Antho	pleura	sola :	and	their	UV	Protection	Mechanism

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Abstract:

Ultraviolet radiation is dangerous to many animals and plants, some of whom have mechanisms of protection. I propose to study whether *Anthopleura sola* covers itself with shell fragments as UV protection. I have preliminarily done both an observational study and an experimental study. The observational study examines the relationship between anemones' exposure to UV light and the percent shell cover they exhibit on the body column. The experimental study tested the alternative hypothesis that shells were a limiting factor in the areas with less shell fragments. Thus, the experimental study consisted of adding shells to the experimental quadrats and observing the change from pre-treatment to post-treatment in terms of percent shell cover. This study found no relationship between UV light and shell cover, but found a significant difference in the timing of shell cover before and after treatment.

Introduction:

Many animals have protective mechanisms against ultraviolet radiation, the shortest wavelengths to reach Earth, which causes damage to nucleic acids (Siebeck and Bohm, 1991). Organisms respond with specific behavioral responses and/or with physiological mechanisms, all of which can be categorized as either covering sensitive cell structures or a recovery capacity (Siebeck and Bohm, 1991). Ozone loss over Antarctica, the Arctic, and high to mid latitudes is a cause for concern because of the increased UV-B radiation effect on marine ecosystems (Häder, D., Kumar, C., Smith, & C. Worrest, 2007). As exposure to UV light increases, aquatic animals will be more vulnerable to harm, especially since UV radiation can negatively affect

productivity, reproduction, and can cause mutation (Häder et al., 2007). For survival, organisms' mechanisms and behavioral responses must compensate, which makes understanding these ultraviolet protections vital.

The rocky intertidal zone is a harsh environment due to abiotic stresses such as waves and their associated forces, the variation of the tides, and frequent and intense exposure to light. The effects of UV radiation on these aquatic animals may be powerful due to their generally exposed locations. Therefore, the rocky intertidal is a model system for the examination of UV light and protection mechanisms.

Anemones are one such organism in the intertidal that must deal with ultraviolet radiation. Anemones are very sensitive to light, which is demonstrated by the fact that all species of anemones were found to be in compliance with the Bunsen-Roscoe law of reciprocity (I×t=Ko, where I is light intensity, t is length of exposure time necessary to elicit a response, and K is a constant which varied between individuals) (North, 1957). The Bunsen-Roscoe reciprocity law means that the sum of the irradiance determines the extent of photochemical effects (Murata & Osakabe, 2013), or in other words, the photochemical reactions that occur depends on how much exposure to photosynthetically active radiation the object receives. Based on average response times, anemones require about a minute of 108 quanta/cm² * sec light stimuli in order to respond by contracting (North, 1957). The intensity of this light stimulus is akin to "roughly the illumination cast by an ordinary street light upon a normal surface about a mile away" (North, 1957). Thus, anemones are very sensitive to light, which they absorb through their nerve rings. The light receptors of Anthozoans have not been identified, however the general mechanisms present in Cnidaria are occili and/or extraocular photosensitivity (Martin, 2002).

Ocelli are photoreceptors that retain some basic features of the vertebrate photoreceptor system such as a cilia, photosensitive membranes, and a cytoplasm and are found on each tentacle bulb of the cnidarians with this visual mechanism (Martin, 2002). Extraocular photosensitivity, sensitivity to light mediated through an extraocular surface, results in behavioral change in response to light (Martin, 2002). A study done on *Anthopleura elegantissima* found that this species, the close relative of *A. sola*, expands within 5-10 minutes of light exposure (Martin, 2002 citing Pearse 1974). Therefore, *A. sola* can reasonably be assumed to be capable of perceiving light accurately and sensitively.

Debris covering, a collection of fragments from mussels, snails, and other intertidal creatures, on anemones has been studied as protection from light and wind. Hart and Crowe (1977) found that the presence of gravel on the body column prevented water loss and that anemones higher in the intertidal zone had more shells than those closer to the water. Informed by Hart and Crowe's study, Dykens and Shick (1984) found that debris cover is an effective sunscreen and that anemones protected from UV rays attach significantly less gravel to themselves than anemones exposed to UV light. Thus, anemones avoid both desiccation and UV radiation using shell/gravel cover.

Anthopleura sola lives in the protected areas of the rocky intertidal zone of the Pacific Coast. In many cases, the rough green surface of the column is hardly visible under debris. Since the surface of the anemone's column is covered by shells, the animal has limited exposure to light. Using A. sola as a possible example of a UV radiation protection mechanism, I investigated whether shell cover is used to shield against UV light in Anthopleura sola. I also investigated the

possibility that some anemones were limited in their body column covering by a low availability of shells.

Methods:

The study includes an observational component and an experimental component. The observational study tested the hypothesis that as exposure to ultraviolet light increases, the percent shell cover on the anemones also increases. The experimental study assessed whether shell availability is a limiting factor in some areas at the test site. Thus the experimental study tests the alternative hypothesis that if shells are added directly to the anemones and the area around them, all anemones will pick up the shells regardless of their exposure to UV light.

For the observational study, I laid out a transect under the Monterey Bay Aquarium, which provides a gradient in shade for organisms under it. This transect included completely sun-exposed area at the edges and the most shaded middle area, as well as a gradation in between. Due to the nature of the terrain and the scattered location of the anemones, I utilized haphazard quadrat tossing to randomly select anemones. I marked the area near the selected anemones with putty so that they could be relocated. I then estimated percent shell cover on each anemone in the quadrat by observation. Next, using a quantum light meter (model LI-250A), I measured the photosynthetically active radiation (PAR) received by each anemone in each quadrat over twenty seconds to get the most accurate value. These measurements took place on a sunny day at around eight-thirty in the morning. Although measurements at noon would have been preferable, I was restricted by time limitations and the necessity of a simultaneously sunny day and a very low tide. I converted the PAR measurements into the total irradiance of the sun

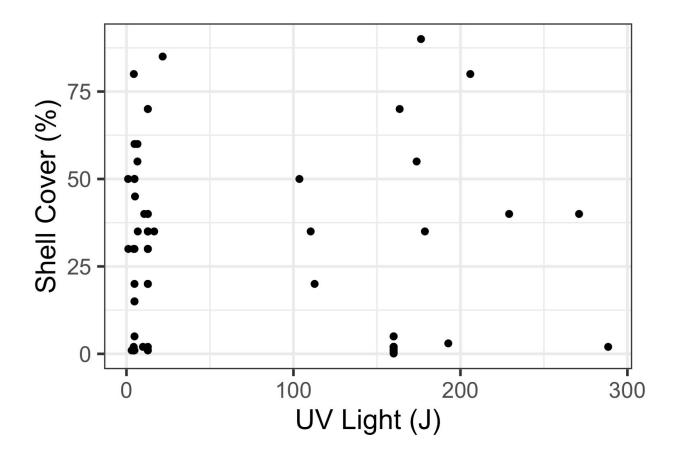
by multiplying the PAR value by two (Jimenez, Kühl, Larkum, & Ralph, 2008). Then, I found the amount of UV light by taking 8% of the total solar irradiance (Moan, n.d.). I hypothesized that the average shell cover of anemones would increase with the amount of UV radiation they experience.

For the experimental study, half of the quadrats under the Aquarium served as the control and half as experimental. I collected shell fragments from Hopkins, moved them to the sites under the Aquarium, and completely covered the experimental sites by dumping shells until the anemone was no longer visible. Control sites did not receive new shells. I monitored and reported the percent shell cover in the same way as I did before, by recording percent shell cover of individuals, after 24 hours. This change in shell cover constitutes timing, which is the difference in shell cover between the two days,

Preliminary Data:

The results of the observational study revealed no relationship between exposure to UV light and shell cover on individual anemones. The number of anemones in each quadrat varied significantly as a result of the randomization of the sites. For example, quadrat one, which had the most exposure to sun, had anemones with only 1% shell cover, while quadrat 10, in the center of the Aquarium shade, contained one anemone with 90% shell cover.

Figure 2: Shell Cover as Function of UV Light



The relationship between UV light and shell cover was not significant ($F_{1,66} = 2.73, p = 0.1$). Thus, shell covering is likely not a mechanism for UV protection. However, shells may be limiting in the anemones' ability to cover themselves. In order to test this idea, I created an experiment to test the hypothesis that shells were a limiting factor. If shells were a limiting factor, then the results would show a clear increase in shells after the experiment in the experimental quadrats alone and the relationship between treatment and timing would be significant.

The experimental design also tested whether anemones utilize shells to protect themselves from UV radiation. If anemones use shells to protect themselves from UV, then exposed anemones would have retained more shells. However, the observational study found no

relationship between UV light and shell cover, and therefore I did not include UV light as a covariate in the analysis of the experimental data.

Although the interaction between treatment and timing was not significant (table 1), both the control and the experiment increased in shell cover after the application of sediment. Therefore, the treatment did not have a significant effect on the shell covering of individual anemones (p = 0.6335). The timing, the difference in shell cover between the two days, accounts for the difference in shell cover (p = 0.0264), since shell cover increased after treatment in both the control sites and the experimental sites. Therefore, the shell cover on the anemones increased for reasons beyond those captured in this study. For example, the timing may be significant because a storm may have washed shells up or the control quadrats could have received added shells from the experimental sites unintentionally.

Figure 3: Shell Cover as a function of Treatment

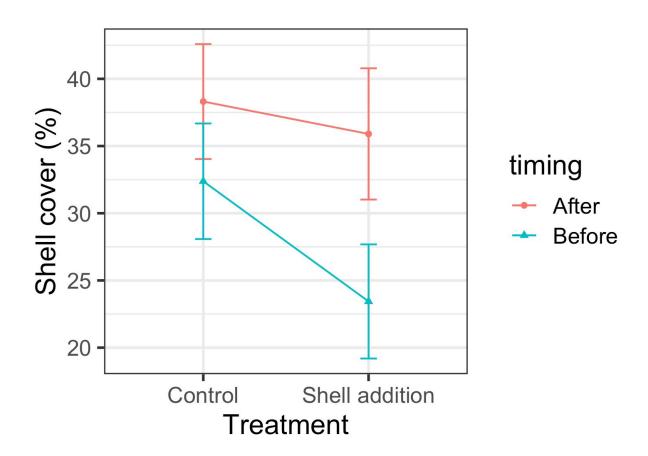


Table 1: Two-way Analysis of Variance for Timing and Treatment

Source	df	SS	ms	F	P
Treatment	1	144	144.2	0.2284	0.633506
Timing	1	3184	3183.6	5.0441	0.026424
Treatment:Ti ming	1	355	354.7	0.5094	0.47668
Residuals	128	80786	631.1		

Proposed Work:

Since my data demonstrated no relationship between UV light exposure and shell covering, I propose to repeat the Dykes and Shick (1984) study that asserts that there is a relationship between these two variables. Dykes and Shick (1984) found that anemones protected from UV light attach significantly less gravel than anemones exposed. To further understand the function of the shell coverings, I would repeat their experiment with *A. sola*, a species unstudied in this regard, and use the anemones from under the Aquarium that yielded such different results. Dykes and Shick (1984) test involved placing anemones in a lab under four conditions: ambient sunlight; outdoors in sunlight, but protected from UV rays; outdoors in sunlight but exposed to an inhibitor of photosynthesis; and indoors in dim lighting. After establishing if their lab results hold for *A. sola*, I would then repeat my field experiment under the aquarium with a longer period observation. Additionally, I would utilize photos taken by a different individual in a blind study design, so that the design eliminates bias in estimating the shell cover. For more precise results, photosynthetically active radiation should be recorded for longer and the shell cover documented through time to record how the percent cover varies through time.

The timing between shell covering observation was significant, which suggests a cause other than UV light as the difference in shell covering. Although protection from UV light is a compelling answer to the function of the shell covering behavior on anemones, there are other explanations for the debris, such as protection from aerial desiccation. Thus, I propose designing an experiment which tests shell covering as a function of the individual anemones' distances from the water in order to test the aerial desiccation hypothesis. If *A. sola* contradicts previous research regarding shell covering as protection, *A. sola* may also contradict the research proving shell cover as desiccation. I would follow the protocol as described in Hart and Crowe (1977),

which involved denuding anemones and simulating natural conditions in a lab to test the rate of evaporative water loss.

I hope to expand the study beyond *A. sola* alone and compare shell covering behavior as a protection mechanism in other anemone species. Observationally, the clonal cousins of *A. sola*, *A. elegantissima*, cover themselves more uniformly and densely with debris. These two species are only differentiable by genetics, with their sole difference being their clonal versus non-clonal reproductive mechanisms and their size. I propose to study the covering behavior of the clonal individuals when placed in different debris availability conditions in comparison with *A. sola's* response to the same conditions. This test will confirm the difference in shell coverings as a genetic difference or as a function of where in the intertidal zone the anemones live and the availability of gravel in those places.

Comparing the results of the UV protection mechanism experiment against the current literature, as well as testing the difference in debris covering in *A. sola* and *A. elegantissima*, lends more insight into UV light protection mechanisms. If UV plays any role in shell coverings, the difference between species may yield insight into these marine creatures and the strength of their protection mechanisms against the broken ozone layer. If UV light plays no role, then in at least this one species this factor of climate change is not a concern. Protection mechanisms, whether against UV or otherwise, provide important information about how the world will adapt as the climate changes.

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