Quantifying Reproductive Behavior in the Sea Anemone *Nematostella vectensis*

William Alexander, UNC Charlotte
Dr. Adam Reitzel and Hannah Justin, Department of Biological Sciences

UNIVERSITY OF NORTH CAROLINA CHARLOTTE

Introduction

The Starlet Sea Anemone Nematostella vectensis (*Nv*) exists widely in coastal estuarine environments across North America. Given its ability to survive in diverse environments, spawn easily, and produce ramets or clones, *Nv* has become a popular cnidarian model organism. Applications for *Nv* are wide-ranging and include evolutionary developmental biology (EvoDevo), cell biology, and genomics.

In order to properly maintain *Nv* in a lab setting it's essential to understand their reproductive behavior patterns. Despite this, research in the field has yet to establish quantitative comparisons of reproductive behavior. As such our work explores how alterations in husbandry mechanisms such as temperature, feeding schedule, and geography impact both sexual and asexual reproduction.

https://acumen.cas.lehigh.edu/content/neurogenesis-sea-anemones

https://www.technologynetworks.com/tn/editors-pics/starlet-sea-anemone-nematostella-vectensis-357928

Objectives

The purpose of this research is to deepen understanding of the reproductive behavior patterns within *Nv*. This project is guided by the following goals:

1) Optimize feeding conditions for reproductive frequency

2) Understand how variations in temperature impact sexual and asexual reproduction.

3) Compare populations from different locations to identify geographic differences in sexual and asexual reproduction.

Additional husbandry mechanisms including salinity and light exposure will be considered in future experiments.

Methods and Data Collection

Stratified Feeding

To determine an optimal feeding schedule for *Nv* the following was carried out:

1) Transfer of anemones from the general lab population into new habitats. Habitats consisted of 10 anemones each in 2.6L Pyrex dishes with Onyx Sand Substrate, and 30 ppt artificial seawater.

2) Artemia feedings of 1mL on a schedule of either 1,3, or 5x/week

3) Population counts 1x/week for 5 weeks

4) Statistical analysis using the SASS software

Temperature and Geography

To investigate the reproductive impact of temperature and geography *Nv* were separated into the following groups:

1) *Nv* collected from a Maryland field site (MD)

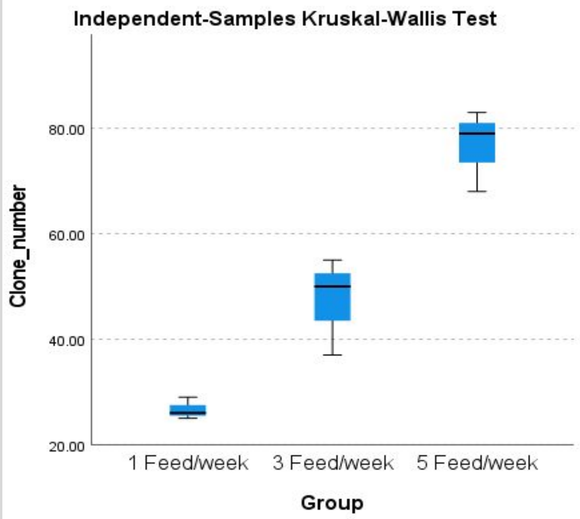
2) *Nv* collected from a Florida field site (FL)

3) FL anemones incubated at 35℃ for one week and at 25℃ for the following two weeks. (FL Incubated)

All groups consisted of five anemones each transferred into a separate 470mL Pyrex dish habitat. Artemia feedings of 250 µL were performed 3x/week along with daily clone counts. Clones were separated into new habitats while eggs were transferred out for fertilization. Data was then analyzed after 21 days.

Preliminary Results

Figure 1



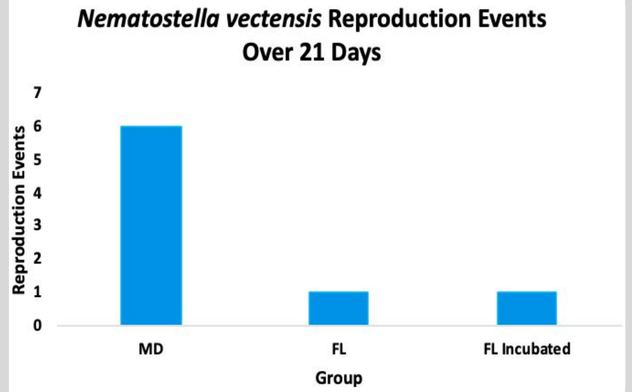
This Kruskal-Wallis boxplot showcases the amount of *Nv* clones produced in each stratified feeding group. Data was collected on three different cohorts for each feeding group. The solid line indicates the average clone number while the box itself shows the upper and lower extremes.

Figure 2

Sample 1-Sample 2	Sig.	Adj. Sig.[a]
1 Feed/week-3 Feed/week	.180	.539
1 Feed/week-5 Feed/week	.007	.022
3 Feed/week-5 Feed/week	.180	.539

The shown pairwise alignment compares the three stratified feeding groups. Each row evaluates our null hypothesis that the sample distributions are the same statistically. The significance (P) value is shown for each comparison along with a P value adjusted by the Bonferroni correction. The second row shown in red yielded a significant P value (<.05) .

Figure 3



This figure shows the number of reproduction events for three experimental groups over 21 days. The Maryland group saw 6 events while both Florida groups only saw one.
The Maryland group is also the only group to have an instance of egg production.

Conclusions

The following conclusions can be drawn from analysis of the collected data:

- Despite initial expectations that all feeding groups would have significant differences only the difference between the 1x/week and 5x/week groups was significant.
- A 5x/week feeding schedule yields the highest rate of clone production in *Nv* when compared to the 1x/week and 3x/week groups
- Within the first 21 days, reproductive frequency remains low in the Florida groups. These data suggest that *Nv* from our Maryland population reproduce faster than those from the Florida site. Additional data is necessary to confirm this trend and to understand the impact that temperature has on reproduction.