

The Effect of Flow Rates on Predation of *Donax variabilis* by *Luidia clathrata*

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ABSTRACT

The slender armed sea star (*Luidia clathrata*) and coquina (*Donax variabilis*) are found along the shorelines of the temperate waters of Tampa Bay. Sea stars have heightened reception to chemical cues and the intermediate disturbance hypothesis was tested in this study. Different induced flow rates similar to those in Tampa Bay (stagnant, low flow, and high flow) were tested on predation choice of sea stars on coquina clams. This experiment was achieved by using a flow bar in a ten-gallon tank of synthetic seawater kept at 34 parts per thousand (ppt). One dead and one live coquina were placed on opposite ends of the tank, and the seastar was placed in the center. Flow rates were changed over a series of treatments, and each feeding regime was timed. The findings of this study suggest that there is no significant difference in flow rate when compared to predation time, as well as no association between prey choice (dead vs. alive) and flow rate. Additionally, behavior on choice was observed and found that there was no significant difference in flow rate and foraging behavior. These findings indicate that predation on coquina by slender armed sea star is not dependent on flow rate and that there is no association of prey choice. Results should be used to propagate further research on other observed predators to determine if these organisms purposely choose the scavenging technique in order to expend less energy on feeding behaviors.

1 INTRODUCTION

Sea stars, such as the slender armed sea star (*Luidia clathrata*) are carnivorous echinoderms that roam the intertidal in search of food. Purely marine, sea stars require a constant level of salinity as very few can live in brackish water. Their main prey usually consists of other echinoderms and bivalves including *Donax variabilis*, the basis for this study. They use their many tube feet to pick up their prey and pry them open. They then expel their stomach through the oral cavity onto the prey to begin the digestion process. Due to sea stars possessing eyespots, they utilize their highly sensitive chemoreception to detect chemical cues from other organisms which allows them to be good predators (Nat. Geographic, 2018).

Tampa Bay is a unique system to study predator-prey interactions. There are more than 10,000 organisms per square meter throughout the bay, indicating its high level of diversity and food availability and primary productivity for predators. In addition, there are more than 100 tributaries constantly flowing into Tampa Bay which allows for continuous mixing and introduction of new organisms (Tampa Bay Estuary Program, n.d.). The mixing is essential to consider when analyzing the prey choice of top predators in the area, considering the amount of food availability. Prey choice here is defined as the predator being given a choice and things like size, strength, and specific behavioral and physiological traits, are taken

into account for not only the predator but also the prey (Menge, 1972).

Chemical cues released from damaged or dying clams have been known to initiate a foraging response from sea stars as opposed to the natural diffusion of chemical release from live clams (Brewer & Konar, 2005). Various factors may also heighten or hinder chemical cues from prey to sea stars. One of these factors is the flow rate. In a study by Drolet & Himmelman (2004), it was found that sea stars were unsuccessful at finding prey when there was no current in the water. Gagnon et al. (2003) found that sea stars can locate prey in back-and-forth water movement using chemoreception. This ability can be beneficial for sea stars which live in areas with some semblance of wave action. However, zones that have high flow rates may become too hard for sea stars to pick up any chemical cues due to the constant moving of the water. Low flow zones may also cause a problem, due to the water being stagnant and minimizing the permeance of chemical cues to move throughout the water. Moderate flow, however, may follow the intermediate disturbance hypothesis. The intermediate disturbance hypothesis is the idea that ecological communities are always trying to achieve equilibrium (Townsend et al., 1997). It is possible that the hypothesis can be applied to flow rate and predation, where the “perfect” amount of movement of chemical cues through the water can allow for best predation by sea stars.

Studies (e.g. St. Pierre & Gagnon, 2015) have shown that the displacement of sea stars is related to wave action and can, therefore, change the abundance of predator and prey interactions. This test analyzed the low flow rate as the control to represent the intermittent wave action along the Tampa Bay coastline. In comparison, no flow and high flow were then compared in order to study this dynamic further. Displacement was found to decrease gradually as velocity increased which could be indicative of a change in feeding parameters that were investigated during this study. Likewise, the location of *Donax variabilis* is dependent on wave action, in addition to *Donax variabilis* shape. According to Ellers (1995), the flow speed of the ocean water must be fast enough that the friction between the clam and the sand can be overcome to ensure movement along the beach, but slow enough that backwash does not displace the clam. This relationship between flow rate and clam habitat may influence species richness on a beach.

In one study (Brewer & Konar, 2005), researchers concluded that the particular species of sea star detected, moved to, and preyed upon the damaged prey while the live prey was left alone, indicating a strong preference for dead clams over live ones. The study was conducted over a down current flow. These observations would be considered and taken into account when testing this hypothesis against flow rates. A correlation between fluid dynamics may change the chemical cue detection of sea stars over the entire

system's flow instead of a constant downstream effect in the study as mentioned earlier.

The purpose of this experiment was to look closer at sea stars, more specifically *Luidia clathrata*, and determine if the flow rate of seawater would affect their predation on *Donax variabilis* as well as to see if there was a definitive choice of prey. This was supported by data collected based on different induced flow rates similar to those in Tampa Bay (stagnant, low flow, and high flow) and was tested against predation choice of sea stars on coquina clams. Within this experiment, our alternative hypothesis states that flow rate will have an effect on predation of *Donax variabilis* by *Luidia clathrata*, and the other treatments will also have a significant and a variety of effects on predatory tactics. The null hypothesis was that the flow rate would not affect predation of *Donax variabilis* by *Luidia clathrata*.

2 MATERIALS AND METHODS

The organisms used in this experiment are slender armed sea stars (*Luidia clathrata*) and coquina (*Donax variabilis*). *Luidia clathrata* are commonly found in the subtropical waters of the Atlantic Ocean in depths as deep as 40 meters (Florida Fish and Wildlife Conserv. Commission, n.d.). They exhibit sexual and asexual reproduction. *Luidia clathrata* prey on bivalves and gastropods. *Donax variabilis* are found commonly in intertidal waters in the east coast of Florida, as well as some beaches on the west coast. *Donax* live in large aggregates due to being broadcast spawners (Encyclopedia Britannica, 2007, bivalves entry). *Donax* are filter feeders and prey on algae, phytoplankton and small bacteria.

The slender armed starfish (*Luidia clathrata*) were collected from Ft. Desoto, Florida during the week of March 10, 2019, to March 17, 2019. Collection of coquina (*Donax variabilis*) took place on Indian Rocks Beach, Florida and Pass-A-Grille Beach, Florida between March 29, 2019, and March 30, 2019. The experiment was run at The University of Tampa Marine Science Field Station over March 31, 2019 and April 1, 2019. The sea stars were held in a saltwater holding tank with salinity 34 parts per thousand (ppt) with continuous circulation. The coquina were held in buckets at the same salinity and containing air stones for circulation.

The utilization of three large holding tanks, one which held the synthetic sea water, one held the sea stars before being exposed to the treatments, and one held the seastars post-experiment. The creation of synthetic seawater was necessary in order to exclude chemicals found in natural seawater that may act as confounding variables in the experiment and also to minimize the stress inflicted on the sea stars so there would be no acclimation period. This seawater was created using deionized water and instant ocean and designed to be maintained at a salinity of 34 ppt.

Twenty-one total sea stars were collected, seven replicates over three treatments. The three treatments were the control which was a low flow environment (average low flow was 3.79 cm/s) still water, and a high rate of flow (average high flow was 13.29 cm/s). Low flow environments are considered the "normal flow" of wave action for the Tampa Bay area. However, since it is an estuary, there are pockets of stagnant water as well as areas of high disturbance due to anthropogenic influences. Forty-two coquinas were needed, one dead and one alive for each treatment and were held in two buckets with air stones to provide a constant flow. A ping-pong ball was used

to calculate flow rate, in addition to a ruler and timer. Four flow bars were needed, one for each tank to create flow. Tubing, T-tubes, and a flow control box were needed for the flow set-up. A metric ruler was needed for organism and tank measurements.

The sea stars fasted for two to three days before observation. Sea stars were transported to test tanks safely in clean buckets which contained the manufactured seawater. Four, ten-gallon test tanks contained 8000 mL of synthetic seawater to minimize the need for an acclimation period.

The coquina were removed from the bucket and separated into four individual petri dishes. In their original water, the coquina was microwaved until open to ensure the coquina was dead and release their chemical cues. Four separate coquina were placed in additional petri dishes as the live coquina. The length and width of each coquina were obtained. One dead coquina and one live coquina were placed at either end of each test tank. The placement of dead and live coquina was alternated for each trial to remove possible bias.

Sea stars were measured from oral opening to the tip of one arm to obtain radial arm length. One seastar was placed in the center of each tank. Sea stars were able to move within the tank to choose prey freely. After sea stars chose their prey, they were returned to the bucket containing synthetic sea water, then placed in the final holding tank. Uneaten coquina were returned to their original bucket.

The flow rate was determined using a ping pong ball. The ball was released near the flow bar at one end of the tank and allowed to travel across the tank to the opposite side. The width of the tank was divided by the time it took the ping pong ball to travel in order to determine the flow rate. Seven tests were run for each condition: no flow, low flow, and high flow.

Data was recorded in a chart, including the measured flow rate in the tank, the length and width of the dead and live coquina, the radial arm length of the sea star, the amount of time it took the sea star to choose its prey, the prey choice, and observations regarding the movement of the sea star in the tank.

The online software JMP was used to perform a 1-way ANOVA test to determine if there was a significant difference in prey choice between dead or live coquina. A contingency test was also performed using JMP to show the preferred feeding of a sea star at the different flow rates. The two groups used for the contingency table include "Flow Rate" and "Prey Choice." Another contingency table was performed using JMP to show if the sea stars' behavior (wandering before feeding or moving directly to prey) affected by flow rate. Two groups used for the contingency table include "Flow Rate" and "Behavior."

3 RESULTS

When placed in a ten-gallon tank containing synthetic seawater at the three rates of flow (no flow = 0 cm/s, average low flow = 3.79 cm/s, average high flow = 13.29 cm/s), the predation choice of sea stars was found to have no association with flow rate (Figure 1., $X^2 = 1.725$, $df = 2$, $p > 0.4220$). When the sea stars were placed in the center of the enclosure and allowed to feed on either the dead or live coquina, their behavior was observed. The behavior of sea stars before selecting a prey choice was found to be not associated with flow rate (Figure 2., $X^2 = 0.382$, $df = 2$, $p > 0.8260$). The

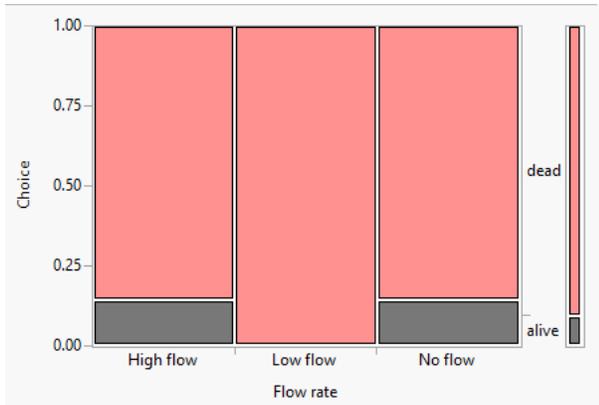


Fig. 1. Contingency Analysis Table - Flow rate vs. Prey choice (dead or alive)

Test	F-Ratio	P-Value
Flow Rate vs. Feeding Time (s)	0.7791	0.4737
Flow rate vs. Dead clam length (cm)	0.2812	0.7581
Flow rate vs. Alive clam length (cm)	3.2133	0.0641
Flow rate vs. Radial length seastar arm (cm)	9.1424	0.0018

Table 1. F-Ratios and p values for flow rate vs. feeding time, flow rate vs. dead clam length, flow rate vs. alive clam length, and flow rate vs. radial length of sea star arm.

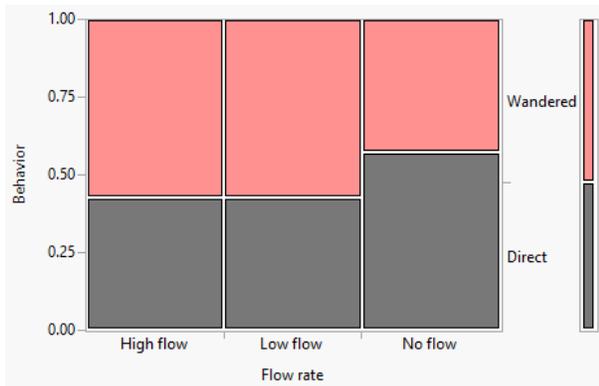


Fig. 2. Contingency Analysis Table - Flow rate vs. Behavior (direct attack or wandering/sensing behavior)

Test	DF	R ²	P value
Flow rate vs. Prey choice (dead or alive)	2	0.1306	0.4220
Flow rate vs. Behavior (direct attack or wandering/sensing behavior)	2	0.0132	0.8260

Table 2. DF, R², and P value for flow rate vs. prey choice (dead or alive) and flow rate vs. behavior (direct attack or wandering/sensing behavior).

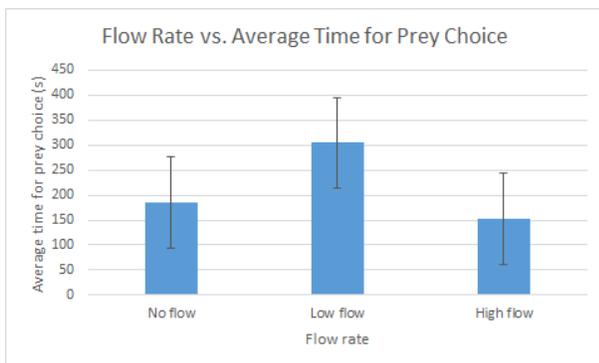


Fig. 3. Flow Rate vs. Average Time for Prey Choice. Mean time in prey choice (dead or alive *Donax variabilis* by *Luidia clathrata*) represented across three treatments indicating no significant difference in flow rate and feeding behavior ($\sigma_M = 90.978$, $\sigma_{\bar{x}} = 0.07127$).

time it took sea stars to select their prey choice was also recorded and was found to be independent of flow rate ($F_{2,18} = 0.7791$, $p = 0.4737$). The lengths of coquina were recorded in each trial, and no significant difference was found in the lengths of dead coquina used in each trial ($F_{2,18} = 0.2812$, $p = 0.7581$) or the lengths of live coquina used in each trial ($F_{2,18} = 3.2133$, $p = 0.0641$). A significant difference was found in the radial length of sea star arms used in each trial ($F_{2,18} = 9.1424$, $p = 0.0018$).

4 DISCUSSION

It was hypothesized that flow rate would affect predation of *Donax variabilis* by *Luidia clathrata*, however, after this experiment we found that to not be the case. Because of this we reject our alternative hypothesis and accept our null hypothesis that flow rate does not affect predation of *Donax variabilis* by *Luidia clathrata*. The null hypothesis was supported. This can be shown by looking at the three different flow rates and the average time it took for the

sea star to pick a clam. There was no significant difference in this case (Figure 3 & Table 1: $F_{2,18} = 0.7791$, $p = 0.4737$). Even though there was no significant difference, there was an observed faster predation time in low flow, with one outlier, which was the cause for the calculated p-value. This could have been due to the intermediate disturbance hypothesis, that the flow with the “middle” amount of flow would allow prey cues to be picked up more easily. However, there was not a significant difference due to the outlier so that no findings could have been verified by this. Feed time is important for prey because of the time it takes for predators to forage actively and to find and consume prey, therefore impacting prey abundance in that local area (Glaspie & Seitz, 2018).

When the sea stars were placed in the tank, their behavior was observed to see if there was a correlation with flow rate and either wandering or direct attack. However, there was no correlation between the two (Figure 2: $p = 0.8260$). This could be due to the fact that no single flow seemed to have direct or any disturbance of chemical cues to the sea stars, causing them to not directly attack the clams but instead have a mix of wandering and attack. Also, when placed in laboratory tanks, sea stars tend to move up the walls of the tank and orient near the surface (Feder, 1963). This was observed in our sea stars, which could also be the reason for the lack of direct attack on clams and more of the wandering behavior.

Another factor that could have come to play was energy conservation. In this experiment, our sea stars were withheld from food for a few days, making them hungry and causing them to use the last of the energy they had to choose a clam. Prey choice could have chosen the cues that signified the dead clams due to it being more metabolically efficient than expending their energy trying to open a live clam. When looking at the prey choice, however, they did choose the dead clams more than they did the live ones. This could be due to the chemical cues that are given off by dead clams versus live. Even though they did show to choose the dead over the live clam, there was no correlation between choice when compared to flow rate (Table 2: $p = 0.4220$). Sea stars have heightened reception to chemical cues and the intermediate disturbance hypothesis was tested in this study. Chemical cues released from damaged clams have been known to initiate a foraging response from sea stars as opposed to the natural diffusion of chemical release from live clams. There are different chemicals at a different strength which are released from the damaged tissues of dead clams (?). This may be indicative of different chemical cues released from the live coquina once the threat of a predator was introduced. The sea stars actively chose the dead clams which could have something to do with the distaste for the active release of these chemicals from the live coquina.

Different fluid regimes vary in comparison with particular sensory mechanisms and behavior. Organisms adapt to local flow environments in order to successfully locate and consume prey (Weissberg, 2000). This is indicative of the experiment at hand to investigate if there was a difference in predatory and scavenging behaviors based on flow rates of an environment. In order to locate prey, there must be substantial chemoreception done by the sea star. Another study following *A. Rubens* discovered that wave action is one of the key indications that sea stars have a higher ability to explore their environment to localize prey. In this case, *A. Rubens* had an increase in activity in search of prey more frequently in warmer temperatures than cooler ones (St. Pierre & Gagnon, 2015).

Behavioral ecologists also investigated the learned behaviors due to chemoreception in the wild. Damage-released alarm cues are often expelled from dying prey items to warn others that there is a predator nearby. This is crucial in learned recognition of the diffusion of chemical detection in predators (Chivers et al., 2013). In the case of local and other species of sea stars, the evidence that sea stars are facultative scavengers as opposed to solely acting as predators is supported by this notion. Since there is large support of evidence that regardless of the environmental conditions the sea stars are placed in, they will actively locate and consume the dead clam over the live one may have to do with the learned behavior that dying clams expel certain chemicals when injured or dying.

A significant difference was found in the radial length of sea star arms used in each trial (Table 1, $p = 0.0018$). While the size may not have had the most significant contribution to prey choice, it is still important to note for future studies to obtain sea stars that were more close in size.

This experiment emphasized the findings from previous studies that sea stars are facultative scavengers, different from what most textbooks indicate. Also, this experiment suggests that flow rate may not affect predation of *Donax variabilis* by *Luidia clathrata* emphasizing this prey choice no matter the local environment they exist in. This can be supported by our non-significant p values in every experiment when compared to the flow rate. Although our study did not focus on the behavior of the prey on organism, future works could look into flow rate and how the prey organisms respond to chemical cues. Not only do predators show response to prey cues, but the prey is also known to show changes in behavior and morphology due to being preyed upon. Prey organisms often respond to risks by changing their conspicuous behaviors and may reduce their feeding when the prey senses a predator. Other responses to predator cues include avoidance, increased hiding and reduced activity (Kats & Dill, 1998). These responses should be observed with relation to the presence of predators in a range of environmental conditions to thoroughly test the feeding behaviors of sea stars in all capacities.

We would like to thank Dr. Heather Masonjones for helping us craft this experiment as well as provide our group with the correct equipment to carry it out. We would like to also show thanks to Dr. Louis Ambrosio who collected our *Luidia clathrata* as well as take care of them for the duration of our experiment. And lastly, we would like to extend a thank you to The University of Tampa for allowing us to use the Marine Lab to conduct our experiment.

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