Oregon Undergraduate Research Journal
20.1 (2022) ISSN: 2160-617X (online)
blogs.uoregon.edu/ourj
DOI: 10.5399/uo/ourj/20.2.4

Activation of Defense Mechanisms in the Nudibranchs *Peltodoris nobilis* and *Hermissenda crassicornis*

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ABSTRACT

Nudibranchs are marine invertebrates that use various defenses to deter predators. *Peltodoris nobilis* is a member of the superfamily Doridoidea and uses *de novo* chemical synthesis for defense. *Hermissenda crassicornis* is a member of the superfamily Aeolidioidea and uses nematocyst sequestration for defense. Past research explains the function and evolution of defenses in nudibranchs; however, it is unknown whether these defenses are active or passive. The goal of this research was to determine if the defense mechanisms in *P. nobilis* and *H. crassicornis* are active or passive and if one method is more effective at preventing predation than the other. It was hypothesized that the activation of defense mechanisms in both nudibranch species was active and that the defenses were equally effective at preventing predation. This hypothesis was tested by comparing the contact times of a juvenile *Glebocarcinus oregonensis* crab—a predator of nudibranchs—with an anesthetized and non-anesthetized nudibranch. There was no statistical difference in crab contact times between the anesthetized and control *P. nobilis* nudibranchs; however, the anesthetized *H. crassicornis* contact times with the crabs were statistically higher than the control. This suggests that the release of chemicals produced *de novo* in *P. nobilis* is passive, while the firing of sequestered nematocyst by *H. crassicornis* is active. The results also indicated that the control *H. crassicornis* contact times with the crabs were statistically lower than those of *P. nobilis*; however, the crabs demonstrated little predatory behavior across all trials. Therefore, this study cannot conclusively determine which defense mechanism is more effective at preventing predation.

1. INTRODUCTION

Nudibranchs ("nudi" [naked] "branch" [gill]) are marine invertebrates found in subtidal and intertidal zones around the world. Commonly known as sea slugs, they are members of the phylum Mollusca, class Gastropoda, and order Nudibranchia (WoRMS 2021). The order Nudibranchia consists of marine slugs that, unlike most other members of the class Gastropoda, do not have a shell for defense as adults. Biologists have posited that over time, the evolution of new defense mechanisms in nudibranchs made the protection offered by the shell unnecessary (Faulkner 1983). These new defense mechanisms are derived from nudibranchs' prey: specifically, nudibranchs use chemicals or organelles obtained from their prey for their own defense.

There are several superfamilies within the order Nudibranchia, including Doridoidea and Aeolidioidea, each with distinguishing features and defense mechanisms. Doridoidea (dorid) nudibranchs have an exterior gill plume at the posterior of the animal used for respiration and papillae (tiny bumps similar to those on the human tongue) on their dorsal side (Figure 1). *Peltodoris nobilis* is a dorid nudibranch characterized by their bright yellow coloring (Figure 2). Aeolidioidea (aeolid) nudibranchs have

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tentacle-like dorsal papillae called cerata along their body that resemble anemone tentacles (Figure 1). Cerata are responsible for respiration and contains outfoldings of the digestive diverticula (Pechenik 2015). *Hermissenda crassicornis* is an aeolid nudibranch with bright red, orange, and blue on their cerata (Figure 3).

Dorids use *de novo* chemical synthesis to deter predators by creating new chemicals from the chemicals obtained from their prey. *De novo* chemical synthesis refers to the production of complex molecules from simple molecules. Dorids prey on sponges (phylum Porifera) and use the chemicals produced by their prey in a couple of ways (Faulkner and Ghiselin 1983). First, they retain pigments from the prey to use as camouflage when feeding on that prey. This is considered a passive defense, since body coloration does not change in response to the nudibranch's environment (Faulkner and Ghiselin 1983). Second, they use *de novo* chemical synthesis to convert the chemicals from their food into different chemicals for defense (Faulkner and Ghiselin 1983). When chemicals are found in the tissue or glands of an animal but not in their digestive gland, it provides evidence that *de novo* synthesis has occurred (Cimino 1999). In dorids, these chemicals are stored in the skin glands.

Instead of using *de novo* synthesis like dorids, aeolid nudibranchs use nematocyst sequestration for their defense, which entails the storage and firing of nematocysts produced by prey to defend the nudibranch from predators. Aeolid nudibranchs prey on members of the phylum Cnidaria, which includes jellyfish, sea anemones, and hydroids among others. Members of this phylum have organelles called cnidae (meaning "a nettle" or "a stinging thread") that are used for prey capture, defense, and sometimes locomotion (Pechenik 2015). There are several types of cnidae; however, aeolids sequester the most common category of cnidae called nematocysts (meaning "thread bag") (Pechenik 2015). The sting from the cnidae kills or immobilizes the animal's target long enough for the cnidarian to escape predators or consume prey. Aeolid nudibranchs feed on hydroids and sequester their preys' nematocysts to use for their own defense (Goodheart 2016). The nudibranchs store the nematocysts in their functional state in the tips of their cerata, and, when attacked by a predator, the nudibranchs forcibly eject the nematocysts through a small pore on the cerata tip, rupturing the cell membrane and releasing the nematocysts in the form of mucus, which stings the target (Anthony 2016).

Existing research describes the evolution and function of de novo chemical synthesis and nematocyst sequestration. It also suggests that the adaptation of chemical defenses is a driving evolutionary force for nudibranchs, providing insight into their adaptive radiation (Cimino 1999). There is no research, however, on whether the release of chemicals produced *de novo* and the firing of sequestered nematocysts are voluntarily activated by the animal in response to the environmental (active) or are constantly functional (passive). There is also no research comparing the effectiveness of defense mechanisms in dorid nudibranchs to aeolid nudibranchs. Exploring these questions will increase our understanding of the adaptation of chemical defenses over time and their benefits to the animal, which could provide insight into the direction of future adaptations and predator-prey relationships.

2. LITERATURE REVIEW

Hermissenda crassicornis (Eschscholtz, 1831) and *Peltodoris nobilis* (MacFarland, 1905) are found along the Pacific coast from Alaska to California. They are both found in the low intertidal zone, on rocky shores, and in marinas. The common names of *H. crassicornis* are the thick-horned aeolid and the northern opalescent sea slug. These slugs can reach up to 80 mm long and are extremely

aggressive (and occasionally cannibalistic) nudibranchs (Wheeling 2002). The common name of *P. nobilis* is the false sea lemon. They can reach up to 260 mm and give off a lemon scent when disturbed (Rudman 2000).

Existing research describes defense mechanisms used by *P. nobilis, H. crassicornis*, and related species (Edmunds 2016, Faulkner & Ghiselin 1983, Goodheart & Bely 2016). Camouflage is a common defense mechanism in dorids, and there is no evidence to support coloration as a warning to predators (Edmunds 2016). Acid secretion as a defense against predators has also been observed in some dorid species, such as *Anisodoris stellifera* (a genus to which *P. nobilis* previously belonged). The most important defensive glands in *A. stellifera* are large subepidermal acid glands, and other non-mucus skin glands are mostly absent. Most dorids are also unpalatable to fish, potentially due to the non-mucus skin glands (Edmunds 1968). Further, the genus *Phyllidia* is known to produce a poisonous secretion; however, the defensive glands of other dorids are not well studied (Edmunds 1968). *P. nobilis* does produce a fruity odor when disturbed, but it is only speculated as to whether this deters predators (Rudman 2000). *P. nobilis* also possess the secondary metabolite 1-Methylguanosine, which potentially serves as a defense or signaling molecule, and a degraded sesquiterpenoid that acts as an odiferous compound, which may also be used for defense (Dean & Princep 2017). One study also found that aqueous extracts of the digestive gland of *P. nobilis* were lethal to shore crabs and mice when injected with it, indicating that *P. nobilis*' tissue is toxic (Fuhrman et al. 1979).

H. crassicornis uses ceratal autotomy and nematocyst sequestration as defense mechanisms against predators (Miller 2005). Ceratal autonomy occurs when a nudibranch releases cerata from their body to avoid capture by a predator. Nematocyst sequestration is extensively researched in aeolid nudibranchs, particularly in *H. crassicornis* (Anthony 2014, Goodheart & Bely 2016), although it is unknown if *H. crassicornis* is one of these species. It is also unknown how long *H. crassicornis* retains these sequestered nematocysts (Anthony 2016).

The function and composition of cnidae in Cnidarians is well-studied. Cnidae are organelles in cells called cnidoblasts (or nematoblasts), and there are three main groups of cnidae. Nematocysts are the best-studied and most common group of cnidae, with over 30 different types of nematocysts recorded in literature. While some literature uses "nematocyst" and "cnidae" interchangeably, it is more common to use the term nematocyst only when referring to this most common group. All cnidae are composed of a proteinaceous rounded capsule with an opening covered by a hinged operculum. In the capsule, there is a coiled tube that rapidly everts and shoots out of the cell during discharge, triggered by surface chemoreceptors on nearby cells and by chemical and tactile stimulation sensed by a cluster of cilia, called the cnidocil, that project from the cnidoblast (Figure 4). Osmotic pressure is the primary force behind the discharge of cnidae (Pechenik 2015).

Over the years, there have been some noteworthy changes made in the taxonomy and nomenclature of these nudibranchs. *P. nobilis* was previously referred to as *Montereina nobilis, Anisodoris nobilis,* and *Diaulula nobilis*, names which still appear in the literature. "*Peltodoris nobilis*" is the currently accepted name (Rudman 2000). *H. crassicornis* was previously referred to as *Phidiana crassicornis* and *Cavolina crassicornis*; however, these names are currently unaccepted (WoRMS 2022). Both superfamilies are in the infraclass Opisthobranchia, which commonly appears in literature. However, this term is currently an abandoned concept, and its status is uncertain (WoRMS 2021).

3. PURPOSE OF INVESTIGATION AND PROJECT DESCRIPTION

There already exists extensive research into the chemical ecology and evolution of defense mechanisms in nudibranchs; however, no research has been done on the activation of defenses. Activation and effectiveness of defense mechanisms are crucial elements of predator defenses in organisms. Knowing if activation is active or passive and if one method is more effective than the other will provide deeper insight into the mechanisms of evolution and the adaptive radiation of nudibranchs, increasing our knowledge of driving evolutionary forces, diversity, and potentially, the direction of future adaptations of defense mechanisms in these species. Understanding these dynamics is of particular importance as climate change continues to cause rapid deterioration and change in ocean ecosystems. Increasing our knowledge of evolution and adaptive radiation in species that are at the intermediary trophic level will allow scientists to predict the effects of climate change on many different species that live in a similar ocean habitat.

My research sought to answer two questions. First, is the release of defensive chemicals produced *de novo* in *Peltodoris nobilis,* and is the firing of sequestered nematocysts in *Hermissenda crassicornis* active or passive? Active defense mechanisms are voluntarily released based on contact with a predator, while passive defense mechanisms are constantly functional and do not depend on contact with a predator. Second, is chemical defense or sequestration of cnidae a more effective means of preventing predation? The more effective defense mechanism should result in a statistically lower contact time between the control nudibranch and the predator, thus better preventing predation than the other. I hypothesized that the defense mechanisms for both nudibranchs were active (indicated by a statistical difference between the control and anesthetized contact times) and equally effective at preventing predation.

These hypotheses were tested by comparing the contact time of an anesthetized and control nudibranch with a predatory crab. If the amount of contact time was greater for the anesthetized nudibranch than the control, the release of defense chemicals was considered active. This means that the animal voluntarily releases the chemicals or fires the nematocysts sequestered in their cerata. If there was no difference in the contact times, then the defense was considered passive. This means that the chemicals produced *de novo* in dorids are constantly being released, or that the sequestered nematocysts stored in the cerata of aeolids are still fired when the nudibranch is unable to voluntarily fire them. To determine if one defense mechanism was more effective, the total amount of time the crab spent in contact with the control dorid and aeolid nudibranchs was compared. If there was no significant difference between the control contact times with the crab, neither was more effective. If the crab spent less time in contact with the control of one species than the other, that species was assumed to have the more effective defense mechanism.

4. METHODS

The dorid nudibranch used for this experiment was *Peltodoris nobilis* and the aeolid nudibranch was *Hermissenda crassicornis*. Specimen and data collection occurred from September 28, 2021 to December 3, 2021. Nudibranchs were collected from E dock or the Shanks light trap at the Charleston Marina in Charleston, Oregon (Figure 5). Nudibranchs were collected up to one week prior to an experiment and kept in separate containers in a sea table with sponges or hydroids for food. The crabs used for this experiment were juvenile *Glebocarcinus (Cancer) oregonensis*, also known as the Pygmy Rock Crab. Crabs were collected from the Shanks light trap or buoys

attached to F dock (Figure 5). They were collected on the day of the experiment or the day prior. Juvenile crabs were used because their mouth parts are not as well-defended and are more susceptible to injury than those of adult crabs, resulting in a stronger reaction to nudibranch defenses. For each trial, 1 crab and 2 nudibranchs (1 control and 1 anesthetized) were used. No crabs or nudibranchs were reused for a different trial.

For the *P. nobilis* trials, 2 nudibranchs of roughly the same size were transferred from the sea table into separate glass containers. One glass container was filled with 200 mL of sea water (control) and the other with 100 mL of sea water and 100 mL of 7% Magnesium Chloride (MgCl2) (anesthetized). Lids were placed on top of the containers to prevent escape, and the container with the nudibranch in the treatment was labeled using a piece of paper towel (Figure 6). Nudibranchs were left in the containers for at least 2 hours or until they were fully anesthetized. The nudibranchs were considered fully anesthetized when they no longer recoiled when the gill plume was touched. During this time, crabs were collected from the Charleston Marina or the sea table (if they had been collected the day prior) and placed into separate, smaller glass containers.

Once the nudibranch in MgCl2 was fully anesthetized, the control or anesthetized nudibranch was transferred to a separate glass container with 200 mL of sea water. A crab was placed right next to the nudibranch in the container, so that they were close to or barely touching, and a timer was set for 5 minutes. The time the crab and nudibranch remained in contact was observed during those 5 minutes by watching during the trial and recorded with a stopwatch. If the crab and nudibranch began the trial touching, the stopwatch was started as soon as either individual moved while remaining in contact. Contact included any part of the crab and nudibranch, whether the crab was entirely on the nudibranch or just a claw was touching. After 5 minutes, the crab was removed from the container, and the carapace (shell) length was measured. The crab was then placed back in the same container used during the preparation for the experiment for another 5 minutes. During this time, the length of the control and anesthetized nudibranchs was measured and recorded. After 5 minutes, the second nudibranch was transferred with the same crab to a new container—again, with 200 mL of sea water—for another 5 minutes. Contact was observed and recorded, and once the time was up, the crab was removed.

This process was repeated for all trials, alternating whether the control or anesthetized nudibranch was exposed to the crab first. The behaviors of the crabs and nudibranchs during the trials were also recorded. Predatory behavior in crabs was indicated when they repetitively moved their front chelae towards their mouth while in contact with the nudibranch. After all trials for the day were completed, the nudibranchs and crabs were released on the same dock from which they were collected, far enough (43.345614, -124.323141) from the collection site (43.345048, -124.323034) that there was no chance of reusing a nudibranch (Figure 5).

H. crassicornis trials were completed in an identical manner, with two slight differences: the nudibranchs were transferred into a smaller glass container with 75 mL of sea water during the trial due to their smaller size. Also, *H. crassicornis* nudibranchs were considered anesthetized when they no longer moved in response to touch or laid upside down on the bottom of the container.

18 trials were completed for *H. crassicornis*, and 10 trials were completed for *P. nobilis*. The number of trials across the two species differed due to sampling limitations (differences in the number of specimens found in the field). The data were analyzed with Microsoft Excel. A one-way ANOVA compared the contact time with a crab between the control and anesthetized nudibranchs. A separate one-way ANOVA was also

used to compare the sizes of the control and anesthetized nudibranchs used in experiments for the same purpose. A graph with standard error compared the control contact time with a crab between *P. nobilis* and *H. crassicornis* trials. One-sample t-tests compared the length of the control nudibranchs, the anesthetized nudibranchs, and the carapace size of the crabs used in the experiments to determine if variation in sizes influenced results.

5. RESULTS

5.1. *PELTODORIS NOBILIS*

Predatory behavior—crabs moving their front chelae to and from their mouths while in contact with the nudibranchs—was observed in 3 of the 10 trials and only with anesthetized nudibranchs. In 8 of the 10 trials, the crabs hid their back legs under the control nudibranchs; in the other 2 trials, there was little to no contact with either the anesthetized or control nudibranchs. In 2 trials, the control nudibranchs released mucus, and in 1 trial, the anesthetized nudibranch had previously released mucus. In 1 control trial where mucus was observed, the crab avoided the nudibranch after coming into contact with the mucus. Crabs did not express predatory behavior in trials where mucus was present (Table 1).

A one-way ANOVA indicated that there was no significant difference in the contact times with crabs between the anesthetized and control *P. nobilis* ($F_{1,18} = 0.23$, $p = 0.632$) (Table 2). The average contact times with crabs for the control and anesthetized nudibranchs were 167 seconds (SD ± 106 seconds) and 193 seconds (SD ± 129 seconds), respectively (Table 3).

The average carapace length of the crabs used in these trials was 10.3 mm (SD ± 3.59 mm). The average control and anesthetized nudibranch lengths were 7.55 cm (SD ± 1.98 cm) and 7.24 cm (SD ± 1.84 cm), respectively (Table 2). To confirm that variations in nudibranch and crab sizes did not affect results, one-sample t-tests found no significant difference for the crab carapace lengths ($t9 = 0$, $p = 0.5$), control nudibranch lengths ($t9 = 0$, $p = 0.5$), and anesthetized nudibranch lengths ($t9 = -0.017$, $p = 0.5$). A one-way ANOVA also found no significant difference between the control and anesthetized nudibranch lengths ($F_{1,18} = 0.13$, $p = 0.72$) (Table 2).

5.2. *HERMISSENDA CRASSICORNIS*

Predatory behavior was observed in 10 of the 18 trials, and no predatory behavior was observed in the remaining 8. Predatory behavior was observed between the crab and anesthetized nudibranchs in 8 of those 10 trials, with the control and anesthetized nudibranch in 1 trial, and with just the control nudibranch in the remaining trial. There was no predatory behavior in 16 of the 18 control trials. In 4 of the 16 trials, the nudibranch initiated the contact, and in 2 of those trials, the crab moved to avoid the nudibranch. In 3 control trials, the nudibranchs moved to avoid the crab. In 7 trials, the crab avoided or ignored the control and anesthetized nudibranch. In 1 trial, cerata were observed to continue moving after they were removed from the body of the nudibranch—either by ceratal autotomy or the crab—which could have implications for future research (Table 4).

A one-way ANOVA indicated that the crab's contact time was significantly higher for the anesthetized *H. crassicornis* than the control ($F_{1,34}=9.37$, $p=0.004$) (Table 5). The average contact times with crabs for the control and anesthetized nudibranchs were 7 seconds (SD ± 13 seconds) and 86 seconds (SD ± 108 seconds), respectively (Table 6).

The average carapace length of the crabs used in these trials was 9.66 mm (SD ± 3.55 mm). The average control and anesthetized nudibranch lengths were 2.26 cm (SD ± 0.90 cm) and 2.11 cm (SD ± 0.70 cm), respectively. To confirm that variations in nudibranch and crab sizes did not impact results, one-sample t-tests found no

significant difference in crab carapace lengths (t17 = 0.007, p = 0.5), control nudibranch lengths (t17 = 0.031, p = 0.5), and anesthetized nudibranch lengths (t17 = 0.006, p = 0.5). A one-way ANOVA found no significant difference between the control and anesthetized nudibranch lengths (F1,34 = 0.57, p = 0.57) (Table 5).

6. DISCUSSION

6.1. ACTIVATION OF DEFENSE MECHANISMS

6.1.1. *PELTODORIS NOBILIS*

There was no significant difference between the control and anesthetized nudibranch contact times with the crabs (F1,18=0.23, p>0.05). This indicates that the release of chemicals produced *de novo* is passive in *Peltodoris nobilis*. Overall, the crabs exhibited little predatory behavior with the anesthetized nudibranchs and no predatory behavior with the control nudibranchs. The average contact time for the control and anesthetized nudibranchs was 167 seconds (SD ±106 seconds) and 193 seconds (SD ± 129 seconds), respectively—more than half the duration of the trials. This lack of predatory behavior coupled with the high contact time suggests that while the chemicals produced by *P. nobilis* via *de novo* chemical synthesis appear to prevent predation, they do not prevent contact with predators. It is possible that the crabs did not demonstrate predatory behavior due to their placement in an unfamiliar environment; however, since members of the same species demonstrated predatory behavior with *Hermissenda crassicornis* after experiencing the same treatment, this is not very likely.

The prevention of predation without the prevention of contact may be due to chemicals produced *de novo* that signal that the nudibranch is poisonous or tastes bad. Previous studies have identified 1-Methylguanosine, which potentially serves as a defense or signaling molecule, and a degraded sesquiterpenoid, which acts as an odiferous compound in *P. nobilis* (Dean & Princep 2017), as possible culprits. The presence of these molecules, coupled with evidence from previous studies supporting the toxicity of *P. nobilis* tissue, could potentially explain the lack of predation by the crabs (Fuhrman et al. 1979). Crabs have excellent senses of taste and smell; once they come into contact with potential prey, they can taste and smell them using setae on their appendages, and both pairs of their antennae are primarily sensory (Pechenik 2015). Crabs could therefore interpret if the chemical signals produced by nudibranchs indicated that they were poisonous or tasted bad. These chemicals do not appear to prevent predation by all predators, however, as one nudibranch was taken out of the collection container and consumed by a seagull during collection at the docks.

Nudibranchs released mucus in 3 of the trials (2 by the control nudibranch and 1 by the anesthetized). In trial 4, the crab got caught in the mucus and actively tried to get it off and escape. After escaping the mucus, the crab did not attempt contact again. In all 3 trials where mucus was released, there was no contact between the crab and nudibranch. This suggests that mucus also works as a predator deterrent for *P. nobilis*, and it appears to be more effective at preventing any contact with a predator than released chemicals. The presence of toxins in mucus could potentially explain this observation; however, the composition of the mucus produced by *P. nobilis* is unknown. Sea slugs in the superorder Sacoglossa produce mucus with ichthytoxic activity, but it is unknown if the mucus produced by dorids is toxic (Di Marvo et al. 1993).

6.1.2. *HERMISSENDA CRASSICORNIS*

The contact times between the crabs and the control nudibranchs were significantly lower than those between the crabs and the anesthetized nudibranchs (F1,34 = 9.37, p = 0.004). This

supports the hypothesis that the firing of nematocysts obtained through nematocyst sequestration is active. The crabs exhibited predatory behavior in 10 trials, indicating that they would eat the nudibranchs given the opportunity. However, in 2 control trials, the *Hermissenda crassicornis* initiated contact with the crabs, and the crabs moved to avoid the nudibranch. The predatory behavior of crabs in some trials coupled with the crab's avoidance of nudibranchs in others suggests that this defense mechanism is successful at preventing predation. Control nudibranchs also used movement to avoid the crabs; however, the crabs moved much faster, so this method was not very effective if the crab pursued the nudibranch.

6.2. DETERMINING EFFECT OF SPECIMEN SIZE VARIATION ON RESULTS

One-sample t-tests found no significant difference in the sizes of the crabs, the control, or the anesthetized nudibranchs used for the *P. nobilis* and *H. crassicornis* trials. Variations in crab size could potentially influence results because the mouth parts of larger—and consequently, older—crabs are better-defended than those of younger crabs. Larger crabs would therefore be less affected by defense mechanisms, which could increase contact time for the larger crabs. Inversely, larger nudibranchs could contain more chemicals or nematocysts than smaller nudibranchs, increasing their defensive capabilities and consequently decreasing contact time. However, since no statistical differences in size were found, it can be assumed that the differences in contact time were strictly due to the effectiveness of the defense mechanism and not influenced by size variation. For both species, there was no significant difference between the control and anesthetized nudibranch lengths. This indicates that variation in size between the control and anesthetized groups did not influence the results.

6.3. EFFECTIVENESS OF DE NOVO CHEMICAL SYNTHESIS VS. NEMATOCYST SEQUESTRATION

I hypothesized that the average control nudibranch contact time with the crab would be lower for the more effective defense mechanism. It was determined that the average contact time for the control *H. crassicornis* trials was significantly lower than *P. nobilis* (Figure 9). This suggests that the *H. crassicornis* defense mechanisms were more effective at preventing predation. However, minimal predatory behavior was observed in the crabs during the *P. nobilis* trials, despite the high contact time. Consequently, the control contact times of the *P. nobilis* trials do not accurately reflect the effectiveness of the release of chemicals produced *de novo* at preventing predation, since little predatory behavior was observed during contact. Therefore, while *H. crassicornis* defense mechanisms did a better job of preventing contact between the predator and the nudibranch, this study cannot determine if nematocyst sequestration is more effective at preventing predation than *de novo* chemical synthesis.

7. CONCLUSION

My research sought to answer two questions. First, is the release of defensive chemicals produced *de novo* in *Peltodoris nobilis*, and is the firing of sequestered nematocysts in *Hermissenda crassicornis* active or passive? Second, is chemical defense or sequestration of cnidae a more effective means of preventing predation?

There was no significant difference in crab contact time between the control and anesthetized nudibranchs for *P. nobilis* ($F_{1,18} = 0.23$, $p > 0.05$). I therefore concluded that the release of chemicals synthesized *de novo* as a defense mechanism is passive in this species. The high contact time with the crab for the control and anesthetized nudibranchs, coupled with a lack of predatory

behavior, indicates that the chemicals produced *de novo* do not prevent contact between the predator and prey but may prevent predation. For *H. crassicornis*, the contact time between the anesthetized nudibranchs and the crabs were significantly higher than the contact time with the control nudibranchs ($F_{1,34} = 9.37$, $p = 0.004$), suggesting that nematocyst firing is active in this species.

Predator defenses and the activation of these defenses are important to the survival of an individual and the species as a whole; therefore, understanding these defenses can provide deeper insight into the mechanisms of evolution and adaptive radiation of nudibranchs. This, in turn, increases our knowledge of driving evolutionary forces, diversity, and potentially, the direction of future adaptations in these species. Determining that members of the order Nudibranchia possess both active and passive defense mechanisms is indicative of high levels of adaptive radiation, which is also reflected in the morphological and prey preference differences between species. This research, coupled with other research on nudibranchs, also provides a point of reference for the current diversity of nudibranchs. This will allow for the comparison and continuation of research on adaptive radiation in these species as ocean ecosystems face rapid deterioration due to climate change. As intermediary trophic level species, nudibranchs are of particular interest in determining the effects of climate change in marine habitats, since changes in these species can impact or reflect changes in entire ecosystems.

In a few trials, *P. nobilis* released mucus and *H. crassicornis* moved to avoid contact with the crab, and in other trials, cerata were detached from the nudibranch's body but still moved. Future experiments could study mucus as a potential defense mechanism of dorid nudibranchs. Another future area of research could focus on comparing the effectiveness of defense mechanisms. The findings from such studies could provide insight into the direction of future adaptations in nudibranchs and their interactions with predators. For *P. nobilis*, it may be more beneficial to complete this experiment with adult crabs instead of juveniles due to their larger size and the lack of predatory behavior displayed by the juvenile crabs. It would be useful to see if the lack of predatory behavior changed with adult crabs. A final future area of study could be devoted to determining if the cerata of *H. crassicornis* can still fire nematocysts when separated from the nudibranch's body. Previous research on *H. crassicornis* concludes that ceratal autotomy, or the release of cerata, is a method used by nudibranchs to avoid predation (Miller 2005). There is no research, however, on the movement of cerata after separating from the body or whether the cerata can still fire nematocysts in this state.

8. FIGURES

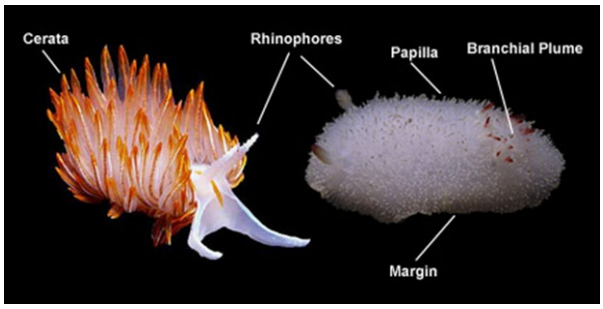

Figure 1: Anatomy of Aeolid and Dorid Nudibranchs. The basic anatomy of an aeolid (left) and dorid (right) nudibranch. For dorid nudibranchs, the margin is also referred to as the mantle, the branchial plume as the gill plume, and the papilla as dorsal papilla.

Figure 2: Image of *Peltodoris nobilis*. Photo taken off San Miguel Island, California by Bruce C. Wight (2000).

Figure 3: Image of *Hermissenda crassicornis*. Photo taken in Cape Flattery, Washington by Brooke Reiswig (2006).

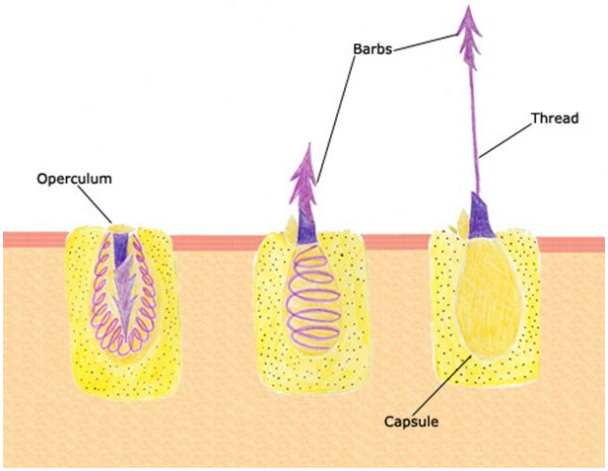

Figure 4: Diagram of a nematocyst firing. An image depicting the stages and anatomy of a nematocyst firing. The stages are the unfired (left), firing (middle), and fired (right) nematocyst. Nematocysts also contain cnidocils, or clusters of modified cilia, on the outside of the cnidoblast (the cell) that sense chemical or tactile stimulus and trigger discharge.

Figure 5: Image of specimen collection and release locations. A Google Maps image of the Boat Basin in Charleston, Oregon (43°20'45.5"N 124°19'24.6"W). The blue oval represents the collection location of the nudibranchs, the yellow oval represents the release location, and the green oval represents the location of the Shanks light trap on F dock where the crabs and some *Hermissenda crassicornis* were collected. Google Maps (n.d) [Boat Basin Road, Oregon 97420] Retrieved January 20, 2022, from https://www.google.com/maps/place/Boat+Basin+Rd,+Oregon+97420/@43.3440301,-124.3296136,17z/data=!3m1!4b1!4m5!3m4!1s0x54c382312fa9a005:0xbc04d6ebdd655934!8m2!3d43.3440262!4d-124.3274249

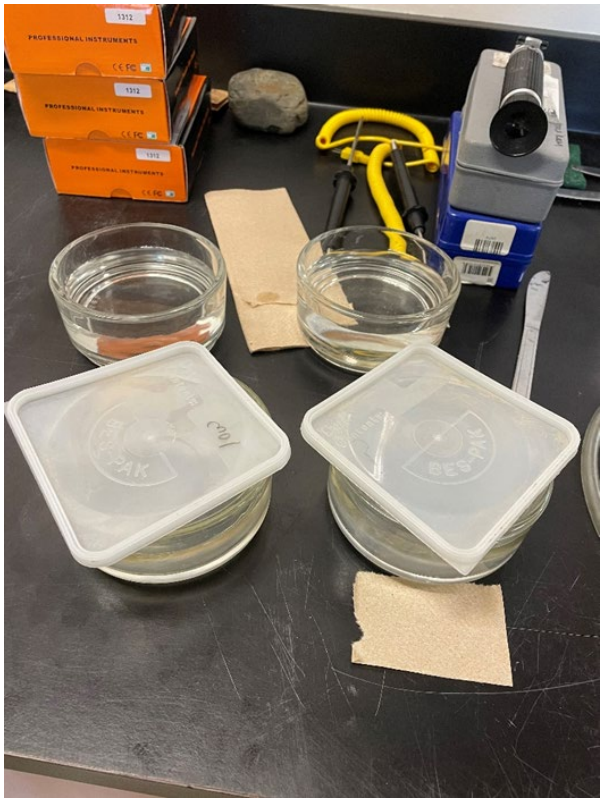

Figure 6: Image of experimental set up for *Peltodoris nobilis.* The nudibranchs are in the covered glass containers, and the brown paper marks the anesthetized nudibranch. The glass containers behind the covered containers were used for the data collection. The *Hermissenda crassicornis* experimental set up looked the same, except the containers were not covered and the glass containers used for data collection were smaller. The knife was used to move the crabs to and from the containers.

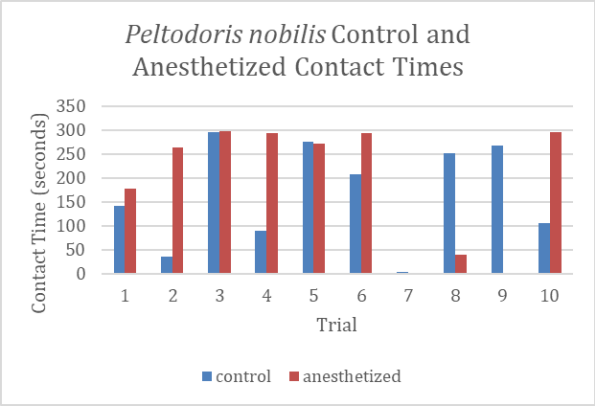

Figure 7: *Peltodoris nobilis* control and anesthetized contact times. The amount of time the crabs contacted the control and anesthetized nudibranch in each *Peltodoris nobilis* trial.

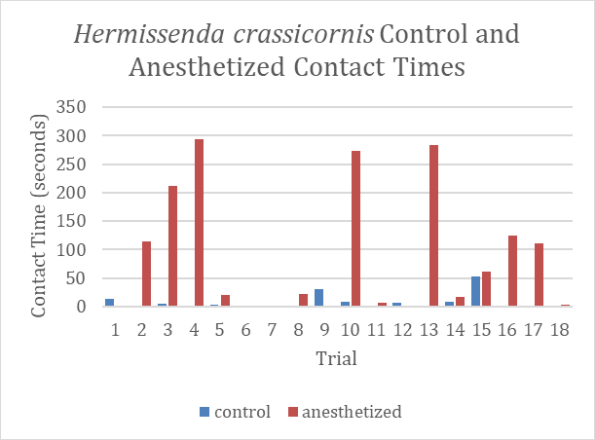

Figure 8: *Hermissenda crassicornis* Control and Anesthetized Contact Times. The amount of time the crabs contacted the control and anesthetized nudibranchs in each *Hermissenda crassicornis* trial.

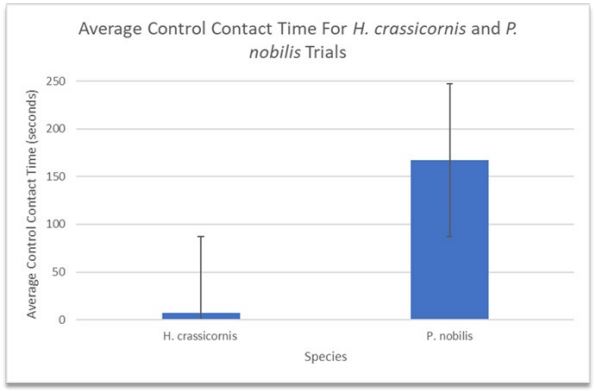

Figure 9: Average Difference in Control Contact Time for *H. crassicornis* and *P. nobilis* Trials. The average (SE) difference in the control contact times with the crabs *for Hermissenda crassicornis* and *Peltodoris nobilis* trials. *Peltodoris nobilis* had a significantly higher contact time between the control nudibranchs and the crab than *H. crassicornis*.

9. TABLES

Table 1. A table listing the behavior of the crabs when in contact with the nudibranchs. "Nudi" is short for nudibranch.

	Crab 1	**Crab 2**	**Crab 3**	**Crab 4**	**Crab 5**
Control Observations	Crab hiding under nudi, no predatory behavior, nudi releasing mucus	Crab hiding under nudi, no predatory behavior	Nudi not avoiding contact, no predatory behavior	Visibly releasing mucus, crab avoided contact w nudi, moving to the opposite side of dish, no predatory behavior	Crab hiding back legs under nudi, no predatory behavior
Anesthetized Observations	Crab on top of upside down nudi, no predatory behavior	Crab on top of nudi, some predatory behavior	Crab back legs under nudi, no predatory behavior	Nudi released mucus before trial, no predatory behavior	Crab on top on nudi, no predatory behavior
	Crab 6	**Crab 7**	**Crab 8**	**Crab 9**	**Crab 10**
Control Observations	Crab hiding back legs under nudi, no predatory behavior	Crab avoided nudi	Crab hiding under nudi, no predatory behavior	Crab hiding under nudi, no predatory behavior	Crab hiding under nudi, no predatory behavior
Anesthetized Observations	Crab on top of nudi, no predatory behavior	Crab ignored nudi	Crab on top of nudi, some predatory behavior	Crab avoided contact	Crab on top of nudi, some predatory behavior

Table 2. A table listing the statistics for a one-way ANOVA comparing the contact times with the crab between the control and anesthetized *Peltodoris nobilis* and a one-way ANOVA comparing the lengths of the control and anesthetized nudibranchs in the experiment. Also, the statistics for the one-sample t-tests comparing the sizes of the control *P. nobilis*, the sizes of the anesthetized *P. nobilis*, and the carapace length of crabs used in the trials are included.

	Control vs Anesthetized Contact Time	**Crab Carapace Length**	**Anesthetized Nudibranch Length**	**Control Nudibranch Length**	**Control vs Anesthetized Nudibranch Length**
Type of Test	One-way ANOVA	One-sample t-test	One-sample t-test	One-sample t-test	One-way ANOVA
p- value	0.63	0.50	0.50	0.50	0.72
Standard Deviation	N/A	3.59 mm	1.84 cm	1.98 cm	N/A
Mean	N/A	10.3 mm	7.24 cm	7.55 cm	N/A
t	N/A	0	-0.017	0	N/A
F	0.23	N/A	N/A	N/A	0.13
Degrees of Freedom	19	9	9	9	19

Table 3. Raw data for the *Peltodoris nobilis* trials.

	Crab 1	**Crab 2**	**Crab 3**	**Crab 4**	**Crab 5**	**Crab 6**	**Crab 7**	**Crab 8**	**Crab 9**	**Crab 10**	**Mean**
Control Contact Time (seconds)	141	35	295	89	276	208	4	252	268	106	**167**
Anesthetized Contact Time (seconds)	177	263	297	294	271	293	1	40	0	296	**193**

Table 4. Observations of crab and nudibranch behavior during *Hermissenda crassicornis* trials.

	Crab 1	Crab 2	Crab 3	Crab 4	Crab 5	Crab 6	Crab 7	Crab 8	Crab 9
Control Observations	Nudi initiated contact	Crab ignored nudi	crab mostly ignored nudi	Crab actively avoiding nudi while nudi moving towards crab	Nudi initiated contact, touched crab w front antennae than both went to opposite sides and stayed there	Nudi and crab avoiding e/o	Nudi clung to surface of water out of crab's reach	Crab actively avoiding nudi when it got close	Predatory behavior when in contact, nudi moving to avoid crab
Anesthetized Observations	Crab ignored nudi	Predatory behavior when in contact	Predatory behavior when in contact	Predatory behavior when in contact	Crab stayed on opposite side of container as nudi	Crab ignored nudi	Crab ignored nudi	Predatory behavior when in contact	Crab mostly trying to escape, ignored nudi
	Crab 10	**Crab 11**	**Crab 12**	**Crab 13**	**Crab 14**	**Crab 15**	**Crab 16**	**Crab 17**	**Crab 18**
Control Observations	Crab and nudi mostly avoided e/o	Crab ignored nudi	Nudi looks unhealthy, crab mostly ignored nudi	Crab and nudi avoided e/o	Tendrils separated from nudi still moving, mostly avoided contact	Predatory behavior when in contact, nudi moving to avoid crab	Crab and nudi ignored e/o	Crab and nudi ignored e/o	Crab and nudi ignored e/o
Anesthetized Observations	Predatory behavior when in contact	Crab ignored nudi	Crab ignored nudi	Predatory behavior when in contact	Crab ignored nudi	Predatory behavior when in contact	Predatory behavior when in contact	Predatory behavior when in contact	Crab mostly ignored nudi

Table 5. P-values for *Hermissenda crassicornis* trials.

	Control vs Anesthetized Contact Time	Crab Carapace Length	Anesthetized Nudibranch Length	Control Nudibranch Length	Control vs Anesthetized Nudibranch Length
Type of Test	One-way ANOVA	One-sample t-test	One-sample t-test	One-sample t-test	One-way ANOVA
p- value	0.004	0.50	0.50	0.50	0.57
Standard Deviation	N/A	3.55 mm	0.70 cm	0.90 cm	N/A
Mean	N/A	9.66 mm	2.11 cm	2.26 cm	N/A
t	N/A	0.007	0.006	0.031	N/A
F	9.37	N/A	N/A	N/A	0.32
Degrees of Freedom	35	17	17	17	35

Table 6: Raw data for the *Hermissenda crassicornis* trials.

	Crab 1	Crab 2	Crab 3	Crab 4	Crab 5	Crab 6	Crab 7	Crab 8	Crab 9	Mean
Control Contact Time (seconds)	13	0	5	1	3	0	0	0	31	--
Anesthetized Contact Time (seconds)	1	115	212	294	20	0	0	22	2	--
	Crab 10	**Crab 11**	**Crab 12**	**Crab 13**	**Crab 14**	**Crab 15**	**Crab 16**	**Crab 17**	**Crab 18**	
Control Contact Time (seconds)	8	0	7	1	8	53	0	0	0	7
Anesthetized Contact Time (seconds)	273	7	0	284	17	62	124	111	4	86

ACKNOWLEDGEMENTS

I would like to thank Professors Alan Shanks, Maya Watts, and Lisa Munger for serving on my thesis committee and for helping me to fully examine the specific topic and consider the various perspectives and contexts related to this subject matter. I would also like to thank Professor Brian Bingham for assisting with the experimental design for this research. I express my sincerest gratitude for having the privilege of having excelling professors in the Clark Honors College who were willing to guide me through my educational career at the University of Oregon.

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