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# The Impact of Bacterial Association on the Regenerative Properties of *Nematostella vectensis*

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# Abstract

The sea anemone, *Nematostella vectensis,* is a marine invertebrate capable of regeneration. *Nematostella vectensis* is associated with a bacterial consortium (microbiome). This research seeks to understand the role of the bacterial community in regenerative development. We hypothesize that the bacterial community will either (1) help the organism, (2) harm the organism, or (3) be indifferent. To test these hypotheses, we conducted two experiments: one comparing the effects of antibiotics on regenerative growth and another on the addition of bacteria. Twenty *N. vectensis* were kept in a container filled with artificial seawater (ASW) at 15 parts per thousand (ppt) and fed *Artemia salina*. After being fed, *N. vectensis* were left to grow for 48 hours. For the first experiment, we treated nine bisected specimens of *N. vectensis* with a cocktail of antibiotics and kept non-treated bisected individuals in sterile ASW. This cocktail consisted of neomycin, kanamycin, ampicillin, and chloramphenicol at 50µg/mL each. Twenty *N. vectensis* were used in the second experiment, with one group of 10 bisected individuals treated with the antibiotic cocktail and the other remained in sterile ASW. Both were exposed to 10^5 *Vibrio alginolyticus* cells. Groups in both experiments were given 7 days to regenerate. Phenotypic observations were performed to judge regeneration. Upon observation, it was seen that antibiotics positively impacted recovery of the bisected individuals. Additionally, the natural community helped protect the organism against the invading *V. alginolyticus*. Findings from this study lend some insight into the role bacteria have on their host organism.

# Introduction

## 1.0 Objective

*Nematostella vectensis* (*N. vectensis*) resides on the coasts of countries such as the United Kingdom and United States; they have also been seen in parts of Canada. Informally known as the 'Starlet Sea Anemone', they have been noted for their regenerative abilities (Mossman, 2000).

Bacteria are known to be ubiquitous. Various strains of bacteria exist that, when applied to living organisms, can serve to either 1) help it, 2) harm it, or 3) form a commensalistic relationship with it and neither benefit from the relationship nor harm the host. This project aims to take a look into the regenerative abilities of *N. vectensis* and the role bacterial communities play.

## 1.1 Hypothesis

Prior to this project, we formed some hypotheses about how bacterial association with *N. vectensis* may affect organismal regenerative development. We hypothesized that bacterial association with *N. vectensis* will either inhibit, promote, or have no effect on organismal regeneration.

To prove this hypothesis, two experiments were performed. One investigated the regeneration of *N. vectensis* under antibiotic conditions. The other looked into the addition of vibrio bacteria and its effect on those treated with or without antibiotics.

## 1.2 Background

*Nematostella vectensis* belongs to the phylum Cnidaria, class Anthozoa, order Actiniaria, and family Edwardsiidae. They exist in the wild as free-floating, isolated marine polyps and

burrow themselves in the sediment of the ocean floor (Mossman, 2000). Various populations of *N. vectensis* possess the unique ability to reproduce both sexually and asexually. The summer and fall seasons mark the times when the organism sexually divides, while year-round they are believed to possess the ability to reproduce asexually (Mossman, 2000). Reproducing asexually involves the organism splitting in half, this process is known as cloning. Sexual reproduction, on the other hand, requires a male and female breeding, resulting in genetically-diverse offspring. *Nematostella vectensis* relies on dispersive fertilization for successful reproduction (Reitzel, et al., 2007).

Regeneration has been an item of interest for researchers in evolutionary developmental (evo-devo) biology for some time. Bilaterians are a group of widely diverse animals (Layden, et al., 2016). To uncover how this group grew to become very diverse, as recorded in a literature review by Layden, et al. (2016), scientists sought to uncover traits of its most common ancestor, the urbilaterian, by studying the Cnidarian animal groups. The Cnidarian animals, a close relative of the bilaterians, were sought to study (Dunn, CW, et al., 2008; Ryan, JF, et al., 2013, and Hejnol, A, 2009; Layden, et al., 2016). *Nematostella vectensis*, a member of the phylum Cnidaria, was chosen to study because of how developed it was and how familiar as a model organism it was for anthozoan Cnidarians (Layden, et al., 2016). For this reason, our research decided to focus on *N. vectensis* for the purpose of investigating its regenerative abilities.

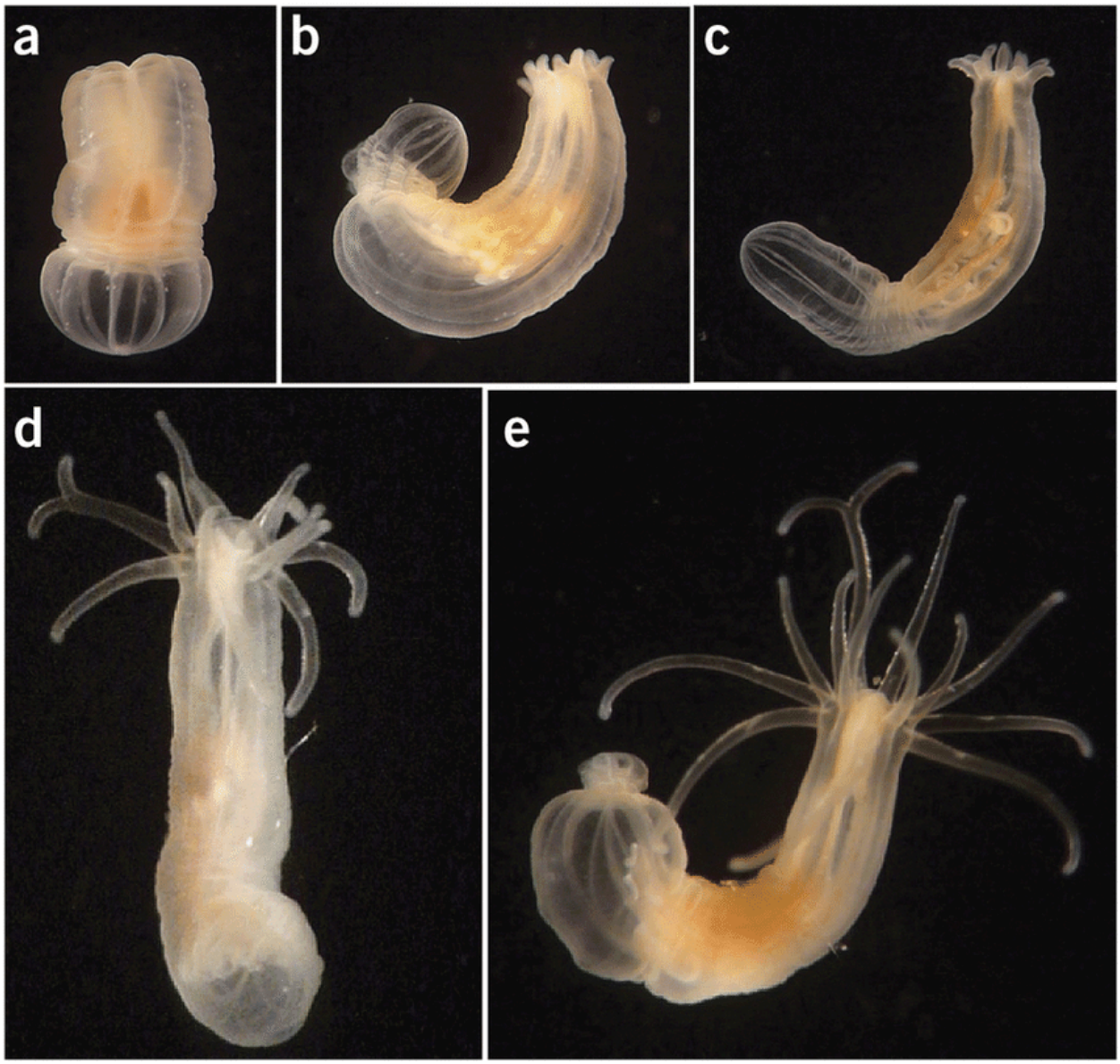
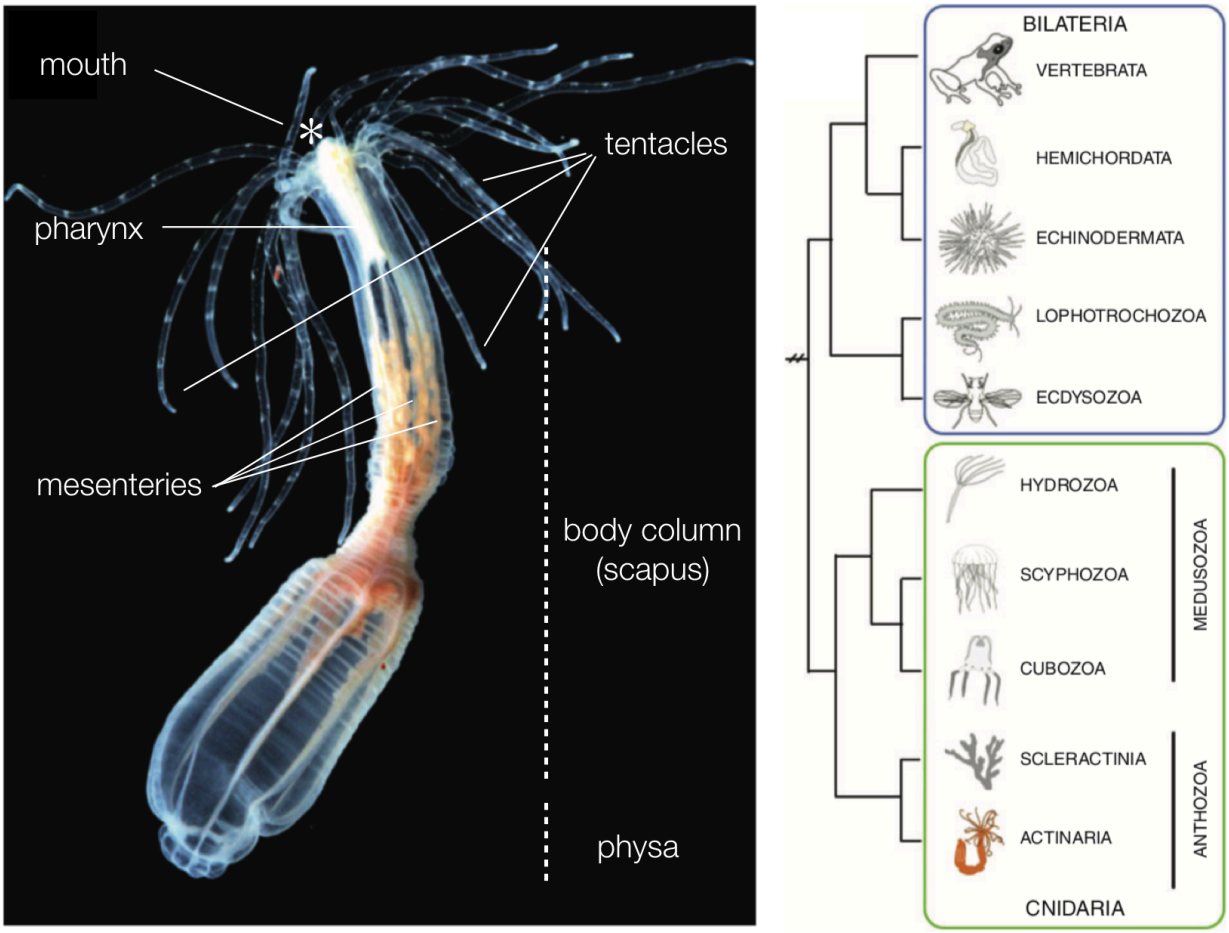



Figure 1.0: This image shows the stages of regeneration within the *N. vectensis* sea anemones. Obtained from Stefanik, et al., 2013.

The physical structure of *N. vectensis* can be categorized into an oral and an aboral end. The body column makes up the oral end and the phsya makes up the aboral end (Layden, et al., 2016). The body column makes up most of the organism, and is composed of the mouth, tentacles, pharynx, and mesenteries. In our experiment, we called this section the ‘head’. The

physa of the sea anemone is the clear looking portion of the end of the organism (Layden, et al., 2016). For our experiment, we called this the 'tail' end of the organism.




*Nematostella vectensis* is an anthozoan cnidarian sea anemone and a adult polyp is shown on in the left panel. Phylogeny showing the sister relationship of cnidarians to bilaterians and *Nematostella's* position within the cnidarians. From Layden et al., 2016.

Figure 1.1: The anatomy of *N. vectensis*. This image shows the various parts of the starlet sea anemone, as well as the phylogenetic tree of the organism. Obtained from "Nematostella: About *Nematostella vectensis*", n.d.

# Materials and Methods

## 1.3 Sample Preparation and Procedures

To perform this experiment, we utilized a pyrex dish and 15 parts per thousand (ppt) Instant Ocean artificial sea water (ASW) for storing *N. vectensis*. We fed *N. vectensis* up to three times *Artemia salina*, commonly known as brine shrimp. These organisms were fed in order to have the necessary amount of energy to fully grow and regenerate in the future.

### A) Antibiotic experiment

First, 18 individuals were manually pippetted to a glass plate and fed some *Artemia salina*. After letting them sit and grow for 48 hours, two glass bowls of 15 ppt ASW were used to separate the experimental group from the control group. Each group consisted of 9 individuals (n=9). These organisms sat in the bowls for three days, in order for us to make sure all of the preexisting bacteria were dead. After 3 days, they were rinsed one hour before cutting procedures began. The control group remained in untainted 15 ppt instant ASW. The experimental group was treated with a cocktail of antibiotics, which included 50 micrograms (µg)/milliliters (ml) of neomycin, 50 µg/ml of kanamycin, 25 µg/ml of ampicillin, and 20 µg/ml of chloramphenicol. Forceps and scalpels were used to cut the organisms in half along the physa, effectively separating the oral body column from the aboral end. Three six well plates were used to separate all bisected anemones into individual wells. They were separated and labelled as either AH and NaH, denoting antibiotic and nonantibiotic heads, or AT and NaT, denoting antibiotic and nonantibiotic tails. Those classified as AH were destined to regrow their physa. Those in the AT group were determined to regrow back their upper body column including their tentacles. Each well was filled with 5 mL of 0.2µm sterile filtered 15 ppt ASW. The duration of this experiment lasted 7 days. On days 2, 4, and 7, individual wells were checked under a light microscope, and photos were recorded at the observation intervals. A standard phone camera was used to take pictures of the creatures for personal reference. Growth observed over the course of days were recorded in a google spreadsheet.

B) Bacterial Experiment

The bacterial experiment was performed on a different population of *N. vectensis* than the antibiotic group to reduce any unforeseeable confounding variables. Two groups (n=10) were utilized for this separate experiment; they were separated into a control and experimental group. The control group was left in untainted 15 ppt sterile instant ASW. The experimental group was associated with a cocktail of antibiotics at concentrations of 50 (µg)/milliliters (ml) for neomycin, 50 µg/ml for kanamycin, 25 µg/ml for ampicillin, and 20 µg/ml for chloramphenicol. These organisms sat in the bowls for 48 hours to ensure weakening of the organismal microbiome. Overnight cultures of *Vibrio alginolyticus* were grown in Heart Infusion Broth for experimentation the following day. This culture was inoculated into fresh media 3 hours before exposure. To wash the bacterial cells, they were spun at 5,000G for 5 minutes, supernatant decanted and washed in 1mL sterile Phosphate Buffered Saline (PBS). This mixture was pelleted at 5,000G for 5 minutes, decanted again, and subsequently resuspended in 1mL sterile PBS.

After about 48 hours, *N. vectensis* were rinsed from the antibiotic exposures, if applicable, and then cut along the physa. They were pipetted into designated wells filled with 5 mL of sterile 15 ppt instant ASW. The bacteria used in this experiment were *Vibrio alginolyticus*, and $10^5$ cells were pipetted into the wells. These sea anemones were left over the 3 day weekend. The duration of this experiment lasted 1 week, or 7 days.

Observations followed and organisms were checked on days 3, 5, and 7. A standard phone camera was used to take pictures of the creatures for personal reference. Growth observed over the course of days were recorded in a google spreadsheet.

# Results

## 1.4 Evaluating *N. vectensis* growth

### A) Antibiotic Experiment

Upon observation and analysis of the photos taken, there was a notable difference in growth in organisms of the experimental group when compared to those in the control group. Those immersed in a solution of 15 ppt ASW and the cocktail of antibiotics fared better in terms of tentacle regeneration and general survivability than those in regular 15 ppt ASW aquatic environments. Due to this reason, it was believed that antibiotics positively impacted the recovery of bisected individuals. These results were interpreted as due to the harmful or indifferent effects of bacteria on the regeneration of the sea anemones. Refer to figure 1.2 for a graphical comparison of the results.

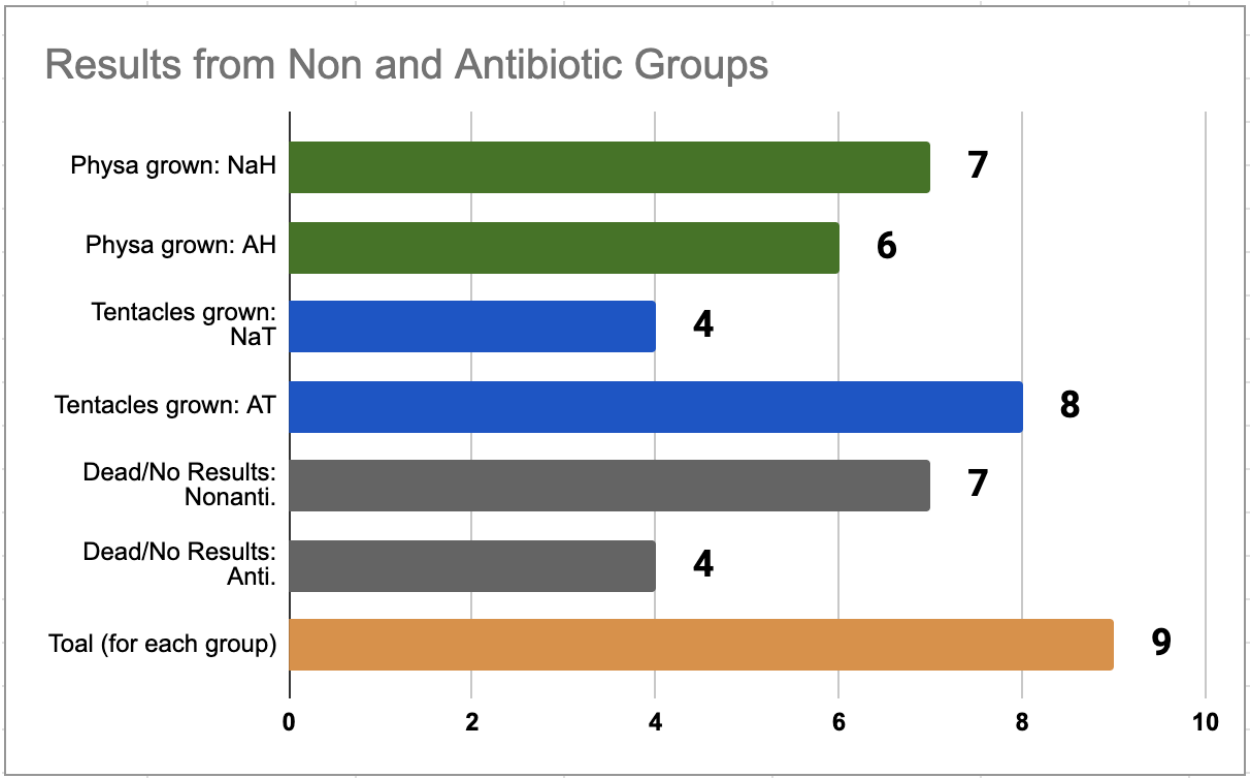



Figure 1.2: This bar graph shows the results obtained from both groups involved in the antibiotic experiment.

**B) Bacterial Experiment**

Upon observation and analysis of the animals, there was a slight difference in the growth of the sea anemones in the control group when compared to those in the experimental group. Every well contained $10^5$ cells of *V. alginolyticus*, however only the wells in the experimental group contained the cocktail of antibiotics. Due to this reason, it is hypothesized that the native microbiome acted as a buffer against invading bacteria.Refer to figure 1.3 for a graphical comparison of the results.

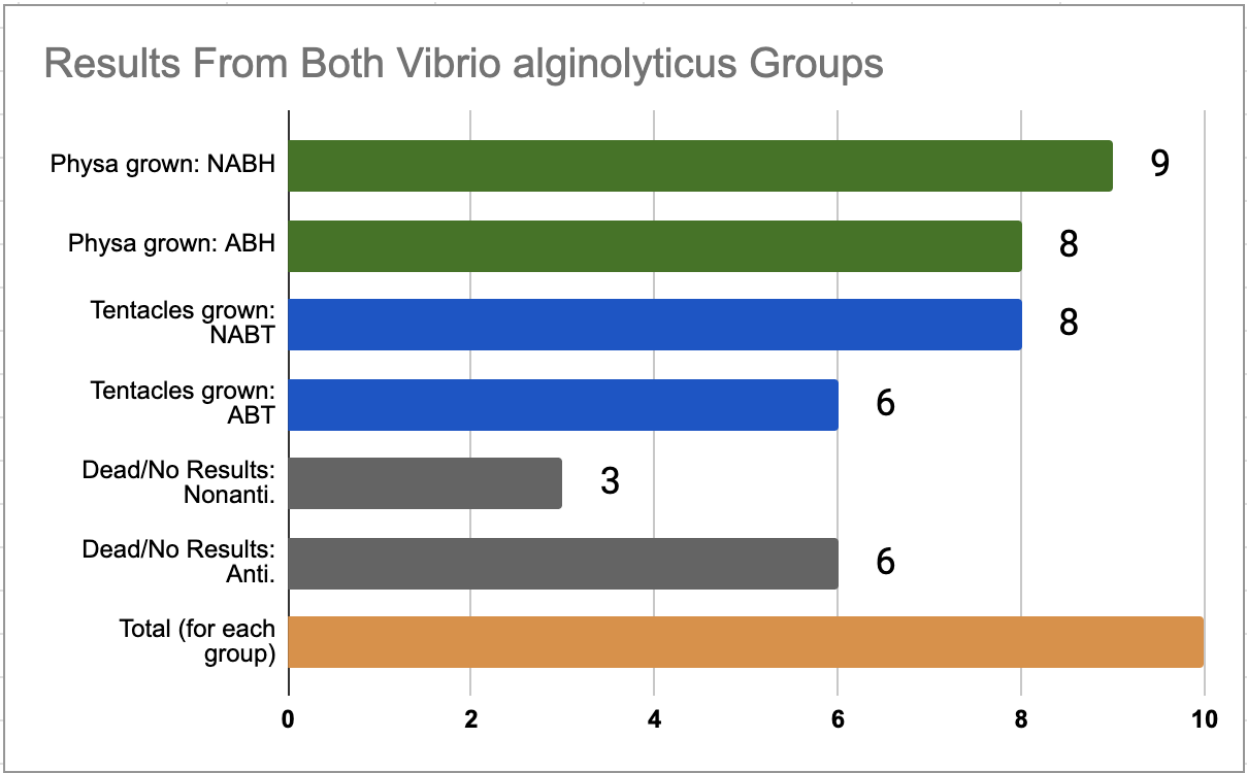



Figure 1.3: This bar graph references the results obtained from both groups involved with the bacterium *V. alginolyticus* in the bacterial experiment.

# Conclusion

## 1.5 Discussion

The starlet sea anemone, *Nematostella vectensis*, is a marine invertebrate with the unique ability to regenerate various parts of its body. The mechanism for this is not entirely known, however there is some indication that bacteria in the microbiome of the organism may have a role in this. For this experiment, we sought to investigate the effects bacteria have on *N. vectensis* during periods of regeneration through wound healing. Photos of phenotypic observations were taken to compare the growth of individuals between certain time frames within the timespan of seven days.

This study was split into two small experiments defined by the use of antibiotics and the addition of bacteria into the organismal habitat. From the study, we were able to infer a possible role bacteria may play in regenerative development. More studies and experiments will need to be conducted in order to get more accurate results.

## 1.6 Implications of this Research

Research into the regeneration of *N. vectensis* sea anemones lend some insight into the role bacteria may play in the act of regeneration. More research is needed to quantitatively understand the effect bacteria have on regrowth. Future understanding of organismal growth in connection to a bacterial microbiome has the potential to contribute to the field of regenerative medicine.

## 1.7 Note from the Author

Special thanks to Dr. Adam Reitzel for accepting me into his lab, and allowing me to partake in this project. I would also like to thank Quinton Krueger, a PhD student in the biological sciences, for his direct guidance. I would also like to say a word of gratitude to the individuals in the Reitzel lab who gave me a warm welcome and acted to assist me in the project.

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