DISPLAYS OF DEFENSE: BEHAVIORAL DIFFERENCES IN ANTAGONIST AVOIDANCE IN FOUR OPISTHOBRANCH MOLLUSKS

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Abstract. The defensive behaviors of four opisthobranchs (*Glossodoris cincta*, *Risbecia imperials*, *Stylochelius striatus*, and *Dolabrifera dolabrifera*) were observed and categorized. The displays studied were mantle flexation, mucus production, mantle secretion, inking, and rearing. Members of each species were placed in two laboratory situations containing two different antagonists. The antagonists (*Dardanus lagopodes* and *Lutjanus fulvus*) were chosen because they were carnivorous, abundant, and found in the same ecology as the opisthobranchs studied. Additionally, they were chosen because they differed phylogenetically, physiologically, and behaviorally and, therefore, represented two very different predators. In some cases, individuals exhibited different defensive behaviors in the presence of different antagonists. Differential responses could reflect physiological, biological, or phylogenetic differences between the four observed opisthobranch species. In some instances, defensive displays were observed across lineages.

Key words: defense; inking; mantle flexations; rearing; opisthobranch; nudibranch; sea hare; Chromodorididae; Aplysiidae; *Moorea; French Polynesia*

INTRODUCTION

Although nudibranchs lack the protective shell characteristic of numerous gastropods, many possess chemical signals or toxins to deter predators (Mallet & Joron 1999, Penney 2004). Evidence suggests that many opisthobranchs use sequestered chemical compounds from food sources as a means for defense (Faulkner & Ghiselin 1983, Avila 1995). In some cases, the compounds involved in defense are known to be toxic, and in other cases they are not, but still appear to act as effective deterrents for predators (Gimenez-Casalduero et al. 1999, Long & Hay 2006). Additionally, the bright colors of many opisthobranch species may have evolved to warn predators against attacking these soft-bodied marine invertebrates (Rosenberg 1989, Tullrot 1994, Lindstrom 2001). Since opisthobranchs cannot see color, evidence suggests that it is unlikely that bright markings and bold patterns would have evolved from sexual selection or species recognition (Servedio 2000, Behrens 2005). Aside from the present theories surrounding chemical defense and aposematic coloration, the multiple behaviors associated with many defensive displays are still poorly understood.

This study is aimed at examining some of the behavioral responses exhibited by opisthobranchs when in the presence of an antagonist. This paper centers on two major questions. The first and preliminary question being, what are the different predator defense mechanisms exhibited by four opisthobranch species found in Moorea? This study identifies and compares the behaviors exhibited by various opisthobranchs in the presence of an antagonist. Many behaviors such as the release of mucus or ink should be observed only in the presence of an antagonist, since the likely cost of producing the compounds for these compounds is great.

My second major question is how do these opisthobranchs behave when presented with different antagonists? That is, does defensive behavior change in the presence of antagonists that differ phylogenetically, physiologically, and behaviorally? I expect prey subjects to display a variety of defensive behaviors, some of which will only be apparent in the presence of a particular antagonist. If this is the case, I expect to observe differential response to antagonists because of phylogenetic, developmental, or physiological differences between the opisthobranch species. I expect defensive displays to be lineage-specific and to observe the same behaviors in organisms that are more closely related.

METHODS

Study site

All opisthobranch species were collected while snorkeling at Cook's Bay (FIG. 1) in Moorea, French Polynesia. Opisthobranchs were found in close proximity to the Gump Research Station, primarily between the waterfront bungalows and the small grove of trees just north of the station. All subjects were collected in the late morning or early afternoon.

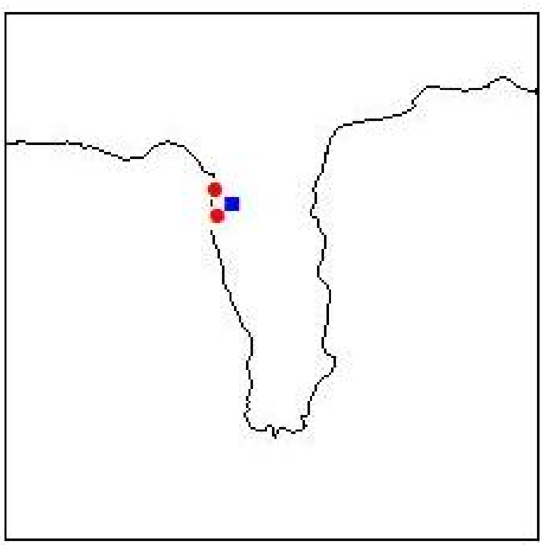

FIG. 1 Site map of Cook's Bay (grove and bungalows noted by ● and field site noted by ■).

Description of species

Two nudibranch species (Chromodorididae) and two species of sea hares (Aplysiidae) were observed in this study (FIG. 2).

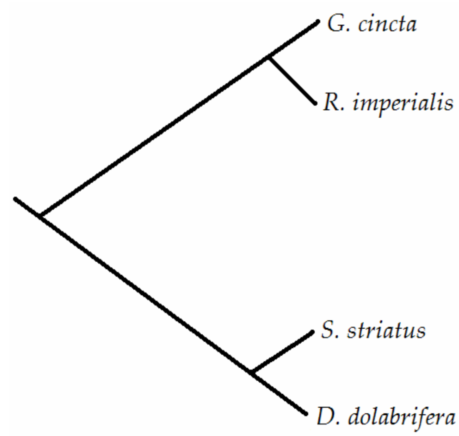

FIG. 2. Phylogeny of opisthobranch species (adapted from Johnson 1994).

Glossodoris cincta (Bergh, 1888) (FIG. 3) is the first of two conspicuously colored opisthobranch species used in this study. This nudibranch is characterized as having three colored bands around the mantle border: an outermost pale blue, middle black, and innermost yellow or khaki band (Rudman 2000). Most of its body is mottled dark brown and light pink in color. Its rhinophores and gills appear to be dark brown speckled with light pink. *Glossodoris* nudibranchs excrete some secondary metabolites from their diets which are thought to deter some though not all potential predators (Rogers 1991). Subjects were found feeding on algae on rock rubble. This species was the most abundant at the collection sites.

FIG. 3. *Glossodoris cincta*

Risbecia imperialis (Pease, 1860) (FIG. 4) is the second brightly colored opisthobranch species used in this study. This species was also found feeding on algae growing on rocks and on the sandy substrate near coral heads. These nudibranchs were often found occurring in pairs, and were observed tailing—a behavior in which one opisthobranch follows the mucus trail of another, using the sensory cells in its oral tentacles (Behrens 2005). This nudibranch is characterized as having a primarily white mantle and foot with yellow dots. Its gills are white edged with dark blue, and its rhinophores are dark blue with white flecks. The zigzagged mantle border of *R. imperialis* is dark blue and encompasses small yellow dots.

FIG. 4. *Risbecia imperialis*

Stylochelius striatus (Quoy and Gaimard, 1832) (FIG. 5) is the first of two inconspicuous opisthobranch species examined in this study. This sea hare can be characterized as having a mottled green and brown body. Also characteristic of *S. striatus*, are dark longitudinal lines and scattered royal blue dots along its mantle. This species was found feeding on blue-green algae that forms film over rocks or muddy surfaces.

FIG. 5. *Stylochelius striatus*

Dolabrifera dolabrifera (Cuvier, 1817) (FIG. 5) is the second cryptic opisthobranch observed in this study. The body of *D. dolabrifera* is flattened and a mottled green in color. The posterior half of the organism is rounded and narrows closer to the head. Its parapodia are fused except for a portion in the posterior midline where two flaps form a respiratory opening (Rudman 2003). This species is known to have glands incapable of producing ink or its associated anti-predator proteins (Prince 2006). The species can be found attached to rock rubble.

FIG. 6. *Dolabrifera dolabrifera*

To address my primary question, does defensive behavior in opisthobranchs change when different antagonists are present, I used two antagonists in this study. I chose hermit crabs (*Dardanus lagopodes*) (Forskål, 1775) because they represent a predator that crushes and chews its prey. It is likely that an opisthobranch would have a lower probability of surviving an encounter with a hermit crab than with a predator that swallows its prey whole (Penney 2004). This is also why I chose the blacktail snapper (*Lutjanus fulvus*) (Scneider, 1801) for my second antagonist. This snapper is representative of fish predators that mouth and swallow their prey. While neither of these species may represent natural opisthobranch predators, they still posed a potential threat and were expected to trigger a defense mechanism in the prey subject. Furthermore, both antagonists are carnivorous, abundant, and share the same habitat as the four opisthobranch species used in this study. There is also very little information available on natural opisthobranch predators.

For the purposes of this study, six hermit crabs were collected while snorkeling in Cook's Bay. Eight blacktail snappers were also caught off of the Gump Station dock using lunchmeat for bait and a bamboo fishing pole. Antagonists were not injured during collection and were maintained in tanks with continuously flowing seawater. They were returned to the site of capture at the conclusion of the experiment.

Experiment design

This experiment was conducted between 10/1/06 and 11/12/06. While in captivity, all opisthobranchs were kept in plastic containers. The sides of each container were punctured using a piece of heated metal to allow for adequate water flow through the container. The container lid was replaced with a rubber-banded mesh sheet to generate additional flow between the water in the container and the water in the tank. Each container contained one opisthobranch and one algae-covered rock to provide the subject with food. The containers were placed in a large outdoor tank. This was necessary because sunlight was needed for the algae to photosynthesize. Rocks were changed out twice weekly and were collected at the sites where opisthobranchs were found.

Each observation tank contained only natural rocky substrate collected where the opisthobranch was found, one antagonist, and one opisthobranch. The tanks used in the crab trials were large plastic containers with the lids removed. The tanks used in the fish trials had fresh salt water flowing though them constantly to ensure that the water contained enough oxygen to sustain the fish. Six subjects of each opisthobranch species were exposed to antagonists in three-hour intervals. I observed the actions of the prey subjects for the first and last half hour of each trial. Chi-square analyses were used to test for significant differences between crab and fish trials.

The opisthobranchs were first exposed to the hermit crabs. The hermit crab was placed in an observation tank and allowed to acclimate for a half hour. The opisthobranch was then placed in the tank in close proximity to the antagonist. Upon completion of the hermit crab trials, the same opisthobranch subjects were then exposed to the second group of antagonists, the blacktail snappers. The fish were also allowed to acclimate for a half hour before the trial. The fish were released after being kept in the lab for one to two days. The control experiment for both sets of trials followed the same time frame and set-up. The opisthobranch was left in the observation tank with a rock while no antagonist was present. The control trial lasted for three hours and opisthobranch behaviors were recorded for the first and last half hour of the trial.

RESULTS

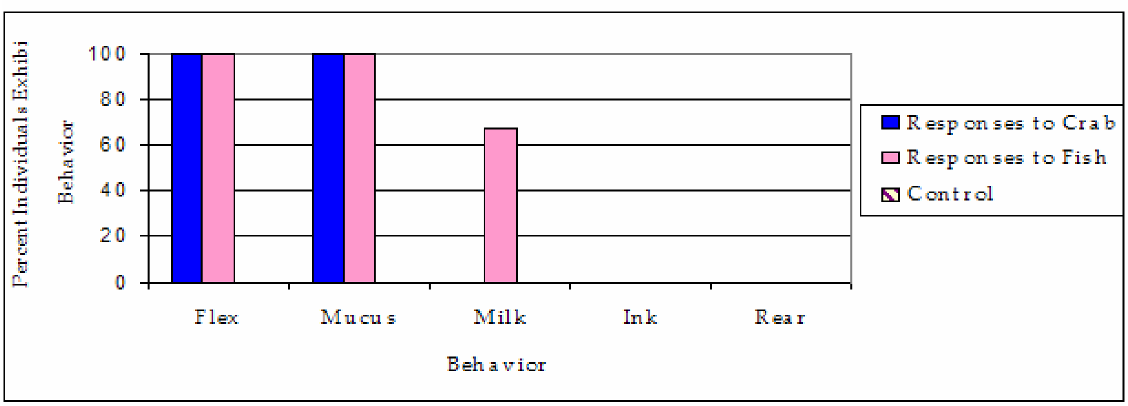

FIG. 7. Behaviors observed in *G. cincta*.

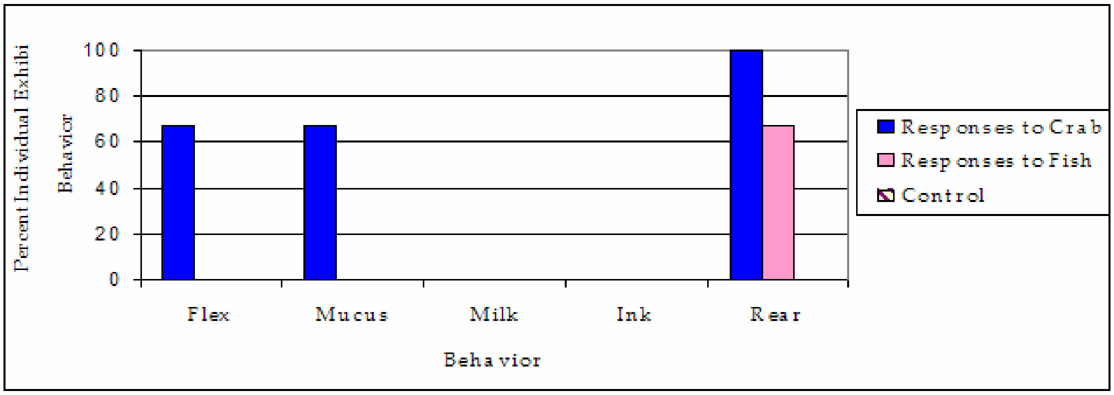

FIG. 8. Behaviors observed in *R. imperialis*.

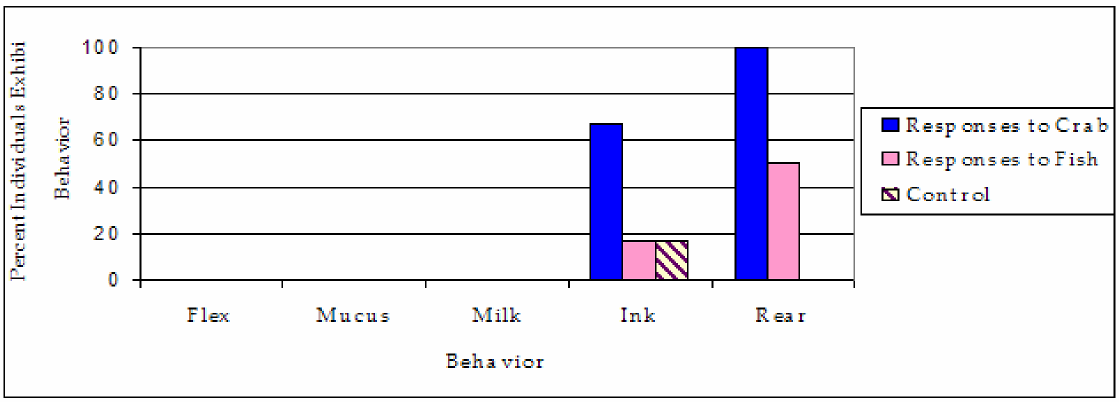

FIG. 9. Behaviors observed in *S. striatus*.

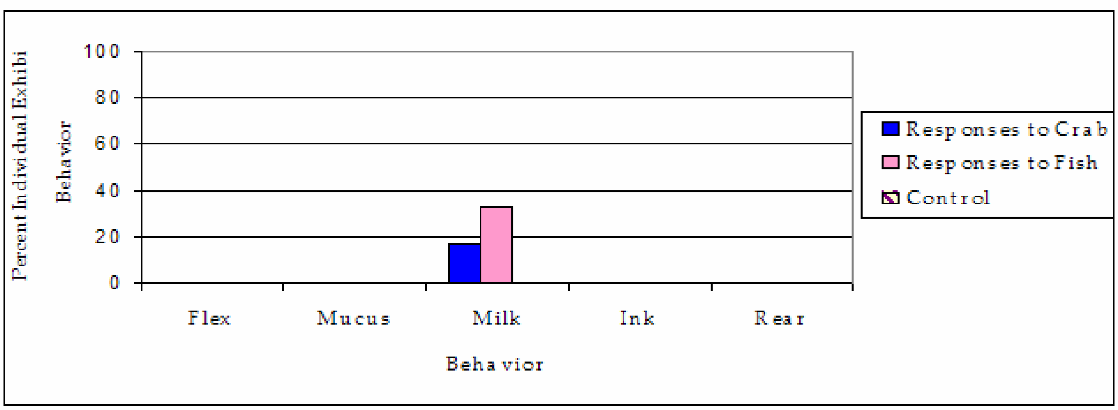

FIG. 10. Behaviors observed in *D. dolabrifera*.

In *G. cincta*, mantle flexations and mucus production occurred in all individuals during both crab and fish trials (FIG. 7). The secretion of a milky substance from the organism's mantle was seen in 67% of the fish trials and was not present in the crab trials (FIG. 7). Inking and rearing were absent in all trials (FIG. 7).

Mantle flexing and the production of mucus was observed in 67% of the *R. imperials* specimens during the crab trials (FIG. 8). Both behaviors were absent in the fish trials (FIG. 8). Rearing was seen in both crab and fish trials, but was more frequent in the crab trials (FIG. 8). Inking and mantle secretions were not present in any *R. imperialis* individuals (FIG. 7).

In *S. striatus*, rearing and inking were present in both crab and fish trials, though both behaviors were more prevalent in the crab trials (FIG. 9). Inking also occurred once in the control setting (FIG. 9). Mantle flexations, mucus production, and mantle secretions were absent from all trials (FIG. 9).

The only behavior recorded in *D. dolabrifera* was the secretion of a white substance from the organism's mantle (FIG. 10). This occurred more frequently in the fish trials, but was also present during the crab trials (FIG. 10).

Chi-square analyses reflected no significant differences between crab and fish trials.

DISCUSSION

Defensive displays

The displays I observed are mantle flexation, mucus production, mantle secretion, inking, and rearing.

Mantle flexations were only observed in the two nudibranch species (*G. cincta* and *R. imperialis*). Although *G. cincta* appeared to react to both antagonists, it is interesting that *R. imperialis* only displayed this behavior in the presence of the crab antagonist. A mantle flexation involved the nudibranch lengthening and twisting its body. It appeared to flex so that its head was directed downward, foot twisted, and the body's posterior portion faced up. During this display, the organism's gills and rhinophores were alert and moving. This may indicate that the organism was taking in sensory information about its environment and, perhaps, its antagonist. Little has been written on mantle flexations, though I speculate that the behavior may be a defensive display that allows individuals to appear longer or bigger in the presence of an antagonistic force. This theory is supported an earlier observation that when a nudibranch is disturbed there is a general contraction, which causes the mantle edge to appear more prominent (Crozier 1916).

Like mantle flexing, mucus production also occurred only within the two nudibranch species. It appeared to co-occur with the flexing display in both *G. cincta* and *R. imperialis*. This is interesting because perhaps the flexing display is a necessary physiological component of mucus production in these nudibranch species. Mucus production is known to be element of locomotion in opisthobranchs, but its role in predator defense has been understudied. The distinct mucus trails left by *G. cincta* and *R. imperialis* appeared to be entirely different from the clear, gel-like substance usually associated with locomotion (Behrens 2005). Additionally, *R. imperialis* produced mucus only during the crab trials, possibly indicating that the organism was more stressed in the situation with the more tactile of the two antagonists.

Both *G. cincta* and *D. dolabrifera* were observed secreting a white, milky substance from their mantles when an antagonist was present. This is interesting because *G. cincta* is a nudibranch and *D. dolabrifera* is a sea hare. It is unclear as to whether the two excretions contained the same compound, though it is thought that such secretions are associated with the sponges that the opisthobranchs feed on (Becerro et al. 2006). Additionally, opisthobranchs are known for secreting chemical compounds that deter predators (Gosliner et al. 1996). I grouped both occurrences as one behavior, since they appear to be the product of the same situational cause—the milky substance was only secreted in the presence of an antagonist. Interestingly, the substance was secreted more often in the presence of the fish antagonist. Perhaps a chemical cue from the fish triggered this reaction. This theory is supported by the observation that mantle secretions occurred almost immediately after the opisthobranch was placed in the tank with the antagonist.

Inking was observed only in *S. striatus* and occurred more frequently in the crab trials than in the fish trials. This could suggest that the organism was more stressed in the situation with the more exploratory and tactile antagonist. Inking is a known defense mechanism in sea hares, and it is thought that the ink distracts a predator long enough for the prey to flee (Rodhouse 1998). This behavior is noted in many other mollusks, perhaps most famously in octopuses (Rodhouse 1998).

Rearing is a behavior associated with repulsion or escape (Behrens 2005). The posture involved the posterior region of the opisthobranch remaining attached to a surface while its mantle and anterior region was lifted up. During this display the organism's oral tentacles were highly active. Because of this, I speculate that this display does not only signal repulsion, but may also be involved in obtaining sensory information about the organism's environment. The behavior was only seen in *R. imperialis* and *S. striatus*. Based on a fundamental understanding of the body plans of all of the observed species, this is not surprising. Both of these species have a defined neck-like region of the mantle, which is absent from the other two species. Interestingly, rearing was seen in all *R. imperialis* and *S. striatus* individuals during the crab trials, but was less prevalent in the fish trials. Perhaps the sensory information about the crab was more accessible, and therefore, specimens were more active during these trials.

Conclusions

Because many behaviors are poorly understood in opisthobranchs, information regarding why these animals act as they do in the presence of antagonistic organisms is relevant to better understanding the physiology and effectiveness of defensive displays.

I expected to observe a variety of defensive behaviors in the four opisthobranch species studied. It is interesting that no species exhibited every behavior recorded in this study. This may be due the differing physiologies of each species.

Though I expected to see the same behaviors exhibited by more closely related species, this was only true some of the time. For example, mantle flexing and the production of distinct mucus trails were only seen in the two nudibranch species (*G. cincta* and *R. imperialis*). Perhaps this behavior is physiologically impossible for the other two species to exhibit, or perhaps they excrete a less visible form of mucus that does not co-occur with a highly visible display. On the other hand, the secretion of a milky discharge was observed in both lineages (in *G. cincta* and *D. dolabrifera*). The secreted compounds could indicate that the nudibranch and sea hare feed on similar sponges and may be parts of similar trophic systems. Or perhaps the display evolved in more than once because it proved to be an effective defense against predation. Rearing was also seen across lineages (in *R. imperialis* and *S. striatus*). Perhaps the behavior evolved in both species because their similar physiologies allowed for sensory information to be collected in a similar way.

I also expected that the defensive behaviors exhibited would be different in the presence of different antagonists. While there was a differential response for many species between the two antagonists, the particular frequency of responses was species-specific. It was interesting to find that in certain cases, there was a differential response of prey to different antagonists. For example, *G. cincta* only exhibited a milky secretion in the presence of the fish antagonist, and *R. imperialis* only flexed and produced mucus in the presence of the crab antagonist. This might be attributed to the organism experiencing more stress in one particular interaction.

For the most part, none of the defensive behaviors occurred in the absence of an antagonist. This suggests that all the observed behaviors are defensive displays, or at the very least, are in response to the presence of another organism in the tank. Only one individual inked in at the start of the control trial, indicating that the individual was stressed during the transfer from the holding tank to the observation tank.

Although chi-square analyses revealed no significant differences between crab and fish trials, differences in behavioral responses are clearly visible in graphs of the original data. Perhaps a larger sample size and further analysis would show a more statistically sound trend.

This paper sought to examine the various defensive behaviors exhibited by four species of opisthobranchs. Further research on the natural predators of these species, if any exist, is necessary in better understanding how they fit into their trophic system and ecology. Additionally, it would be interesting to see if any of the recorded displays are prevalent in behavioral arenas other than defense, such as mating, predation, and intraspecific communication.

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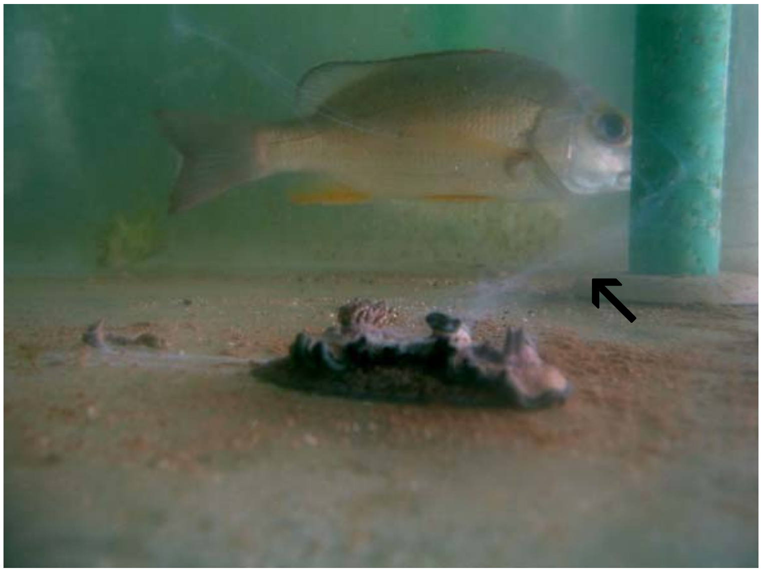
Appendix A

The following list provides photograph and brief description of some of the defensive displays observed in this study.

Mantle flexing by *G. cincta* in the presence of *D. lagopodes*.

One *D. lagopodes* inspects a distinct mucus trail left by *G. cincta*.



A *G. cincta* specimen secretes a milky discharge in the presence of *L. fulvus*.

Rearing display by *S. striatus* in the presence of *D. lagopodes*.

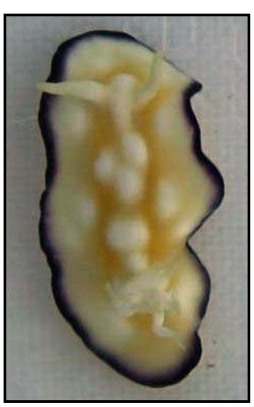
Appendix B

The following list provides a photograph (left) and brief description (right) of every opisthobranch species I encountered during my time on Moorea, and is intended as a reference for future students. I do not presume that these are the only species present around the island, or that these species cannot be found at different depths or locations than I have specified.

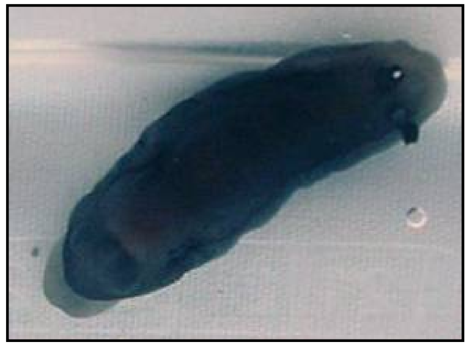
***Cyerce elegans* (Bergh, 1870)**
Found: 5-10 feet, Cook's Bay
Length: 15mm
Primary color: Pale yellow/white
Distinguishing characteristics: bifurcate enrolled rhinophores; opaque cerata

***Chromodoris lochi* (Rudman, 1982)**
Found: 25-30 feet, Cook's Bay
Length: 35mm
Primary color: Light blue/white
Distinguishing characteristics: Black longitudinal lines; orange gills and rhinophores

Chromodoris sp.1 (possibly closely related to *C. virbrata*)
Found: 5-10 feet, Cook's Bay
Length: 30mm
Primary color: White/yellow
Distinguishing characteristics: Dark purple mantle border, gills, and rhinophores

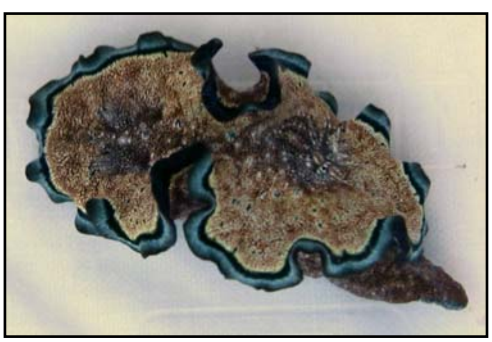


Chromodoris sp.2
Found: 5-10 feet, Cook's Bay
Length: 30mm
Primary color: Yellow
Distinguishing characteristics: White marks, gills, and rhinophores; dark purple mantle border

***Dendrodoris nigra* (Stimpson, 1855)**
Found: 5-10 feet, Cook's Bay
Length: 25mm (adult)
Primary color: Black (above); Dark brown (juvenile, left)
Distinguishing characteristics: White tips of rhinophores; white spots along mantle; red mantle border (juvenile)

***Dolabrifera dolabrifera* (Cuvier, 1817)**
Found: 5-10 feet, Cook's Bay
Length: 20mm
Primary color: Mottled green
Distinguishing characteristics: Flat; rounded; body narrows closer to head



***Glossodoris cincta* (Bergh, 1888)**
Found: 5-10 feet, Cook's Bay
Length: 50mm
Primary color: Mottled pink/brown
Distinguishing characteristics: Dark black/blue mantle borders; rhinophores and gills are dark brown speckled with pink

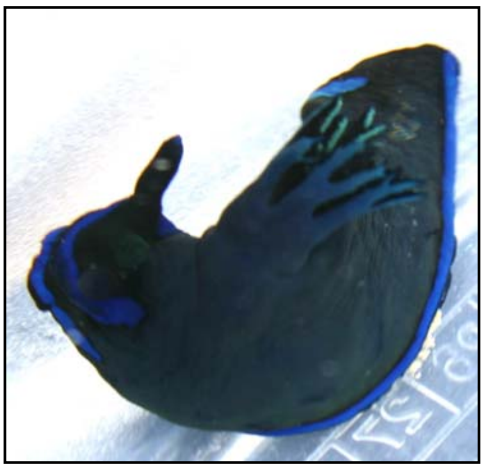
***Hexabranchus sanguineus* (Ruppell & Leuckart, 1828)**
Found: 5-10 feet, Cook's Bay
Length: 35cm
Primary color: Red
Distinguishing characteristics: Swimmer; grows to large sizes; white mantle border; red with white gills
Photo courtesy of Sarah Chinn

***Hypselodoris zephyra* (Gosliner & Johnson, 1999)**
Found: 4-6 feet, Opunohu Bay
Length: 25mm
Primary color: Beige/lavender
Distinguishing characteristics: Black longitudinal lines; orange rhinophores and gills

***Risbecia imperialis* (Pease, 1860)**
Found: 5-10 feet, Cook's Bay
Length: 45mm
Primary color: White
Distinguishing characteristics: Yellow dots along mantle and in zigzagged blue mantle border; gills are white edged with dark blue; rhinophores are dark blue speckled with white

***Risbecia tryoni* (Garrett, 1873)**
Found: 30 feet, Cook's Bay
Length: 60mm
Primary color: Crème/white
Distinguishing characteristics: Purple mantle border; dark spots on mantle; gills are crème edged in brown.

Stylochelius striatus **(Quoy and Gaimard, 1832)**
Found: 5-10 feet, Cook's Bay
Length: 55mm
Primary color: Mottled green/brown
Distinguishing characteristics: Small royal blue dots along mantle; dark longitudinal lines



Tambja morosa **(Bergh, 1877)**
Found: 30 feet, Cook's Bay
Length: 70mm
Primary color: Black
Distinguishing characteristics: Royal blue markings near mouth and along mantle border; green/blue gills