SEQUESTERING OF NEMATOCYSTS: TURN OVER RATE IN *Aeolidia papillosa*

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Abstract: Nudibranchs rely on assimilating the defensive mechanisms like toxicity and nematocysts from their pry in order to secure themselves from attack. In aeolid nudibranchs, the nematocysts they sequester at the tips if their cerata, in specialized glands known as cnidosacs, are believed to degrade over time. In this study the nudibranch *Aeolidia papillosa* was observed to see if a rate of nematocyst turnover could be identified. After a four day study of examining nematocyst expression in six specimens of *A. papillosa* exposed to the prey *M. senile*, a significant linear rate of increase was observed (R^2=0.9885).

INTRODUCTION

Upon first glance, nudibranchs seem rather unlikely creatures. Akin to other gastropods, nudibranchs are typically slower than most potential predators. However, unlike other gastropods and most other mollusks for that matter, nudibranchs have evolutionarily lost their defensive shell. Their unlikely-ness is capitalized by the conspicuous nature many of them seem to exhibit. Yet, despite all these perceptible setbacks, there are few reports of predation on nudibranchs in nature and have no known primary predators (Frick, 2003). What is not immediately forthcoming within nudibranch biology is their resourcefulness to assimilate other fauna's defensive faculties. For many nudibranchs, largely in aeolids, "klepto-cnidea" is observed to take place in many species as the nematocysts of their cnidarian fare are sequestered and functionally expressed in the nudibranch's cerata.

As aeolids eat their cnidarian food-stuff, they are able to consume and digest nematocysts whole an unfired. Though the full explanation as to why nematocyst arenot triggered during predatory encounters by the nudibranch is unresolved, it is well agreed

the mucus coating it secretes possess seemingly species specific, anti-firing properties (Mauch, 1997). As their cnidarian food-stuff is ingested through the mouth and passes through the pharynx, nematocysts are transported by ciliary and peristaltic contractions from the stomach to the cerata lumen. In the cerata, nematocysts are separated from the liquid and dry food, drawn to the tips of the cerata and stored in specialized compartments known as *cnidosacs* (**Fig 1**) (Martin, 2003).

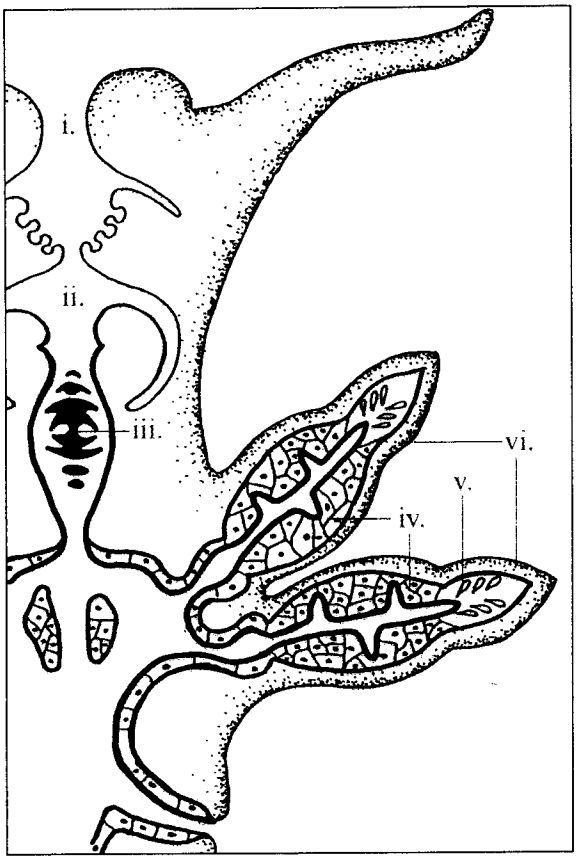

Figure 1: Artistic rendition of an aeolid cross section. i.: lips, ii.: pharynx, iii.: radula, iv.: digestive glands, v.: cnidosacs, vi.: cerata.

Interestingly, the sequestered nematocysts are not believed to be permanent additions, even if unfired. Recent studies have suggested that nudibranchs have a great deal of control over what kind of nematocyst they sequester. Nematocyst expression to be

contingent on food availability: where specimens gradually express an increased rate of the nematocysts founds within their most recent food source. No matter how they are attained, nematocyst must be continually be replaced as studies have shown nematocyst firing-ability to be compromised and compositional degradation over time (Day, 1978).

The exact species-specific nematocyst turnover rate has been poorly studied, though there is evidence that expression of nematocysts in some species as son as two hours (Day, 1978). A species of Pacific Northwestern nudibranch commonly referred to as the shaggy-rug nudibranch, *Aeolidia papillosa*, is an interesting subject. *A. papillosa* is known to commonly eat a wide variety of anemone species (Kramer, 2004). I hypothesize that specimens of *A. papillosa* will show a noticeable, constant turnover in nematocyst types when presented with a species different from those they had previously been feeding on.

METHODS

Specimens of *A. papillosa* used in were collected from Cape Arago and the floating docks comprising the Charleston Marine docks in Charleston, OR., during late July. Eight specimens were collected altogether. Upon capture, note of all anemone species within one meter were noted, initial nematocysts types analyzed and separated in plastic Tupperware® containers with holes and placed in a holding tank with running seawater and starved seven days prior to testing. Fortunately, all six specimens collected at Cape Arago were both found around either *Epiactis*, or *Anthopleura* and expressed nematocyst types and morphologies found in these two. In this experiment *Epiactis* and *Anthopleura* are not differentiated as they both have the same two nematocyst that are

expressed in *A. papillosa:* basitrichous isorhiza and holotrichous isorhiza (Day, 1974) Their type and morphologies cannot be distinguished from each other without use of an electron microscope (Muscatine, 1974).

After the starving duration, specimens of *A. papillosa* were placed in a 32x16cm plastic CritterKeeper® with flowing seawater and three <4cm tall *Metridium senile* collected from the Charleston Marine docks for four days. *M. senile* was used as it is a known food source of *A. papillosa,* but the two nematocyst it has which are expressed by *A. papillosa* are distinguishable from those of *Epiactis* and *Anthopleura. M. senile* has microbasic amastigophore and basitrichous isorhiza that are twice the length of those found in *Epiactis* and *Anthopleura.*

After being "fed" *M. senile*, a sample of cerata was seize and extended using a pair of laboratory forceps and trimmed at the base with a scalpel. The tip of the cerata was placed over a clean slide where it was cut once longitudinally and repeatedly laterally with a scalpel. A single drop of 0.1% methylene blue was used to stain and enhance visualization of the cells. A coversheet was placed over the diced sample. Applying force in concentric circles, the blunt end of an eye-dropper was used to "smudge." Sample sets were fashioned by randomly selecting a portion of the smattered cerata tip under 100x magnification and counting all capsules within view and their respective origins, noted. This was repeated for each individual at the beginning of each day for four days.

RESULTS

I had hypothesized that a positive, constant rate of nematocyst turnover from the initial species to the more recently exposed one. A linear increase in the expression of *M. senile* specific nematocysts (R^2=0.9885) was observed between six specimens over the course of four days (**Fig 2**) with a projected 100% turnover in nematocysts taking place just shy of six days. Presented with these results, I fail to reject my hypothesis. Two *A. papillosa* were found toward the end of the experiment on the Charleston Marine docks. This area is interesting as *M. senile* is the only common anemone apparent. For two day their cerata were examined for nematocyst types and both days they showed 100% *M. senile* types.

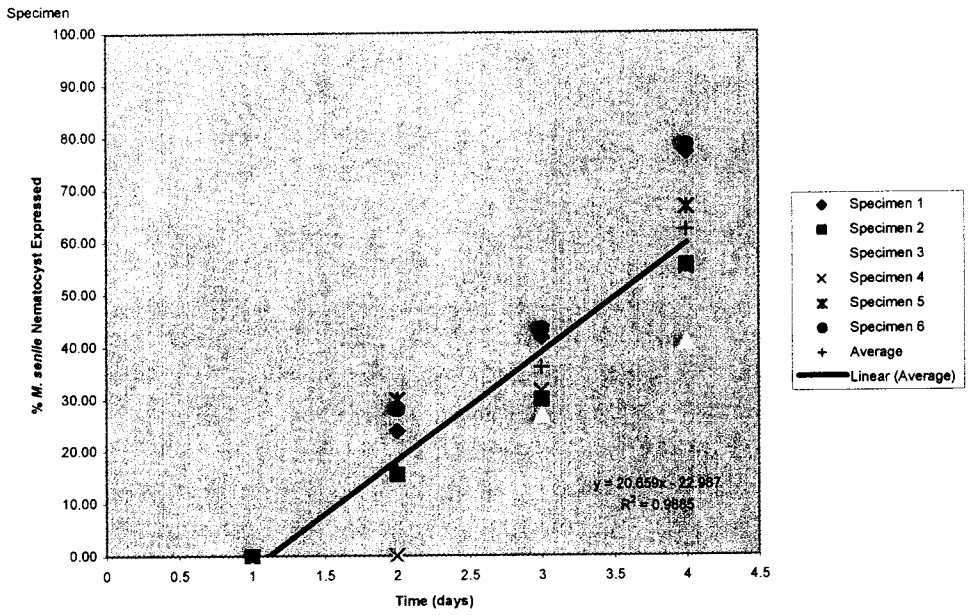

Figure 2: The percent *M. senile* specific nematocysts found in samples of six different *A. papillosa* that previous to the experiment had not been feeding on *M. senile*.

Figure 3: Example of a sample set taken from *A. papillosa* after 4 days of feeding on *M. senile* (40x magnification).

DISCUSSION

A. papillosa showed a fairly high rate of turn over, however it is not obviously clear why this may be necessary considering it is believed to take two weeks before most nematocyst have become nonfunctional (Day, 1978). In a study done by Frick (2003), the expression of different nematocyst types by nudibranch *Flabellina verrucosa* correlated with the type of predator they were exposed to. In this study the presence of a predator was not an expressed factor. However, it should noted that a technicality during the last 24 hour of Specimen-1 may support Frick's findings. Another experiment involving a roughly, 24cm diameter pycnipodia had been set on top of Specimen-1's experimental environment and allowed to drain water into it. Specimen-1 on the last day was the only specimen in this study to have microbasic amastigophore >120m m in length. All other microbasic amastigophore observed in this study were <65m m in length. *M. senile* is quite interesting in that microbasic amastigophore are found on both catch tentacles and acontia threads, yet can be differentiated in that those found on acontia threads are typically almost twice as long (in a previous study, I found microbasic amastigophores on

acontia threads to between 120 and 124m m). These absurdly large microbasic amastigophore of *M. senile* acontia threads are thought to have co-evolved with the presence of *A. papillosa* as a deterrent. In fact, an experiment done by Edmunds (1976) looking at the feeding of *A. papillosa* on M. senile found that all *A. papillosa* which had fed from the anemone's column not only died, by were found to have large numbers of large, discharged microbasic amastigophore in their pharynx and stomach. Perhaps the pycnipodia presented itself to be such a threat that a potentially deadly-in-itself defense was taken up. However, more direct studies are needed to accurately asses the factors of risky food choice in *A. papillosa.*

REFERENCES

Day, R. M. and L. G. Harris (1978). Selection and turnover of coelenterate nematocysts in some aeolid nudibranchs. *J. The Veliger* **21**(1): 104-109.

Edmunds, M., G. W. Potts, R. C. Swinfen, and V. L. Waters (1974). The feeding preferences of *Aeolidia papillosa* (L.) (Mollusca, Nudibranchia). *J. Mar. Biol. Assoc. UK.* **54**: 939–947.

Frick, K. (2003). Response in nematocyst uptake by the nudbrach *Flabellina varicose* to the presence of various predators in the southern Gulf of Maine. *J. Biol. Bull* **205**: 367-376.

Kramer, A. and L. Francis (2004). Predation resistance and nematocyst scaling for *Metridium senile* and *M. farcimen. J. Biol. Bull.* **207**: 130-140.

Martin, R. (2003). Management of the nematocysts in the alimentary tract and in the cnidosacs of the aeolid nudibranch Cratena peregrina. *J. Mar. Biol* **143**: 533-541.

Mauch, S. and J. Elliot (1997). Protection of the nudibranch *A. papillosa* from the discharge of the sea anemone *Antheleura elegantissima. J. The Veliger* **40**(2):148- 151.

Muscatine, L. and H. M. Lenhoff (1974). *Coelentrate Biology: Reviews and New Perspectives*, Academic