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

by

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Nudibranchs ("sea slugs") are marine invertebrates (Phylum Mollusca, Class Gastropoda) that use various chemical defenses to deter predators. *Peltodoris nobilis* is a member of the superfamily Doridoidea and uses *de novo* chemical synthesis, converting simple molecules into complex molecules, for defense. *Hermissenda crassicornis* is a member of the superfamily Aeolidioidea and uses nematocyst sequestration, the storage and firing of nematocysts obtained from prey, for defense. Past research explains the function and evolution of defenses in nudibranchs; however, it is unknown if these defenses are active or passive. Active defense mechanisms are voluntarily activated in response to the environment while passive defense mechanisms are constantly functional. 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, or works better, 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 with an anesthetized and non-anesthetized (control) nudibranch. There

was no statistical difference in the 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. Results indicated that the control *H. crassicornis* contact times with the crabs were statistically lower than *P. nobilis*. Based on the initial experimental design, this suggests that nematocyst sequestration is more effective at preventing predation. However, the crabs demonstrated little predatory behavior, or attempt to harm, the *P. nobilis* when in contact, so this study cannot conclusively determine which defense mechanism is more effective at actually preventing predation.

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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 phylum Mollusca is highly diverse and includes a wide variety of species such as squids, octopuses, clams, snails, and slugs. The class Gastropoda includes all freshwater, marine, and terrestrial snails and slugs. The order Nudibranchia consists of marine slugs that, unlike most other members of class Gastropoda, do not have a shell for defense as adults. Overtime 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 prey, where 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 on their dorsal side (Figure 1). *Peltodoris nobilis* is a dorid nudibranch characterized by its bright yellow coloring (Figure 2). Aeolidioidea (aeolid) nudibranchs have 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. First, they retain pigments from the prey to use as camouflage when feeding on that prey. This is a passive defense since the 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 an animal, but not in the digestive gland, *de novo* chemical synthesis is indicated (Cimino 1999). These chemicals are stored in the skin glands of dorids.

Aeolid nudibranchs use nematocyst sequestration for their defense. This refers to the storage and firing of nematocysts produced by prey to defend the nudibranch from predators. They 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). Nematocysts (meaning "thread bag") are the most common category of cnidae (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 prey's 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 nematocysts are forcibly ejected through a small pore on the cerata tip, rupturing the cell membrane and releasing the nematocyst in mucus, which stings the target (Anthony 2016).

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. *H. crassicornis* can reach up to 80 mm long while *P. nobilis* can reach up to 260 mm (Sea Slug Forum 2021). The common name of *P. nobilis* is the False Sea Lemon and it is commonly confused with *Doris montereyensis*, common name the Sea Lemon (Cooper, 1862). They are distinguishable because *P. nobilis* has rhinophores and gill plumes that are lighter than the dorsum, unlike *D. montereyensis*. *Hermissenda crassicornis*, common names the Thick-horned Aeolid and the Northern Opalescent Sea Slug, are extremely aggressive and occasionally cannibalistic nudibranchs. They appear similar to *H. opalescens* (Cooper, 1862), common name the Southern Opalescent Sea Slug. These two species are commonly confused in literature, however *H. crassicornis* is distinguishable from *H. opalescens* by the presence of a longitudinal white band along the anterior side of the cerata (Sea Slug Forum 2021).

Previous research describes defense mechanisms used by *Peltodoris nobilis*, *Hermissenda crassicornis*, and related species (Edmunds 2016, Faulkner & Ghiselin 1983, Goodheart & Bely 2016). Camouflage is a common defense mechanism in dorids, however there is no evidence supporting 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). The genus *Phyllidia* is also known to produce a poisonous secretion, however the defensive glands of other dorids are not well studied (Edmunds 1968). *Peltodoris nobilis* does produce a fruity odor when disturbed, but it is only speculated whether this deters predators (Sea Slug Forum 2021). The secondary metabolite 1-Methylguanosine, which potentially serves as a defense or signaling molecule, and a degraded sesquiterpenoid acting as an odiferous compound have been observed in *P. nobilis* (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 their tissue is toxic (Fuhrman et al. 1979).

Ceratal autotomy is another defense mechanism used by *Hermissenda crassicornis* against predators (Miller 2005). This refers to the separation of cerata from the nudibranch's body. There is also extensive research into nematocyst sequestration in aeolid nudibranchs, particularly *H. crassicornis* (Anthony 2014, Goodheart & Bely 2016). Some aeolid species demonstrate selectivity among the different types of nematocysts found in Cnidarians, 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 3 main groups of cnidae. Nematocysts are the best studied and the most common group, with over 30 different types. Several different types of nematocysts can be found in one organism. Some literature uses nematocyst and cnidae interchangeably, although it is more

common to use the term nematocyst only when referring to this most common group. 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 (Figure 4). Discharge is 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. Osmotic pressure is the primary force behind the discharge of cnidae (Pechenik 2015).

Past 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 behind nudibranchs, providing insight to their adaptive radiation (Cimino 1999). There is no research 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 versus aeolid nudibranchs. Exploring these questions will increase our understanding of the adaptation of chemical defenses over time and their benefits to the animal.

There are some noteworthy changes in the taxonomy and nomenclature of these nudibranchs. *Peltodoris nobilis* was previously referred to as *Montereina nobilis*, *Anisodoris nobilis,* and *Diaulula nobilis*, which still appear in literature. *Peltodoris nobilis* is the currently accepted name (Sea Slug Forum 2021). *Hermissenda 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).

Purpose of Investigation and Project Description

There is extensive research into the chemical ecology and the evolution of defense mechanisms in nudibranchs; however, there is no research 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 further 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.

My research sought to answer two questions: 1) Is the release of defensive chemicals produced *de novo* in *Peltodoris nobilis* and the firing of sequestered nematocysts in *Hermissenda crassicornis* active or passive? 2) Is chemical defense or sequestration of cnidae a more effective means of preventing predation? The more effective defense mechanism would work better at preventing predation than the other. Active defense mechanisms are voluntarily released based on contact with a predator. Passive defense mechanisms are constantly functional and do not depend on contact with a predator. I hypothesized that the defense mechanisms for both nudibranchs were active 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 are constantly being released or that the sequestered nematocysts stored in cerata are not fired while the nudibranch is unable to voluntarily fire them. To determine if one defense mechanism was more effective, the total amount of time the crabs 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 had the more effective defense mechanism.

Methods

The dorid nudibranch used for this experiment was *Peltodoris nobilis* and the aeolid nudibranch was *Hermissenda crassicornis*. Specimen and data collection occurred from 9/28/2021-12/03/2021. Nudibranchs were collected from E dock or the Shanks light trap at the Marina in Charleston, Oregon (Figure 5). Nudibranchs were collected up to one week prior to an experiment and were kept in separate containers in a sea table with sponge or hydroids for food. The crabs used for this experiment were juvenile *Glebocarcinus (Cancer) oregonensis,* common name 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, compared to adults, their mouth parts are not as well defended and more susceptible to injury, 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 *Peltodoris 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 (anesthetized). Lids were placed on top of the containers to prevent escape and a piece of paper towel was used to mark the nudibranch in the treatment (Figure 6). Nudibranchs were left in the containers for at least 2 hours or until the individuals in $MgCl_2$ 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 slug in $MgCl_2$ 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 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. 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 length was measured. The crab was then placed back in its original container 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 crab to a new container with 200 mL of sea water for another 5 minutes. Contact was observed and recorded and once 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 recorded. Predatory behavior in crabs was when the crab repetitively moved its front chelae towards its mouth while in contact with the nudibranch. After all trials for the day were completed the nudibranchs and crabs were

released in a location far enough from the collection site so there was no chance of reusing a nudibranch (Figure 5).

Hermissenda crassicornis trials were completed in an identical manner except 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 were upside down on the bottom of the container.

18 trials were completed for *Hermissenda crassicornis* and 10 trials were completed for *Peltodoris nobilis*. The number of trials was different due to limitations in field collection. Data were analyzed with Microsoft Excel. A one-way ANOVA compared the contact time with a crab between the control and anesthetized nudibranchs. 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. A one-way ANOVA was also used to compare the sizes of the control and anesthetized nudibranchs used in experiments for the same purpose.

Results

Peltodoris nobilis

Predatory behavior, the crab moving its front chelae to and from its mouth while in contact with the nudibranch, was observed in 3 of the 10 trials and only with anesthetized nudibranchs. In 8 of the 10 trials the crab hid its 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. There was no predatory behavior in the trials where mucus was present (Table 1).

A one-way ANOVA indicated no significant difference in the contact times with the crab between the anesthetized and control *Peltodoris nobilis* ($F_{1,18}$=0.23, p=0.632) (Table 2). The average contact time with the crabs for the control and anesthetized nudibranchs was 167 seconds (SD ±106 seconds) and 193 seconds (SD ± 129 seconds), respectively (Table 3). The average carapace length of crabs used in 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 (t_9=0, p=0.5), control nudibranch lengths (t_9=0, p=0.5), and anesthetized nudibranch lengths (t_9=-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).

Hermissenda crassicornis

Predatory behavior, the crab moving its front chelae to and from its mouth or pulling at cerata while in contact with the nudibranch, was observed in 10 of the 18 trials and no predatory behavior was observed in the remaining 8. Predatory behavior was observed in the crab with anesthetized nudibranchs in 8 of those 10 trials, with the control and anesthetized nudibranch in 1 trial, and with just the control in the other. There was no predatory behavior in 16 out of 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 being separated from the body of the nudibranch, 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 *Hermissenda crassicornis* than the control ($F_{1,34}$=9.37, p=0.004) (Table 5). The average contact time with the crabs for the control and anesthetized nudibranchs was 7 seconds (SD ±13 seconds) and 86 seconds (SD ± 108 seconds), respectively (Table 6). The average carapace length of crabs used in 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 (t_{17}=0.007, p=0.5), control nudibranch lengths (t_{17}=0.031, p=0.5), and anesthetized nudibranch lengths (t_{17}=0.006, p=0.5).

A one-way ANOVA found no significant difference between the control and anesthetized nudibranch lengths ($F_{1,34}$=0.57, p=0.57) (Table 5).

Discussion

Activation of Defense Mechanisms

Peltodoris nobilis

There was no significant difference between the control and anesthetized nudibranch contact times with the crabs ($F_{1,18}$=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 time 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.

The prevention of predation without preventing contact is possibly due to chemicals produced *de novo* signaling that the nudibranch is poisonous or tastes bad. The presence of the secondary metabolite 1-Methylguanosine, which potentially serves as a defense or signaling molecule, and a degraded sesquiterpenoid acting as an odiferous compound coupled with the evidence supporting the toxicity of *P. nobilis* tissue could potentially explain the lack of predation (Dean & Princep 2017, 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 are poisonous or taste bad.

Mucus was also produced by nudibranchs in 3 of the trials. In trial 4, the crab got caught in the mucus and actively tried to get it off and escape. Once the crab escaped the mucus, it did not attempt contact again. In all 3 trials, there was no contact between the crab and nudibranch. This suggests that mucus also works as a predator deterrent for *Peltodoris nobilis* and 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).

Hermissenda crassicornis

The contact times with the crabs and the control nudibranchs were significantly lower than with the anesthetized nudibranchs ($F_{1,34}$=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 will eat the nudibranchs given the opportunity. However, in 2 trials, the crab avoided the nudibranch after the initial contact. 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, since crabs will try to eat the nudibranchs. Control nudibranchs also used movement to avoid the crabs;

however, the crabs moved much faster so this was not very effective if the crab pursued the nudibranch.

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 *Peltodoris nobilis* and *Hermissenda crassicornis* trials. Variations in crab size could potentially influence results because the mouth parts of larger, and consequently older, crabs are better defended than younger crabs. Larger crabs would therefore be less affected by defense mechanisms, which could increase contact time for the larger crabs. Larger nudibranchs could also contain more chemicals or nematocysts than smaller nudibranchs, increasing their defense and consequently decreasing contact time. However, since no statistical difference was found, the difference in contact times 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 results.

Effectiveness of *de novo* Chemical Synthesis vs. Nematocyst Sequestration

It was 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 *Hermissenda crassicornis* trials was significantly lower than *Peltodoris 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, comparing control contact times does 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 nudibranch, this study cannot determine if nematocyst sequestration is more effective at preventing predation than *de novo* chemical synthesis.

Conclusion

My research sought to answer 2 questions: 1) Is the release of chemicals produced *de novo* in *Peltodoris nobilis* and the firing of sequestered nematocysts by *Hermissenda crassicornis* active or passive? 2) Is one defense mechanism more effective than the other?

There was no significant difference with the crab contact time between the control and anesthetized nudibranchs for *Peltodoris nobilis* ($F_{1,18}$=0.23, p>0.05). It was 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 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 *Hermissenda 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. *Hermissenda crassicornis* initiated contact with the crabs in some control trials, and in 2 such trials the crabs moved to avoid the nudibranch. This behavior supports the effectiveness of nematocyst sequestering and firing in defense.

In a few trials, *Peltodoris nobilis* released mucus and *Hermissenda crassicornis* moved to avoid contact with the crab and in other trials, cerata were detached from the nudibranch's body but still moved. Future research could study mucus as a potential defense mechanism of dorid nudibranchs, as well as the potential toxicity of the mucus produced by *P. nobilis* and other dorids. Another future area of research could compare the effectiveness of defense mechanisms. This could provide insight to 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 by the juvenile crabs. \It would be interesting to see if the lack of predatory behavior changed with adult crabs. A final future area of research could determine 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 and if the cerata can still fire nematocysts in this state.

Figures

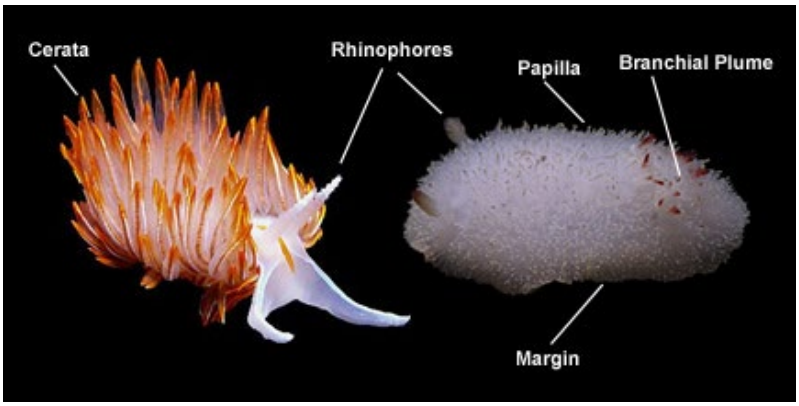

Figure 1: Anatomy of Aeolid and Dorid Nudibranchs

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. Diagram taken from Emeralddiving.com.

Figure 2: Image of *Peltodoris nobilis*.

Photo taken off San Migul Island, California by Bruce C. Wight (2000).

Figure 3: Image of *Hermissenda crassicornis*.

Photo taken in Cape Flattery, Washington by Brooke Reiswig (2006).

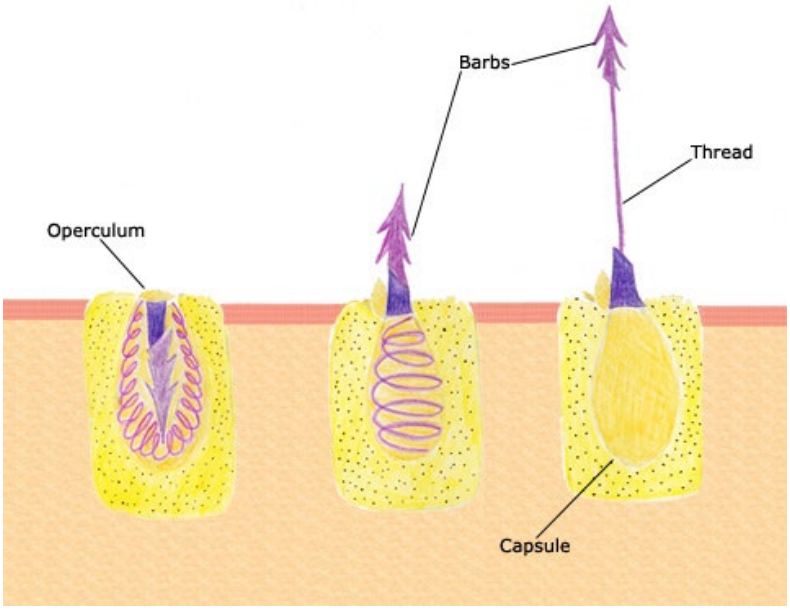

Figure 4: Diagram of a nematocyst firing

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. Photo taken from NOAA website page " Nematocyst Cell".

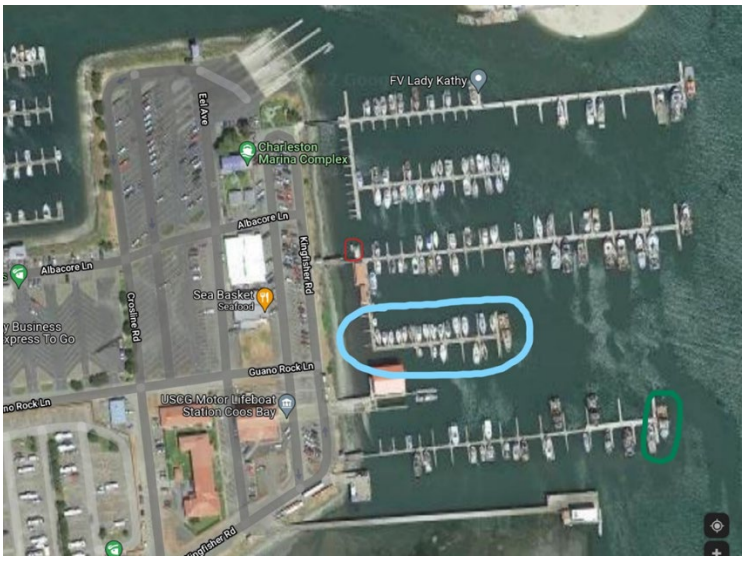

Figure 5: Image of specimen collection and release locations

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 red 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.

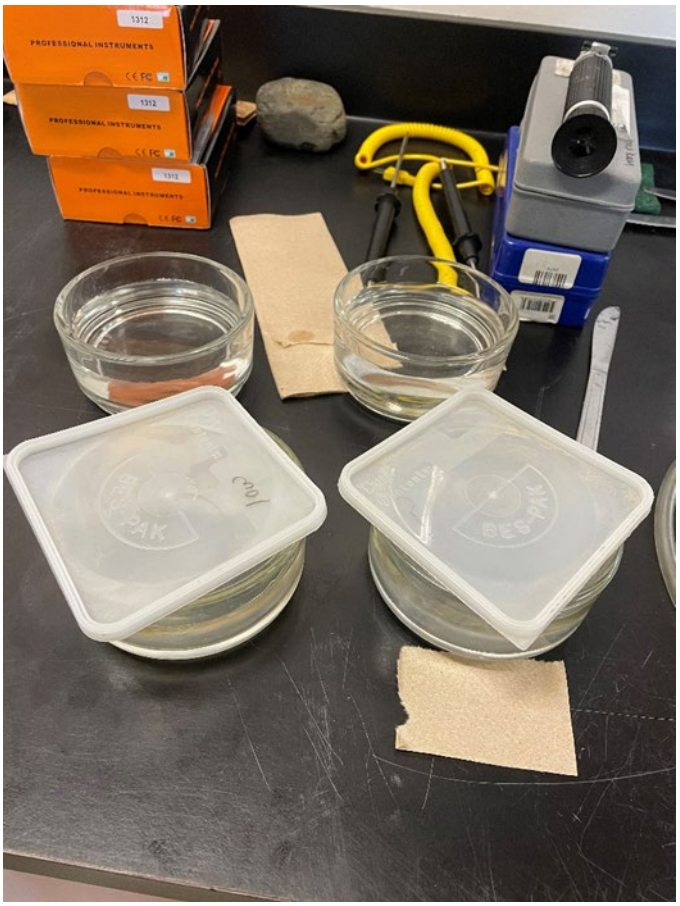

Figure 6: Image of experimental set up for *Peltodoris nobilis*.

The nudibranchs are in the covered glass containers, and the brown torn 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.

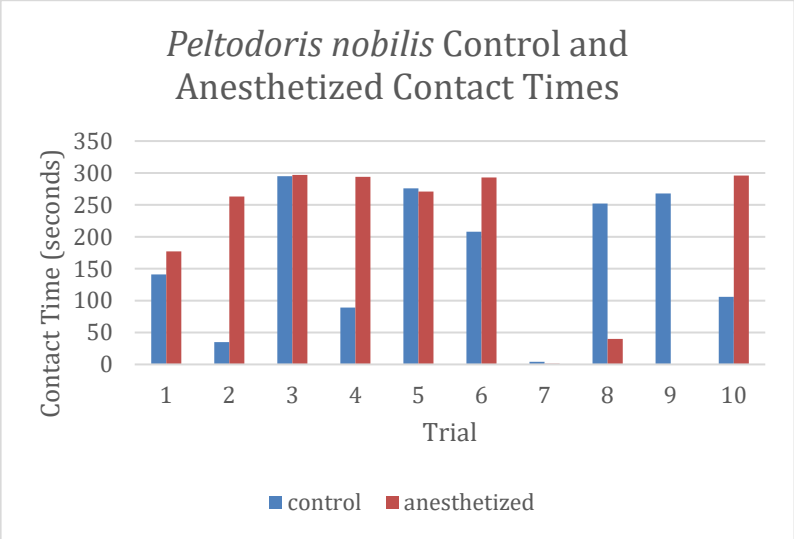

Figure 7: *Peltodoris nobilis* Control and Anesthetized Contact Times

The amount of time the crabs contacted the control and anesthetized nudibranch in each *Peltodoris nobilis* trial.

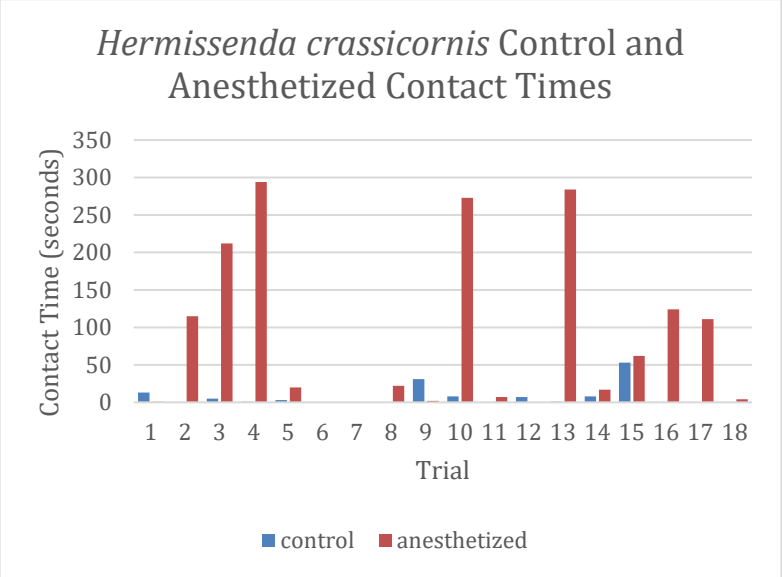

Figure 8: *Hermissenda crassicornis* Control and Anesthetized Contact Times

The amount of time the crabs contacted the control and anesthetized nudibranchs in each *Hermissenda crassicornis* trial.

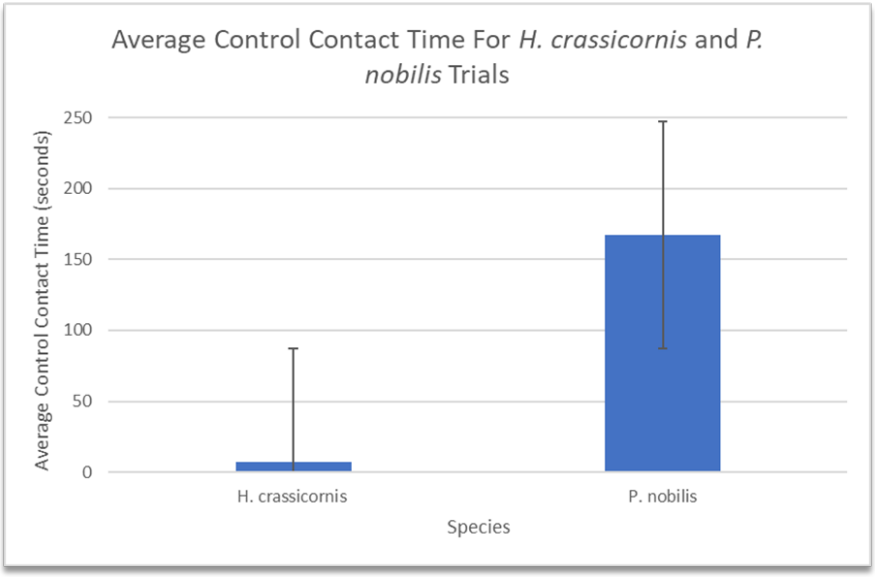

Figure 9: Average Difference in Control Contact Time for *H. crassicornis* and *P. nobilis* Trials

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.*

Tables

	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 1: Observations of Crab and Nudibranch Behavior During *Peltodoris nobilis* Trials

A table listing the behavior of the crabs when in contact with the nudibranchs. “Nudi” is short for nudibranch. Of the 10 trials, predatory behavior in the crab was observed in 3 trials, all between the crab and anesthetized nudibranch. In the remaining 7 trials, no predatory behavior was observed. Predatory behavior was considered to be whenever a crab moved its front claws when stationary and in contact with the nudibranch. 3 nudibranchs were observed to have released or were currently releasing mucus.

	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 2: Statistics for the *Peltodoris nobilis* trials

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. These p-values indicate whether the contact time or sizes are significantly different. A p-value greater than 0.05 indicates that the data are not statistically significant and a p-value less than 0.05 indicates that the data are statistically significant. There was no significant difference between the control and anesthetized contact times with the crabs, supporting passive activation. There was also no significant difference between the carapace lengths of the crabs and between the lengths of the nudibranchs used in the trials. This indicates that any variation in the sizes of the nudibranchs or crabs used in this experiment did not impact results.

	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 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**
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 4: Observations of Crab and Nudibranch Behavior During *Hermissenda crassicornis* Trials

A table listing the behavior of the crabs and the nudibranchs in the *Hermissenda crassicornis* trials. "Nudi" is short for nudibranch. Of the 18 trials, predatory behavior was observed in 10 of the crabs. This predatory behavior was observed only with the anesthetized nudibranch for 8 of the trials. In the remaining 2 trials, predatory behavior was observed with both the control and anesthetized nudibranch in one, and just the control in the other. In the remaining 8 trials no predatory behavior was exhibited by the crab. Predatory behavior was whenever a crab moved its front chelae to and from its mouth when in contact with the nudibranch.

	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 5: p-values for *Hermissenda crassicornis* trials

A table listing the statistics for a one-way ANOVA comparing the crab contact times between the anesthetized and control nudibranchs and a one-way ANOVA comparing the sizes of the control and anesthetized nudibranchs. Also, the statistics for the one-sample t-tests comparing the sizes of the control *H. crassicornis*, the sizes of the anesthetized *H. crassicornis*, and the carapace length of crabs used in the trials. A p-value greater than 0.05 indicates that the differences in sizes or contact time are not statistically significant and less than 0.05 indicates that the differences are statistically significant. The difference in contact time with the crab was significantly higher for the anesthetized nudibranchs than the control. This supports an active defense mechanism. The difference between crab carapace lengths was not significant and the difference in the lengths of the nudibranchs was also not significant. This indicates that the differences in the sizes of the crabs and the nudibranchs did not impact results.

	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

Table 6: Raw data for the *Hermissenda Crassicornis* trials

Bibliography

Anthony, S.E. (2014). Selective cnida sequestration in the aeolid nudibranch Hermissenda crassicornis: ecology and mechanism of defense acquisition [Master of Science thesis]. Department of Biological Sciences, University of Albert.

Britannica, T. Editors of Encyclopaedia. (2020). nudibranch. *Encyclopedia Britannica*. https://www.britannica.com/animal/nudibranch.

Edmunds, M. (1968). Acid Secretion in Some Species of Doridacea (Mollusca, Nudibranchia). *Journal of Molluscan Studies.* 38: 121-133.

Edmunds, M. (2016). Defensive mechanisms in some nudibranchs [PhD thesis]. University of Oxford.

Faulkner, D.J., & Ghiselin, M.T. (1983). Chemical defense and evolutionary ecology of dorid nudibranchs and some other opisthobranch gastropods. *Marine Ecology Progress Series*. 13: 295-301.

Goddard, JHR. (1983). The Opisthobranchs of Cape Arago, Oregon with Notes on Their Natural History and a Summary of Benthic Opisthobranchs Known from Oregon [Master Thesis.] Department of Biology, University of Oregon.

Goodheart, J.A., & Bely, A.E. (2016). Sequestration of nematocysts by divergent cnidarian predators: mechanism, function, and evolution. *Invertebrate Biology*. 136(1): 75-91.

Cimino, G., & Ghiselin, M. (1999). Chemical defense and evolutionary trends in biosynthetic capacity among dorid nudibranchs (Mollusca: Gastropoda: Opisthobranchia). *Chemoecology* 9:187–207.

Dean, L.J., & Prinsep, M.R. (2017). The chemistry and chemical ecology of nudibranchs. *Royal Society of Chemistry.* 34: 1359-1390.

Di Marzo, V., Marin, A., Vardaro, R.R. et al. (1993). Histological and biochemical bases of defense mechanisms in four species of *Polybranchioidea ascoglossan* molluscs. *Marine Biology*. 117: 367–380. https://doi.org/10.1007/BF00349312.

Fuhrman, F.A., Fuhrman, G.J., & Deriemer, K. (1979). Toxicity and Pharmacology of Extracts From Dorid Nudibranchs. *The Biological Bulletin*. 156(3):289-299. https://doi.org/10.2307/1540918.

Miller, J., & Byrne, M. (2005). Ceratal autotomy and regeneration in the aeolid nudibranch *Phidiana crassicornis* and the role of predators. *Invertebrate Biology*. 119: 167-176.

MolluscaBase eds. (2021). MolluscaBase. Hermissenda Bergh, 1879. Accessed through: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=367881 on 2021-10-31

Rudman, W.B. (2000). *Peltodoris nobilis* (MacFarland, 1905). [In] Sea Slug Forum. Australian Museum, Sydney.

Wheeling, R. (2000). *Pelotdoris nobilis* (MacFaland, 1905). [In] Inverts.wallawalla. Walla Walla University