 33.6°C in *A. elegantissima* from San Juan Island. The large variation in maximum recorded body temperatures shows that temperatures experienced *in situ* are highly variable, and that intertidal animals likely exist over "thermal mosaics" (Helmuth et al. 2011).

There was evidence of such thermal mosaics, as body temperature was positively correlated with position in the intertidal zone. This is likely due to the duration of aerial exposure, which explains why anemones in the lower intertidal zone are able to remain cooler than upper intertidal anemones. In the lower intertidal zone, anemones can be exposed for a few minutes up to several hours. However, at +2 m above tidal datum, anemones can be exposed to air 90% of the time (Ricketts 1934). In the upper intertidal, anemones are also less likely to experience wave splash, which can reduce desiccation and promote cooling.

Unlike patterns reported for *A. elegantissima*, body size did not affect the warming rates of *A. xanthogrammica*. During daytime low tides, *A. elegantissima* with a larger body size maintain lower internal body temperatures than smaller individuals (Dingman 1998). This effect can be multiplied through aggregation of individuals, as is common in *A. elegantissima* (Bingham et al. 2001). Bingham et al. (2011) found that aggregations of anemones respond to thermal changes like a single larger individual, remaining below ambient temperatures and not exhibiting the same temperature extremes as isolated individuals. Since *Anthopleura* spp. are dependent on evaporative cooling when aerially exposed, their rate of warming is highly dependent on water content. Once desiccated,

anemones heat quickly (Bingham et al. 2011). Anemones with a larger body size are able to retain more water, and ultimately reduce the risk of desiccation while evaporatively cooling. In this study, body size of *A. xanthogrammica* was not correlated with body temperature. This was likely because *A. xanthogrammica*, with their much larger body sizes, were able to maintain a high water content throughout their aerial exposure. This reduced desiccation, and enhanced their ability to maintain temperature below ambient levels.

Work with *A. xanthogrammica,* and previous studies on *A. elegantissima*, indicate that both species experience periods of hyperthermic stress *in situ*. The temperatures experienced by *A. elegantissima* and *A. xanthogrammica* may regularly exceed the 22°C threshold for stable photosynthetic efficiency in *E. marina,* indicating that zoochlorellate anemones may experience temperatures high enough to stress the symbiotic association for short periods of time. However, the symbiotic association between zoochlorellae and their hosts appears to be resilient to short-term temperature stressors that are associated with low tide. In the Pacific Northwest, densities of zoochlorellae tend to remain constant seasonally (Dingman 1998, Bergschneider and Muller-Parker 2008, Dimond et al. 2011), suggesting that the holobiont can rebound from brief temperature stress events.

Broader implications

Temperature and light stress have profound effects on the photophysiological performance of *E. marina*, and sustained temperatures above 22°C resulted in symbiont photoinhibition. Maximum yield measurements are generally a strong indicator of the general

fitness of photosynthetic organisms (Maxwell and Johnson 2000). A decreased maximum yield could influence the competitive balance between zoochlorellae and zooxanthellae in *Anthopleura* spp. that host both (LaJeunesse and Trench 2000). With increasing global temperatures, species of symbionts better adapted for warmer environments will out-compete those less adapted (Saunders and Muller-Parker 1997, Rowan et al. 1997). Both species of zooxanthellae (*S. muscatinei* and *S. californium*) are able to maintain higher levels of photophysiological performance than *E. marina* under similar conditions (Verde and McCloskey 2001, Verde and McCloskey 2002). While *E. marina* appears to have a thermal limit of 22°C, *S. muscatinei* maintain constant rates of photosynthesis until 26°C (Muller-Parker et al. 2007). The thermal limit of *S. californium* is even higher, persisting until over 30°C (Muller-Parker et al. 2007). Due to these differences in thermal tolerances, an increase in temperature would likely lead to a shift in the distribution of zoochlorellae and zooxanthellae at a local (microhabitat) scale, and at a latitudinal scale.

Under thermal stress, *Anthopleura* may adaptively bleach and switch their symbiont complement, shifting towards more temperature tolerant symbionts (Bates 2000, Secord and Muller-Parker 2005). Symbiont compliment shifts are generally due to resident populations of zooxanthellae inside the anemones, which are able to outcompete and outgrow the native zoochlorellae population (Weis and Levine 1996). This switch from zoochlorellate to zooxanthellate complement has been termed “browning”, and has been documented in the field with transplantation studies (Saunders and Muller-Parker 1997, Bates 2000). This switch in symbiont complement, from the relatively sensitive zoochlorellae to the more tolerant zooxanthellae, has potential implications for community biodiversity in intertidal

areas, as *A. elegantissima* and *A. xanthogrammica* are the only anemones that host *E. marina* (Muller-Parker and Davy 2001).

The physiological tolerances of the host play an important role in the function of a symbiosis and may ultimately be what limits the distribution of the symbionts. I found that *A. xanthogrammica* was less tolerant of sustained high temperatures than *A. elegantissima*. However, even with the lower heat tolerance, *A. xanthogrammica* are able to maintain higher percentages of symbionts (*A. elegantissima*: 96.7 ± 4.6 % loss; *A. xanthogrammica*: 84.0 ± 18.1 % loss), even during periods of prolonged high temperatures. In the field, *A. xanthogrammica* is able to buffer their symbionts due to their large body size and thicker host tissues (Dimond et al. 2012). These morphological features may explain why zoochlorellate *A. xanthogrammica* are able to persist higher in the intertidal zone than zoochlorellate *A. elegantissima*, as they provide a more favorable environment to the sensitive *E. marina*. This supports the idea that temperature and light stressors are the primary factors limiting the distribution of anemones hosting zoochlorellae to higher latitudes and cooler microhabitats (Saunders and Muller-Parker 1997, Bergschneider and Muller-Parker 2008), and indicates that *A. xanthogrammica* may be a more favorable host than *A. elegantissima* in the context of a changing global climate.

LITERATURE CITED

Bates, A. (2000). The intertidal distribution of two algal symbionts hosted by *Anthopleura xanthogrammica* (Brandt 1835). *Journal of Experimental Marine Biology and Ecology*, *249*(2), 249-262.

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.

Baird, A. H., Guest, J. R., & Willis, B. L. (2009). Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. *Annual Review of Ecology, Evolution, and Systematics*, *40*, 551-571.

Bergschneider, H., & Muller-Parker, G. (2008). Nutritional role of two algal symbionts in the temperate sea anemone *Anthopleura elegantissima* Brandt. *The Biological Bulletin*, *215*(1), 73-88.

Bhagooli, R., & Hidaka, M. (2003). Comparison of stress susceptibility of *in hospite* and isolated zooxanthellae among five coral species. *Journal of Experimental Marine Biology and Ecology*, *291*(2), 181-197.

Bingham, B. L., Freytes, I., Emery, M., Dimond, J., & Muller-Parker, G. (2011). Aerial exposure and body temperature of the intertidal sea anemone *Anthopleura elegantissima*. *Invertebrate Biology*, *130*(4), 291-301.

Brown, B. E. (1997). Coral bleaching: causes and consequences. *Coral Reefs*, *16*(1), S129-S138.

Brown, B. E., & Zamani, N. P. (1993). Mitotic indices of zooxanthellae: a comparison of techniques based on nuclear and cell division frequencies. *Marine Ecology - Progress Series*, *89*, 99-99.

Carpenter, K. E., Abrar, M., Aeby, G., Aronson, R. B., Banks, S., Bruckner, A., Chiriboga, A. et al. (2008). One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science*, *321*(5888), 560-563.

Coles, S. L., & Brown, B. E. (2003). Coral bleaching—capacity for acclimatization and adaptation. *Advances in Marine Biology*, *46*, 183-223.

Dayton, P. K. (1971). Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. *Ecological Monographs*, *41*(4), 351-389.

Dayton, P. K. (1973). Dispersion, dispersal, and persistence of the annual intertidal alga, *Postelsia palmaeformis* Ruprecht. *Ecology*, 433-438.

Dimond, J. L., Bingham, B. L., Muller-Parker, G., 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.

Dimond, J. L., Holzman, B. J., & Bingham, B. L. (2012). Thicker host tissues moderate light stress in a cnidarian endosymbiont. *The Journal of Experimental Biology*, *215*(13), 2247-2254.

Dingman, H. C. (1998). Environmental influence on algal symbiont populations in the sea anemone *Anthopleura elegantissima*. M.S. thesis, Western Washington University.

Dubinsky, Z., & Jokiel, P. L. (1994). Ratio of energy and nutrient fluxes regulates symbiosis between zooxanthellae and corals. *Pacific Science*, *48*(3), 313-324.

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.

Fitt, W. K., Gates, R. D., Hoegh-Guldberg, O., Bythell, J. C., Jatkar, A., Grottoli, A. G., Gomez, M. et al. (2009). Response of two species of Indo-Pacific corals, *Porites*

cylindrica and *Stylophora pistillata*, to short-term thermal stress: The host does matter in determining the tolerance of corals to bleaching. *Journal of Experimental Marine Biology and Ecology*, *373*(2), 102-110.

Gilman, S. E. (2006). The northern geographic range limit of the intertidal limpet *Collisella scabra*: a test of performance, recruitment, and temperature hypotheses. *Ecography*, *29*(5), 709-720.

Hand, C. (1955). The sea anemones of Central California. Part II. The endomyarian and mesomyarian anemones. *Wasmann Journal of Biology*, *13*, 37-99.

Harley, C. D., & Helmuth, B. S. (2003). Local-and regional-scale effects of wave exposure, thermal stress, and absolute versus effective shore level on patterns of intertidal zonation. *Limnology and Oceanography*, *48*(4), 1498-1508.

Helmuth, B. (1998). Intertidal mussel microclimates: predicting the body temperature of a sessile invertebrate. *Ecological Monographs*, *68*(1), 51-74.

Helmuth, B. (2002). How do we measure the environment? Linking intertidal thermal physiology and ecology through biophysics. *Integrative and Comparative Biology*, *42*(4), 837-845.

Helmuth, B., Carrington, E., Kingsolver, J. G. (2005). Biophysics, physiological ecology, and climate change: Does mechanism matter? *Annual Review of Physiology, 67*, 177–201.

Helmuth, Brian, Broitman, B. R. Blanchette, C. A., Gilman, S., Halpin, P., Harley, C. D. G., O'Donnell, M. J., Hofmann, G. E., Menge, B., & Strickland, D. (2006a). Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. *Ecological Monographs*, *76*(4), 461-479.

Helmuth, B., Mieszkowska, N., Moore, P., & Hawkins, S. J. (2006b). Living on the edge of two changing worlds: forecasting the responses of rocky intertidal ecosystems to climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*, 373-404.

Helmuth, B., Yamane, L., Lalwani, S., Matzelle, A., Tockstein, A., & Gao, N. (2011). Hidden signals of climate change in intertidal ecosystems: what (not) to expect when you are expecting. *Journal of Experimental Marine Biology and Ecology*, *400*(1), 191-199.

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.

Holden, C. (1995) Reef bleaching spreads in Caribbean. *Science, 270*, 919.

Jones, R. J., Kildea, T., & Hoegh-Guldberg, O. (1999). PAM Chlorophyll Fluorometry: a new *in situ* technique for stress assessment in scleractinian corals, used to examine the effects of cyanide from cyanide fishing. *Marine Pollution Bulletin*, *38*(10), 864-874.

LaJeunesse, T. C., & Trench, R. K. (2000). Biogeography of two species of *Symbiodinium* (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *The Biological Bulletin*, *199*(2), 126-134.

Levine, M. R. (2010). The effects of season and microhabitat on the distribution and nutritional contributions of two algal symbionts in the intertidal anemone *Anthopleura xanthogrammica*. M.S. thesis, Western Washington University.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*(1), 265-275.

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany*, *51*(345), 659-668.

McCloskey, L. R., 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.

Muller-Parker, G., & Davy, S. K. (2001). Temperate and tropical algal-sea anemone symbioses. *Invertebrate Biology*, *120*(2), 104-123.

Muller-Parker, G., Pierce-Cravens, J., & Bingham, B. L. (2007). Broad thermal tolerance of the symbiotic dinoflagellate *Symbiodinium muscatinei* (dinophyta) in the sea anemone *Anthopleura elegantissima* (cnidaria) from northern latitudes. *Journal of Phycology*, *43*(1), 25-31.

Muscatine, L. (1961). Symbiosis in marine and fresh water coelenterates. *The Biology of Hydra*, 255-268.

Muscatine, L. (1967). Glycerol excretion by symbiotic algae from corals and Tridacna and its control by the host. *Science*, *156*(3774), 516-519.

Muscatine, L., & Pool, R. R. (1979). Regulation of numbers of intracellular algae. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *204*(1155), 131-139.

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*, 221-225.

Pearse, V. B. (1974). Modification of sea anemone behavior by symbiotic zooxanthellae: expansion and contraction. *The Biological Bulletin*, *147*(3), 641-651.

Ricketts, E. F. (1934). The tide as an environmental factor chiefly with reference to ecological zonation on the California coast. *The Outer Shores. Part 2,* 63-68.

Rowan, R., Knowlton, N., Baker, A., & Jara, J. (1997). Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature, 388*, 265-269.

Sampayo, E. M., Ridgway, T., Bongaerts, P., & Hoegh-Guldberg, O. (2008). Bleaching susceptibility and mortality of corals are determined by fine-scale differences in

symbiont type. *Proceedings of the National Academy of Sciences*, *105*(30), 10444-10449.

Saunders, B. K., & Muller-Parker, G. (1997). The effects of temperature and light on two algal populations in the temperate sea anemone *Anthopleura elegantissima* (Brandt, 1835). *Journal of Experimental Marine Biology and Ecology*, *211*(2), 213-224.

Seavy, B. E., & Muller-Parker, G. (2002). Chemosensory and feeding responses of the nudibranch *Aeolidia papillosa* to the symbiotic sea anemone *Anthopleura elegantissima*. *Invertebrate Biology*, *121*(2), 115-125.

Sebens, K. P. (1982). Asexual reproduction in *Anthopleura elegantissima* (*Anthozoa: Actiniaria*): seasonality and spatial extent of clones. *Ecology*, 434-444.

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.

Secord, D., & Muller-Parker, G. (2005). Symbiont distribution along a light gradient within an intertidal cave. *Limnology and Oceanography*, *50*(1), 272-278.

Shick, M. J. (1991). Functional biology of sea anemones. Chapman & Hall, London.

Shick, J. M., & Dunlap, W. C. (2002). Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annual review of Physiology*, *64*(1), 223-262.

Shick, J. M., & Dykens, J. A. (1984). Photobiology of the symbiotic sea anemone *Anthopleura elegantissima*: photosynthesis, respiration, and behavior under intertidal conditions. *The Biological Bulletin*, *166*(3), 608-619.

Stillman, J., & Somero, G. (1996). Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus *Petrolisthes*): correlation of

physiology, biochemistry and morphology with vertical distribution. *Journal of Experimental Biology*, *199*(8), 1845-1855.

Tchernov, D., Gorbunov, M. Y., de Vargas, C., Yadav, S. N., Milligan, A. J., Häggblom, M., & Falkowski, P. G. (2004). Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(37), 13531-13535.

Tomanek, L., & Somero, G. N. (1999). Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: implications for limits of thermotolerance and biogeography. *Journal of Experimental Biology*, *202*(21), 2925-2936.

Verde, E. A., & McCloskey, L. R. (1996). Photosynthesis and respiration of two species of algal symbionts in the anemone *Anthopleura elegantissima* (Brandt)(*Cnidaria; Anthozoa*). *Journal of Experimental Marine Biology and Ecology*, *195*(2), 187-202.

Verde, E. A., & McCloskey, L. R. (2001). A comparative analysis of the photobiology of Zooxanthellae and Zoochlorellae symbiotic with the temperate clonal anemone *Anthopleura elegantissima* (Brandt) I. Effect of temperature. *Marine Biology*, *138*(3), 477-489.

Verde, E., & McCloskey, L. R. (2002). A comparative analysis of the photobiology of zooxanthellae and zoochlorellae symbiotic with the temperate clonal anemone *Anthopleura elegantissima* (Brandt). *Marine Biology*, *141*(2), 225-239.

Warner, M. E., Fitt, W. K., & Schmidt, G. W. (1999). Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proceedings of the National Academy of Sciences*, *96*(14), 8007-8012.

Weis, V. M. (2008). Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *Journal of Experimental Biology*, *211*(19), 3059-3066.

Weis, V. M., & Levine, R. (1996). Differential protein profiles reflect the different lifestyles of symbiotic and aposymbiotic *Anthopleura elegantissima*, a sea anemone from temperate waters. *Journal of Experimental Biology, 199*(4), 883-892.

Yellowlees, D., Rees, T. A. V., & Leggat, W. (2008). Metabolic interactions between algal symbionts and invertebrate hosts. *Plant, Cell & Environment, 31*(5), 679-694.

Zamer, W. E., & Shick, J. M. (1989). Physiological energetics of the intertidal sea anemone *Anthopleura elegantissima*. III. Biochemical composition of body tissues, substrate-specific absorption, and carbon and nitrogen budgets. *Oecologia*, 117-127.