Adaptations of marine animals
Exploratory 2
July 31, 2005

Variation in ultraviolet blockers within Pisaster ochraceus.

Pisaster ochraceus is the most common seastar in the intertidal zones of the Pacific Northwest (Sept 2004). It can be found in color morphs of orange, purple, yellow and brown. *P. ochraceus* is commonly found on exposed and protected rocky shorelines in the mid to low intertidal zones. This sea star is a dominant predator and has a range unrestricted by other predators. *P. ochraceus* feeds on muscles, abalone, chitons barnacles and snails (Sept 2004). The upper limits of the *P. ochraceus's* range are set by desiccation. At the upper limits of its distribution the sea star is subject to large amounts of Ultraviolet radiation. To protect itself UV radiation damage *P. ochraceus* has mycosporine-like amino acids (MAAs) in its tissue. High concentrations of MAAs have been shown to protect cellular processes from UV damage (Franklin 2002).

In this project I looked at the levels of MAAs in several different versions of *P. ochraceus*. Brown juveniles, purple adults and orange adults were compared and their levels of MAAs were examined. In exploring the intertidal zones I noticed that there seemed to be a distribution specific to each color of *P. ochraceus*. The orange morph seemed to be in more exposed locations and the purple morph was generally less exposed. The brown juveniles were never present in exposed sunlight and I only noticed them under rocks. I wanted to test for a relation between distribution and the levels of MAAs in the tissue of each group of *P. ochraceus*. Also I wanted to look to see if MAA levels increased through development. Knowing the levels of MAAs in these different morphs and ages would help to explain the distribution of *P. ochraceus* in the intertidal zone. My hypothesis is that in *P. ochraceus* there is a relation between the levels of MAAs in and the amount of exposure to sunlight. I expect that juveniles will have the lowest level of MAAs and the orange morph will have the greatest level of MAAs.

Methods

The *P. ochraceus* specimens were collected from various locations. The adults were collected from the Boat Dock in mid July 2005. The juveniles were collected from under rocks on Fossil Point in early July 2005. They were kept in a water table with water and airflow and the table was placed out of direct sun exposure.

In order to test the levels of MAAs in *P. ochraceus* the UV absorbance levels were collected from tissue samples. To collect tissue samples, three *P. ochraceus* of each category (juvenile or adult orange or purple) were scraped with a razor blade. The sample sizes were approximately .1ml. Due to the scraping process the tissue particles were very fine, large samples should be minced. The samples were placed in centrifuge tubes along with 1.0ml 100% methanol. They were placed in a centrifuge for 3 minutes on a 13,000 cycle. Using a pipette supernatant was then removed, another 1.0ml 100% methanol was added and the sample was placed in the centrifuge again. The supernatants were combined and placed in a cuvette. Each cuvette was then placed in a spectrophotometer and the level of absorbance was tested at 313nm and 340nm. Blanks of 2.0ml 100% methanol were used to "zero" the machine.

To get UV absorbance in relation to the amount of protein in each sample they were put through a protein assay. This was necessary because the sample size influences the amount of

UV absorbance. To begin a standard solution of bovine serum albumin was created to compare to the tissue samples. The standard was 125 ug/ml. For the tissue samples I began by pipetting 0.1ml of the standard and each sample into a cuvette. Then 2.0ml of working reagent (50 :1 Kit reagent A to B) was added to each cuvette. The cuvettes were then sealed using parafilm and allowed to incubate for 2 hours at room temperature. After incubation the solutions were analyzed in a spectrophotometer at 562nm. The amount of protein in each sample was calculated using the ratio given by the standard. For example the standard was .344 abs units per 125 ug/ml this ratio was 0.002752. The absorbance amount of each sample was then divided by the standard ratio to give absorbance unit per ug.

The relative destruction of *P. ochraceus* was also examined in this project. A survey of the *P. ochraceus* and their location at the Boat Dock was taken. The survey classified each orange or purple *P. ochraceus* as exposed, un-exposed or partly exposed to sunlight. The survey was taken at the afternoon low tide on July 31, 2005. When collecting the juveniles for the project their exposure was noted.

Results

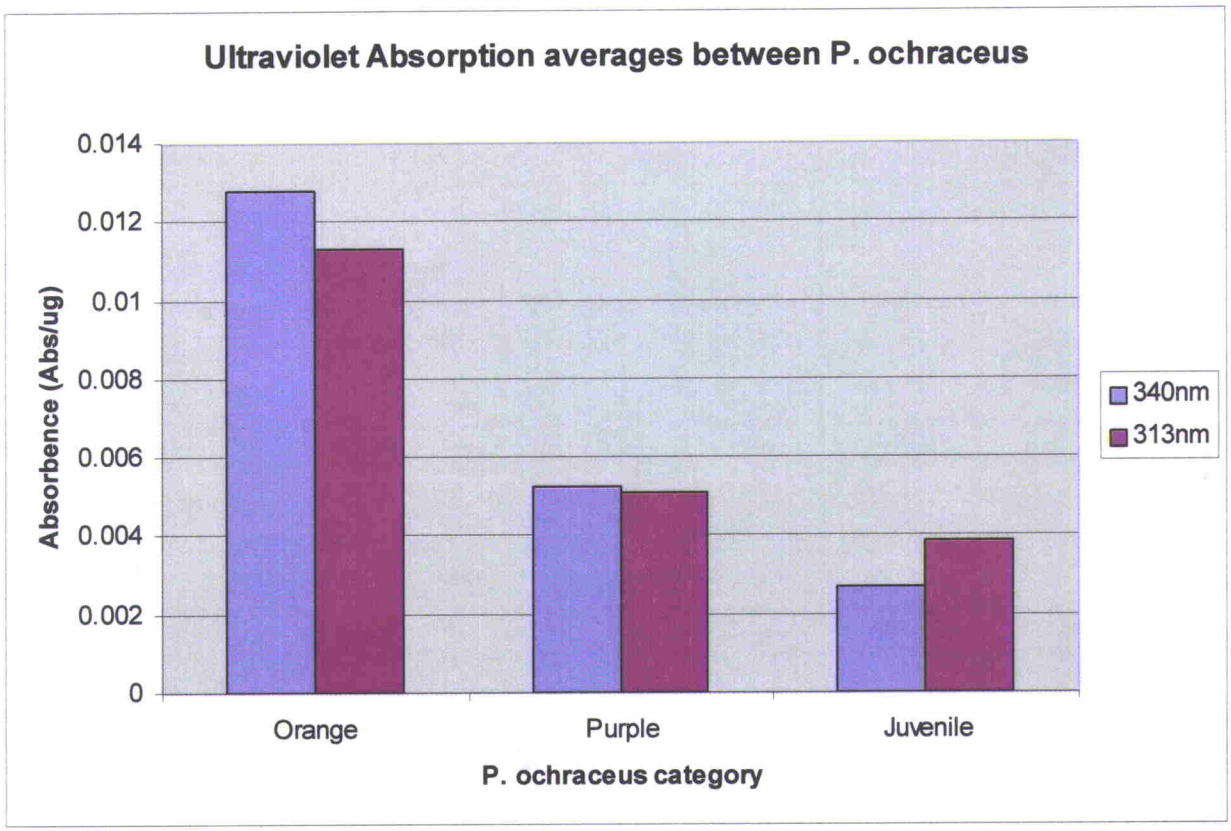

Figure 1. Ultraviolet absorbance per ug of protein in *P. ochraceus*. The groups are classified as adult orange, adult purple and juvenile. Values are from measurements taken in a spectrophotometer at 313nm and 340nm. The standard deviations were: at 313nm 0.000206 for orange, 0.008183 for purple and 0.002034 for the juveniles. At 340nm 0.001865 for orange, .000233 for purple and 0.001831 for juveniles.

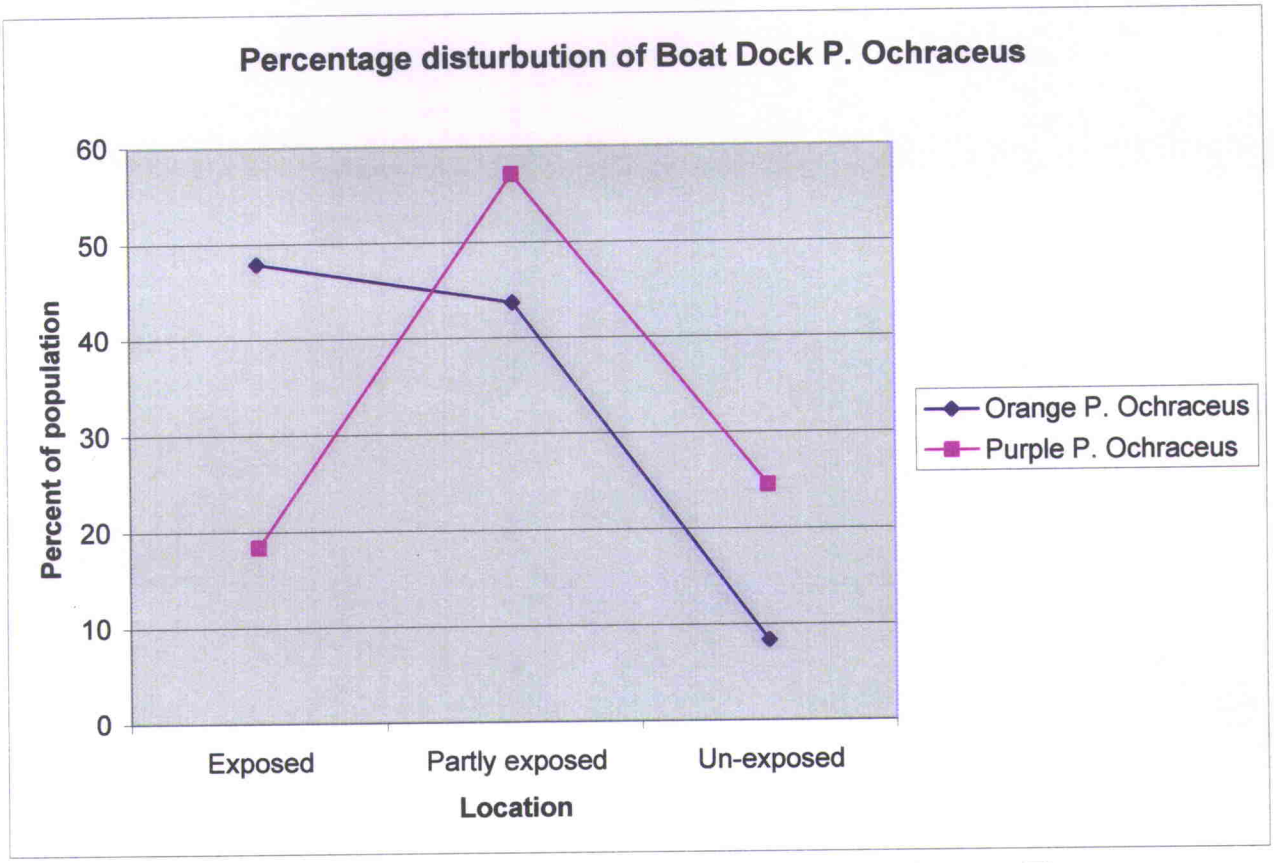

Figure 2. Relative distribution of adult purple and adult orange *P. ochraceus.* The percentage was calculated by the dividing number of individuals of in each location by the total number of that color counted. No juveniles were found.

Figure 1 shows adult orange *P. ochraceus* to have the highest level of UV absorbance per protein ug for both 313nm and 340nm. The juveniles were shown to have lower levels than the adult purple *P. ochraceus* at both 313nm but only slightly lower levels at 340nm. Figure 2 shows the majority of the adult orange *P. ochraceus* counted at the Boat Dock were exposed to sunlight and less than 8.3% of the population was unexposed. The majority of adult purple *P. ochraceus* were partly exposed, 18% were exposed and 25% were un-exposed. In collecting the juveniles at Fossil Point 100% of the 36 observed were protected from sunlight..

Dsicussion

My hypothesis was that there would be a relation between the level of exposure and the levels of MAAs in *P. ochraceus*. I also hypothesized that the adult orange *P. ochraceus* would have the highest levels of MAAs. Figure 1 shows orange *P. ochraceus* to have the highest levels of UV absorbance and the juveniles showed the lowest. Figure 2 confirms my observation of orange *P. ochraceus* being more exposed to sunlight than the purple morph. These two figures create a relation between UV absorbance and the category of *P. ochraceus* (adult orange, adult purple or juvenile. This supports my hypothesis that the levels of exposure are related to the levels of MAAs and would seem to imply a relation between the level of sunlight and the levels of MAAs. This seems logical but it is unknown if animals such as *P. ochraceus* can synthesize

MAAs. MAAs can be found in algae, marine phytoplankton, and zoozanthellae living in symbiosis with corals (Laurion 2002, Newman *et al.* 2000). This seems to make a direct relation between levels of sunlight and levels of MAAs in *P. ochraceus* impossible. A study looking at MAA intake in sea hares (*Aplysis dactylomela*) showed that MAA levels are a reflection of MAA levels in the diet (Carefoot 2000), so perhaps there is a correlation between the levels of exposure and diet. *P. ochraceus* commonly preys on mussels and these mussels are filter feeders therefore able to take in MAAs from food sources such as phytoplankton or algae. Perhaps there is a relation between the levels of MAA intake in mussels and the level of exposure, say more exposed mussels are subject to more wave action and are able to filter in more MAA containing food. Then the *P. ochraceus* in the more exposed areas eat these mussels with elevated MAA levels and as result those *P. ochraceus* have higher MAA levels. This theory would be difficult to test and it does not explain why the orange *P. ochraceus* have higher MAA levels than the purple morph. It is possible that the exposed and un-exposed *P. ochraceus* have radically different diets and this is causing the difference in MAAs. The low levels of MAAs in juveniles is logical because they have are young and have not ingested as large amounts of MAA containing food as the adult purple and orange. It would be very interesting to try to determine why the orange and purple *P. ochraceus* have different levels of MAAs in their tissue. There are many possibilities as to how these adult *P. ochraceus* are acquiring different levels of *P. ochraceus.* It could even be possible that they have developed a pathway that allows them to synthesize MAAs, this could be a very important discovery. Many new and interesting experiments could come out of determining the cause of this relationship. I would also like to repeat this experiment because with the small sample size there are large potentials for error. In my juvenile samples the standard deviation was half the size of the actual value and one of the data points seemed abnormally high. More samples would help make my data stronger. Despite this high standard deviation there is still a large difference in the MAA levels in the orange and purple morphs and I am very confident in this data.

References

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