Predator-prey interactions of *Pugettia gracilis* (Dana, 1851) and *Leptasterias* spp. (Verrill, 1866) and two species of *Lacuna* (Turton, 1827)

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Abstract:

This study focused on the predator prey interactions of two predators, the crab *Pugettia gracilis*, and sea stars of the genus *Leptasterias* and two low intertidal / shallow subtidal zone herbivorous snails, *Lacuna vincta* and *Lacuna variegata*. *L. vincta* and *L. variegata* graze on macroalgae, including *Ulva* spp.and S*accharina sessile*, as well as microalgae such as diatoms that occur on the eelgrass *Zostera marina*. The feeding rates of *P. gracilis* and *Leptasterias* spp. on these two species of *Lacuna* were determine by in lab experiments, which included no choice and preference experiments. We found that *P. gracilis* ate significantly more *L. variegata* per unit time than *Leptasterias* and that the two predators had the same feeding rate on *L. vincta*. In choice tests *P. gracilis* preferred *L. variegata.* Seastars showed no preference. Neither predator was size selective and the size of the predator did not have a meaningful impact on the number of eaten snails or the size of snails eaten. The vulnerability of *Lacuna vincta* and *Lacuna variegata* in eelgrass and macroalgae habitats in the presence of the two predators was tested through mesocosm experiments. Because of the low recapture rate in controls, we were unable to detect significant differences in feeding of the two predators on the two species of *Lacuna* in the two habitat types.

Introduction:

The intertidal zone is an excellent place to study ecology due to the frequent changes of the environment throughout the day (Molles 2008). These areas generally experience two high tides and two low tides within a 24 hour period. Here, terrestrial and marine communities integrate and a separation between vertical zones (e.g., littorind zone, balanoid zone, and sublittoral fringe, Stephenson and Stephenson 1949) defines the spatial distribution and abundance of organisms. Abotic factors such as temperature, salinity, and dissolved oxygen often influence the distribution of organisms within each zone (Stephenson and Stephenson 1949, Helmuth et al. 2006). Intertidal zone invertebrates and other marine fauna have evolved to tolerate the constant changes of such harsh and remodeling environments. These evolutionary changes in orgamismal physiology provide modern perspectives on the relationship between terrestrial and aquatic habitats (Helmuth et al. 2006).

Direct and indirect interactions between organisms in the intertidal zone community showcase the physical and biotic processes that limit the distribution of local organisms (Bertness and Leonard 1997). Previous studies on predator-prey interactions in the intertidal zone have demonstrated the important role of predation on distribution patterns in these complex communities. For example studies on the predation of *Mytilus californianus* by *Pisaster ochraceus* illustrate the renewal of primary space for organisms with limited locomotion (Paine 1966, Menge 1976).Therefore, the benefit of variation in predation intensity is that it allows nearby species to take advantage of cleared areas (Menge 1976). As a result, predation plays an important role in community structure by preventing a single species from dominating the area and thus creating greater species diversity in the surrounding community (Paine 1966).

This study focused on the predator prey interactions of two predators, *Pugettia gracilis*, commonly known as the graceful kelp crab, and sea stars of the genus *Leptasterias* feeding on low intertidal / shallow subtidal zone snails *Lacuna vincta* and *Lacuna variegata*. *L. vincta* and *L. variegata* are small herbivores that graze on macroalgae, including *Ulva* spp.and S*accharina sessile*, as well as microalgae such as diatoms that occur on the eelgrass *Zostera marina*. These two species of gastropod can be found in the low intertidal / shallow subtidal zone across the eastern Pacific shores of Washington, USA and British Columbia, Canada (Kozloff 1996). Both species have a life span of 6-14 months and their typical body size ranges from 1-9 mm (Grünbaum and Padilla 2014). They lay gelatinous egg masses that can be found throughout the year, and are commonly spotted on the surface of macrophytes. Veliger larvae hatch from these egg masses after about one week and spend 4 to 12 weeks in the plankton before they settle and metamorphose in either eelgrass or macroalgal habitats (Martel and Chia 1991, Padilla 2001).

Pugettia gracilis is a major predator of the two *Lacuna* species. Crabs of this species generally do not grow larger than 3 cm in carapace length. They have distinctive blue chelae with orange tips and have noticeable sharp spines on the upper dorsal side of their carapace. Individuals live in eelgrass beds and easily blend in by covering their carapace with small algae (Kozloff 1973) and are also found in macroalgal habitats (Padilla unpublished data). Gender can be easily identified by a simple inspection of the abdomen. Males have a narrow, white triangular abdomen whereas females have a more rounded underside. Sea stars of the genus *Lepstasterias* are other well known predators of *Lacuna* and are easily distinguished by their set of six arms (Chia 1966, Menge 1972a). *L. hexactis* is a commonly found species in the rocky intertidal zone of the northern Pacific coast. They are often abundant in areas where loose rocks cover the shore. These brooding sea stars hold their eggs near the mouth until they develop into juveniles, which thereafter migrate away (Kozloff 1973). Recent work suggests that there may be a number of cryptic species of *Leptasterias* in the San Juan Archipelago (Cohen unpublished data). Cross fertilization is known to occur between individuals of different species resulting in hybrid offspring (Chia 1966).

Studies were conducted at the University of Washington, Friday Harbor Laboratories (FHL), Washington to quantify and assess the feeding rates of *P. gracilis* and *Leptasterias* spp. on the two species of *Lacuna* in two different habitats (eelgrass and macroalgae). Differences in habitat (e.g., substrate type, sunlight exposure, and flora) can change the foraging behavior of predators and can either aid or hinder the efficiency of predation. Our goal was to examine the vulnerability of *L. vincta* and *L. variegata* in eelgrass and macroalgal habitats and observe how feeding rates of *P. gracilis* and *Leptasterias* spp. changed between the habitats. Specifically, we determined the maximum feeding rate of each species of predator on each species of *Lacuna*. We tested whether there was size specific predation by these predators and if they had a preference or potential for greater impact on each species of prey. Finally, we tested whether their feeding efficiency might be different for each species of snails in the two habitat types.

Methods and Materials:

The Salish Sea, the waters in which our study organisms inhabit, is bounded by a network of coastal waterways. Average water temperatures fluctuate between 9-11°C and the typical salinity is 30. Runoff through the Fraser River in British Colombia causes short occurrences of warm (15°C), low salinity (20-25) water, which has been known to affect the shores of the San Juan Archipelago. Events like this have become more frequent in recent years, likely due to climate changes. Studies on the developmental processes of various marine invertebrate larvae, including *Pisaster ochraceus* (Pia T.S. et al. 2012), have been conducted to determine the response of affected species to the decrease in salinity.

Field Sites

Seven different sites throughout San Juan Island, Washington, were visited during the months of June and July 2015. Field sites included: False Bay (48°29'09.9"N 123°04'07.2"W), Mar Vista (48°29'02.9"N 123°03'50.7"W), Fourth of July Beach (48°28'05.4"N 123°00'11.4W), Reuben Tarte County Park (48°36'45.0"N 123°05'57.8W), Jackson Beach (48°31'07.7N 123°00'53.4"W), Merrifield Cove (48°30'13.4"N 123°01'07.3'), and Pile Point (48°28'56.5"N 123°05'43.5"W).

Figure 1. Map of San Juan Island. Sites ranged from Reuben Tarte on the north eastern side of the island to Pile Point on the western side of the island.

All collected organisms were kept at the University of Washington Friday Harbor Laboratories (FHL) (48°32'44.5"N 123°00'46.9"W). Animals were placed in tanks with flow through sea water. Water temperature was relatively consistent with that of the sea water from the harbor, approximately 11°C, and salinity of 30. *L. vincta* and *L. variegata* were collected from visits to False Bay, Fourth of July Beach, Reuben Tarte, and the FHL dock. These species of snails are susceptible to desiccation and avoid warm waters; therefore, field days were scheduled around low tides. Abundance of *Lacuna* at our selected sites was lower than initially expected. In previous years, densities have been documented as high as 3,000/ m^2 (Padilla unpublished data). *Lacuna* were separated by species and contained in 437 ml plastic boxes with 1 mm screening on 4 sides of each box. Due to resource limitations and the size of *Lacuna*, each box contained 50-60 snails. They were given *Ulva* spp. as their main source of food and it was replenished when needed.

Crabs were collected from False Bay (N = 11), Mar Vista (N = 12), Reuben Tarte (N = 1), Jackson Beach (N = 7), and 4th of July Beach (N = 1). Few *Leptasterias* were collected from False Bay (N = 3), Mar Vista (N = 3), 4th of July Beach (N = 1) and Merrifield Cove (N = 8). Stars could not be identified to species, and at the end of the study were sent for molecular identification.

Predators were kept individually in 437 ml plastic boxes with 1 mm screening on 4 sides of each box. Each predator was given a number as a form of identification and their size (± 0.01 mm) was determined with electronic digital calipers. For *P. gracilis*, both the diameter (including spines) and length of the carapace were recorded. Measurements for *Leptasterias* spp. were made from one arm tip to the corresponding opposite arm tip. During their time in the laboratory, crabs were fed *Ulva* spp. Sea stars were also kept with a piece of *Ulva* although it was not provided as food. Both sea stars and crabs were periodically provided with small *Lacuna* for food. Predators were checked daily and food was replenished as needed.

Feeding in the Field

To determine what *P. gracilis* feeds on in the field, we captured crabs in either eelgrass or macroalgal habitats, measured their carapace length and placed them individually into Ziploc® bags with only sea water. Since these crabs are inefficient digesters, we were able to identify what individuals were feeding on by crushing their feces between glass slides and examining them under a compound microscope. For crabs collected at 4th of July Beach (N = 1) and Mar Vista (N= 12), all bags were labeled with the time of capture and habitat location. They were then brought back to the FHL campus and set into sea tables until they produced fecal pellets, which were then frozen and later examined. For crabs collected at Pile Point (N = 11), crabs were placed into Ziploc® bags with sea water and labeled with time of capture. They were given three hours to produce fecal pellets and then were removed, measured and released back to where they were found. Crab feces were brought back to the lab for later examination.

To determine what *Leptasterias* feeds on in the field, we examined the arms and mouth of each collected sea star from Merrifield Cove (N = 8), Mar Vista (N = 5) and Pile Point (N = 6) and noted any source of prey that the sea stars were feeding on at the time of capture.

Feeding in the Lab

The feeding rates of *P. gracilis* and *Leptasterias* spp. were obtained through laboratory experiments conducted over a span of one month. Prior to each trial, the size of all the participating snails were measured with electronic calipers (± 0.01 mm). Each predator was used only once in each experiment. Any previously provided *Ulva* was removed from the predator's cage before ten snails, either 10 *L. vincta* or 10 *L. variegata*, were added. A single predator (*P. gracilis* or *Leptasterias*) was allowed per cage. Trails ran for three hours, such that the organisms were not disturbed until the three hours were over. At the end of each trial, cages were checked and the number of consumed snails was recorded. The remaining snails were measured such that the size of consumed snails was known. In the case of *P. gracilis,* snails were eaten whole with only pieces of shell leftover. However, feeding for *Leptasterias* was based upon the number of snails in the mouth, the number of empty shells in the box and the snails retained in the arms of the sea star. For *P. gracilis*, there were 16 trials for each species of snail. In the case of *Leptasterias* spp., there were 13 trials for each species of *Lacuna*.

To determine if predators had a preference, separate trials were preformed where five *L. vincta* and five *L. variegata* were placed in the cages. This allowed the predator to have a choice between the two species. A different predator (crabs N = 13; sea stars N = 13) was used for each trial.

Mesocosm Studies

Our goal was to determine whether there was a difference in snail vulnerability in two environments for the two predators. Mesocosm studies were conducted in order to test the feeding rates of *P. gracilis* and *Leptasterias* on each species of *Lacuna* in the two habitat types, eelgrass and macroalgal. Habitats were set up in such a way that predator-prey interactions were easily analyzed. Eelgrass used for the mesocosms was collected from Fourth of July Beach while macroalgae, whole *Saccharina sessilis* thalli with their holdfast intact, were collected at Mar Vista, or were found in the drift algae at Pile Point. Six outdoor tanks (T1-T6) were divided by a barrier of eggcrate panels covered with 1 mm screening into two sections. Tanks had a height of 63 cm, length of 112 cm and a width of 50 cm. The depth of water was 35 cm. Pots were filled with sand and planted with either eelgrass or macroalgae (total area of approximately 1,000 cm^2). Pots were placed on either side of the eggcrate barrier in each tank such that each habitat type was isolated from the other and animals did not interact with neighboring individuals. Tanks had flow through seawater with flow rates adjusted to 1 L/min during experimental trials. The inflow water tube was placed so that water came into the tank on the right edge of the right half of the tank, and the standpipe for the outflow was on the left half of the tank to the left of the test plant. There were 12 treatments, each with one habitat type, eelgrass or macroalgae, one species of *Lacuna, L. vincta* or *L. variegata*, and a predator (crab, sea star or a no predator control). A total of 6 replicates of each treatment were run. For each replicate, each tank had eelgrass on one side and the kelp on the other (eelgrass was in the right side of alternate tanks and on the left of the others), *L. vincta* on one side and *L. variegata* on the other. For successive replicates, the side of the tank with *L. vincta* and *L. variegata* were switched so that any snails not found in the previous replicate would be found. Each treatment was run once in each tank. Tables 1-3 show the different treatments.

Table 1. Treatments for *L. vincta*			
Prey	**Predator**	**Habitat**	**Treatment #**
L. vincta	*P. gracilis*	Eelgrass	1
L. vincta	*P. gracilis*	Marcoalgae	2
L. vincta	*Leptasterias*	Eelgrass	3
L. vincta	*Leptasterias*	Marcoalgae	4

Table 2. Treatments for *L. variegata*			
Prey	**Predator**	**Habitat**	**Treatment #**
L. variegata	*P. gracilis*	Eelgrass	5
L. variegata	*P. gracilis*	Macroalgae	6
L. variegata	*Leptasterias*	Eelgrass	7
L. variegata	*Leptasterias*	Marcoalgae	8

Table 3. Controls for both *L. vincta* and *L. variegata*. No predator.		
Prey	**Habitat**	**Treatment #**
L. vincta	Eelgrass	9
L. vincta	Macroalgae	10
L. variegata	Eelgrass	11
L. variegata	Macroalgae	12

For each replicate of each treatment, 10 *Lacuna* (*L. vincta* or *L. variegata*) were placed at the base of the test plant species and allowed to attach to the plant for 10 minutes. The water flow was adjusted to 1 L/min. A predator (crab or sea star, or neither for controls) was then placed at the base of the test plant. Treatments were left undisturbed for 6 hour. At the end of the trial predators were removed and the remaining snails were collected from each treatment. When removing predators from the treatments, their location was documented along with any other notable data. In the case of *Leptasterias*, snails that were found on the mouth and on arms were also documented. If all snails were not found after the first inspection, tanks were drained and searched thoroughly for all test snails. Replicates were run with one day in-between each other in order to retrieve any test snails that were found the next day. The identity and number of snails retrieved was checked with microscope and any snails that had clearly been eaten during the trial recorded.

Results:

Feeding in the Field

We were able to collect feces from 23 crabs in the field; these individuals were collected at MarVista (N = 11), Pile Point (N = 11), and 4th of July Beach (N = 1). When inspected under a compound microscope, we found that all 23 crabs were feeding on macroalgae and diatoms. Only 6 crabs (23%) had *Lacuna* shell in their feces; these snails were from all sites except the eelgrass at Mar Vista (Table 4). A few were known to be feeding on other prey items such as sponges and arthropods (Table 4). In the case of *Leptasterias*, we found that none of the sea stars (N = 19) were feeding on *Lacuna* when collected in the field. However, about 15.8% were feeding on barnacles and 5.2% on bryozoans (Table 5). Most were not feeding at the time they observed (79%) (Table 5).

Table 4. Taxa identified in the feces of *P. gracilis* collected from three different sites (N = 23)

Site	Macroalgae	Diatoms	*Lacuna*	Other
MarVista (Kelp)	9	9	2	3
MarVista (Eelgrass)	2	2	0	0
Pile Point	11	11	3	6

4th July Beach	1	1	1	0
Total	23	23	6	9
Percent Feeding (%)	100	100	26	39

Table 5. Food items found in the mouth and arms of *Leptasterias* spp. at three different sites (N = 19)

Site	**Barnacles**	**Bryozoans**	***Lacuna***	**Nothing**
Merrifield Cove	0	0	0	8
Mar Vista	2	1	0	2
Pile Point	1	0	0	5
Total	3	1	0	15
Percent Feeding (%)	15.8	5.2	0	79

Feeding in the Lab

P. gracilis

For the no choice experiments, paired t-tests (two tailed) were conducted in order to determine if there was a difference in the mean size of snails eaten and the mean size of snails not eaten for both species of *Lacuna* by *P. gracilis*. The results indicated no statistically significant difference in the mean size of snails eaten and the mean sizes of snails not eaten for *L. vincta* (N = 13, P = 0.21) or *L. variegata* (N = 16, P = 0.37). Crabs were not selective in the size of *Lacuna* they ate and therefore were able to eat all sizes when given a single species of *Lacuna*.

Ordinary Least-Squares (OLS) regressions were used to determine if there was a relationship between *P. gracilis* carapace length and the size of snails that were eaten and not eaten for both species of *Lacuna* (Table 6). There was no significant linear relationship between size of *L. vincta* eaten and crab carapace length (N = 46, P = 0.172, Figure 2). A slight negative linear relationship was found between the size of *L. vincta* not eaten and crab size (N = 124, P = 0.012, Figure 3). In the case of *L. variegata* and *P. gracilis*, a positive linear relationship was found between the size of snails eaten and the *P. gracilis* size (N = 79, P = 0.038, Figure 4). No linear relationship was found between size of snails not eaten and predator size (N = 102, P = 0.080, Figure 5). A summary of regression equations is in Table 6.

Table 6. Regression Summary for *Lacuna* Shell Size and *P. gracilis* Carapace Length in the No Choice Experiments

	Dependent Variable	**Independent Variable**	**r^2**	**Slope**	**y-Intercept**	**P value**	**N**
***L. vincta* Eaten**	Snail Shell Size	Carapace Length	0.0419	0.074	3.370	0.172	46
***L. vincta* Not Eaten**	Snail Shell Size	Carapace Length	0.0831	-0.137	8.105	0.001	124
***L. variegate* Eaten**	Snail Shell Size	Carapace Length	0.0722	0.072	2.597	0.0169	79
***L. variegate* Not Eaten**	Snail Shell Size	Carapace Length	0.0305	0.057	3.123	0.079	102

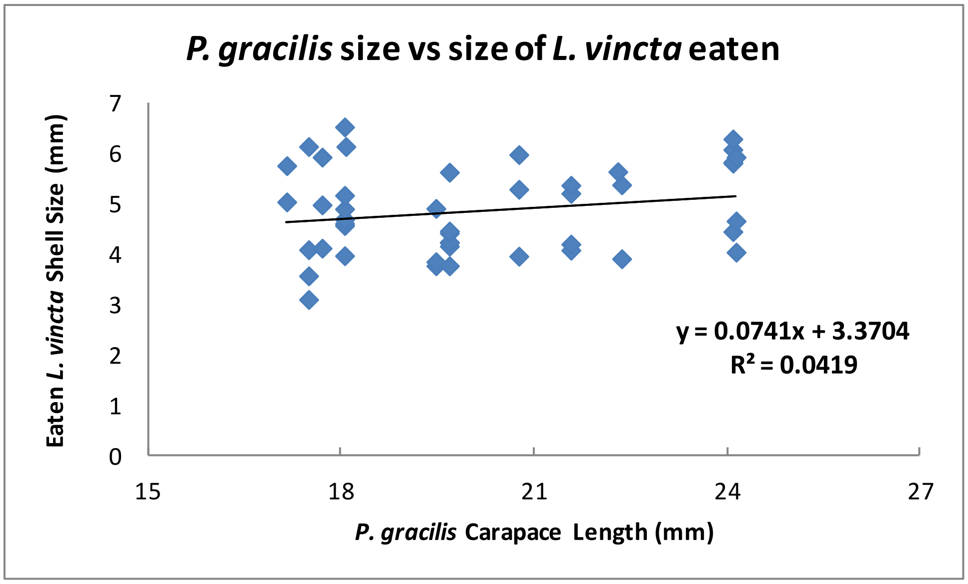

Figure 2. *P. gracilis* carapace length and size of *L. vincta* eaten.

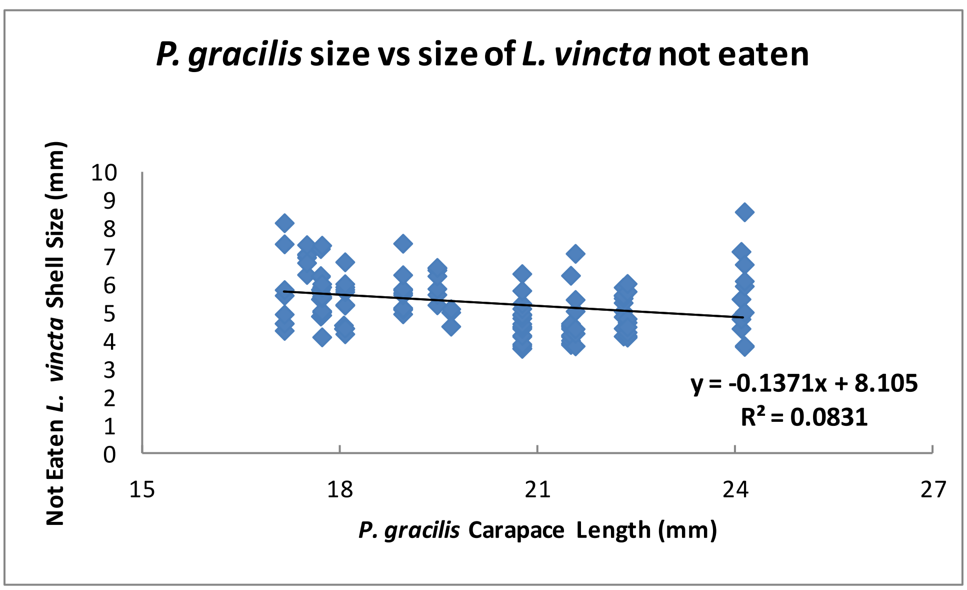

Figure 3. *P. gracilis* carapace length and size of *L.vincta* not eaten.

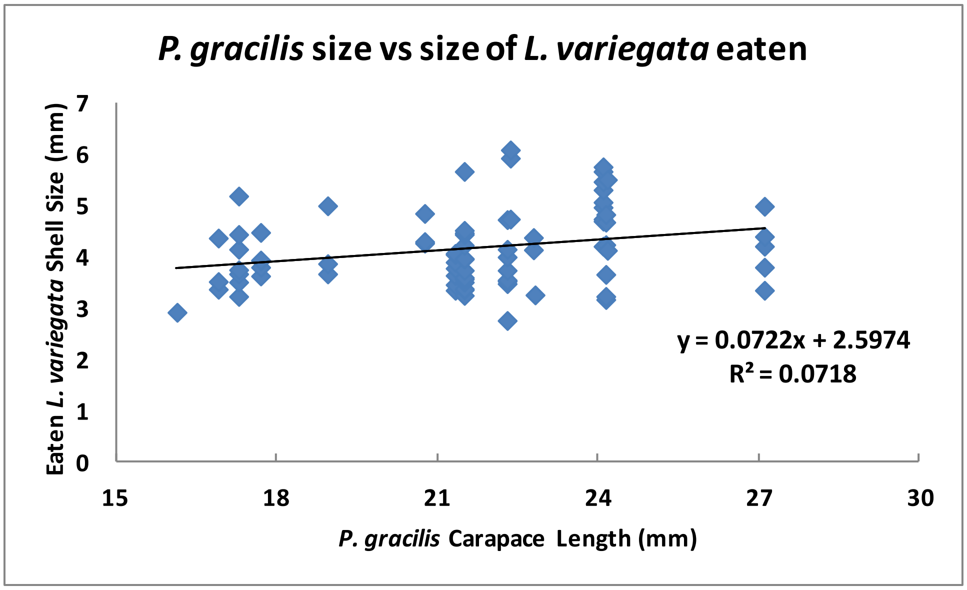

Figure 4. *P. gracilis* carapace length and size of *L. variegata* eaten.

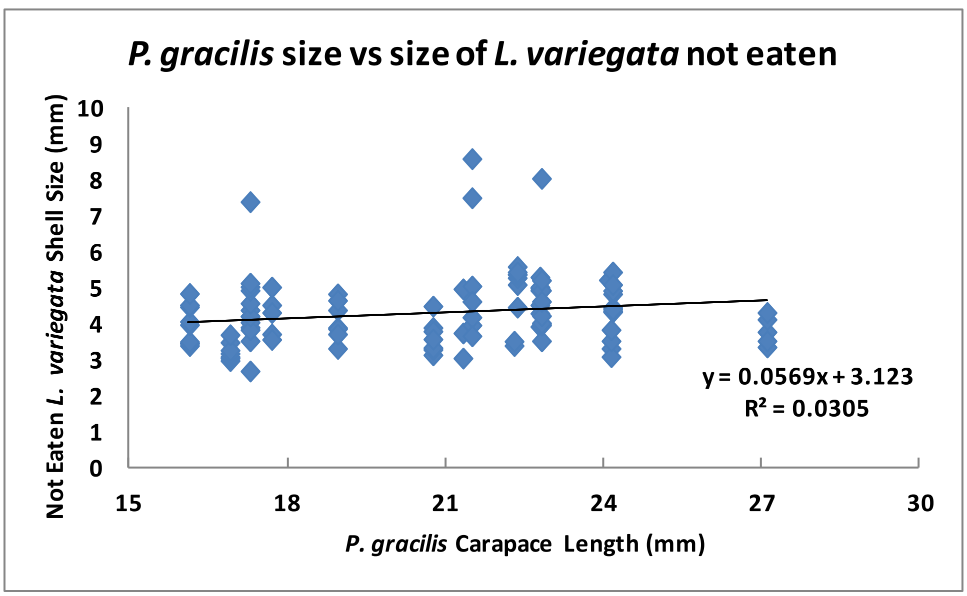

Figure 5. *P. gracilis* carapace length and size of *L. variegata* not eaten.

OLS analyses were also done to determine if there was a relationship between *P. gracilis* size and the number of *Lacuna* eaten (Table 7). There was no statistically significant relationship between the number of *Lacuna* eaten for either species, *L. vincta* (N = 16, P = 0.968, Figure 6) and *L. variegata* ((N = 16, P = 0.191, Figure 7) and *P. gracilis* carapace length.

Table 7. Regression Summaries for the Number of *Lacuna* Eaten and *P. gracilis* Carapace Length in the No Choice Experiments

	Dependent Variable	**Independent Variable**	**r^2**	**Slope**	**y-Intercept**	**P value**	**N**
***L. vincta* Eaten**	Number of Snails Eaten	*P. gracilis* Carapace Length	0.0001	0.011	2.590	0.968	16
***L. variegata* Eaten**	Number of Snails Eaten	*P. gracilis* Carapace Length	0.119	0.329	-2.684	0.191	16

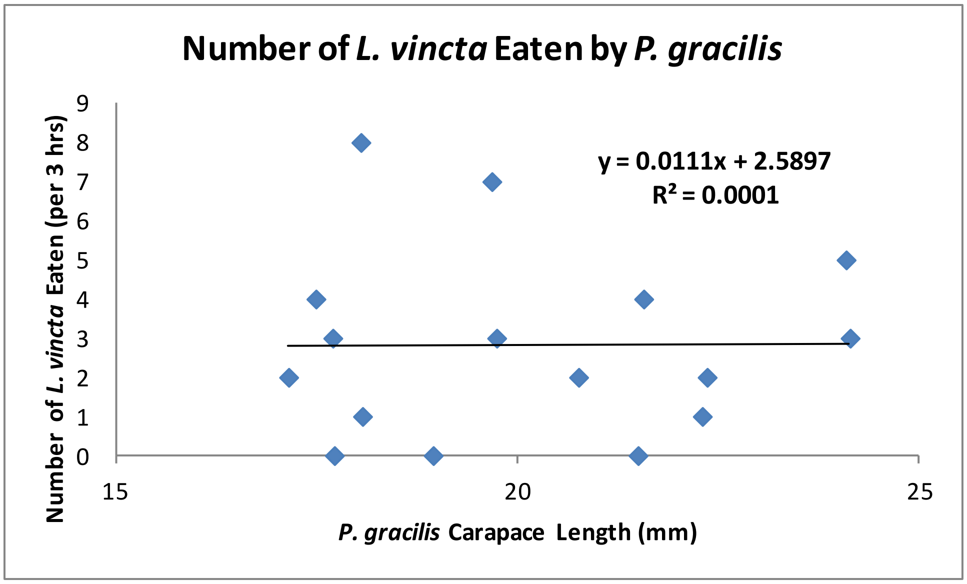

Figure 6. The number of *L. vincta* eaten by *P. gracilis* for crabs of different sizes.

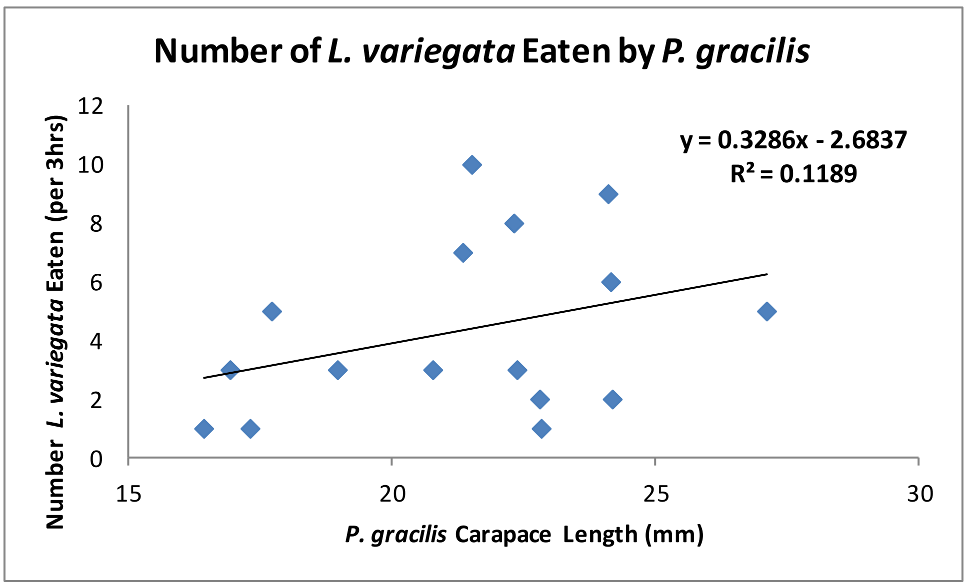

Figure 7. The number of *L. variegata* eaten by *P. gracilis* or crabs of different sizes.

We found that in the no choice experiments, when crabs were presented with one species of *Lacuna*, the average number of *L. vincta* eaten by *P. gracilis* was 2.8 ± 0.59 per three hours and for *L. variegata* it was an average of 4.3 ± 0.73 (Table 8). Two way G-tests were conducted to determine if there was a difference in the number of *L. vincta* and *L. variegata* eaten for the no choice experiments. When given only one species of *Lacuna*, crabs ate more *L. variegata* than *L. vincta* (G-test, df = 1, G = 7.89, $P < 0.005$).

Table 8. Summary for the Number of *L. vincta* and *L. variegata* eaten over 3 hours by *P. gracilis* in the No Choice Experiments

	L. vincta	***L. variegata***
Average	2.813	4.313
Standard Error	0.593	0.734
Sample Size	13	13

In the preference experiments where both species of *Lacuna* were presented to the predator, the average number of *L. vincta* eaten was 1.42 ± 0.30 and the average for *L. variegata* was 2.75 ± 0.46 (Table 9). When given a choice between the two species of snails, the result of the two way G-test indicated a significant difference in the number of *L. vincta* and *L. variegata* eaten (G-test, df = 1, G = 9.53, $P < 0.005$). We found that *P. gracilis* preferred to prey on *L. vareigata* rather than *L. vincta*. There was no statistically significant difference in the proportion of *L. vincta* and *L. variegata* that were eaten between the no choice experiments and preference experiments. That is to say that the feeding rates of *P. gracilis* for each species of *Lacuna* did not differ between the two experiment types (G- test, df = 1, G = 3.44, $0.10 < P < 0.5$).

Table 9. Summary for the Number of *L. vincta* and *L. variegata* Eaten by *P. gracilis* in the Preference Experiment

	L. vincta	***L. variegata***
Average	1.417	2.750
Standard Error	0.301	0.460
Sample Size	13	13

Leptasterias spp.

Two tailed paired t-tests were conducted to determine if there was a difference in the mean size of snails eaten and the mean size of snails not eaten by *Leptasterias* for both species of *Lacuna* in the no choice experiments; *Leptasterias* spp. was not size selective. For *L. vincta*, no statistically significant difference was found in the mean size eaten and not eaten by *Leptasterias* (N = 11, P = 0.259). These same results were found for *L. variegata* (N = 11, P = 0.191). When given only one species of snail, *Leptasterias* spp. were able to eat all sizes and individuals were not size selective.

OLS regression analyses were also used to determine the relationship between the size of *Lacuna* eaten and not eaten and size of *Leptasterias* spp. (Table 10). There was no significant linear relationships between the size of *L. vincta* that were eaten N = 37, P = 0.197, Figure 8) or size not eaten (N = 153, P = 0.463, Figure 9) and the size of sea star.

A positive relationship was found between the size of *L. vincta* captured and the size of *Leptasterias* spp. (N = 10, P = 0.007, Figure 10). Linear relationships for *L. variegata* eaten (N= 42, P = 0.023, Figure 11) and captured (N = 21, P = 0.021, Figure 13) were found, but there was no significant linear relationship between sea star size and the size of *L. variegata* not eaten (N = 147, P = 0.067, Table 12).

Table 10. Summary of Regression Analses for *Lacuna* shell size and *Leptasterias* spp. Size in the No Choice Experiments

	Dependent Variable	**Independent Variable**	**r^2**	**Slope**	**y-Intercept**	**P value**	**N**
***L. vincta* Eaten**	Snail Shell Size	*Leptasterias* spp. Size	0.047	0.015	4.693	0.197	37
***L. vincta* Not Eaten**	Snail Shell Size	*Leptasterias* spp. Size	0.004	-0.003	5.425	0.463	153
***L. vincta* Captured**	Snail Shell Size	*Leptasterias* spp. Size	0.620	0.066	2.510	0.007	10
***L. variegata* Eaten**	Snail Shell Size	*Leptasterias* spp. Size	0.122	0.014	3.370	0.023	42

***L. variegata* Not Eaten**	Snail Shell Size	*Leptasterias* spp. Size	0.0230	0.006	3.727	0.067	147
***L. variegata* Captured**	Snail Shell Size	*Leptasterias* spp. size	0.230	0.026	3.048	0.021	21

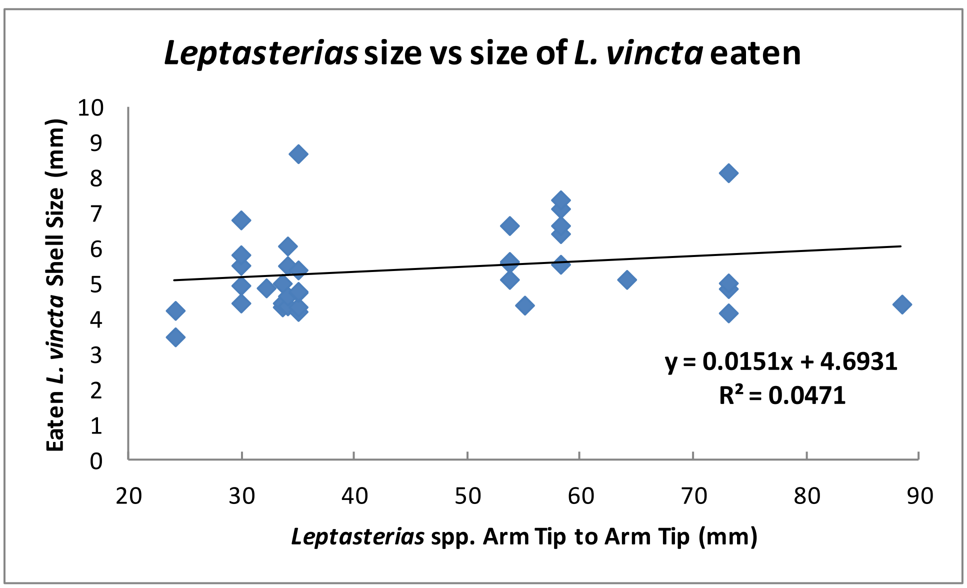

Figure 8. *Leptasterias* size and size of *L. vincta* eaten.

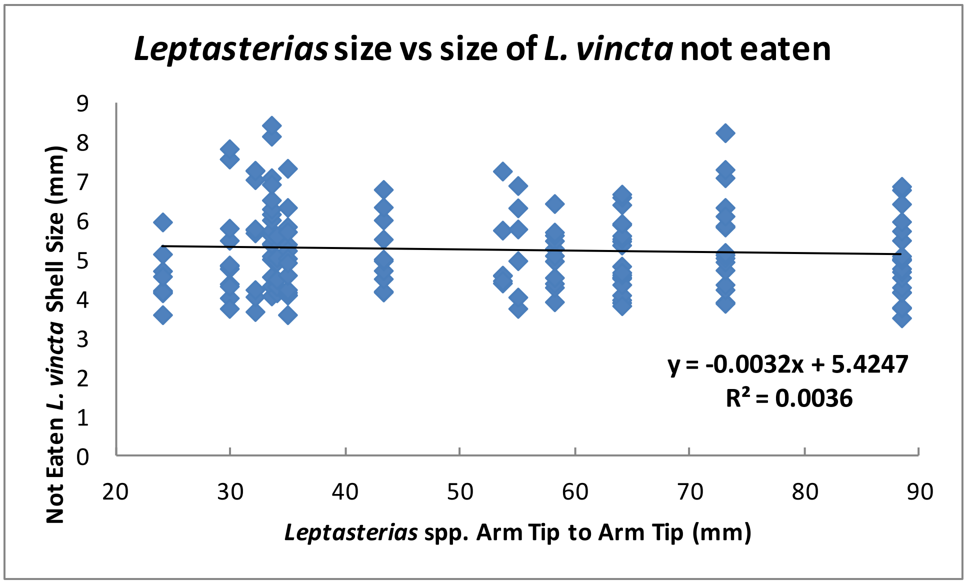

Figure 9. *Leptasterias* size and size of *L. vincta* not eaten.

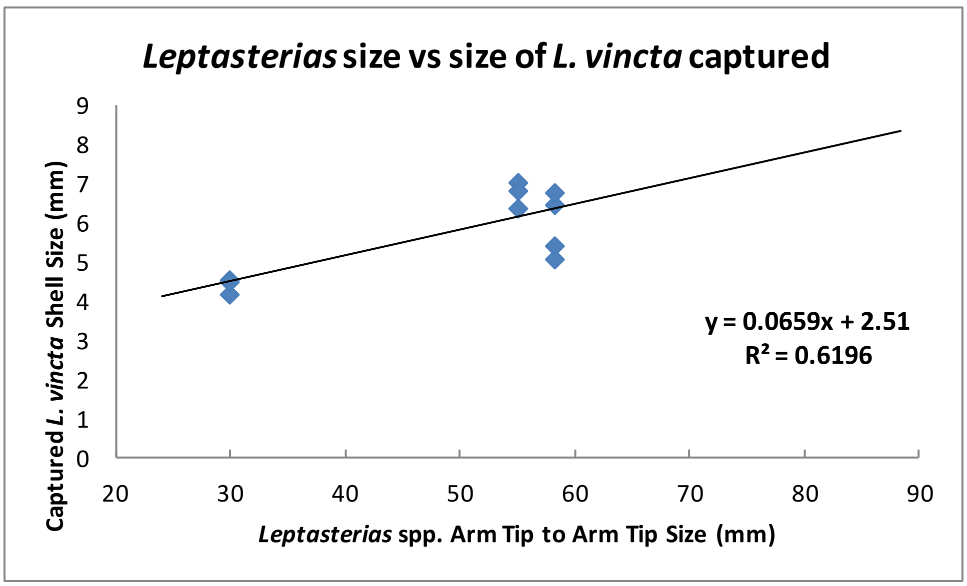

Figure 10. *Leptasterias* size and size of *L. vincta* captured.

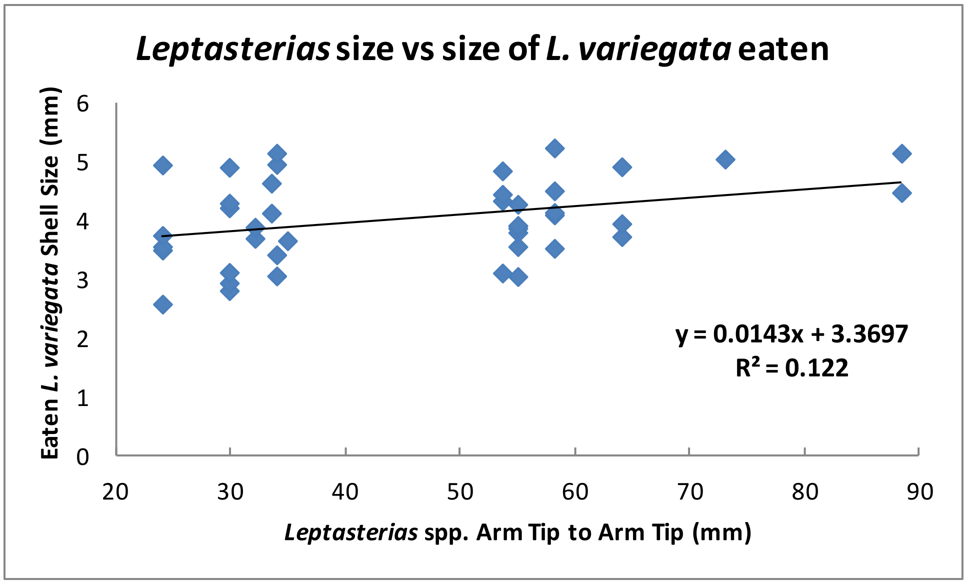

Figure 11. *Leptasterias* size and size of *L. variegata* eaten.

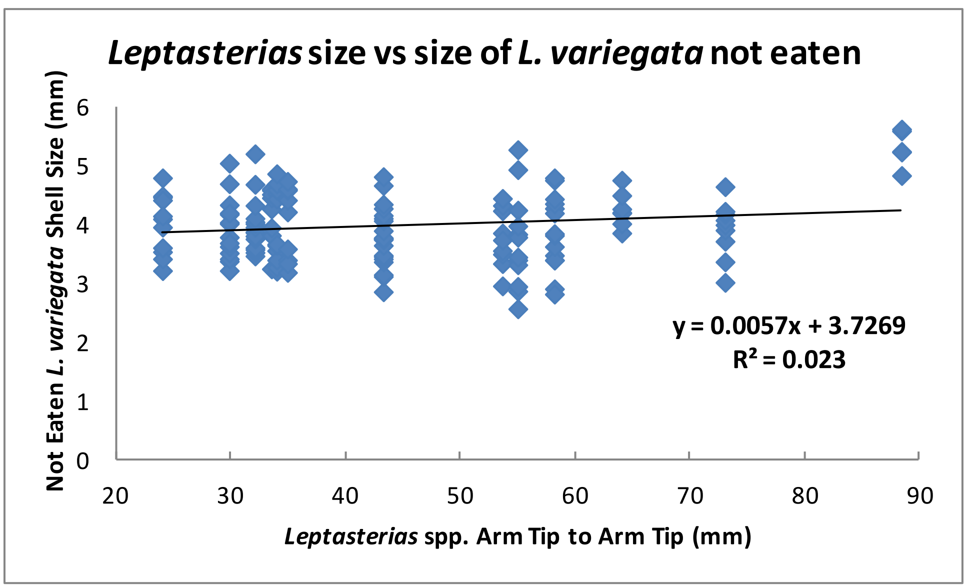

Figure 12. *Leptasterias* size and size of *L. variegata* not eaten.

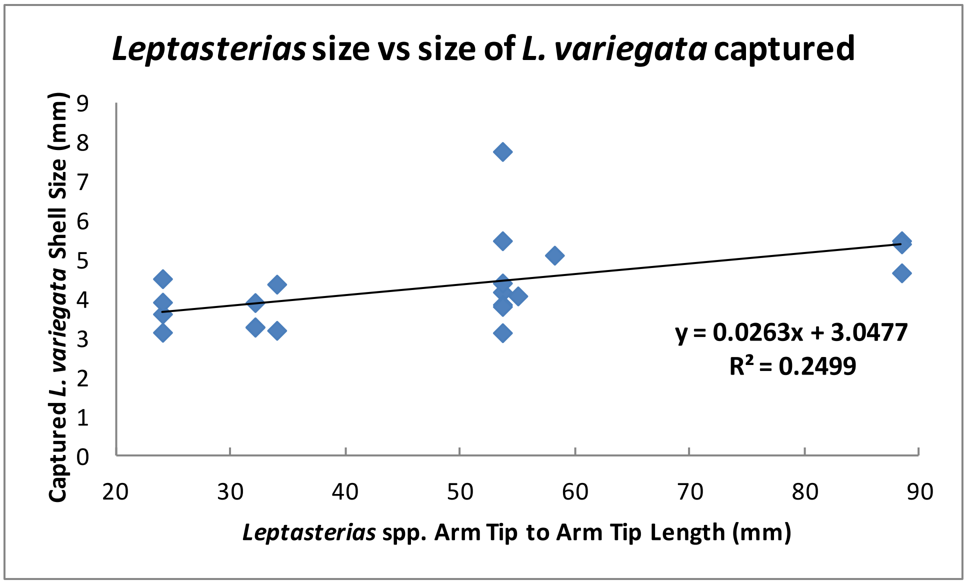

Figure 13. *Leptasterias* size and size of *L. variegata* captured.

OLS regression analyses were used to determine if there was a linear relationship between *Leptasterias* size and number of *Lacuna* eaten (Table 11). There was no statistically significant relationship between the number of *L. vincta* eaten and sea star size (N = 13, P = 0.265, Figure 14). Similarly, no statistically significant relationship between the number of *L. variegata* eaten and star size was found (N = 13, P = 0.514, Figure 15).

Table 11. Regression Summaries for Number of *Lacuna* eaten and *Leptasterias* spp. Size in the No Choice Experiments

	Dependent Variable	**Independent Variable**	**r^2**	**Slope**	**y-Intercept**	**P value**	**N**
***L. vincta* Eaten**	Number of Snails Eaten	*Leptasterias* spp. size	0.111	-0.026	3.168	0.265	13
***L. variegata* Eaten**	Number of Snails Eaten	*Leptasterias* spp. size	0.040	0.014	1.327	0.513	13

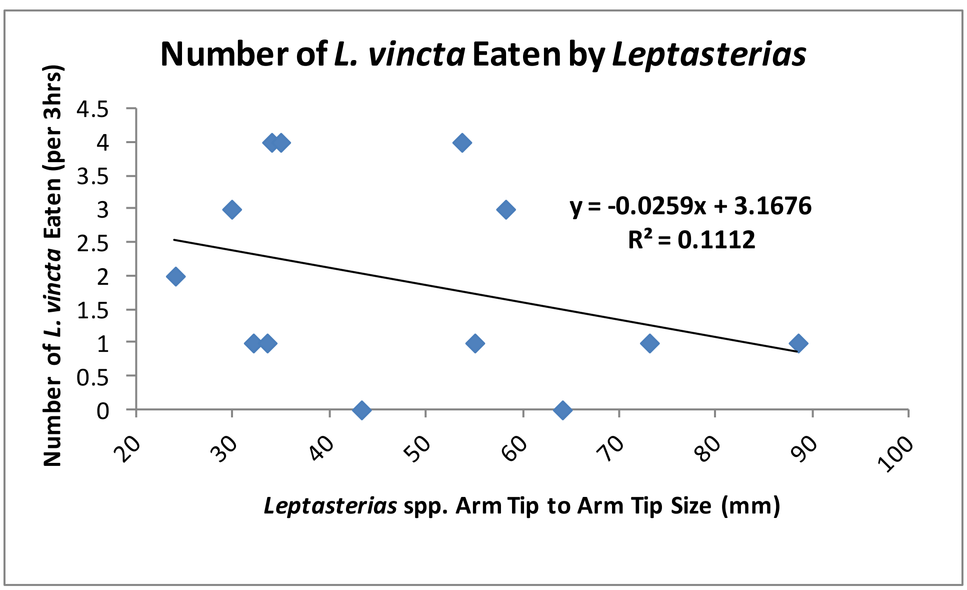

Figure 14. The number of *L. vincta* eaten and *Leptasterias* size.

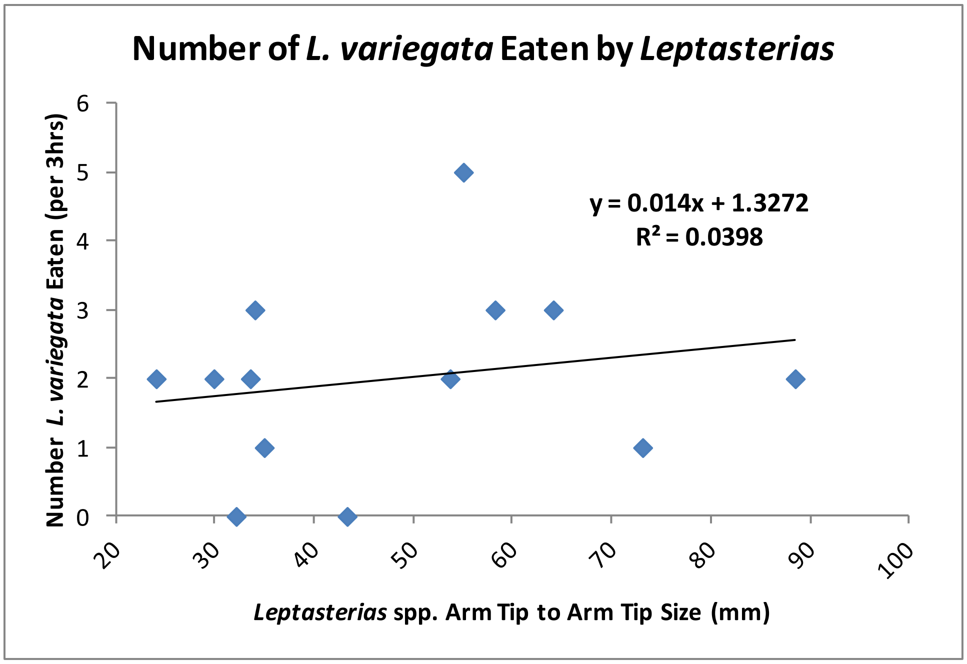

Figure 15. The number of *L. variegata* eaten by *Leptasterias* of different size.

In the no choice experiments, where sea stars were presented with one species of snail, the average number of *L. vincta* eaten was 1.92 ± 0.42 in three hours (Table 12). For *L. variegata*, the average number of snails eaten was 2.00 ± 0.38 (Table 12). When sea stars were given one species of *Lacuna*, there was no significant difference in the number of *L. vincta* eaten and the number of *L. variegata* eaten by the predator (G-test, df = 1, G = 3.841, P < 0.75). We found that when given both species of *Lacuna* the average number of *L. vincta* eaten was 0.54 ± 0.22 and the average number of *L. vareigata* was 0.92 ± 0.21 (Table 13). The two way G-test indicated no significant difference in the number of *L. vincta* and the number of *L. variegata* eaten by *Leptasterias* for the preference trails (G-test, df = 1, G = 2.08, 0.10 < P < 0.25). Additionally, there was no significant difference in the proportion of *L. vincta* eaten and *L. variegata* eaten in the single choice and preference experiments (G- test, df = 1, G = 1.26, P > 0.75).

Table 12. Summary for the Number of *L. vincta* and *L. variegata* eaten by *Leptasterias* spp. in the No Choice Experiments.

	L. vincta	***L. variegata***
Average	1.92	2.00
Standard Error	0.415	0.375
Sample Size	13	13

Table 13. Summary for the Number of *Lacuna* eaten by *Leptasterias* spp. in the Preference Experiments.

	L. vincta	***L. variegata***
Average	0.538	0.923
Standard Error	0.215	0.211
Sample Size	13	13

<u>*Mesocosm Studies*</u>

A low recovery rate in the controls prevented us from finding any statistically significant differences in the number of snails recovered for either species of *Lacuna* between: predator treatments, predator treatments and controls, and habitat types. On average, controls were missing 0.5-1 snails per replicate, ranging from 8 - 10 snails found in each replicate of each control treatment (Figure 16). In the treatments with *P. gracilis* as the predator, eelgrass as the environment and *L. vincta* as the prey, the average number of snails known to be eaten was 0.17 ± 0.17 and the average number of snails lost was 0.33 ± 0.21. For the treatments with *L. variegata* as the prey, none of the snails were known to be eaten and the average number of snails lost was 0.83 ± 0.65. In treatments with *P. gracilis* as the predator, macroalgae as the habitat and *L. vincta* as the prey, again none of the snails were known to be eaten, and the average number of snails lost was 0.17 ± 0.17. For treatments with *L. variegata* as the prey, the average number of snails known to be eaten was 0.17 ± 0.17 and the average number of snails lost was 2 ± 0.45.

In the treatments with *Leptasterias* as the predator, eelgrass as the habitat type and *L. vincta* as the prey, the average number of snails known to be eaten was 1 ± 0.63 and the average number of snails lost was 0.5 ± 0.22.For treatments with *L. variegata* as prey the average number of snails known to be eaten was 1± 0.45 and the average number of snails lost was 0.67 ± 0.33. The treatments with *Leptasterias* as the predator, macroalgae as the habitat, *L. vincta* as the prey had an average of 1.67 ± 0.49snails known to be eaten and the average number of snails lost was 0.33 ± 0.33. In treatments with *L. variegata* as the prey the mean number of snails known to be eaten was 1 ± 0.52 and the average number of snails lost was 0.83 ± 0.40.

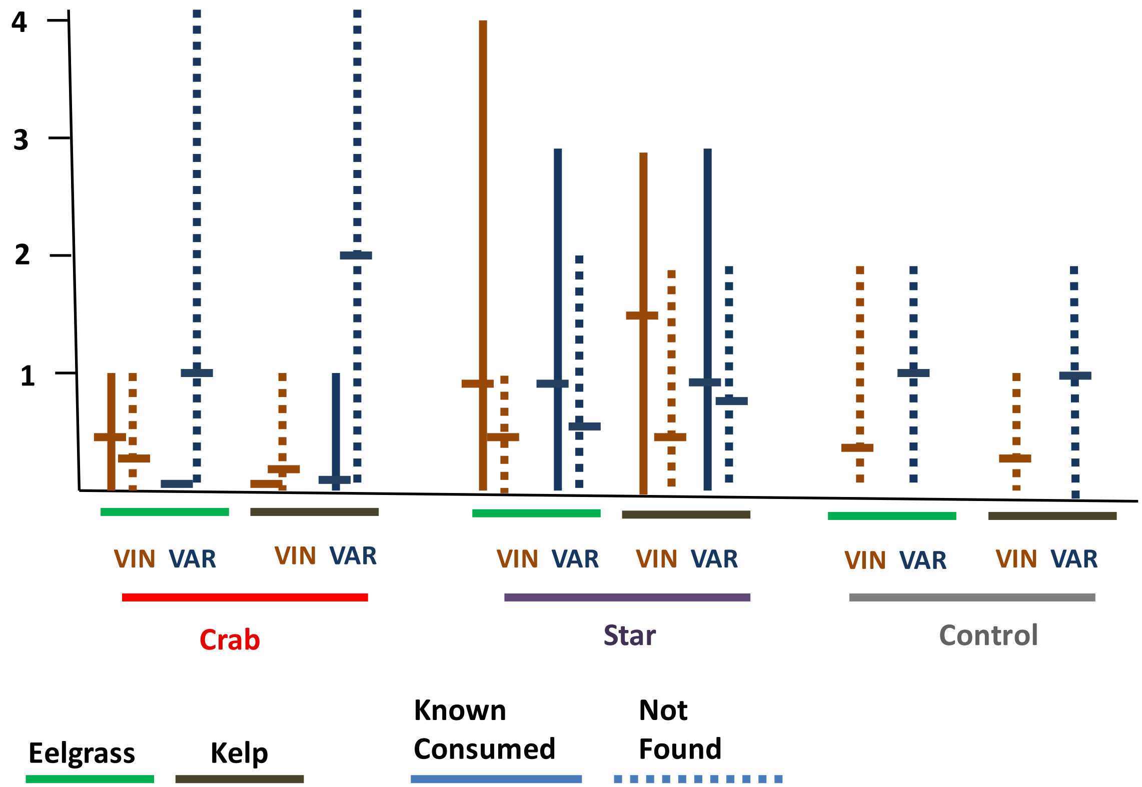

Figure 16. The recorded known consumed snails and snails not found for all treatment types for all six replicates. Cross bars are the means, solid lines are the range of the snails known to be eaten in each treatment, and dashed lines are the range of snails not found in each treatment. VIN = *Lacuna vincta*, VAR = *Lacuna variegata*. There were 6 replicates of each treatment.

Discussion:

__*Feeding in the Field*__

Lacuna populations vary considerably year to year with typical densities as high as 3,000 / m^2 (Padilla unpublished data). In the summer of 2015, the density of both species of *Lacuna* was lower than previous documented years at all sites except 4th of July Beach. We found that 23% of *P. gracilis* were feeding on *Lacuna* at some sites, including: Mar Vista, Pile Point and 4th of July Beach. While not much is known about the predation rates of *P. gracilis* on *Lacuna* in the field, we did find that these crabs do inhabit the same environments as *Lacuna* and prey on a variety of macroalgae, microalgae and marine invertebrates such as sponges and arthropods in addition to *Lacuna*.

None of the *Leptasterias* at our field sites were found to be feeding on *Lacuna*. We were able to found some individuals with barnacles in their mouth and others resting over bryozoans. Historic work has shown that *L. hexactis* is an important predator on *Lacuna*. Menge (1972b) found that 18-41% of sea stars in the field were actively feeding on *Lacuna*. The low densities of *Lacuna* at our sites in 2015 may be attributed to the lack of evidence of feeing by *Leptasteris* and the low rate of feeding by *P. gracilis.* More data for more years on feeding by *P. gracilis* and *Leptasteris* spp. in the field would provide important information on how the feeding behavior of these two predators impacts *Lacuna* populations, and whether snail densities affect feeding rates in the field.

Feeding Rates in Lab

We found that *P. gracilis* ate significantly more *L. variegata* per unit time than *L. vincta* in both no choice and choice experiments. When given a choice, *P. gracilis* preferred to eat *L. variegata* and had a higher feeding rate than *Leptasterias* on *L. variegata*. The two predators had the same feeding rate on *L. vincta*. *Leptasterias* had no preference between the two species of *Lacuna*. The difference in selectivity between the two predators is interesting. Further work would be needed to determine the basis for this selectivity (e.g., visual cues, snail behavior, or chemical differences between the two species), and whether the two species of snails differ in food quality for these predators. The fact that crabs are visual predators may be a plausible reason as to why they ate more

L. variegata than *L. vincta*. In our preference experiments, crabs were able to distinguish between the two species of *Lacuna* and selected *L. variegata*. Sea stars did not have this advantage and therefore had similar feeding rates for both *L. vincta* and *L. variegata*.

There were no linear relationships between the size of either predator and the size of *L. vincta* eaten. From the regression analyses we found a positive linear relationship between crab carapace length and size of *L. variegata* eaten. However, because of the small slope (0.072) and regression coefficient (0.072) we did not consider this association biologically significant. Likewise, in the case of star size and the size of *L. variegata* eaten we obtained a p-value of 0.02 but due to the shallow slope (0.014) and small regression coefficient (0.012) we did not consider this to have biological significance. Due to the small values of the slopes and regression coefficients we concluded that larger predators (crabs and sea stars) did not eat larger snails and that larger predators did not eat more snails per unit time. Therefore, neither predator was size selective nor did predator size have a meaningful impact on the number of prey they ate or the size of prey.

Mesocosms

Mesocosms are tools that are typically used to test questions about the importance of natural communities, but in a way that is more manageble for experimentation. In our mesocosm experiments for *P. gracilis*, there was large number of *L. variegata* that were not retrieved at the end of each replicate trial for both habitat types; however, we cannot attribute this to predation because of the number of snails not retrieved at the end of tests in the controls. In addition, the lack of recovery of *Lacuna* in the controls did not allow us to confirm any significant differences in predation by *Leptasterias*. But, we were able to determine know predation rates for the animals that were found. In the eelgrass habitat, the largest number of *L. vincta* eaten by *Leptasterias* was 4 snails over six hours. In the macroalgal habitat, the greatest number of *L. vincta* eaten was 3 snails in six hours. The largest number of *L. variegata* eaten by *Leptasterias* in both habitat types was 3 snails per replicate. Average predation rates for *L. vincta* by the *Leptasterias* did not differ for the two habitat types, but was higher for *L. variegata* on average in the macroalgal habitat, but the range was greater in the eelgrass habitat type. If snail loss rates in the crab treatments reflect predation, these results suggest higher predation on *L. variegata* in both habitat types. Based on these estimates, the feeding rates of both predators in the mesocosms were less than half those found in the lab experiments.

To obtain a greater sense of *P gracilis* predation in the mesocosm studies, inspection of their feces for *Lacuna* shell would have provided more information as to how their feeding rates changed from one habitat to another.

Because we were unable to find all the snails in the controls, it was difficult to distinguish the difference between predation and failure to find snails. More replicates need to be conducted in order to determine if there is a significant difference in predation by *P. gracilis* and *Leptasterias* spp. on *Lacuna* in the two habitat types, and whether the higher feeding rate and preference for *L. variegata* by *P. gracilis* is maintained in the more complex mesocosm habitats. In addition, the design of tanks must be refined to facilitate recovery and decrease the loss rate of *Lacuna*.

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