Effects of Ultraviolet Radiation on the Behavior of the Sea Anemone *Anthopleura elegantissima*

Theresa M. DiMarco

Abstract

Ultraviolet radiation penetrates our atmosphere and causes cellular damage to many marine intertidal species. Photosynthetic marine species in particular are affected by high levels of ultraviolet radiation, as it can inhibit photosynthesis by the formation of reactive oxygen species. These reactive oxygen species oxidize DNA, preventing DNA synthesis, and inhibit photosynthesis. The aggregating sea anemone, Anthopleura elegantissima, *has symbiotic relationships with photosynthetic zooxanthellae and zoochlorellae, both of which are vulnerable to the effects of ultraviolet radiation. Aggregations of* A. elegantissima *contain several polyp morphologies, including outer warriors and central reproductives. These morphologies differ in their structure and their function: outer warrior polyps engage in competition for space with neighboring species, while the reproductive polyps remain well protected in the center of the colony. In order to determine the effects of ultraviolet radiation on* A. elegantissima*, polyps were exposed to varying levels of radiation both outdoors in approximate environmental conditions and indoors under an artificial ultraviolet source. For indoor and outdoor studies I controlled different levels of ultraviolet radiation by shading polyps with various materials, and observed behavioral responses based on the different levels of shading There were no large differences in position behavior among polyps of the same morphology. However, significant behavioral differences between polyp morphologies suggest that reproductives are more sensitive than warriors to the effects of ultraviolet radiation.*

Introduction

Intertidal organisms are subject to a variety of stresses such as desiccation, nutrient limitation, space competition and predation. One major stressor is ultraviolet (UV) radiation, which can cause severe cellular damage by creating reactive oxygen species that oxidize DNA and disrupt membrane lipids (Adams and Shick, 2001). The portions of UV radiation that most impact organisms fall into the spectrum at 280-380 nm. Though UV-A and UV-B constitute only 5% of total global radiation on a sunny day and range from 280-380 nm, they cause the most damage; marine organisms in particular are affected by UV radiation due to the inhibition of photosynthesis and the inhibition of DNA synthesis (Banszak et al., 1995a).

A. elegantissima, the aggregating anemone, occurs in the high to low intertidal and develops into dense aggregations by asexual reproduction via longitudinal fission of a single polyp (Morris et al., 1980). *A. elegantissima* is also capable of sexual reproduction, as each aggregation is either male or female. The green pigmentation of most individuals is a result of the symbiotic relationship with microscopic algae composed of zoochlorellae and zooxanthellae (Morris et al., 1980). Depending on the geographical location of the colony, symbionts may be composed of either or both of these types of algae. In Northern California two major species found in polyps of *A. elegantissima* are a *chlorella*-like chlorophyte, which is a zoochlorellae, and a zooxanthellae, dinoflagellate *Symbiodinium muscatinei* (Shick et al., 2002).

Within a single *A. elegantissima* aggregation there are different polyp morphologies, including smaller defensive "warriors" around the outer edge, and larger gonad-rich "reproductives" in the center. Warriors have few gonads and an abundance of tentacles packed with large stinging cells called acrorhagi, which are used to attack neighboring species with which *A. elegantissima* competes for space (Ayre et al., 1995). Reproductive polyps contain more gonads and fewer acrorhagi, leaving them more vulnerable, but able to readily produce gametes.

It has been shown that the differences between warriors and reproductives are not merely morphological. A physiological difference between the two morphologies is the level of heat shock protein 70 produced in response to stress (Rossi and Snyder, 2001). Warriors produce higher levels than do larger reproductives. Warriors and reproductives are behaviorally different in that warriors participate in spatial competition with neighboring organisms. Reproductives are protected within an aggregation and have little interaction with non-conspecifics (Ayre and Grosberg, 2005). There may be further behavioral differences in the responses of these polyps to UV radiation.

While many other studies have examined the effects of UV radiation on *A. elegantissima* and its symbionts (e.g., Shick et al., 1984; Stochaj et al., 1993), very little has been done to determine whether behavioral responses to UV exposure are found in these species. It is also unknown how these responses affect distribution of individuals within a colony and of an aggregation in the rocky intertidal. Behavioral responses may indicate either an aversion to UV radiation or a defense against the damaging effects of UV radiation on *A. elegantissima* and its algal symbionts. These behavioral changes may in turn influence where a colony expands on a substrate or determine where new recruits settle. In this study I examined the effects that varying levels of UV radiation had on *Anthopleura elegantissima* behavior in both warrior and reproductive polyps.

Materials and Methods

Specimens of *A. elegantissima* were collected from the north arm of the Bodega Harbor Jetty (Figure 1), from boulders located in the mid intertidal. Samples of warriors and reproductive polyps were taken from separate clones, so that all warriors were genetically identical and all reproductives were genetically identical. All polyps were brought back to the lab, placed in the center of glass finger bowls, and allowed

Figure 1. Sample site located at the entrance to Bodega Harbor on the north arm of the jetty.

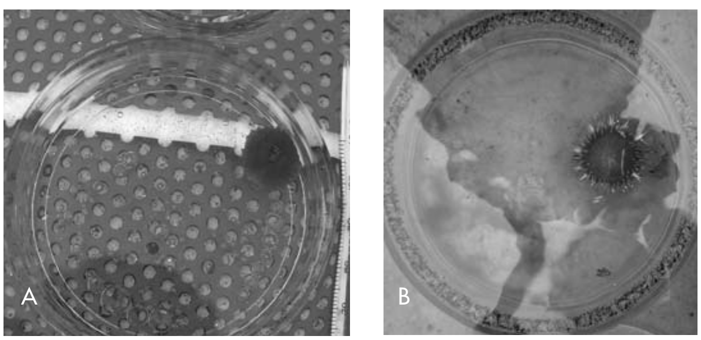

Figure 2. A) Openness stage 1. B) Openness stage 5.

to settle. Any debris attached to the column of each polyp was removed by hand and polyps were left undisturbed overnight in an indoor sea table. Warrior polyps, which were proportionally smaller than reproductives, were placed in small finger bowls and reproductives in large finger bowls. This ensured that the amount of available space to move was proportional between polyp types. In order to keep all polyps contained in finger bowls a ring of artificial turf was cemented to the edge of each bowl with DAP® 100% silicon aquarium sealant. These were cured overnight and allowed to sit in flowing sea water for at least 6 hours before animals were settled into each finger bowl enclosure.

To determine the variations in behavior under different levels of UV exposure, I utilized various shading materials. The control treatment was completely exposed to ambient sunlight and UV radiation. A plexi-glass shade was used to block UVA and UVB radiation, effectively reducing UV radiation by half. A Mylar shade was used to block out UVB and all light was blocked out by completely shading with aluminum foil. In addition the effects of a shaded refuge were tested by only partially shading one treatment with aluminum foil.

Observations of anemone behavior were recorded every three hours from approximately 6 AM to 10 PM over a four day period. Observations of oral disc exposure were recorded on a scale from one to five, one being closed and five being open (Figure 2A and 2B). Position in the enclosure was broken down into four categories: center, edge, corner and side (Figures 3A-3D, respectively).

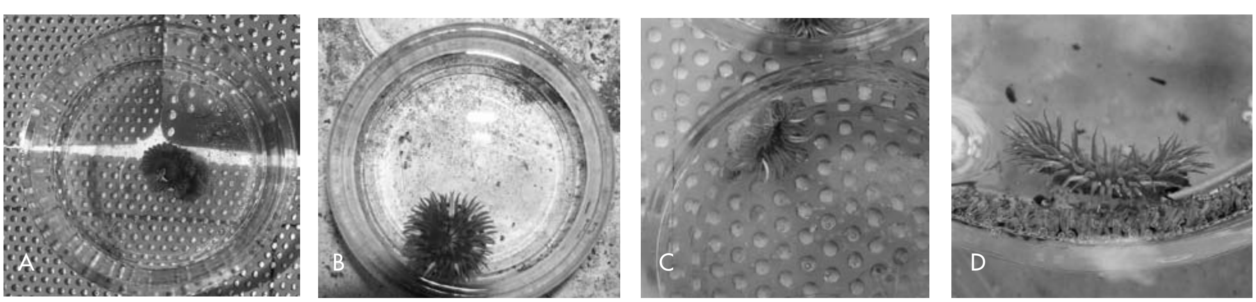

Figure 3. A) Center position. B) Edge position. C) Corner position D) Side position.

Position under shading for the partial cover treatment was recorded along with water temperature and weather conditions at every observation time. UV radiation levels were quantified using a Mannix™ UV Light Meter, which measures UVA and UVB radiation between 290-390 nm.

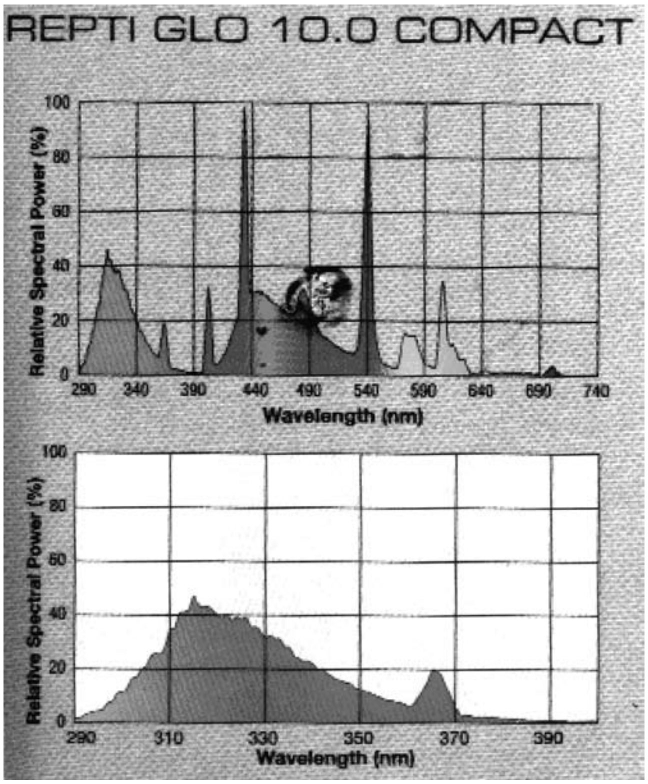

Figure 4. UV Lamp Spectrum as displayed on the packaging.

OUTDOOR STUDY

All shading treatments were performed on warriors and reproductives in replicates of three in an outdoor sea table. The sea table had a continuous flow of fresh sea water from the Bodega Marine Laboratory sea water system. UV readings were taken daily at noon and three in the afternoon.

INDOOR STUDY

New animals were collected and subjected to the same treatments in a Thermo Electron Corporation low temperature incubator at 12°C on warriors in replicates of three. As continuous water flow was not possible within the incubator, sea water in each bowl was replaced daily before each exposure. To simulate UV radiation a ReptiGlo 10.0 UVB fluorescent bulb with a spectrum range of 290 nm to 390 nm (Figure 4) was hung 18 cm above the polyps. Polyps were kept on a 12 hour light, 12 hour dark schedule for the duration of the study. Observations were taken every 3 hours.

DATA ANALYSIS

All data were processed and graphed in Microsoft® Excel. Data were transformed using either a square root function or an arcsine function. Statistics were completed using SYSTAT® 11 ANOVA with Kruskal-Wallis and Tukey post-hoc tests.

Results

OUTDOOR STUDY

On the sunny days of the study ambient UV levels were recorded at approximately 530 $\mu W/cm^2$ and on the cloud cover days approximately 130 $\mu W/cm^2$. Warriors within each treatment spent more time open than closed (Figure 5; $df= 1$, $F \leq 106.199$, $p \leq 0.001$). Across the different levels of shading the percent of time open varied, with the most time open occurring during the total cover treatment ($df= 4$, $F=3.979$, $p= 0.035$). Openness in reproductives revealed that within each treatment polyps also spent more time open than closed, although the amount of time open was different in each case (Figure 6). Polyps in the total cover treatment spent more time open than those in any other treatment ($df= 4$, $F= 13.471$, $p \leq 0.0001$).

There was no difference in the number of times each polyp was found in any given position ($df= 4$, $F \leq 67.570$, $p \leq 0.0001$). Within the control treatment, however, warrior polyps spent more time in the center position of the enclosure (Figure 7; $df= 3$, $F= 17.887$, $p= 0.001$).

There was no difference across the treatments in the number of times reproductive polyps were observed in any given position. Reproductive polyps in the control, plexi-glass and Mylar treatments were never in the

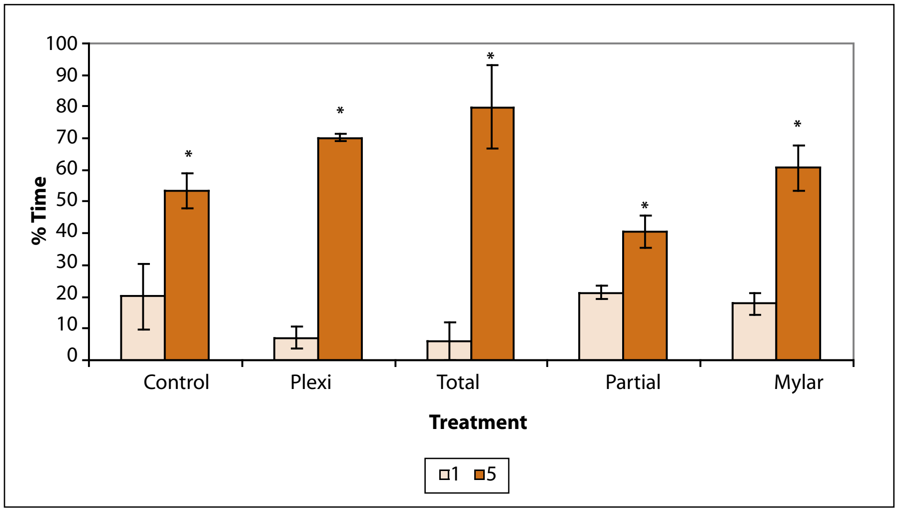

Figure 5. Openness of warrior polyps in the outdoor study. Fully open or fully closed polyps were found in all treatments. Overall polyps spent more time open especially those in the total cover treatment.

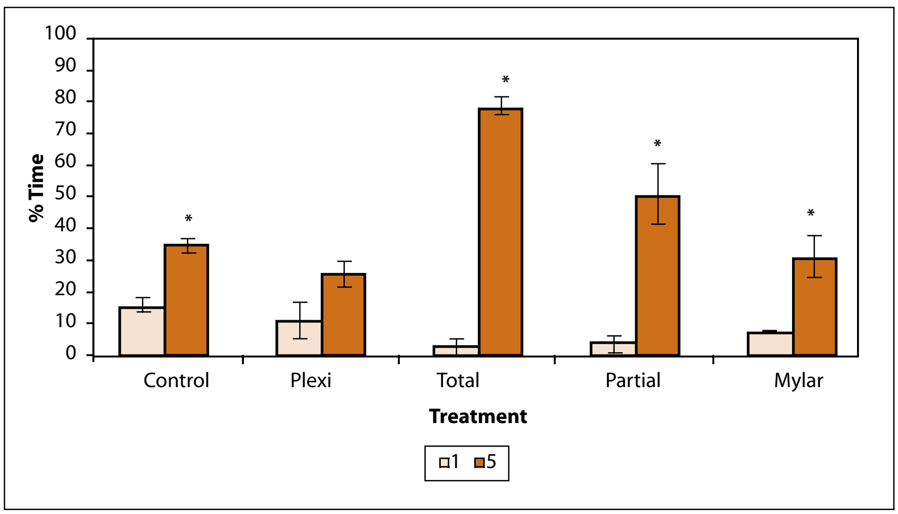

Figure 6. Openness of reproductive polyps in the outdoor study. Polyps were open for a larger percent of time across all treatments; however, the total cover treatment was again open for the longest period of time.

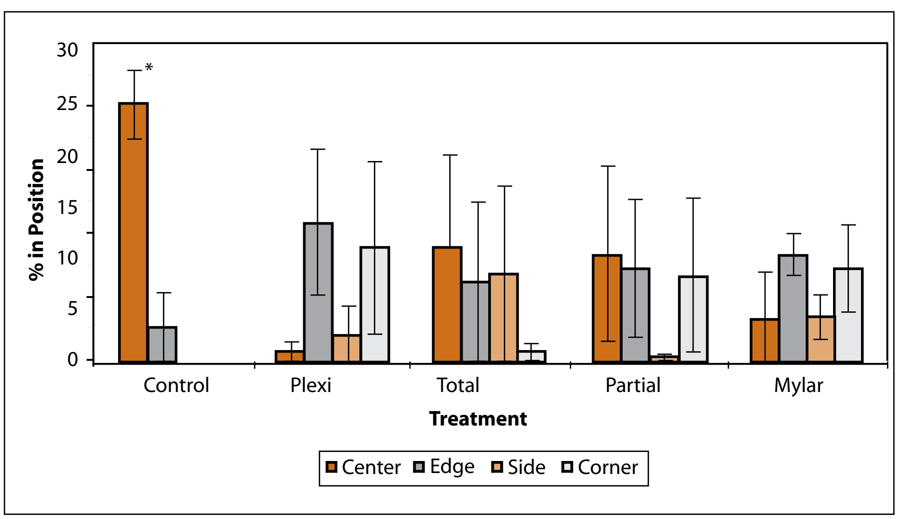

Figure 7. Position of warrior polyps in the outdoor study. In the control treatment polyps were found most often in the center position. In all other treatments polyps were not found in any one position more than another.

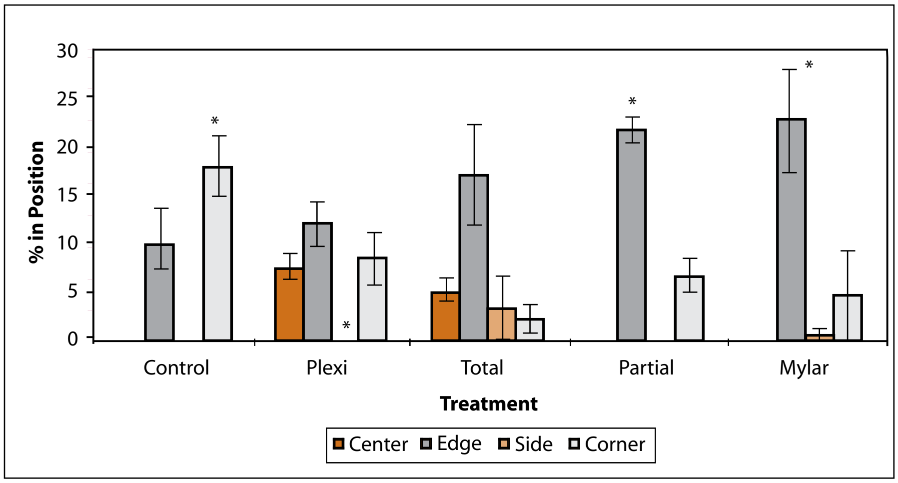

Figure 8. Position of reproductive polyps in the outdoor study. In the partial cover and Mylar treatments polyps were found most often in the edge position and in the control treatment in the corner position.

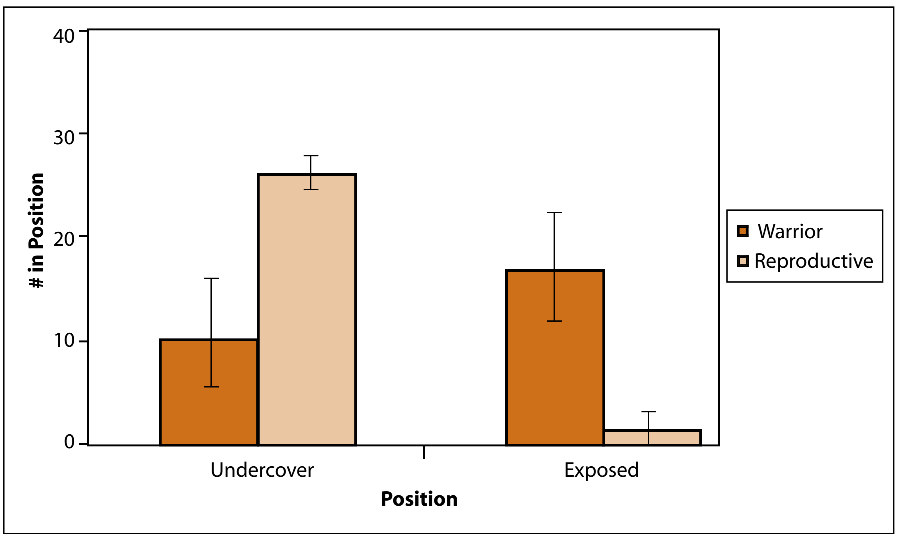

Figure 9. Position of warrior and reproductive polyps in outdoor partial cover treatment. Reproductives were found most often under cover and warriors were found most often exposed to the UV radiation.

center position (Figure 8; df= 4, F= 67.570, p≤ 0.0001). For the Mylar and partial cover treatments, reproductive polyps were more frequently found in the edge position (df= 3, F= 8.351, p= 0.008; F=173.440, p≤ 0.000). In the plexi-glass treatment, polyps were most often found in the center, the edge, or the bottom corner positions of enclosures (df= 3, F= 20.333, p≤ 0.0001), and the control treatment showed polyps most frequently in the corner of enclosures (df= 3, F= 41.294, p≤ 0.0001).

In the partially shaded treatment no difference was observed in the number of times warrior polyps were under cover or exposed (df= 1, F= 0.850, p= 0.409). Reproductive polyps, however, were found more frequently under cover (df=1, F= 32.928, p= 0.005). When compared with reproductives, warriors were found to spend more time exposed (Figure 9; df= 1, F= 10.338, p= 0.032) and reproductives spent more time under cover (Figure 9; df= 1, h= 9.000, p= 0.046).

INDOOR STUDY

In the indoor study UV output from the ReptiGlo UVB bulb was recorded at 20 μW/cm^2 and stayed at that level for the duration of the study. However, this bulb gave out purely UV light and is therefore somewhat comparable to outdoor conditions. The percent time closed for polyps in all treatments was about the same (df= 4, F= 2.014, p= 0.168), and the percent time open did not show statistical variance (Figure

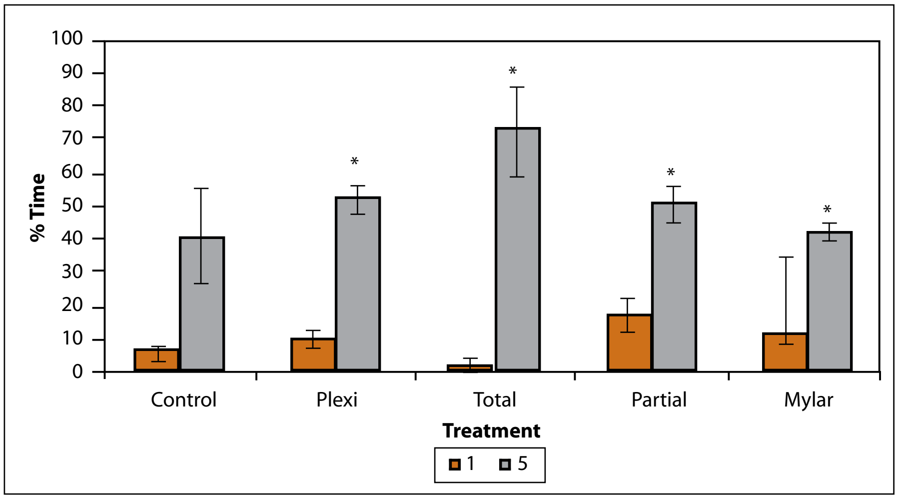

Figure 10. Openness of warriors in the indoor study. Polyps in all treatments spent more time open.

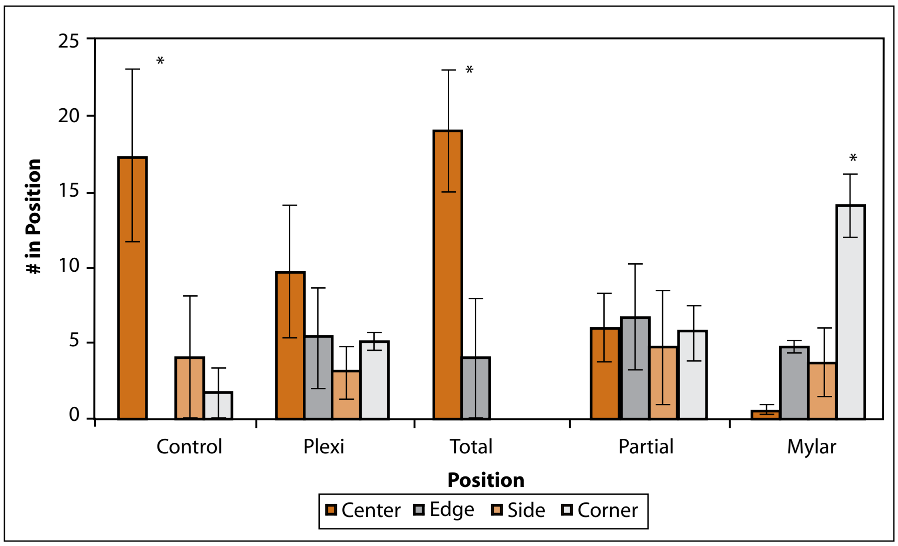

Figure 11. Position of polyps in indoor study. In the Mylar treatment polyps were found mostly in the corner position while polyps in the total cover treatment were found more often in the center position.

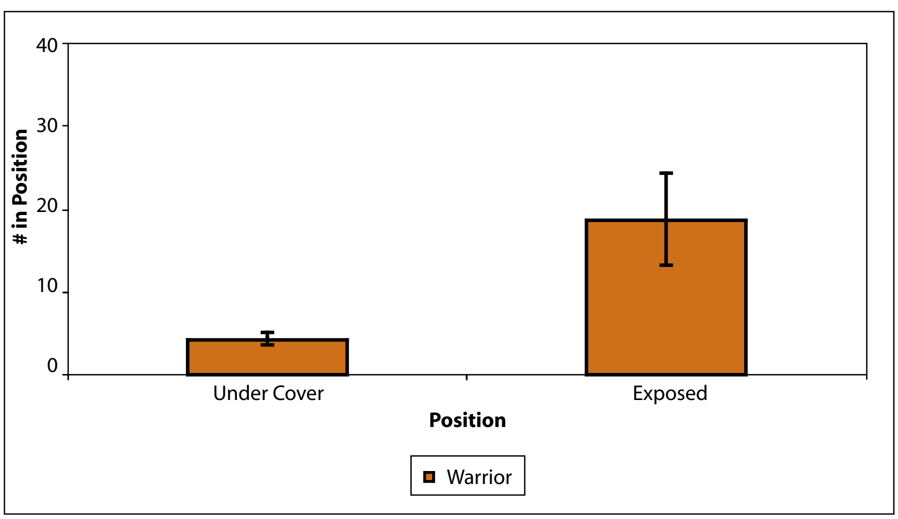

Figure 12. Position of polyps in indoor partial cover treatment. Polyps were found most often exposed to the light source and consequently to UV radiation.

10; df= 4 F= 2.9140, p= 0.078). However, the post-hoc test revealed that polyps in the plexi-glass treatment spent more time open than polyps in the control treatment (Tukey pairwise comparison df=10, p= 0.057). Within each treatment except the control, polyps spent a larger percent of their time open (df= 1, F≤ 76.241, p≤ 0.001). In the Mylar treatment polyps were found most often in the corner position of enclosures (df= 3, F= 12.774, p= 0.002), and in the total cover treatment, polyps were found most frequently in the center position of enclosures (Figure 11; df= 3, F= 10.489, p= 0.004). In the partial cover treatment polyps spent more time exposed than shaded (Figure 12; df= 1, F= 7.353, p= 0.053).

Discussion

The outdoor study revealed that both reproductive and warrior polyps spent more time open in all treatments. While polyps are sensitive to light as seen in other studies (Pearse, 1974; Shick, 1984), they were observed to be open most of the time. Both warrior and reproductive polyps that were completely shaded spent the most time open among all treatments. Position of polyps in the outdoor study revealed warrior polyps to be found most often in the center position of enclosures when exposed to sunlight. This is especially interesting given that these polyps spent more time open than closed, making both anemone and symbionts more vulnerable to potential damage. Reproductive polyps in the control treatment were found most often in the corner of dishes where there was some shading due to the side of the dish. This suggests that UV radiation has little effect on polyp behavior and that there is no need to protect themselves against possible damage. Polyp behavior may change according to specific geographical location, however, as *A. elegantissima* is distributed over a wide range of latitudes and UV radiation exposures. Their behavior while exposed might be explained by the presence of mycosporine-like amino acids, either produced by the algal symbionts or obtained in the diet of *A. elegantissima,* which protect the organism from UV damage (Banaszak et al., 1995b). The data may also imply that warrior polyps are better adapted to UV exposure. Among all other treatments, the position of warriors within enclosures did not reveal any trends. Reproductive polyps, however, spent the most time in the edge position. This may have resulted from the larger enclosure provided them or from the shade offered by a position at the edge of the enclosure or by the artificial turf surrounding it. In the partially shaded treatment, warriors were most often found exposed to sunlight and UV, while reproductives were more frequently found under cover. This again suggests either that warriors are less prone to UV damage or that reproductives are more sensitive to UV radiation.

While the indoor treatment only tested behavioral responses of warrior polyps, the results were consistent with outdoor studies. Polyps in all treatments except the control spent a large percent of time open. The polyps in the plexi-glass treatment in particular were open more than those in the control treatment, which may indicate that reduced UV allows the polyp to spend more time open than closed. Position in enclosure did not vary across or within treatments; however, the total cover treatment showed that polyps were most often in the center position, while the Mylar treatment showed them more frequently in the corner position. This observation confirms the results seen in the outdoor treatments and demonstrates further that position in enclosure was not a behavior shared among polyps in different treatments. In the partial cover treatment, results were similar to the outdoor study: warrior polyps were found most often exposed to the UV light source.

These results show some interesting trends that imply that behavioral responses in *A. elegantissima* depend on the level of UV radiation. UV levels recorded during the outdoor study were not always consistent, as the weather was overcast and cloudy for three of the four days of the study. Overcast weather can block as much as three-fourths of UV radiation, and therefore behavioral responses in the outdoor study may have been altered by the reduced level of radiation. Therefore, further outdoor study conducted on sunny days may be beneficial and may yield different results. However, these data from cloudy days also show how polyps in this particular region, which is often shaded by fog or clouds, behave under natural conditions. Under these circumstances, it may be that distribution of *A. elegantissima* aggregations is less impacted by UV radiation. In general, more replication of both the indoor and outdoor studies would be useful in analyzing and revealing strong trends in the data. An alternative form of shading that completely blocks both UVA and UVB and allows only photosynthetic light should be researched in order to conclusively determine the effects of UV radiation. Another option to further the indoor study would be to use a different UV light source that would ensure equal exposure of all polyps to the artificial UV source. Another possibility would be that a different light intensity may be necessary in order to better approximate natural conditions. If possible, an indoor study on the behavior of reproductive polyps would be valuable in comparison with the results of the outdoors study. In order to truly determine whether these observed behaviors are due to the presence of algal symbionts, however, further study should be conducted using aposymbiotic polyps with both warriors and reproductives. In the absence of algal symbionts, one would hope to see a negative response to UV radiation.

Acknowledgments

First, I would like to thank my advisors Dr. Steven Morgan and Dr. Gary Cherr at the UC Davis Bodega Marine Laboratory, without whose help and guidance this project would have been lost. I would also like to thank two wonderful TAs: Amanda Newsom, for holding my hand and helping fight through my statistics; and Carol Vines, for all the help, suggestions and Starbucks. Thank you to Dr. Ernie Chang and Dr. Jim Clegg at the Bodega Marine Laboratory for being wonderful teachers and helping to introduce me to the world of marine research. To all the Animal Resources Group and physical plant staff, thanks for always helping me find just what I needed. To all my nighttime flashlight holders–Megan Young, Nick Bosch, Beth Lenz and Michelle So—thank you for venturing out to the scariest corner of the lab in full darkness with only one flashlight. And finally, thank you to the entire Spring Class of 2007 who made my quarter at Bodega the best in my entire college experience. I will miss you all.

References

Adams, N.L., Shick, J.M., Dunlap, W.C., 2001. Selective accumulation of mycosporine-likeamino acids in ovaries of the green sea urchin *Strongulocentrotus droebachiensis* is not affected by ultraviolet radiation. Marine Biology 138, 281-294.

Ayre, D.J., Grosberg, R.K., 1995. Effects of social organization on inter-clonal dominancerelationships in the sea anemone *Anthopleura elegantissima*. Anim. Behav. 51, 1233-1245.

Ayre, D.J., Grosberg, R.K., 2005. Behind anemone lines: factors affecting division of labor inthe social cnidarian *Anthopleura elegantissima*. Anim. Behav. 70, 97-110.

Banaszak, A.T, Trench, R.K., 1995a. Effects of ultraviolet (UV) radiation on marine microalgalinvertebrate symbioses. I. Response of the algal symbionts in culture and *in hospite*. J. ofExp. Mar. Biol. and Ecol. 194, 213-232.

Banaszak, A.T., Trench, R.K., 1995b. Effects of ultraviolet (UV) radiation on marine microalgalinvertebrate symbioses. II. The synthesis of mycosporine-like amino acids in response toexposure to UV in *Anthopleura elegantissima* and *Cassiopeia xamachana*. J. of Exp.Mar. Biol. and Ecol. 194, 233-250.

Dykens, J.A., Shick, J.M., 1984. Photobiology of the symbiotic sea anemone, *Anthopleuraelegantissima*: defenses against photodynamic effects, and seasonal photoacclimatization.Biol. Bull. 167 (3), 683-697.

Morris, R.H., Abbott, D.P., Haderlie, E.C., 1980. Intertidal Invertebrates of California. StanfordUniversity Press, Stanford, California.

Pearse, V.B., 1974. Modification of sea anemone behavior by symbiotic zooxanthellae: phototaxis. Biol. Bull. 147 (3), 630-640.

Rossi, S., Snyder, M.J., 2001. Competition for space among sessile marine invertebrates: changes in HSP70 expression in two pacific cnidarians. Biol. Bull. 201, 385-393.

Shick, J.M., Dykens, J.A., 1984. Photobiology of the symbiotic sea anemone *anthopleuraelegantissima*: photosynthesis, respiration, and behavior under intertidal conditions.Biol. Bull. 166 (3), 608-619.

Shick, J.M., Dunlap, W.C., Pearse, J.S., Pearse, V.B., 2002. Mycosporine-like amino acid content in four species of sea anemones in the genus *Anthopleura* reflects phylogenetic but not environmental or symbiotic relationships. Biol. Bull. 203, 315-330.

Stochaj, W.R., Dunlap, W.C., Shick, J.M., 1993. Two new UV-absorbing mycosporine-likeamino acids from the sea anemone *Anthopleura elegantissima* and the effects ofzooxanthellae and spectral irradiance on chemical composition and content. MarineBiology 118, 149-156.