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Potential arm preference within starfish species including *Asterias vulgaris* and *Luidia clathrata*: A study and summary

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Introduction

Side preference has not been limited to humans who are right and left handed. Right or left hand dominance have been observed in mice and primates including bonobos and lemurs (Collins 1975, Hanson, Thorpe, & Chappell 2017, Bennett, Ward, Milliken, & Stafford 1995). Even different invertebrates are known to have side preferences, from green crab claw allometry to snail genera having dextral or sinistral shells (Juanes, Lee, McKnight, & Kellogg 2008, Checa & Jimenez-Jimenez 1997). The majority of animals that have been found to display a side preference have bilateral symmetry. Echinoderms have bilateral symmetry in their larval form and retain certain bilateral tendencies (Ji, Wu, Zhao, Wang, & Lv 2012). Sea stars, having pentamerous symmetry as adults, do not have a specific left or right side, making it harder to observe if one arm can be classified as the dominant arm. A dominant arm is defined as one arm that the sea star more often than not has in front, leading the way. Since potential side preference or arm dominance tendencies are rarely studied, it is beneficial to look at previous studies that describe how and what direction sea stars move based on different stimuli. These studies focus on the mechanisms of the tube feet along the body and arms, direction of movement with no stimulus, and directionality based on shading (Kerkut 1954, Cole 1913, Yoshida & Ohtsuki 1968).

Kerkut (1954) conducted a study to look at what mechanisms control the direction tube feet, whether the central nervous system or just tube feet tension impact how the sea star propels and orients the arms when moving along the bottom. Observing sea stars in both ~~in~~ the field and in laboratory settings, Kerkut first established a baseline for normal locomotion, analyzing the process of the tube feet while accounting the direction of each arm every couple minutes for nearly 2 hours. When observed in the upside down position, the majority of tube feet ended up pointed in a specific direction with over ten specific orientations throughout the experiment, however, the tube feet showed a statistical significance towards one of the five radial directions. An arm was chosen as dominant if the tube feet were pointed towards the end of the arm. It was observed that prior to movement in a specific direction, the tube arms located elsewhere would point towards the direction before the tube feet on the lead arm. This illustrated that sea star movement is not based solely on tension. When dominance changed arms, the new primary arm normally became an adjacent arm. When a singular cut to the central nerve ring was made on some sea stars, the tube feet on either side still reacted in similar ways as when no cut was made.

If a cut was made up the radial nerve cord, the tube feet beyond the cut did not respond with the other changes, opposite of what was observed when everything but the nerve cord was cut. When an arm was cut multiple times, there were less orientations towards that arm recorded than prior to the cuts, until the sea star healed from the cuts. When connections to the circum-oral nerve ring were severed, isolating the arms, resulting in the sea star not having a dominant arm but instead, the tube feet pointed inward, projecting as circular movement when placed on the ground. After cuts were made on the nerve ring, there was a decrease in coordination between the two adjacent arms in which the cut was between. After a baseline understanding of how the tube feet naturally orient, a stimulus was added. On a healthy star, when a strong stimulus is placed on an arm, the tube feet have a flight reaction by pointing towards the arm opposite of the stimulation. When a cut was placed on two sides of one arm on the circum-oral ring and stimulation was added to the opposite arm, the tube feet still point towards the secluded arm and one adjacent to it. When two arms are stimulated concurrently, the remaining two arms point in opposing directions. This study found that multiple aspects are at play pertaining to sea star movement including a collection of neurons and sensory stimulation.

Cole (1913) did a study to determine arm dominance without any external stimulus that could influence the direction traveled. The sea stars were placed in a circular dish in a room with one bright light right above the testing area. Five hundred trials were recorded using then sea stars of varying species. Fifty trials per sea star were performed in one or two days. With each trial run, the sea stars were rotated by one fifth of their circumference, where every trial started with a new arm pointing towards the observer to minimize any direction bias. For many of the sea stars, there was individuality in their movements. Some changed lead arms but headed in the same direction during the trial run. Only during four of the 500 trials did the arm and direction of movement change. There were many different ways the sea stars would move, having one, two, or three arms leading and the rest following. Sometimes the entire sea star would be flat and other times, the tips of the arms would be curved up. This study found the arm to the left of the madreporite was observed as the lead arm the majority of the time for the majority of the sea stars (six out of ten). Arms two and three were rarely lead arms, however, it was observed, but not measured, that arms two and three were shorter than arms one and five on the majority of the specimens. It was shown from a previous study conducted at Woods Hole that the length of the arm did have some impact on the frequency in which it was used as the lead arm.

Yoshida and Ohtsuki (1968) studied how light affects *Asterias amurensis* movement focusing on the impact on ocelli and nerve circum-ring. In order to test the different roles, the ocelli were removed and the radial nerves were transected. On some sea stars, one or more arms were removed with either no or some of the nerve ring attached. Three types of movement were described based on the lighting. One was with half of the trough shaded, another was when the sea star was stationary and one arm was stimulated by light. The third was when an arm was stimulated with light on a moving specimen. Potential pre-existing arm dominance was negated by exposing the sea star to air and either being kept in bright light or complete darkness for a specific amount of time. For light sources, two methods were used. When lighting wide areas, a 40W bulb was hung above the trough. For a small, specific area, a 30W microscope lamp was used above the trough. Both light intensities were measured at 200 lux. Black plates were used to create shadows. To find the direction of movements at a light-dark boundary, 21 individual sea stars were placed in a half-illuminated tank. The dark-adapted sea stars initially moved slowly at randome then became more active and started moving towards the illuminated area. Inversely, the light adapted sea stars started out active but then slowly became less active and stayed near the border between the illuminated and shaded areas, but overall stayed positively phototactic. When the majority of arms were shaded, the sea stars moved in the direction of the illuminated arm When the lead arm was shaded, the sea star became immobile for a little bit then moved using the arms on either side of the original lead arm but stayed moving in generally the same direction. Occasionally, once the original dominant arm came out of the shade, it would become the lead arm once again. Similar reactions were seen if the closest arms to the lead arms were shaded. If the shade was placed on the two arms opposite of the dominant arm, no changes in locomotion were observed. When the ocelli, arms, and/or a section of the radial nerve were missing and the entire area was shaded, the operated arm or side was the lead.

There is little evidence of understanding the locomotion of an undisturbed starfish under even lighting with just one type of stimulus. To try to find any arm preference, this study looked at two species, *Asterias vulgaris* and *Luidia clathrata*, primarily *Asterias vulgaris.*

Asterias vulgaris is a cool-water species found in subtidal waters from North Carolina to the southern parts of the Labrador Sea (Gaymer, Himmelman, & Johnson 2001). *A. vulgaris* are opportunistic eaters, feeding on invertebrates such as mussels and other echinoderms (Himmelman, Dutil, & Gaymer 2005, Drolet & Himmelman 2004). As keystone species studies have been done to understand sea star response to stimuli. Some of these studies have looked at the influence of waves, chemical stimuli, and prey odor for the foraging behaviors of *A. vulgaris* (Gagnon, Wagner, Himmelman 2003, Zafiriou 1972, Drolet & Himmelman 2004). Gagnon et al (2003) found that the presence of waves did not significantly impact the sea stars' chances of obtaining prey, Zafiriou (1972) found that acidic stimuli such as hand sweat were attractive to *A. vulgaris* with no full correlation to shellfish tissue that had shown sea star attractiveness as well. Drolet and Himmelman (2004) found that since sea stars orient themselves upstream of prey odor the majority of the time, only 5% of the sea stars studied in still water successfully found their prey.

There were two goals of this study looking at arm dominance when foraging. The first was to find primary arm dominance, if any, within the starfish species *Asterias vulgaris*. The study also looked into potential unique arm preference within *A. vulgaris* and *Luidia clathrata.*

Methods

As a cool water species, *A. vulgaris* was unable to be kept in room temperature tanks. Instead, six starfish were kept in an incubator set at 12ºC within 5 gallon buckets with filters. Frequent water changes were preformed to keep fresh water circulating in addition to the filters. Other containers of seawater were kept in the incubator as well for water changes and were used to fill in a tank when running a trial. The sea stars were received in low health, with some mortalities to sea star wasting disease. They were given two days to acclimate to the new environment; on the second day, food was introduced to see if the sea stars would be food motivated. All reacted and moved towards the food but did not eat. After the acclimation period, trials were started. A trial run classified as a starfish being placed on one side of a tank and a food stimulus of an open clam placed 30 centimeters away. To test preference, the lead arm was recorded. A lead arm was classified as the arm in front based on what direction the sea star was moving. The starting lead arm was chosen for each trial through a randomized number generator; arm one was the one closest to the madreporite and two-five assigned in a clockwise fashion (Figure 1). A time limit of ten minutes was placed on each trial. Two trials were run in one day for each sea star, two trial days. To keep the food source as a viable stimulus, the sea star was allowed to feed only after the last trial of the day. Every run was recorded using the data collection template shown in Table 1. The data were then analyzed and compared to the data collected from four test trials run on an individual *Luidia clathrata* that followed similar procedures. To analyze the data, percentages were taken for the numbers of times each arm was the ending lead arm and for the rotation direction.

Results

Out of twenty trials run, only ten were completed within the ten-minute time period (Table 1). All of the *A. vulgaris* trials resulted in an ending lead arm that was different than the starting lead arm whereas the trials with *L. clathrata* started and ended with the same arm 37.5% of the time. There was no arm that was primarily used for any sea star; *L. clathrata* had a highest percentage of 37.5% of arm 2 and when totaled together, four trials of *A. vulgaris* the highest percentages were 33.3% for both arms 2 and 3 (Table 2). *Asterias vulgaris* rotated clockwise 66.7% of the trial runs and *Luidia clathrata* rotated counterclockwise 75% of the time (Table 3.)

Many of the trials, the sea stars climbed up the side of the tank, and many of the incomplete trials resulted in the starfish either not moving or moving in the opposite direction of the food source (Appendix).

Discussion

Based on the data collected, no patterns were discernable as to a primary lead arm. There was also no significant difference in the direction of rotation the starfish would reorient itself.

Trials were deemed incomplete if the starfish did not reach the food within the allotted ten minutes. Any incomplete trial runs were assumed to be the cause of lethargy due to stress of wasting disease and temperature changes.

In conclusion, based on the usable data collected, there does not appear to be any consistency in which a specific arm was preferred in the species *Asterias vulgaris.* Even with a low sample size, there was no discernable pattern of any arm preference in individual specimens. Other studies have shown different results, including the impact of chemical stimuli tested which may contribute more of an impact on the direction chosen by sea stars (Brewer & Konar 2005, Moore & Lepper 1997, Zafiriou 1972).

During this research, many sources of error came into play including; starfish wasting disease decreasing the number of usable sea stars, sea star lethargy, the lack of water movement, and water temperature difficulties. Preliminary findings from this study suggest the need for more studies with *Luidia clathrata* to determine if lead arms are present within individuals or overall in the species, Additional future studies might also focus on how wasting disease impacts foraging behavior.

Figures and Tables

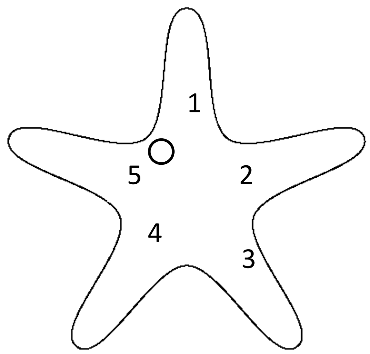

Figure 1: This figure shows how the numbers of each arm was assigned in affiliation with the madreporite.

Table 1: Starting, midway, and ending arm positions and total time for all trials. DNC stands for did not complete. These were based on the trial run descriptions found in the appendix.

A. *vulgaris* A

Trial #	Total Time (min)	Starting Lead	Midway Lead	Ending Lead
1	3	2	2/3	3
2	6	5	3/4/5	2
3	10	4	4/5	DNC
4	7	3	-	DNC

***A. vulgaris* B**

Trial #	Total Time (min)	Starting Lead	Midway Lead	Ending Lead
1	7	4	4	5
2	10	3	2	DNC
3	10	1	5	DNC
4	10	4	3/4	DNC

***A. vulgaris* C**

Trial #	Total Time (min)	Starting Lead	Midway Lead	Ending Lead
1	4	1	2	3
B	10	1	-	DNC
C	9	5	2/3	2
D	10	4	5/1	DNC

***A. vulgaris* D**

Trial #	Total Time (min)	Starting Lead	Midway Lead	Ending Lead
1	10	3	-	DNC
2	10	2	2/3	DNC
3	9	2	4/5	DNC
4	10	1	4/5	4

L. clathrata

Trial #	Total Time (min)	Starting Lead	Midway Lead	Ending Lead
1	9	5	2/3	2
2	1	3	2/3	3
3	2	2	1/2	1/2
4	2	5	4/5	4

Table 2: Percent time when each arm was the ending arm.

***A. vulgaris* A** n=2

Arm	**1**	**2**	**3**	**4**	**5**
Percentage	0	50	50	0	0

***A. vulgaris* B** n=

Arm	**1**	**2**	**3**	**4**	**5**
Percentage	0	0	0	0	100

***A. vulgaris* C** n=2

Arm	**1**	**2**	**3**	**4**	**5**
Percentage	0	50	50	0	0

***A. vulgaris* D** n=1

Arm	**1**	**2**	**3**	**4**	**5**
Percentage	0	0	0	100	0

***A. vulgaris* Total** n=6

Arm	**1**	**2**	**3**	**4**	**5**
Percentage	0	33.3	33.3	16.7	16.7

L. clathrata n=4

Arm	**1**	**2**	**3**	**4**	**5**
Percentage	5	37.5	25	25	0

Table 3: Percent rotation direction

Sea Star	**Clockwise Rotation (%)**	**Counterclockwise Rotation (%)**
***A. vulgaris* A** n=2	50	50
***A. vulgaris* B** n=1	100	0
***A. vulgaris* C** n=2	100	0
***A. vulgaris* D** n=1	0	100
***A. vulgaris* Total** n=6	66.7	33.3
L. clathrata n=4	25	75

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Appendix: Sea star behavioral descriptions for each trial

A. vulgaris A:

- Trial one, the sea star first led with arm two and crawled toward the food with arms two and three in the lead. It then reached the food with arm three.
- Trial two, sea star had arm five as the starting arm. It crawled up the side of the tank using arms three, four, and five as the lead arms then walked towards the food with arm three, moved by, and touched the food with arm two.
- Trial three, the sea star led originally with arm four but turned to lead with two and three for a little bit then crawled up the glass using arms one and two. After that, it used arms four and five to the side and stayed there for the rest of the trial.
- Trial four, sea star was placed with the lead arm as arm one but did not move for the entire trial.

A. vulgaris B:

- Trial one, the sea star led with arm four for nearly the entire run until three quarters of the way when it turned a little and touched the food with arm five.
- Trial two,
- Trial three, sea star led with arm one to walk a bit then walked to the side of the tank using arm five. Did not go to the food.
- Trial four, sea star started with arm four, started walking towards the food with arms three and four then stopped in the middle.

A. vulgaris C:

- Trial one was started out with arm one, the sea star stayed in place for a while, and then walked to the food.
- Trial two, sea star's lead arm was number one until it moved to where arms one and five were grouped together and arms two, three, and four were grouped together with no true lead side. The sea star then stayed in that position for the entire trial.
- Trial three, the sea star led with arm 5 but moved with arms two and three, primarily arm two, to the food. Once it reached the food, it made first contact with arm one.

- Trial four, sea star originally led with arm four but curled arms five and one up the wall and then stayed where it was for the entire trial.

A. vulgaris D:

- Trial one, the lead arm was number three but the sea star did not move the entire trial.
- Trial two, the original lead arm was arm two, the sea star moved to the middle of the tank with arms two and three leading then stopped for the entire trial.
- Trial three, the sea star started with arm two in the lead but used arms four and five to climb up the side of the tank where it stayed for the entire trial.
- Trial four, sea star at first led with arm three, moved up the side with arms four and five, then crawled on the side of the tank to the food and touched the food with arm four.

L. clathrata:

- Trial one, the starting arm was arm five, the starfish crawled up the side of the tank and flipped over. After righting itself, the lead arms stayed as two and three with arm number two coming into contact with the food first.
- Trial two, the starting arm was number three. The entire time, the lead arms were arms two and three, where arm number three was angled more towards the food and made initial contact.
- Trial three, the starting lead arm was two. The starfish moved the entire distance with arms one and two in the front.
- Trial four, the initial lead arm was number five, however, started crawling up the side of the tank with arms three and four, then started crawling towards the food using arms four and five as the leads. The food was initially touched by both arms four and five.