

Prey Sensation in Sea Star *Asterias forbesi*

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I. Introduction

Asterias forbesi, a member of the echinoderm phylum and asteroid class, are common in the rocky intertidal zone from the North Atlantic to the Gulf of Mexico (Castro and Huber, 2005). The diet of these carnivorous sea stars consists mostly of bivalves and gastropods such as mussels and snails, all of which are slow moving animals (Moore and Leeper, 1997).

A. forbesi, like most echinoderms, have pentamerous radial symmetry with bodies consisting of two distinct sides, aboral and oral. The aboral side has a spiny skin used for protection and to clean off debris that may land on them. The oral side consists of hundreds of tube feet on the tips of each arm and along the radiating channels of the

arm called ambulacral grooves (Ruppert and Barnes, 1994). The tube feet often end in a sucker used in many ways, including walking and climbing by pulling the body with tube feet as they use suction cups to adhere to the surface. This ability is important to the sea star because they can live in an area where waves constantly break over them. The suckers can also be used for receiving chemical and mechanical stimuli, an adaptation used for foraging for food (Ruppert and Barnes, 1994). There has been a report indicating that there are seventy thousand sensory cells per square millimeter found in these tentacle-like tube feet at the tip of the arms and along the ambulacral grooves. (Ruppert and Barnes, 1994).

The greater part of the nervous system of an asteroid is connected to the epidermis. In the base of the peristomial epidermis is the circumoral nerve ring. At each point of this pentagonal nerve ring are radial nerves which extend into each arm of the sea star. Both the radial and the circumoral nerve ring bring the movement control to the tube feet (Ruppert and Barnes, 1994). The tube feet are coordinated to walk forwards, backwards, or stay still, depending on the message that a stimulus is picked up and sent to the nerve center. *A. forbesi* always walk with one arm leading straight toward where they are headed. The leading arm, however, is not a specific arm, demonstrating that the sea stars receive stimulation the same in all their arms. The lead arm is determined by the external stimuli that the starfish sense (Campbell, Coppard, D'Abreo, and Tudor-Thomas, 2001).

In order to determine whether *A. forbesi* can use chemoreception to orientate itself around food, an experiment was conducted to test whether the sea stars exhibits negative, positive, or no chemotaxis toward a prey odorant. To begin to test where the chemoreceptors are located, the part of the body the sea stars brought to an odorant was recorded. This experiment was different from testing in the *A. forbesi's* natural environment because there was no filtering of water in the experimental dish, stimulating the high intense seawater flow in the intertidal zone.

Foraging techniques depend on the type of environment in which an animal lives. I hypothesized that *A. forbesi*, would bring the tip of any arm to a prey odorant if present. However, if the odorant is presented as the actual live prey animal, like a mussel, then the amount of times that it brings the tip of an arm toward the odorant will be higher than if just the odorant with no animal was present. *A. forbesi* has a limited amount of time that it can catch food because of high and low tide of the intertidal zone. Sea stars need water to be covering them to move because of their hydrostatic skeleton. Water coverage also allows them to come out from hideouts because the water protects them from the sun and wind preventing desiccation (Menge, 1972). This enables them to forage only during the short period of high tide. The limited amount of time given to *A. forbesi* for foraging favored the evolution of special sensory organs which allows them to find food more quickly. This stress and the fact they live in a zone of high impact waves is the force behind the evolutionary adaptation of sea star foraging strategies, making them good competitors

and keystone species to many ecological habitats (Moore and Leeper, 1997).

This project was done in collaboration with Advanced Marine Biology students 2005 from Wheaton College, Jenny Fisher, Alex Adams, and Maris Madeira. Fisher and I both worked with the same species of sea star *A. forbesi*, and both studied the predation techniques of this animal.

Fisher studied the movement rate of different sized *A. forbesi*. As the tide rises, the level of water eventually reaches the starfish and their prey so that they can begin to forage. The success rate of the sea depends on amount of time that it takes to start moving once high tide covers them, rate of movement, how well they can locate a prey, and then consume it (Menge, 1972).

Adams looked at olfaction in the American lobster, a scav animal. Specifically, he looked at the change in the flicking rate per second of the lobster's antennules when in odorant was added. I tested the orientation of the *A. forbesi*, another predatory animal, when an odorant was added (Adams, 2005). We have collaborated by testing different types of odorant plumes on different species and quantitatively measured the difference in motion of the subject species when the odorants were added.

Madeira worked with *Hemigrapsus sanguineus*, testing for phototactic responses and found that the crab showed negative phototaxis when looking for a crawl space to hide in for protection from predators (Madeira, 2005). During the times of low tide when the *A. forbesi* can not forage, they are negatively phototactic (Ruppert and Barnes, 1994). In a similar way as the *Hemigrapsus sanguineus* the *A. forbesi* crawl into cracks and crevasses in the rocks in order to avoid predators.

◇ II. Materials and Methods

Materials:

- 3 *Asterias forbesi* from the Marine Biological Laboratory in Woods Hole, Massachusetts, kept in a 10 gallon aquarium.
- 1/2 pounds of small live mussels from grocery store. Kept in separate 10 gallon aquarium.
- 5 gallon bucket
- hydrometer
- small aquarium net
- strainer
- 2, 50 ml beakers

- 5 large finger bowls: 18 cm circumference, 3 inch high, round, and glass
- 1 pipette
- stirring rod
- 50 ml container
- molasses
- tweezers
- blender
- small dark rock (size of a small mussel)
- Epson Photo pc 850Z camera with stand
- cold room (to keep animals and run experiments)

Methods:

To make the prey odorant, 4 to 5 mussels were taken out from aquarium. They were all cracked open so that juice poured into a 50 ml beaker and the meat was scraped out with tweezers. 20 ml of mussel juice and meat were measured out and placed into a blender with 20 ml of distilled water. They were blended together for about ten seconds and 20 ml of the resulting slurry was put back into a clean 50 ml beaker. 40 ml of molasses was measured out and added to slurry of mussel meat to make a more viscous prey odorant. This was contained in a small capped container for later use.

The sea water for the experiment was made by mixing a ratio of one gallon of distilled water per half a cup of Instant Ocean so that the salinity was about 34 parts per thousand (ppt). A hydrometer was used to test the salinity. The water was then placed in the cold room at least 4 hours that it would cool to the temperature of the room so that temperature would not be varying throughout the experiment.

An Epson Photo pc 850Z camera was secured on a stand and placed on the experimental bench in the cold room near an outlet so that it faced directly down onto the bench. The straps were secured so that they were out of view of the lens and then the power chord was plugged into the camera and outlet. A piece of white paper was put on the lab bench in the view of the camera lens. This was so that later the sea star movements could be viewed very clearly. The lights over head were dimmed by covering with white paper. This limited the glare of the lights on the dish which created clear pictures.

Labels were made out of paper and marker so that they fit outside the finger dishes and still be seen in the view of the camera lens. This was tested by taking pictures of the bowl and seeing if labels showed up. Labels read: "salt water, molasses, rock, mussel, sea star 1, sea star 2, sea star 3, arm 1&2, arm 2&3, arm 3&4, arm 4&5, and arm 5&1."

These were used constantly through out the experiments to keep track of what experiment was taking place in the movies that were made later on.

Setting up experiment:

1. The camera was turned on to the multi frame selection and the mode was changed until it read "interval" on the screen.
2. The camera was then set to take 1 picture every 10 seconds by pressing the up and down button so that the dish read 0:0:10 (10 seconds) on the screen.
3. The labels that read "seawater," "experiment 1," "starfish 1," and "arms 1 and 2" were placed in the corner of the white paper so it was in the viewing field of camera, but out of the dishes way.
4. 6 finger dishes were filled with one inch of sea water and place on the experiment bench, one being placed on the white paper, not covering the labels.
5. The zoom button on the camera was used to center on one experimental dish so that it was as close as possible to the sea stars.
6. Three stars were taken out of the main "predator aquarium" and placed in one of the experimental dishes out of view of camera. This was the "holding dish."
7. The sea stars were left to settle for 5 minutes in their new environment.
8. All three sea stars were measured into the notebook. Leg one was the closest arm to the right of the madreporite and legs 2,3,4, and 5 were numbered going clockwise from arm 1. Arm 1 was then measured in centimeters from the very tip of the arm to the center of the body. The length was recorded in the chart. This was used as identification and also to measure the sea star's movement.
9. After the camera was set up, it ran from here until the end of the experiment. The labels were placed according to which sea star and arms were being experimented on.

Seawater- Control 1:

10. "Sea star 1" was taken out of the holding dish and placed into the experimental dish under the camera so that the arms experimenting on were faced toward the middle of the dish. As the sea star settled onto the bottom, a pipette was filled with sea water from experimental dish.
11. Immediately after the sea star had settled, the pipette of sea water was squirted between arm1 and 2.
12. After ten minutes of experimenting the same sea star was placed into the "cleaning dish," a finger dish of sea

water used only for this purpose, for ten seconds.

13. While in the cleaning dish, the finger bowl used for experimenting was switched so that it contained clean sea water.
14. The same sea star was put into the new experimental dish and step #11 was repeated with other arms: between 2 and 3, 3 and 4, 4 and 5 (or whatever combination of legs was available).
15. Steps #11-14 were repeated with "sea star 1" and "sea star 2" with the other two star fish, then return all star fish back to holding dish, remembering to switch labels.

Molasses-mussel odorant- Experiment 1:

16. A half of a pipette (.5 ml) was filled with the concoction of molasses and mussel odorant that was made earlier and set aside.
17. Labels were changed accordingly.
18. "Sea star 1" was taken out of the holding dish and put into the experimental dish.
19. Once the star fish had settled onto bottom of the dish .5 ml of odorant was squeezed out between leg 1 and 2 at the bottom of the dish, slow enough so the odorant had the shape of a circle. The odorant reached the sea star within 1 minute.
20. While the experiment was running the used dishes were emptied, cleaned, and refilled with new sea water.
21. After ten minutes of recording with camera the sea star was placed into a "cleaning dish" for 10 seconds while the experiment dish was switch to an unused dish.
22. The same sea star was placed back into the experimental dish and steps #19 to 21 were repeated with the other arm combinations: between 2 and 3, 3 and 4, 4 and 5 (If arms were missing, the whatever combinations of arms are available).
23. Steps #18-22 were repeated with "sea star 2" and "sea star 3."
24. All three star fish were returned to holding dish and all dirty water was changed.

Rock- Control 1:

25. Labels were switched accordingly.
26. One small mussel was taken out of the main aquarium and kept in a finger dish of sea water. Its length, width, and height were recorded into lab notebook.
27. The small rock was washed with distilled water, then with sea water, and was then placed in the center of the

experiment dish.

- 28.** "Sea star 1" was placed in experiment dish so that the small rock was between arms 1 and 2 as close as possible, but not touching. Reaction was recorded with camera for ten minutes.
- 29.** The sea star was placed into the cleaning dish for ten seconds while changing experimental dish to one with new seawater.
- 30.** Steps #27-29 were repeated with "sea star 1" with the other arm combinations: between 2 and 3, 3 and 4, 4 and 5 (If arms were missing, the whatever combinations of arms are available).
- 31.** Steps #27 through 30 were repeated with "sea star 2" and sea "star 3," then all were returned to holding dish.

Mussel- Experiment 2:

- 32.** The live intact mussel set aside was placed in the center of the experiment dish, in the same location as the rock was.
- 33.** "Sea star 1" was placed in dish so that its arms were between legs 1 and 2, but not touching, as done with the small rock.
- 34.** The reaction of the sea star was recorded with the camera for ten minutes.
- 35.** The sea star was placed into the cleaning dish for ten seconds while switching the experimental dish to a clean one.
- 36.** "Sea star 1" was placed back into the experimental dish and steps #33-35 were repeated with the other arm combinations: between 2 and 3, 3 and 4, 4 and 5 (If arms were missing, the whatever combinations of arms are available).
- 37.** Steps # 34-36 were repeated with "sea star 1" and "sea star 2."
- 38.** Camera was turned off and memory card was transferred to the computer in the ICUC lab at Wheaton College, Norton, MA.

Analyzing data

- 39.** The memory card was plugged into computer and the pictures were transfer into a movie through Quick Time Player.
- 40.** The frames not needed, ones before, after, and in between different experiments during set-up were deleted.
- 41.** The video was saved as "contained" onto the computer.

- 42.** Two bar graphs were made. One showing the difference in negative, positive, and no chemotaxis of all the experiments and another showing the center positive chemotaxis versus the tip positive chemotaxis.

Calculating data:

Categorizing the behaviors correctly was important to determine what type of chemotactic responses the sea stars were exhibiting; positive, negative, or none, and therefore obtaining accurate quantitative data. Although the sea stars were given ten minutes to show movement, the first movement was what was looked at. If there was no movement in the first ten minutes, then it was marked down as "no reaction" in the notebook (see table 1). When the odorant was placed in the saltwater experimental dish it created an observant yellow plume, being more concentrated towards the middle of the plume, observed by a darker shade of yellow.

"Positive chemotactic responses" were defined as when either the tip or the center of the sea star moved toward the odorant and there were calculations to determine both of these positive chemotactic responses.

Tip Positive chemotaxis:

- 43.** For each experiment, the initial length between the two arms being tested from tip to tip was measured in centimeters. This length was then divided in half. This number was called the "tip measurement number" and used as a measurement to determine whether positive chemotaxis at the tip was seen.
- 44.** If the distance decreased from the initial placement of the tip of either arm to the initial odorant point (or salt water- control), where the pipette squeezed out the odorant by the "tip measurement number" (from step 43), and the sea star held that position for at least twenty seconds then it was marked as positive chemotactic response to the tip in table, indicating which leg showed positive chemotaxis. (see table 1). This minimum time restriction made sure the sea star wasn't just passing by and happened to pass an arm tip by the odorant.

Center Positive Chemotaxis:

- 45.** The length of the sea stars arm, from tip of the arm to the center of the body, were measured in centimeters for each arm used in the experiment and that number, called the "center measurement number" was used to determine if the sea star exhibited positive chemotaxis toward the center.
- 46.** If the sea star brought the center of its body directly over the odorant point so that it moved the "center measurement number" without bringing an arm to the odorant first, but went straight between the two arms to get to the odorant, and stayed in that spot for at least 20 seconds, then it was counted as positive chemotaxis to the center.

Negative Chemotaxis:

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47. If the sea star's first movement did not spend more than 20 seconds in the positive chemotactic positions and kept walking away from odorant, or walked away in an opposite direction for at least 20 seconds, then it was counted as negative chemotaxis.

If a sea star moved a distance of its arm length toward the odorant so that its center was directly over the odorant source and it stayed in that position for at least 20 seconds, then it was considered as positive chemotaxis, marked in the data table as "pos- center." To get to this position the sea star could not twist the body

Negative chemotaxis was recorded when a sea stars moved backwards from an odorant. Backwards movement counted as moving away from the odorant in any direction as long as it was more than a 95 degree angle or more away from the odorant source.

"No chemotaxis" was recorded when positive and negative chemotaxis were not present in the sea star. If there was no movement of more than 50% of its arm length in any direction in the course of the ten minutes of the experiment or the star fish moved so that the distance form the sea star to the odorant remained the same, then the movement was marked in the data table as "No RXN" and was counted as being no chemotaxis.

Table 1: *A. forbesi* Response to Odorants

Starfish	arm placement	control 1- sea water	experiment 1- molasses/mussel slurry	Control 2- rock	experiment 2- mussel
starfish 1	arms ___				
cm=	arms ___				
	arms ___				
	arms ___				
	arms ___				
starfish 2	arms ___				
cm=	arms ___				
	arms ___				
	arms ___				
	arms ___				
starfish 3	arms ___				
cm=5.0	arms ___				
	arms ___				
	arms ___				
	arms ___				

Table 1: This table was used to organize data collection and to show whether the experiments showed positive, negative, or no chemotaxic movement.

III. Results

Table 2: results "N" values

Starfish	arm placement	control 1- sea water	experiment 1- odorant	Control 2- rock	experiment 2- mussel
starfish 1	arms 1&2	No reaction	pos- Center	no reaction	pos- Tip
cm=3.75	arms 2&3	No reaction	pos- Tip	neg	neg
	arms 3&4	No reaction	pos- Tip	pos- Center	neg
	arms 4& 5	No reaction	No reaction	pos- Center	pos- Tip
	arms 5&1	No reaction	pos- Center	neg	pos- Center
starfish 2	arms 1&2	No reaction	pos- Tip	neg	pos-Tip
cm=2.75	arms 2&3	No reaction	neg	pos- Tip	pos-Tip
	arms 3&4	No reaction	neg	pos- Tip	pos- Center
	arms 4&1	No reaction	No reaction	no reaction	no reaction

Table 2: This table shows the amount of times that each of the starfish showed a certain chemotactic behavior. For "no reaction" n=14 "positive tip" n=9, "positive center" n=6, and "negative" n=7.

One of the sea stars was not included in the results because of the size difference. *A. forbesi* number one and two show that the control experiment with sea water resulted in all sea stars showing a hundred percent no response. In the experiment with the molasses and mussel slurry odorant, there was an increase in both negative and positive chemotaxis. In the second control experiment with the small rock the sea star showed all three chemotactic responses. In the second experiment with the mussel the negative and no chemotactic responses went down and the positive responses went up. (See figure 1).

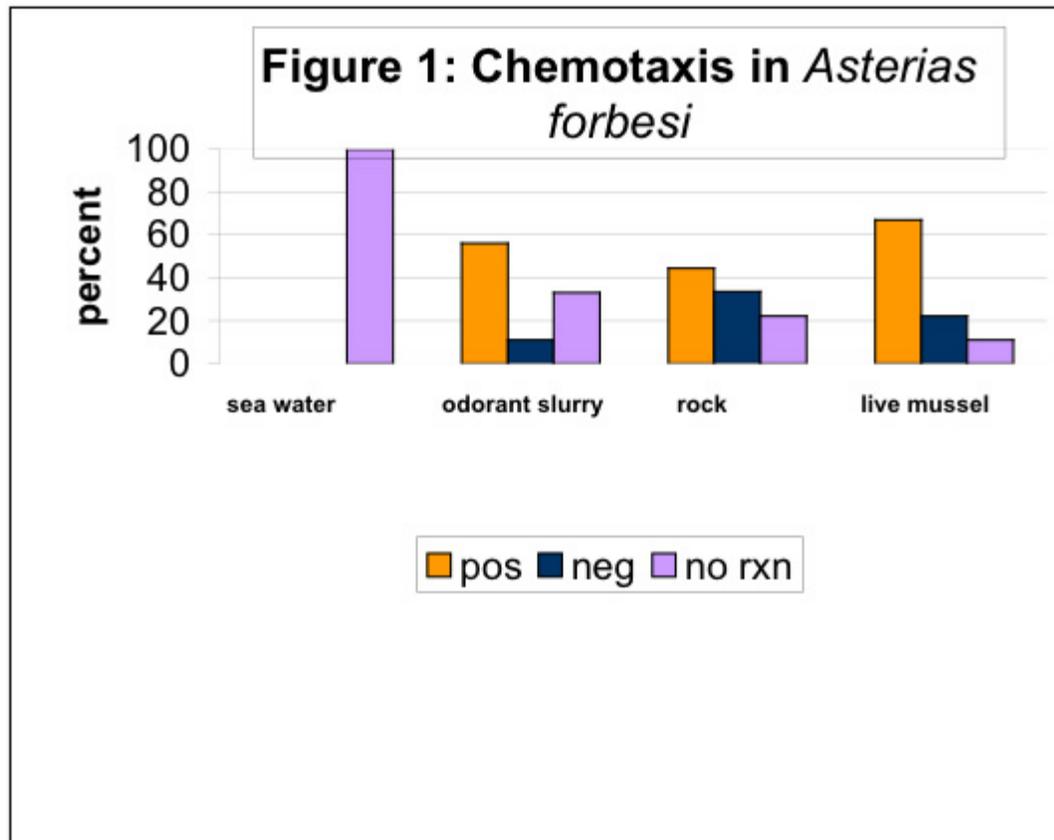


Figure 1: This graph shows the different chemotactic responses of sea stars under different odorant conditions. It compares the addition of seawater, molasses-mussel slurry, a small rock, and a small live mussel and measures the different types of chemotactic behaviors in percent, either being positive (orange), negative (blue), or no reaction (purple).

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Out of all the positive chemotactic responses, the sea stars would either put the tips of their arms or the center of their bodies over the odorant. As seen in Figure 2, they put the tips of their arms to the odorant twenty percent of the time more often than they would center their whole body over it.

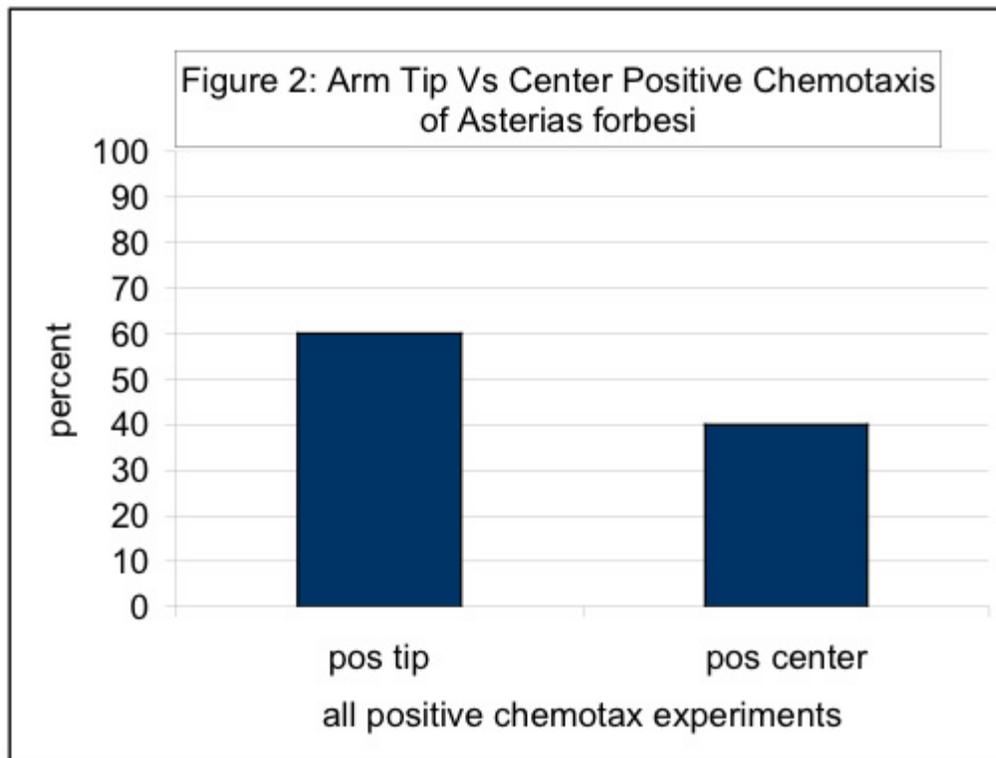


Figure 2: This graph shows the two types of positive chemotactic movement and compares in percent how many of the experiments showed a positive movement to the tips verse a positive movement to the center.

IV. Discussion and Conclusions

A higher percent of positive chemotaxis was displayed by the sea star, *A. forbesi*, when a chemical stimulus was added to its experimental dish, then when there was no chemical stimulus (see fig 1). When *A. forbesi* showed positive chemotaxis, there was a twenty percent higher chance that it would bring a tip of the closest arm to the odorant first, rather than going straight to the middle (see fig 2). This data supports my hypothesis that *A. forbesi* would bring the tip of any arm to the prey odorant if present. Also, when the odor was presented as a whole live mussel, the amount of times that it brought the tip of an arm toward the odorant was higher than when the odorant was presented as a liquid. However, there were instances when the sea star would immediately bring the center of its body over odorant without using the tip of an arm. This could imply that the two tips nearest to the odorant were picking up the chemicals at the same rate and the nervous system was therefore telling the tube feet to walk toward the odorant at the same speed and time. This could also indicate that the chemosensory cells are also located along the ambulacral grooves of the arms or in the center of the sea star near its stomach. If there were sensory cells located in other parts than the arm, then if a part of an arm was cut off, the sea star could still detect odorant stimuli

as the arm regenerated. Also, the amount of positive movement toward the rock in control two, was higher than the positive movement toward the sea water in control one. This could mean that the sea star was using touch as a sense for predation. As the sea star naturally moves around the dish, it could have accidentally touched the rock, and then climb on top of it to examine what it was feeling. Because the sample size that was collected was so small, chance alone could have explained the results of positive chemosensory. Since one of the sea stars was excluded from the data analysis because of size, the results may be in fact really heavily influenced on chance.

Sources of error in this experiment could include the fact that the sea stars were experimented on in a dish that did not represent the fluid flow of its natural environment, the intertidal zone. If *A. forbesi* is in fact sensitive to light and holds photoreceptors in its arms (Barnes and Ruppert, 1994), then the shadows of people entering the room during some of the experiments might have simulated the shadows of natural predators. This could have shielded them away from being aggressive and going after the prey odorant, instead reacting negatively phototactic, looking for dark places to hide.

The size of *A. forbesi* determines how fast the sea star is going to move to capture prey during high tide (Fisher, 2005). The bigger a sea star is the less it moves around. This is because the big sea stars are less susceptible to predators and desiccation than the smaller ones (Fisher, 2005). However, both hold sensory cells that allow them to hunt for their food without being totally "blind." The smaller sea stars may use the sensory cells as well, but they also moved around faster, possibly to locate as much prey as possible in the least amount of time.

Fisher's experiment collaborated with mine in the sense that we both studied the evolutionary adaptations in relation to the limited amount of time during high tide to hunt for food. It was seen in both that the uncontrollable varying light was a factor that may have been a source of error in our experiments. The shadows may have made the sea stars move less than if there were no disruptions.

Adams' experiment showed that the antennules of an American lobster flicked at a faster rate when an odorant was added to the lobster's tank. This experiment showed a behavior change when the odorant was picked up by the chemoreceptors. My experiment was also based on a behavior change based on movement when odorants were added. Adams' experiment showed a similar type of adaptation in another marine animal. This similarity of our results suggests that the lobster and the sea stars locate their food the same way and therefore may have evolved in similar environments.

Improvements to refine this experiment include setting up a tank with higher fluid flow so that the water flow can resemble more of a sea star's natural environment. The high fluid flow could either help the sea star in picking up odorant or dilute it and make it harder for the starfish to sense (Vickers, 2000). The fluid dynamics clearly relate

to the movement of the odorant. Also, I would make sure people walking in and out of the room did not affect the starfish behavior, altering the results of the experiments. To expand the experiment, more tests on the chemoreception could be conducted on the *A. forbesi* by testing the reactions to different types of odorants, such as other prey, predator, and unnatural new odorants. Maybe other types of prey odors could be detected faster, depending on the familiarity of the odorant. Predator odorants could be tested to see if the odor was picked up by other sensory cells and resulting in negative chemotactic response. An odor they haven't had contact with previously in their life, of another animal or an unnatural odor, could be tested to observe what their response would be to an unknown smell. Testing all these odors could test my hypothesis in a greater extent, showing whether if the sea star was actually showing positive chemotaxis toward the predator odorant only, or if it showed positive chemotaxis to any newly added odorant.

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