Sea Anemone *Anthopleura xanthogrammica* with Zooxanthellae Have Higher MAA Levels than *A. xanthogrammica* with Zoochlorellae

Bi 457

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Introduction

Zoochlorellae (green chlorophytes) and zooxanthellae (golden-brown dinoflagellates, *Symbiodinium*) are photosynthetic algae that inhabit the endodermal tissues of their hosts (Weis 1996). A giant green sea anemone, *Anthopleura xanthogrammica*, is a common sea anemone in intertidal zones along the west coast of North America, and it forms symbiotic relationships with the two photosynthetic algae. The color of the algae makes the anemone either bright green or brownish green. Interestingly, the color of *A. xanthogrammica* seems to be related to the distribution of the animal in intertidal zones. Bright green-colored anemones are more likely to be found in the higher intertidal zone, whereas brownish-colored anemones often inhabit the lower intertidal zone (Bates 2000; Secord et al. 2000). Bates (2000) explains that this intertidal distribution is caused mainly by different levels of heat tolerance in zooxanthellae and zoochlorellae. If the distribution was true, *A. xanthogrammica* with zooxanthellae (ZX anemones) would be well adapted to high UV radiation, which is strongest at high intertidal levels. Marine invertebrates living in intertidal zones often have mycosporine-like amino acids (MAAs) which are natural UV-absorbing sunscreens. In order to determine if the ZX anemones are better adapted to UV exposure, I developed the hypothesis that *A. xanthogrammica* with zooxanthellae (ZX anemone) would have higher MAA values than *A. xanthogrammica* with zoochlorellae (ZC anemones), therefore being more tolerant of UV exposure.

Methods and Materials

Sampling

Fourteen tentacle samples were collected at South Cove, Coos County, Oregon, and 8 samples were collected at North spit, Coos Bay, Oregon. I went to South Cove during the lowest tide (Lowest tide =-1.2) and began sampling at the lowest mark of the intertidal zone, and I cut several tentacle samples from a sea anemone. Then, I walked inland until I saw another *A. xanthogrammica.* Because it was difficult to cut out tentacles from contracted anemones, I ignored those highly contracted anemones and kept walking until another animal was found.

At North Spit, sampling was done in a similar manner. I started to sample from the lowest tide mark (Lowest tide: -0.6) and walked toward the center of the jetty (about 25 m from the lowest tide mark). I cut out and collected the tentacle samples on the way to the center of the jetty. Once I reached the center of the jetty, I walked about 10 m along the center line of the jetty and then walked toward the lowest mark. Again, I collected samples on the way down to the lowest tide mark.

All samples were collected from individuals that were exposed to the sunlight. Animals under rocks or in crevice were ignored; since my experiment was to see if there was any difference in UV absorbance in the ZX and ZC anemones, exposed anemones would show the difference more strongly. I preserved the tentacle samples in centrifuge tubes (1.5 ml each) and kept them refrigerated until the MAAs analysis.

MAAs analysis and BCA protein assay

Tips of tentacles of *A. xanthogrammica* were used for MAA analysis and protein assay. All tentacle samples were cut into approximately the same volume and rinsed with filtered water. Then, each sample was minced and put in a centrifuge tube. I added 1.0 ml of 100 % methanol to the tube and let it sit. Twenty minutes later, I centrifuged the sample (8000 rotation per min) for 60 seconds and pipetted off the supernatant. The supernatant was kept in a different centrifuge tube for the later use. Another 1.0 ml of the 100 % methanol was added to the tissue. I let the sample sit for 20 minutes and centrifuged it again. The supernatant was pipetted off and combined with the previously extracted supernatant. I analyzed the UV absorbance at λ=313nm and λ=340nm, by using spectrophotometry. I also performed the BCA protein assay to quantify the absorbance.

Results

The ZX anemones were observed in the higher intertidal zones, while the ZC anemones were distributed in the lower intertidal zone (Figure 1). Mean distances from the lowest tide mark are 39.14m for the ZX anemones and 14.86m for the ZC anemones.

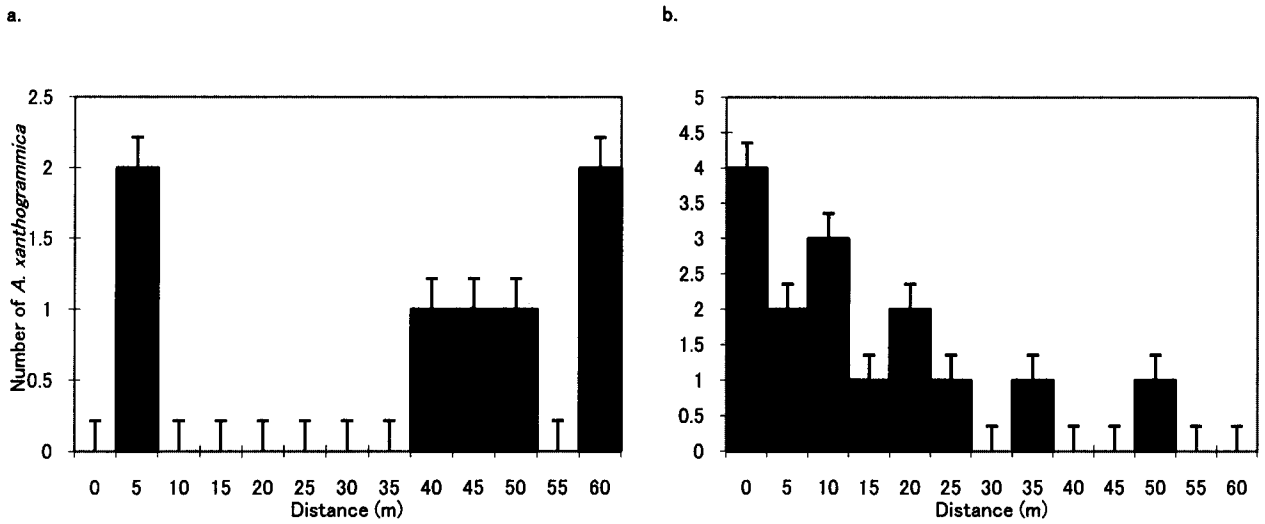

Figure 1. Distributions of *A. xanthogrammica*. **a.** *A. xanthogrammica* with zooxanthellae, **b.** *A. xanthogrammica* with zoochlorellae. Distance (m) = distance from the lowest tide mark; bars on the columns represent SE.

MAA analysis and BCA protein assay produced absorbance unit per ug pretein at 313 nm and 340 nm for each tentacle sample. Correlation between distance from the lowest tide mark and absorbance unit per ug pretein was more clear at 313 nm, but no strong correlation seems to exist at 340 nm (Figure 2). The correlation coefficients were 0.606 and 0.348 for 313nm and 340nm, respectively. When mean absorbance for zooxanthellae and zoochrolellae was compared, zooxanthellae had higher absorbance for both wavelengths (313nm and 340nm) (Figure 3).

Discussion

The distance from the lowest tide mark seems to influence the distribution of the two symbiotic algae; the ZX anemones inhabit the higher intertidal area, and the ZC anemones are more likely to be found in the lower intertidal. This result is consistent with Bates (2000) and Secord et al. (2000). However, an intertidal area is actually a complex, 3-dimentional habitat. *A. xanthogrammica* is often found under rocks and in crevices as well as exposed spaces. These shaded microhabitats may help zoochlorellae survive in higher intertidal areas. When I sampled the tentacle tissues at the intertidal areas, I purposefully chose exposed *A. xanthogrammica* so that I could clearly see how the intertidal distribution affects the ability of *A. xanthogrammica* to produce natural sunscreens. Thus, my results indicate the distribution of the "exposed animals," not *A. xanthogrammica* in

general. If tissue samples were also collected from shaded *A. xanthogrammica,* the result might differ from my result presented above.

There are 4 major MAAs found in *A. xanthogrammica*: mycosporine-taurin, porphyra-334, shinorine, and mycosporine-2 glycine (Shick et al. 2002a). Wavelengths of maximum absorption are 310 nm for mycosporine-taurin and 334 nm for the three other MAAs (Shick et al. 2002b). Therefore, higher absorbance at λ=313nm in both the ZX and the ZC anemones (Figure 3) is most likely due to the higher concentration of mycosporine-taurin. Shick et al. (2002a) report the high concentration of mycosporine-taurin (≈45% of total MAAs) in the ZX anemone as well. The absorbance at λ=313nm seems to be positively correlated with the distance from the lowest tide mark. This result suggests that the exposed sea anemones possess more mycosporine-taurin with increasing distance from the sea. The concentrations of the three other MAAs (porphyra-334, shinorine, and mycosporine-2 glycine) are less likely to increase with increasing distance from the sea. This may indicate that mycosporine-taurin is the primary sunscreen in *A. xanthogrammica.* Production of this MAA may be strongly selected for in the higher intertidal areas.

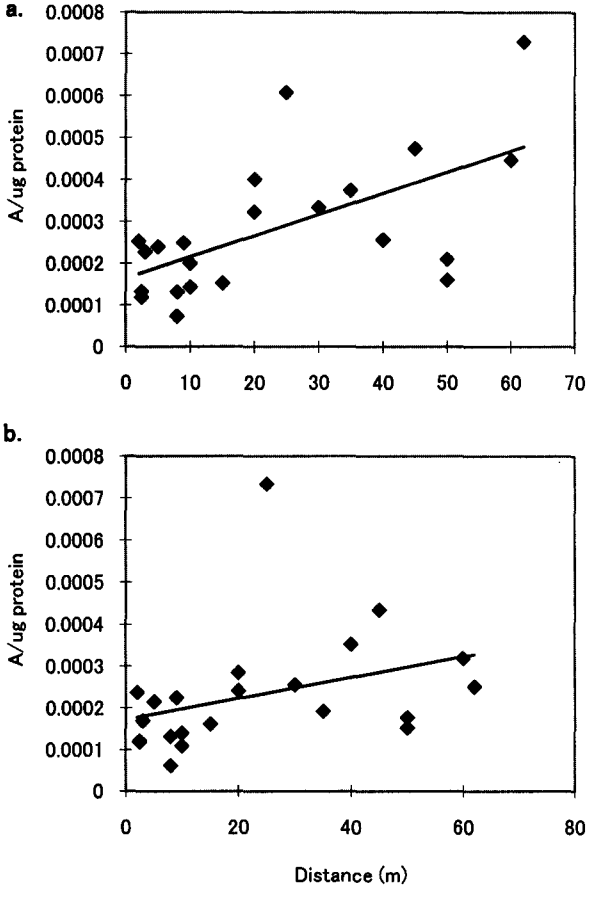

Figure 2. Relationships between the distance from the lowest tide mark and UV absorbance at 2 wavelenghs. **a.** λ=313nm and **b.** λ=340nm

Figure 3 also suggests that ZX anemones have the higher mean absorbance at both λ=313nm and λ=340nm than ZC anemones. This result supports my hypothesis that the ZX anemones have higher MAAs than the ZC anemones. The ZX anemones live in higher intertidals, whereas the ZC anemones live in the lower

intertidals; the concentration of mycosporine-taurin also increases with increasing distance from the sea; therefore, the ZX anemones have higher MAAs than the ZC anemones.

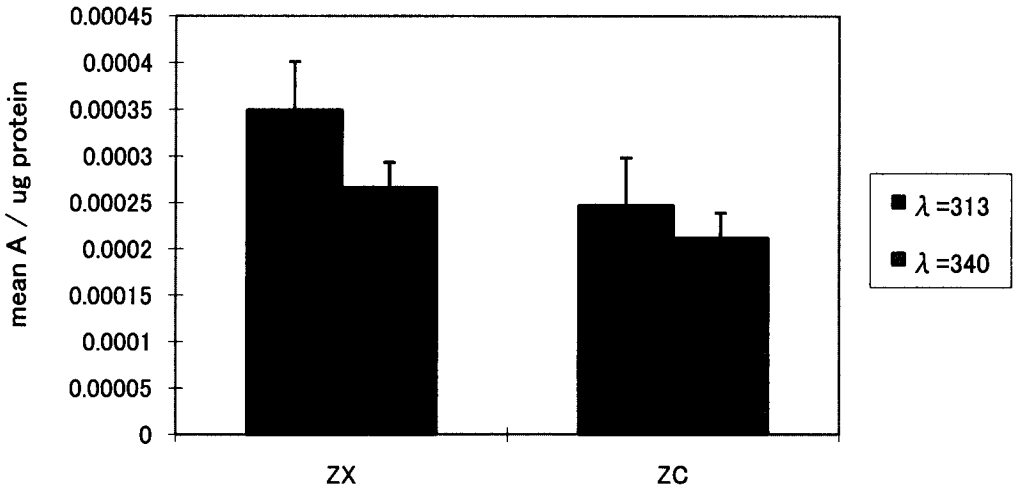

Figure 3. The mean UV absorbance at two wavelengths in the ZX anemones (n=7) and the ZC anemones (n=14). Bars on the columns represent SE.

Even though my results support my hypothesis, there are a few points that should be mentioned. As I described above, all my results were based only on "exposed" *A. xanthogrammica*. If the anemones residing in shaded areas, such as under rocks or in crevices, were taken into account, the distribution of ZX and ZC anemones and mean UV absorbance might be greatly changed. Therefore, I would obtain samples from all the anemones in the next experiment and see if there would be different results. MAA analysis and BCA protein assay are biochemical techniques in which a precise amount of chemicals and an exact timing of chemical reactions are important. Even small carelessness may cause a huge difference in the result; meticulous attention must be paid.

My study revealed the differences in the distribution of ZX and ZC anemones and the amounts of MAAs synthesized in the two anemones. However, what factors contribute to this distribution remains unclear. One possible factor is the capacity of synthesis of heat shock proteins (Hsp), which help organisms live in the elevated temperature environment. Zooxanthellae might induce the host's tissues to produce Hsps so that the host could survive in the high intertidal. In fact, there is endosymbiotic bacterium (*Holospora obtuse*) that enhances heat-

shock gene expression of the host (Hori and Fujitani 2003). Synder and Rossi (2004) revealed that *Anthopleura elegantissima* express more than 3 times the amount of Hsp 70 on a sunny day versus a foggy day. Thus, *A. xanthogrammica* may have the ability to change the expression of Hsps. Furthermore, the gene expression might be facilitated by zooxanthellae. There is still much to learn about the relationship between the symbionts and *A. xanthogrammica*. The relationship seems to be much more than the shelter-nutrition exchange.

References:

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