Nematocyst Concentration in *Anthopleura artemisia*, Variation Between Pigments

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Summer 2008

Introduction

Three species of *Anthopleura* inhabit the Eastern Pacific Coast, *Anthopleura elegentissima*, *Anthopleura xanthrogrammica* and *Anthopleura artemisia*. Of these anemones extensive research and literature has been generated in both morphological descriptions and behavior for *A. elgentissima* and *A. xanothrogrammica*. However, little scientific data has been generated for *A. artemisia*. *A. artemisia* ranges from Alaska to southern California and inhabits sandy areas attached to large shell or rock. It has distinct morphology expressed by tentacles with distinctive white bands (Sept 2002).

In my second exploratory I made observations of aggressive feeding behavior in the *A. artemisia*, particularly a rapid paralysis of prey in comparison to *A. elegentissima* (Sease 2008). Paralysis is likely induced by the firing of nematocyst, which discharge and release toxins into prey upon proper stimuli (Muscatine 1974).

The hypothesis of this study is that the distinct white bands articulated on the tentacles of *A. artemisia* are of higher nematocyst concentrations then that of the darker bands.

Methods and Materials

Three individuals of *Anthopleura artemisia*, of similar oral disk diameter, were sacrificed in order to obtain tentacles of similar length. Dissections occurred within 1 hour of analysis, and anemones were rinsed first in fresh water, then in distilled water.

A comparison of concentration, classification of nematocyst and the percent each band was relative to total tentacle area were used in my analysis. Anemones were kept in outside water tables at 11.5-12°C. *Anthopleura artemisia* samples were collected on July 15^{th} from Fossil Point from excavated boring clam hole. Tentacles were selected on the basis of most distinct pigment variation (clear bands or stripes)(Figure 3).

Each tentacle was initially accessed on total area under the magnification of 4x on a compound microscope. The total area was then used in order to calculate percentage light bands and percentage dark bands. A Fuchs Rosenthal Ultra Plane Hemacytometer was used to estimate area, and percent dark/light pigments. It was comprised of a 1/16sq mm grids. Each specimen was photographed, and then the grid was used to make geometric approximations. Tentacles were compressed by placing 2-3 cover slips over the slide to ensure the thinnest section was analyzed.

Each tentacle was then placed on a new slide (Hemacytometer several cm thick, which did not allow for sufficient magnification for nematocyst analysis) and sections were selected at random for nematocyst concentration survey. Magnification was set to 40x on a compound scope, and 'quadrats' of the diameter (area = $0.0123mm^2$) of the view spot served as parameters of nematocyst enumeration. Methylene blue was applied to the slide in order to enhance the visibility of cnidae.

A clicker was used to count nematocyst in the view spot of the scope, which had the radius of $.0625mm^2$, and an area of $1.23 \times 10^{-2}mm^2$. Each 'quadrat' was given an approximation of dark/light percentage and values collected were magnified (i.e multiplied by 3 if present in 1/3 of the area) to give density estimates for each tentacle. Five 'quadrats' were sampled on each specimen, and average values were then taken to produce a comparison of nematocyst concentrations between individuals and between bands.

Results

The average concentration of nematocyst for dark bands was 24705 nematocyst/mm^2. This value was higher than the concentration found in the light bands of 21300 nematocyst/ mm^2(Figure 1). Dark bands expressed greater percentage of total area (average 10%) for all samples than light bands (average 8.6%) (Figure 2). The nematocyst identified were *Bastitrichous Isorhiza* (Muscatine 1974) (Figure 5).

Figure 1. Comparison of Nematocyst concentrations between the dark and light color bands

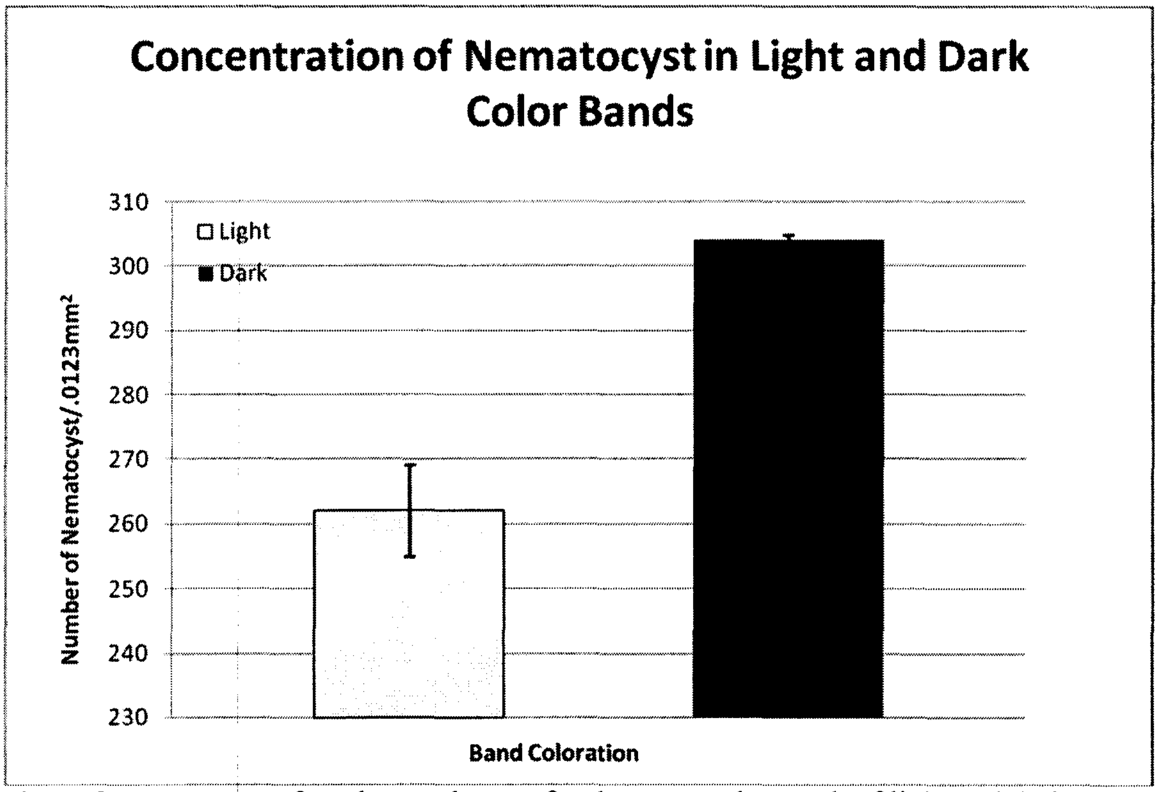


Figure 2. Percentage of total tentacle area for three tentacles used, of light and dark bands.

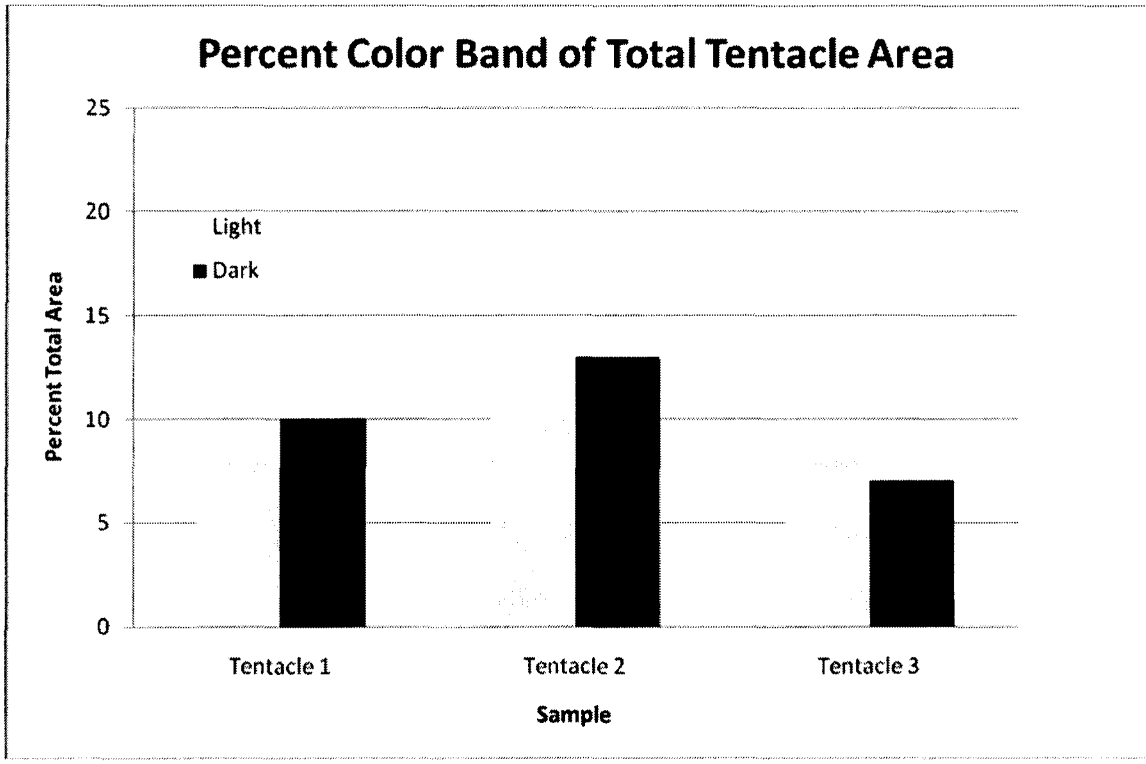


Figure 3. Photograph of *A. artemisia* under dissection scope.

Figure 4. Photograph of *A. artemisia* tentacle under 4x magnification of compound scope.

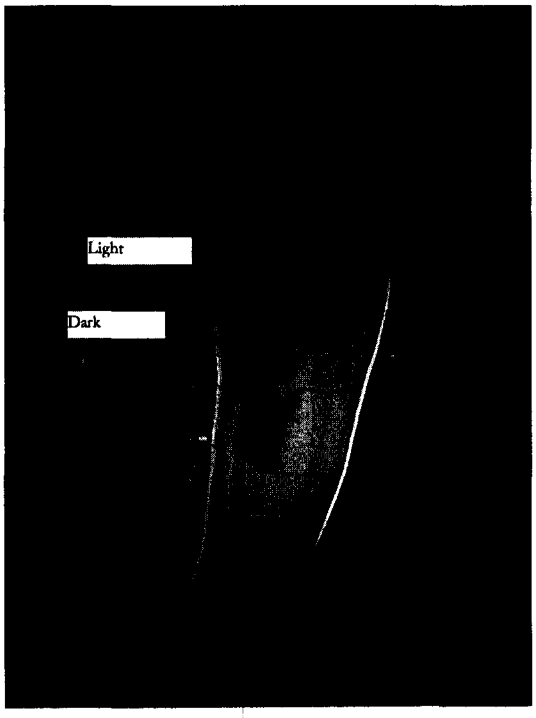
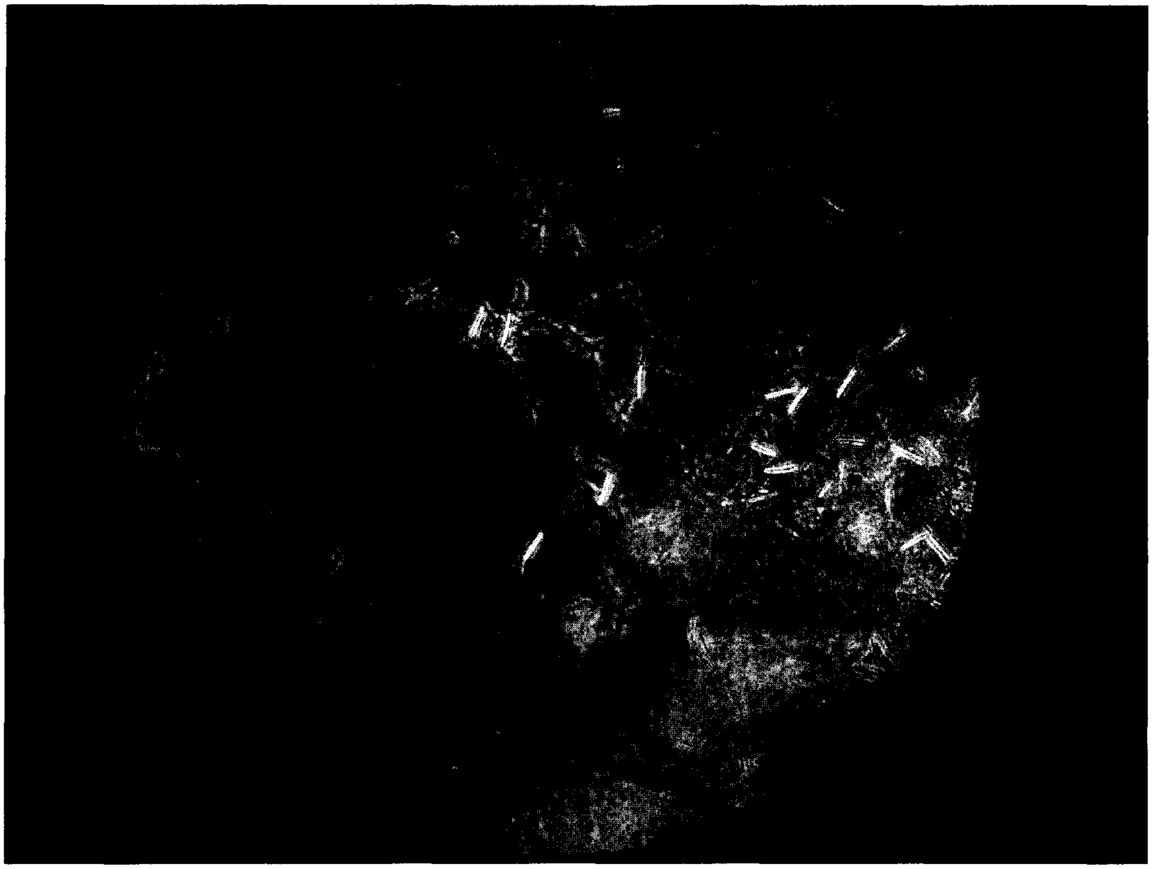

Light
Dark

Figure 5. Image of firing nematocyst, following the addition of methylene blue and acetic acid.



Discussion

My data showed results contrary to my hypothesis. White bands had lower concentrations of nematocyst/mm^2 than that of dark bands. It is not likely that the rapid paralysis of prey exhibited by *A. artemisia* is attributed to the distinctive white bands. The slightly higher concentration of nematocyst in the darker bands may or may not be an influential factor in their aggressive behavior. A comparison between nematocyst concentration with A. *elegentissima* and *A. artemisia* would provide a better reference for density significance. Although the concentrations do not reflect any significant differences within this *Anthopleura* species, it is likely that variation between species might. Anemones have large variety in nematocysts, including regional specialization and substantial differences from species to species (Francis 2004). Composition of the nematocyst could be more responsible for the variation rather than the density for rapid paralysis from *A. artemisia.* Some literature suggests that distinct color morphs within *Anthopleura orientalis,* found in the Sea of Japan, are associated with variation in nematocyst type (Manchenko 2000). This suggests again the possibility that nematocyst composition could be a factor in the coloration of this *Anthopleura* species.

A compound scope with a mounted camera would be beneficial in this survey. Much larger sample sizes and more individuals would also greatly improve the quality of my data. Survey of the tentacle area lacking color bands would serve as a good control, or indicator of basal levels of nematocyst concentration.

A. artemisia exhibits minimal variation between nematocyst densities in light and dark color bands. Errors such as small data sets, inexperience in microbiology, as well as lack of a comparison between two *Anthopleura* species, make it difficult to make any concrete conclusions. Basic morphological observations such as density estimates as well as percent area within the *A. artemisia* tentacles do offer preliminary data in investigating the cause of rapid prey paralysis by the moonglow anemone.

Work Cited

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