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PREDATOR DEFENSE MECHANISMS IN SHALLOW WATER SEA CUCUMBERS (HOLOTHUROIDEA)

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Abstract. The various predator defense mechanisms possessed by shallow water sea cucumbers were surveyed in twelve different species and morphs. While many defense mechanisms such as the presence of Cuvierian tubules, toxic secretions, and unpalatability have been identified in holothurians, I hypothesized that the possession of these traits as well as the degree to which they are utilized varies from species to species. The observed defense mechanisms were compared against a previously-derived phylogeny of the sea cucumbers of Moorea. Furthermore, I hypothesized that while the presence of such structures is most likely a result of the species' placement on a phylogenetic tree, the degree to which they utilize such structures and their physical behavior are influenced by their individual ecologies. The presence of a red liquid secretion was restricted to individuals of the genus *Holothuria* (Linnaeus 1767) however not all members of the genus exhibited this trait. With the exception of *H. leucospilota*, which possessed both Cuvierian tubules and a red secretion, Cuvierian tubules were observed in members of the genus *Bohadschia* (Ostergren 1896). In accordance with the hypothesis, both the phylogenetics and individual ecology appear to influence predator defense mechanisms. However, even closely related species of similar ecology may differ considerably.

Key words: holothurians; defense; toxicity; Cuvierian tubules; Moorea, French Polynesia

INTRODUCTION

Sea cucumbers belong to the phylum Echinodermata and the class Holothuroidea. Echinoderms are distinguished by having a coelomic, water vascular system and pentaradial symmetry. Holothurians in turn are characterized by having feeding tentacles that extend from a circumoral ring. Individuals can range in size from 10 to 2000mm in length (Lawrence, 1897). Holothurians come in many shapes and sizes and inhabit a wide variety of habitats from the intertidal zone to the deep sea (Anonymous, 2003).

Holothurians are important ecologically as well as economically and culturally. Sea cucumbers have been collected and utilized as a food item, especially in Southeast Asia, for generations. Food products include dried gonads, dried body meat and longitudinal muscles, and salted fermented intestine

(Sakthivel et. Al, 1994). Approximately 20 species in two families and five genera, including *Holothuria*, *Bohadschia*, and *Thnelota*, are traded internationally (Anonymous, 2003). The topic of overharvesting and sustainable aquaculture has become increasingly important in recent years.

Studies have noted that predation plays a major role in influencing community structure as well as spatial and temporal distribution of marine invertebrates (Bingham, 1986; Francour, 1997). Predators of holothurians include fish, crustaceans, and most importantly sea stars (Francour, 1997). Because of the shape and structure of sea cucumbers, either a portion of the sea cucumber must be bitten off by the predator, or the individual must be ingested whole (Lawrence, 1987). In response to predation pressures, sea cucumbers possess a wide variety of defense mechanisms. Such defenses include toxicity, thickened body wall,

evisceration, autotomy cryptic behavior, unpalatability, and swimming or other active avoidance of the predator (Bingham, 1986).

A toxic secretion has been identified in species belonging to the genus *Holothuria*, particularly *H. atra*. This secretion is burgundy in color and is thought to be a toxin that was used by inhabitants of the Pacific to poison fish, thus it is most likely utilized as a defense mechanism by the sea cucumber (Delia, 1977).

Possibly the most studied defense mechanism is the expulsion of Cuvierian tubules. Cuvierian tubules are found in several species belonging exclusively to the family Holothuriidae (Flammang et. al, 2005). The tubules are white, intracoelomic caeca attached to the basal part of the left respiratory tree (Van Den Spiegel, 1987). When physically stimulated, the sea cucumbers expel a few tubules through the anus which become adhesive as they elongate and stick to almost any surface (Flammang, 2003). The expulsion is directed toward the point of stimulus and the number of tubules expelled is proportional to the intensity of stimulation (Hamel, 2000). The tubules can elongate to up to 30 times their original length, or approximately 60cm depending on the species, and can maintain both their strength and adhesiveness for several days (Van Den Spiegel, 1995).

Cuvierian tubules are particularly effective against fish as the fish become entangled and immobilized in the tubules (DeMoor, et. al, 2003). Furthermore, it is thought that the presence of Cuvierian tubules is correlated with a higher degree of fish predation observed at lower latitudes (Lawrence, 1987).

This study includes a survey of the sea cucumbers in shallow waters surrounding Moorea, as well as an assessment of their predator defense mechanisms. I investigated the possible relationships between defense mechanisms and habitat and life histories as well as phylogeny and relatedness of species. While an attempt was made to qualify all defense mechanisms exhibited, I focused on the following: presence of Cuvierian tubules and or a liquid secretion, toxicity, body

structure and texture, coloration, and behavior.

I expect that individuals will exhibit multiple defense mechanisms and that not all mechanisms will be expressed to the same extent. Additionally, I expect to see some similarities among closely related species, but it is likely that the individuals' life histories will also influence the way they respond to potential predators.

METHODS

Collection and initial observations

I surveyed shallow water sites on the northern and western sides of Moorea for sea cucumbers. Sites were reached either by swimming, snorkeling, or boat. The collection sites include Temae (1), Cook's Bay directly off of the Gump Station (2), Cook's Bay near Pihaena Point (3), a reef just offshore between PK 14 and PK 15 (4), three sites at the Motu on the northwest side of the island (5), and the Mangrove Marsh near Haapiti (6) (Figure 1). I also observed and collected sea cucumbers on Tetearoa; an atoll approximately 59km to the North of Papeete, Tahiti. I picked up the individual sea cucumbers gently with rubber coated gloves and placed them in a plastic bin while still underwater so as to disturb them as little as possible. They were kept in the bins until I returned to the Gump Station, where they were placed in a large concrete tank (8'x4'x2') with flowing sea water. Ecological factors such as depth, substrate, distance from shore, and proximity to coral and other organisms were noted for each species observed.

Individuals were identified to species whenever possible using Guille, 1986 and Clark and Rowe, 1971. When identifications could not be made based on superficial characteristics alone, ossicles were extracted from the body wall. This was done by soaking an approximately 2cm² segment of body wall in sodium hypochlorite for 48 hours at approximately 7 degrees celcius. The ossicles were collected from the bottom of the small jar, slide mounted, and photographed for identification.



FIG. 1. Study Sites.

Individual and behavioral observations

I observed the sea cucumbers individually in a small, glass aquarium (approximately 25x50x25cm) with flowing sea water. Once placed in the tank, I left the sea cucumbers alone for approximately 15 minutes to acclimate. At this time I took photographs for identification and voucher purposes, and I took physical measurement in an undisturbed state. I measured length and width of the sea cucumbers using a measuring tape and I measured volume using a large graduated plastic container.

I simulated a predator attack by mechanically stimulating the sea cucumbers using large, blunt-tipped, metal forceps to pinch the dorsal integument. I described and recorded the response as well as the effort it took to elicit a response. If Cuvierian tubules were expelled, I removed the tubules and placed them in a plastic cup using forceps and while wearing inverted latex gloves (the adhesive tubules stuck to most surfaces, but I was able to remove them most easily from the smooth latex). I counted the number of times tubules were expelled as well as the approximate number of tubules expelled each time. If tubules were not expelled as a result of pinching with forceps, I turned the sea cucumbers over and poked their ventral side either with forceps or my fingers. I then picked up the individuals and gently constricted along the body with my hands. Lastly, I removed the cucumbers from the water and held them momentarily. For the

individuals that secreted a colored liquid when stimulated, I held them above a plastic cup and squeezed gently in a peristaltic motion to collect the liquid. Lastly, I placed the individuals in plastic freezer bags and placed them in the freezer for a minimum of six hours to sacrifice them before dissection.

Toxicity assessment

I removed the sea cucumbers from the freezer and allowed them to thaw for a few minutes before I dissected them using scissors and a razor blade. I noted the presence or absence of Cuvierian tubules within the body cavity. I then separated the external tegument and flesh from the internal organs, muscles, and connective tissue. If a liquid secretion was present in the plastic bag, I collected it for the toxicity assessment either by pouring it into a graduated cylinder or by removing it from the bag with a syringe.

To test toxicity I performed a series of brine shrimp bioassays. I raised brine shrimp using a salt water solution of 2 tablespoons sea rock salt and 1L of tap water. I measured 0.5mL of either Cuvierian tubules, liquid secretion, external body, or internal body parts by filling a graduated cylinder with 10mL salt water and adding the desired part until the water line read 10.5mL. The salt water solution used was of the same concentration as the brine shrimp were raised in. I then poured the contents of the graduated cylinder into a Petri dish and added 25 live brine shrimp. I recorded the number of live brine shrimp in each Petri dish after one hour and at multiple hours for a 24 hour period.

Analysis

The data collected from the bioassays were analyzed in a number of ways. First I looked at mortality after one hour by subtracting the number of live brine shrimp in each test from the number of live brine shrimp in the control to get the number of brine shrimp that died as a result of the test substance. I then did three one-way Anova analyses comparing the various genera, species, and sea cucumber parts to level of mortality. Additionally, I did a regression

analysis comparing the volume of the individual sea cucumbers with mortality after one hour.

To determine the estimated median lethal time (LT50), I extrapolated when mortality equals 50% for each assay. I repeated the same three one-way Anova analyses for LT50 as with mortality after one hour.

Additional Anova analysis and regression were done with *Holothuria atra* comparing volume and toxicity.

Finally, I used a phylogeny of Moorean sea cucumbers created by K. Tremain, 2005 and adapted it for the species included in this study. I overlaid the presence of Cuvierian tubules, a secretion, and various other physical characteristics onto the tree using *MacClade* software.

RESULTS

Collection and initial observations

I surveyed eight sites with varying habitats and found 15 different species and morphs of sea cucumbers in three different families (Appendix 1). Twelve species and morphs are included in this study. The most abundant species encountered was *Holothuria (Halodeima) atra* Jaeger, 1833. I found *H. atra* at every site and in almost every habitat type. A previous survey of the island conducted by J. Pearse in 1989 encountered this species and noted that it was “probably the most abundant and common echinoderm in French Polynesia” (Pearse, 1989). These small to medium sized sea cucumbers are almost black in color and are often seen with sand stuck to their bodies. When touched, most large individuals secreted a burgundy liquid through their skin at the place of contact. The individuals ranged in size from seven cm to 27cm in length. I split the species into two groups according to size in order to investigate the possible relationship between size and predator defence.

Two other species of *Holothuria* were observed: *Holothuria leucospilota* Clark, 1920 and *Holothuria fuscogilva* Cherbonnier, 1980. I only encountered *Holothuria leucospilota* in the very shallow calcium carbonate composite of Temae. Individuals of this species are almost

black with soft spikes all over. They are thin and can reach a length of over 50cm. Every individual I observed was almost entirely hidden under rock and when I tried to pick them up they became wedged under so tightly that it was impossible to collect them except when I was able to pick up the entire rock itself. Pearse observed *H. leucospilota* at four locations around Moorea; always completely or partially under either rubble or rock.

Two individuals of *Holothuria fuscogilva* were found; one light grayish and the other brownish purple. The lighter one was found on coral sand between coral heads and the darker one was found partially under an overhang of coral. Both were difficult to see as they blended in with their surroundings. Pearse does not list this species, but another study conducted in the Opunohu Bay of Moorea did note its presence (Parmentier, 2005).

The second most abundant species encountered was *Bohadschia argus* (Jaeger 1833). I found three color morphs of this species, but only included two in this study: a light purple with dark spots and a dark burgundy with orange spots. The third color morph was marbled brown with dark brown spots. This species was found in all but two sites. It was also found in all but two habitats which had shallow water less than 0.5m deep with no live coral. Pearse observed the two color morphs included in this study and noted that while both were abundant, the darker morph was encountered more often. During collection I observed many individuals expelling Cuvierian tubules when disturbed either purposely or accidentally by me. About one third to half of the total individuals observed were partially obscured either by sand or by coral.

I also collected individuals of *Bohadschia marmorata?*, *Bohadschia vitiensis marmorata*, and *Bohadschia spp.*. Pearse describes the species I am referring to as *B. vitiensis marmorata* as *B. vitiensis* but notes that it is also referred to as *B. marmorata* in Connon & Silver, 1986. It is possible that these two species are in fact the same species, but for the purposes of this study I consider them two different species. All species of *Bohadschia* were found in areas with sandy substrate and scattered coral

heads. Some individuals were found in areas of predominantly coral and coral rubble and a few were found in shallow water with sandy substrate and with no nearby coral heads. Also, while collecting I observed individuals from each species expell Cuvierian tubules when provoked. All of the above species of *Holothuria* and *Bohadschia* belong to the family Holothuriidae.

Two genera of the family Synaptidae were encountered: *Chiridota* and *Synapta*. I collected *Chiridota sp.*, tiny pink sea cucumbers 0.5 to five centimeters long, by sifting through the fine coral sand with my fingers. They were buried two to six centimeters in the sand. After collecting them my fingers were pink where I had touched them. Pearse describes *C. rigida* from two locations around Moorea, however a study conducted in 2005 describes *C. hawaiiensis* from the same location I encountered them (Tremain, 2005). I believe that the same species, *Chiridota spp.*, was observed in the two previous studies.

The second synaptid encountered is *Synapta maculata*. This species is long, snakelike, and very active. Their integument feels and acts like Velcro hooks when touched; clinging even to latex gloves. In contrast to *Chiridota*, this species was observed to reach lengths of over 130 centimeters.

Thenelota ananas is the largest of the species I encountered and the only member of the family Stichopodidae. They are grayish beige in color with many forked spikes that, as the specific name suggests, resemble a pineapple. They also have a soft, bright pinkish-orange underside.

Individual and behavioral observations

When physically disturbed, large individuals of *H. atra* pulled in their tentacles used for feeding, erected spikes all over their bodies, stuck to the glass of the aquarium, and secreted a burgundy liquid. Some individuals were active and attempted to escape, however most showed little or no movement. Small individuals of *H. atra* also erected spikes all over their bodies and stuck to the glass. They were slightly more active than the larger individuals. I did not observe a liquid being secreted, but when wiped with tissue paper

the paper became burgundy. After being placed in a plastic bag and frozen, a small amount of burgundy liquid could be obtained from the plastic bag.

Like *H. atra*, *H. leucospilota* erected spikes all over and pulled in their tentacles. They became slightly shorter and more robust. One individual was very active and tried to escape, but the other three showed little or no movement when being physically stimulated. A single individual expelled a few very thin Cuvierian tubules when first disturbed. I removed the tubules but could not get it to expel again. I did not observe a secretion during my observations, but after being frozen a secretion as observed in the plastic bag.

I did not observe any of the behaviors exhibited by *H. atra* or *H. leucospilota* in *H. fuscogilva*; except clinging to the substrate. The two individuals of *H. fuscogilva* were extremely hard and I could not deform their shape in any way. During the dissection, I could not cut the tegument with scissors at all and it was very difficult to do so with a razor blade. Their texture was also rough like coarse sandpaper.

All the individuals belonging to the genus *Bohadschia* erected small spikes all over their bodies. These spikes were in the center of their spots, except in *B. vitiensis marmorata* which does not have spots. *Bohadschia sp.* remained soft as it erected its spikes while all others became more rigid. All but one individual expelled Cuvierian tubules when physically stimulated. *Bohadschia argus* (dark), *B. vitiensis marmorata*, and *B. marmorata?* curved their anal end back in an upright "C" position and expelled Cuvierian tubules toward the point where the physical stimulation was being exerted. One individual of *B. argus* (light) made a slight motion and *B. sp.* made no motion while expelling tubules. All individuals pushed water through their anal opening as they were expelling tubules which made the tubules move and appear greater in number.

When disturbed with forceps in a Petri dish full of water and no sand, individuals of *Chiridota sp.* changed their shape slightly and move away from the disturbance, but did not appear to do anything else. In their natural

setting they were observed to burrow under the sand.

Synapta maculata were extremely active and move in a snake-like manner away from the disturbance. When touched they clung to my gloves and often left tiny, hook-like structures behind. I did not observe any other defense mechanisms.

KEY TO SPECIES NAMES

- 1 *Bohadschia argus* (dark)
- 2 *Bohadschia argus* (light)
- 3 *Bohadschia marmorata*?
- 4 *Bohadschia vitiensis marmorata*
- 5 *Bohadschia* spp.
- 6 *Thenelota ananas*
- 7a *Holothuria atra* (small)
- 7b *Holothuria atra* (large)
- 7 *Holothuria leucospilota*
- 8 *Holothuria fuscogilva*
- 9 *Synapta maculata*

Thenelota ananas do not appear to respond to physical stimulation other than by clinging to the substrate and becoming slightly more rigid. However, when the stimulation ceased they moved away from the location where it took place relatively quickly. Fish, belonging to the family Carapidae, were found in every individual of *T. ananas* dissected. One individual had two carapid fish inside it; one of which was 22 cm long, or almost 40% of the total body length.

Toxicity assessment

After one hour, the highest mortality was observed in *T. ananas*, followed by *B. marmorata*, and *S. maculata* and the least mortality was observed in *H. atra* (small) (Figure 2). The highest mortality of all the individual parts was observed in the external body parts of *T. ananas* and internal body parts of *S. maculata*.

The oneway anova analyses for variance showed that there is a significant difference in mortality after one hour at both the genus and species level (p is <0.0001 for both) (Figures 3 and 4). However when comparing only the genera *Bohadschia* and *Holothuria*, there is no significant difference in mortality after one hour ($p=0.32$).

A regression analysis showed that volume of the individual is correlated with mortality after one hour ($p<0.0001$). Additionally, a oneway Anova showed that individual volume and species are highly correlated ($p<0.0001$) as well.

According to the calculated median lethal times (LT50), *H. atra* (large) displayed the highest mortality rate followed by *T. ananas* and *B. marmorata*, and the lowest was displayed by *H. atra* (small). The oneway anova analyses showed a significant difference between LT50s at the species level ($p<0.0001$), a slight difference at the genus

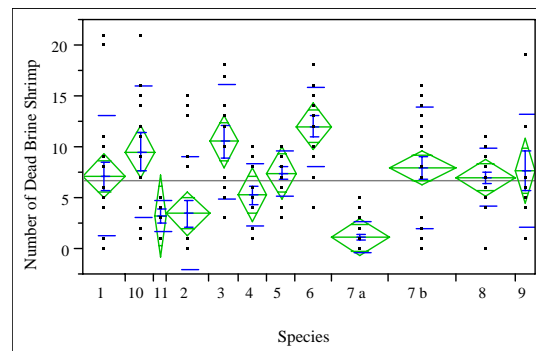


Figure 3: Comparing mortality after 1 hour between species.

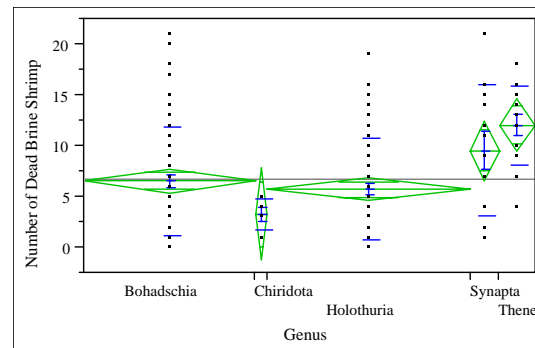


Figure 2: Comparing mortality after 1 hour between genera.

level ($p=0.049$), and very little difference among parts independent of taxa ($p=0.25$). Again, there was no significant difference between the genera *Bohadschia* and *Holothuria*.

There is a significant difference in the toxicity between large and small individuals of *Holothuria atra* observed in both the mortality after 1 hour and LT50 ($p<0.0001$ for both) with larger individuals being much more toxic. A regression analysis showed a

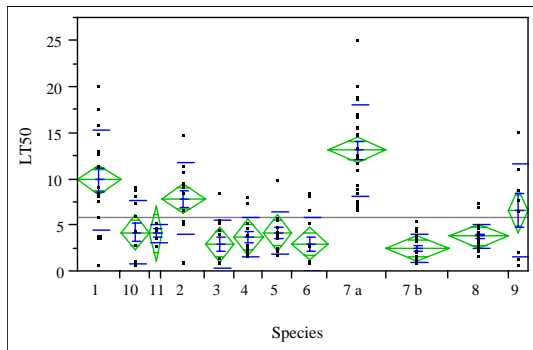


Figure 4: Comparing LT50 between species.

strong correlation ($p < 0.0001$) between size and mortality after one hour. Anova analyses for large individuals showed no significant difference in mortality after one hour between secretions collected off of live individuals and from the bag after freezing ($p = 0.46$). Thus being frozen did not have an effect on toxicity. No significant difference was observed in mortality after one hour between the secretions of individuals characterized as either large or small ($p = 0.16$).

Phylogeny

The presence of Cuvierian tubules, a red liquid secretion, an active flight response, and a thickened tegument were mapped onto the phylogeny adapted from Tremain 2005. The phylogeny was originally created using 27 morphological and ecological characteristics (Tremain, 2005). The placement of *Thenelota ananas* between *Bohadschia* and *Holothuria* is somewhat problematic because *Thenelota* belongs to the family Stichopodidae while the other two belong to the family Holothuriidae. However, for the purposes of this study the phylogeny is still very useful.

Eight of the twelve species in Tremain's study were present in this one. I omitted one species from the phylogeny and substituted a few of the species in this study for closely related species. For instance, I replaced *Holothuria* (*Lessonothuria*) with *Holothuria fuscogilva*, and *Bohadschia* unknown spp. K with *Bohadschia* spp.

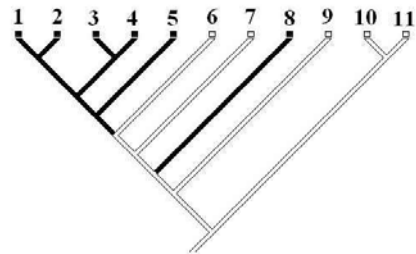


FIG. 5. Presence of Cuvierian tubules.

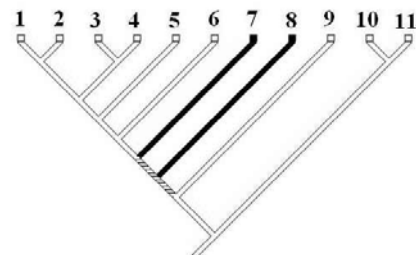


FIG. 6. Presence of a red, liquid secretion.

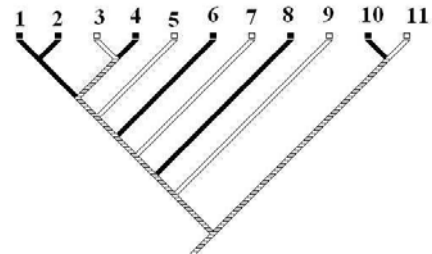


FIG. 7. Presence of an active flight

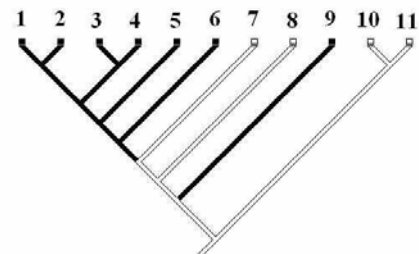


FIG. 8. Presence of a thickened tegument.

DISCUSSION

Sea cucumbers as a group have a wide variety of predator defense mechanisms and most species utilize one or more (Bingham, 1986). I observed that individual species not only possess multiple mechanisms, but they exhibit these mechanisms to varying degrees. The most striking of the defense mechanisms observed was the expulsion of Cuvierian tubules. All members of the genus *Bohadschia* that I observed expelled tubules, but not all the tubules produced the same mortality in the bioassays. For instance tubules from *B. argus* (light) produced relatively high levels of mortality after one hour while tubules from *B. argus* (dark) produced relatively low levels. It is possible that the toxicity of the tubules themselves may not be very important for their function in predator deterrence. It has been noted that while the tubules do possess toxins (Parmentier, 2005), they function primarily by entangling and immobilizing potential predators (DeMoor et. al, 2003).

In addition to Cuvierian tubules, the coloration of *B. argus* may also play a role in predator avoidance. Depending on the substrate, the light or dark color morphs of *Bohadschia argus* may be cryptic. The lighter color morph blends in better with the calcium carbonate sand and light colored corals, while the darker color morph blends in more with darker corals. However both morphs were found out in the open, near all types of coral, and partially buried in the sand. In addition to color, the spots may serve to disrupt the overall shape of the cucumber and make it even more cryptic; or the spots may be aposematic, warning potential predators to stay away. The two are considered different color morphs of the same species and a further look into how color is determined could provide more insight into the relationship between color and predator defense.

Bohadschia vitiensis marmorata did not produce particularly high mortality rates in the toxicity assessment, however it expelled Cuvierian tubules readily and in large quantity. One individual even eviscerated its entire digestive tract when I agitated it after having removed all the Cuvierian tubules. I only observed this behavior in one individual,

but the regeneration of the intestinal tract is one of the most studied in regeneration studies of holothurians (Van Den Spiegel, 2000). Immediately after evisceration, the sea cucumber rapidly crawled away from its intestines. This is an extremely effective defense behavior especially for predators that prefer to only eat part of the cucumber, such as fish or crustaceans (Francour, 1997). The predator is distracted by the intestines and the sea cucumber has a chance to escape, however this is an extremely high energy cost to the cucumber and that is probably why I only observed it after persistent agitation and removal of all Cuvierian tubules.

Holothuria leucospilota, the only species other than members of *Bohadschia* possessing Cuvierian tubules, possessed multiple defense mechanisms yet did not respond very much to agitation. Both Cuvierian tubules and a liquid secretion were present in this species, but neither were utilized to a great extent during this study. A previous study of *H. leucospilota* observed evidence of expulsion of Cuvierian tubules in 2.3-6.1% of individuals in the field (Hamel, 2000), showing that they do in fact utilize this mechanism in the wild. Toxicity of this species is moderate compared to the other species in this study. *Holothuria leucospilota* was observed to wedge itself under rocks to avoid predation. This is most likely a very successful behavior for avoiding larger predators and the moderate toxicity may be enough to deter smaller predators.

A significant difference in the toxicity between large and small individuals of *Holothuria atra* was observed in both the mortality after 1 hour and LT50. This suggests that there is an increase in toxicity as the individual ages. Individuals of all sizes were observed out in the open and were usually covered in sand in the wild. Further study into the ontogeny of the species and any behavioral differences might provide more insight into why larger individuals are more toxic.

Holothuria fuscogilva is the most cryptically colored of all the species observed in this study. I only encountered two, but it is likely that there were more because it was found in a relatively common habitat type. The bioassays showed that it is only moderately toxic,

however it is unlikely that many predators can penetrate its extremely rough, thick integument.

Thenelota ananas, the most toxic of the species studied, exhibited relatively passive forms of predator deterrence by becoming slightly more rigid and clinging to the substrate. It is possible that the spikey appearance suggests to predators that, although it is cryptically colored, *T. ananas* is unpalatable. Another explanation for the spikey texture, in conjunction with a thickened integument, is to make it difficult for potential predators to penetrate into the body. The bright underside may also serve in predator defense by being aposematic, however I did not observe any behaviors that might support this.

Thenelota ananas is also the largest of the species encountered and while a regression analysis showed a strong positive correlation between volume of the individual and toxicity, volume and species are also strongly correlated. Therefore, it is uncertain whether individual size plays a role in toxicity, or if both size and toxicity can be attributed to the species independent of the other.

The four carapid fish found in three specimens of *T. ananas* is probably related to the large size and absence of Cuvierian tubules. A study of carapid fish in the Opunohu Bay of Moorea found fish in 81% of *T. ananas* examined. The fish are generally found in the respiratory tree where they take advantage of the water flow and if the diameter of the respiratory tree is too small the fish cannot penetrate into it. *Thenelota ananas* was observed in this study to have a relatively high level of toxicity, however the carapid fish are particularly resistant to sea cucumber toxins (Parmentier, 2005). Even Cuvierian tubules prove to be an ineffective defense mechanism against these well adapted fish as they are covered in mucus which keeps the tubules from adhering to them (Parmentier, 2005). One small fish was found in a specimen of *Holothuria leucospilota* as well.

Comparing the presence and absence of Cuvierian tubules to a phylogenetic study performed in 2005 suggests that Cuvierian tubules either evolved separately in *Bohadschia* and *H. leucospilota*, or more likely that they

were present in *Holothuria* and then were subsequently lost by multiple species or lineages. A previous phylogenetic study of the family Holothuriidae which included eight species and five genera suggests that Cuvierian tubules were present before the initial radiation of the family and then lost by various members (Kerr et. al, 2005). The results of this study support Kerr et. al's findings. Kerr et. al also state that because a basal lineage of the family possesses Cuvierian tubules which are small, not adhesive, and rarely expelled, Cuvierian tubules may have evolved for some other function than defense (Kerr et. al, 2005).

The red secretion was only observed in the species *Holothuria atra* and *Holothuria leucospilota*. According to the phylogeny examined, this specific secretion probably only evolved once. Thickened skin appears to be characteristic of *Bohadschia* and has evolved multiple times in other species as well. Having an active flight response, on the other hand appears to not necessarily be related to a species' phylogenetic history, but rather it varies from species to species and is most likely a result of the particular ecology and life history of the animal.

A total of 12 species and morphotypes were examined in this study and different defense mechanisms and levels of toxicity were observed in each. Inheritance and phylogenetic relationships appear to play a large role in determining what mechanisms are present. However the expression of the traits and utilization of certain mechanisms is also influenced by the individual's ecology; thus there appears to be a degree of phenotypic plasticity among the species.

Future studies including introducing potential predators such as asteroids, crustaceans, and gastropods into a controlled setting may provide additional knowledge of holothurian defense behavior. For example, a particular species may respond differently to different predators and thus the presence of multiple defense mechanisms within a single species may be explained. Also, many of the phylogenetic relationships within Holothuroidea remain unresolved. This study serves as a baseline for further study into sea

cucumber defense as well their ecology in general.

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APPENDICES

APPENDIX A: Study sites and species encountered.

Genus	Species	Cook's Bay at Gump Station	Cook's Bay Reef Crest	Mangrove Marsh near Haapiti	North side of Motu	East side of Motu Tiahura	Temae	Tetearoa	West of Sheraton b/w PK14 & PK15
Bohadschia	vitiensis marmorata				x			x	
Holothuria	leucospilota						x		
Holothuria	atra	x	x	x	x	x	x	x	x
Thenelota	ananas				x				x
Synapta	maculata	x					x		
Chiridota	spp.					x		x	
Bohadschia	argus (dark)	x	x		x	x	x	x	x
Bohadschia	argus (light)	x	x		x	x	x	x	x
Bohadschia	marmorata				x			x	
Bohadschia	spp.				x			x	
Holothuria	fuscogilva				x				

- 1 Temae: Composite CaCO₃ near shore, predominantly coral with some sandy bottom further.
- 2 Cook's Bay at Gump Station: Sand bottom with coral rubble and scattered coral heads.
- 3 Cook's Bay near Pihaena Point: Sand bottom with coral rubble and scattered coral heads
- 4 West of Sheraton between PK14 & 15: Composite CaCO₃ near shore, predominantly coral with some sandy bottom further.
- 5 a: East side of Motu Tiahura: Fine coral sand.
b,c: North side of Motu: Sand and coral rubble bottom with scattered, large coral heads. Deep water ~1.5m
- 6 Mangrove marsh near Haapiti: Fine sand and sediment bottom. Scattered mangrove trees and occasional coral
- 7 Tetearoa: Fine sand and sediment bottom. Scattered mangrove trees and occasional coral

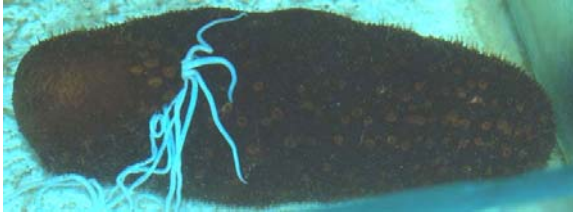
APPENDIX B: Physical and behavioral characteristics observed by species.

Species	Cuverian Tubules Expelled	Secretion Present	Thickened body wall	Active Escape Behavior	Change in Shape/Texture	Retractable mouthparts	Burrowing/Hiding under rock behavior	Volume >500mL	Volume >1000mL
<i>Bohadschia argus</i> (dark)	1	0	1	1	1	1	1	1	1
<i>Bohadschia argus</i> (light)	1	0	1	1	1	1	1	1	1
<i>Bohadschia marmorata</i>	1	0	1	0	1	1	1	1	1
<i>Bohadschia</i> spp.	1	0	1	0	1	1	1	1	0
<i>Bohadschia vitiensis marmorata</i>	1	0	1	1	1	1	1	1	0
<i>Chiridota</i> spp.	0	1	0	1	0	1	1	0	0
<i>Holothuria atra</i> (large)	0	1	0	0	1	1	1	1	0
<i>Holothuria atra</i> (small)	0	1	0	0	1	1	0	0	0
<i>Holothuria fuscogilva</i>	0	0	1	0	0	1	0	1	1
<i>Holothuria leucospilota</i>	1	1	0	1	1	1	1	0	0
<i>Synapta maculata</i>	0	0	0	1	1	1	0	0	0
<i>Thenelota ananas</i>	0	0	1	1	0	1	0	1	1

APPENDIX C: Characteristics of habitats inhabited by species of holothurians.

Species	Shallow (<0.5m) CaCO3 Composite	Shallow (<0.5m) coral sand substrate	~1m predominately coral heads and rubble w/ little sand	~1m coral sand substrate w/ few coral heads	1-2m sand/coral rubble w/ scattered coral heads	4-6 m sandy substrate w/ few coral heads
<i>Bohadschia argus</i> (dark)	0	0	1	1	1	1
<i>Bohadschia argus</i> (light)	0	0	1	1	1	1
<i>Bohadschia marmorata</i>	0	0	0	1	1	0
<i>Bohadschia</i> spp.	0	0	0	1	1	0
<i>Bohadschia vitiensis marmorata</i>	0	0	0	1	1	0
<i>Chiridota</i> spp.	0	1	0	0	0	0
<i>Holothuria atra</i> (large)	1	1	1	1	1	0
<i>Holothuria atra</i> (small)	1	1	1	1	1	0
<i>Holothuria fuscogilva</i>	0	0	0	0	1	0
<i>Holothuria leucospilota</i>	1	1	0	0	0	0
<i>Synapta maculata</i>	1	0	1	1	0	0
<i>Thenelota ananas</i>	0	0	0	0	0	1

APPENDIX D: Species List and Photos



Bohadschia argus (dark)



Bohadschia argus (light)



Bohadschia marmorata?



Bohadschia vitiensis marmorata



Bohadschia spp.



Chiridota spp.



Holothuria atra



Holothuria fuscogilva



Holothuria fuscogilva



Holothuria leucospilota



Synapta maculata



Thelotrema ananas