Sea Star Acrobatics:
Testing the Righting Response of Eight Different Asteroid Species on Two Different Substrata

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Abstract:

Sea stars typically live with their oral side facing the substratum, and the ability to right themselves when turned over is important response to avoid desiccation and predation. The righting time of 8 species of sea stars; *Henricia leviuscula* (3 color variations), *Henricia pumila sp. nov., Leptasterias hexactis*, *Pyconopodia helianthoides*, *Pisaster ochraceus, and Pteraster tesselatus* were tested on two different substrata to determine if it affects righting time. The righting times were measured when sea stars were placed with their aboral side on the substratum in two different tanks, one covered with sand and another with no sediment. Since podia adhesion to the substratum is required for a sea star to right itself, it was expected that substrata with a smaller grain size would cause an increase in righting time compared to a flatter surface. Sea star diameter was measured to determine the correlation between size and righting time. Leading arm identification was also observed to test the initiating arm preference. *P. tesselatus* was the only species that was unable to right itself in sand. In most species, sand increased the average righting time compared to other substrata. Individuals with a larger diameter were found to have

an increased righting time. No significant relationship was found to which arm initiated the response in all individuals. This study helps to add to the minimal knowledge of this unusual behavior of echinoderms.

Introduction:

When placed with their aboral side on the substratum, sea stars (Echinodermata: Asteroidea) respond by using their tube feet to right themselves; the sea stars adhere to the substratum and pull themselves so that they are righted to have their aboral side on the substratum. Asteroids are more vulnerable to desiccation and predation when their oral side is facing upwards as opposed to on the substratum; therefore it is important for sea stars to have the ability to right themselves quickly on a variety of substrata. The ability of a sea star to right itself can also be used as an indication of a specimen's health, by determining if an individual is capable of righting itself or not (Held and Harley 2009). This specific righting response is a characteristic behavior of echinoderms because of they have the ability to turn over due to adhesion with their tube feet and is an important ability to avoid vulnerability (Lawrence and Cowell 1996; Kleitman 1941).

Tube feet are a synapomorphy of echinoderms that allow asteroids to grip to the substratum during periods of heavy wave action in intertidal habitats (Santos et al. 2005). Tube feet are also used in locomotion to lift and support the sea star while it moves across a substratum (Santos et al 2005). Tube feet are generally arranged in 2 rows on each asteroid arm (Migita et al 2005). Using muscle control, tube feet can retract or bend allowing for the flexibility that sea stars need for locomotion and to right themselves when inverted (Migita et al 2005). Sea stars use their tube feet to adhere to the substratum during the righting response, which allows them to have the traction needed to right themselves. They will begin to right themselves by extending their tube feet near the tips of each arm to make contact with the substratum (Migita et al 2005). Once contact with the substratum is made, asteroids will begin to step ahead so that one arm is turned over first and the other arms will slowly follow (Migita et al 2005).

Previous studies have described the overall variation in the righting response and showed that there were three different ways that a sea star rights itself (Jennings 1907). One way is by somersaulting so that a species places one of its arms on the substratum then bends three arms over and then brings the other arms over its central disk to be righted (Jennings 1907; Polls and Gonor 1975). Another way a sea star can right itself is by folding over three of its arms and bringing the other two over its central disk to complete the righting response (Jennings 1907; Polls and Gonor 1975). The final way that Jennings (1907) described for a sea star species to flip over was for it to first raise all of its arms and then bend them over to begin the righting response (Polls and Gonor 1975).

Other studies have measured the difference in asteroid righting response time in stressful environments such as increased temperature or salinity, however the results showed no significant difference between treatments (Lawrence and Cowell 1996; Held and Harley 2009). Additional studies tried to determine if sea stars show a preference for which arm initiates the righting response, but again, didn't find significant trends in the species used (Polls and Gonor 1975). In contrast, Ji et al (2012) found that *Asterias amurensis* had a specific righting response, which indicated a tendency towards bilateral movements as well as a preference towards specific arms when initiating the righting response. However, none of those studies examined the differences in righting response on different substrata.

Eight species of sea stars were used in this study: Three different color variations of *Henricia leviuscula (*blood red, yellow, and gray arm pitted*)*, *Leptasterias hexactis*, *Pyconopodia helianthoides*, *Pisaster ochraceus*, *Pteraster tesselatus,* and *Henricia pumila sp. nov*. All species are common in the intertidal along the Pacific coast. *H. leviuscula* is has five slender arms, varies in color from yellow to blood red, and can grow as large as 20 cm (Lambert 2000). *L. hexactis* has six arms which tend to be wider than the arms of *H. leviuscula. L. hexactis* can reach 8 cm in diameter and are therefore generally smaller than *H. leviuscula* (Lambert 2000). A large asteroid species with 18-24 arms, which can be up to 40 cm long, around a broad central disc covered with soft slimy skin is *P. helianthoides* and it is a voracious predator of the intertidal (Lambert 2000). *Pteraster tesselatus* has five stubby arms, can grow up to 12 cm, and is able to secret mucus as a defense mechanism (Lambert 2000). *P. ochraceus* is the most common intertidal sea star, has 5 stout arms, and calcareous plates that make the body stiff (Lambert 2000). *Henricia pumila sp nov*. is a species that is currently being described. It resembles *Henricia leviuscula* but has shorter, wider arms and mottled coloring (Eeernisse et al. 2010). It is the only brooding species of *Henricia* found in the Puget Sound and is wide spread along the Pacific Coast (Eernisse et al. 2010). All these species are common in the intertidal where they might come in contact with both types of substrata that will be used. These species were chosen for this project to test the righting response of a wide range of different sized sea stars and species with a variety of number of arms

Asteroid species are found in several different habitats and it would be expected that individuals have different righting times on different substrata due to the variation in the traction available. The species in this study were obtained from the rocky intertidal areas so they should not be used to moving on sandy surfaces in their habitat. Given the importance of the interaction between tube feet and the substrate during righting behavior, it was expected that substrata with a smaller grain size, such as sand, will cause an increase in the time required for sea stars to right themselves compared to a flatter and more uniform surface such as a sea table. The correlation between size and the righting response time was also measured and it was expected that an increase in size would cause an increase in righting time. Leading arm preference was observed using the madreporite as a landmark on the aboral side. It was expected that individuals within the same species would behave similarly and show a preference for which arm was used first to initiate the righting response.

Methods:

Study organisms:

Eight species of sea stars were used in this study: Three different color variations of *Henricia leviuscula (*blood red, yellow, and gray arm pitted*)*, *Leptasterias hexactis*, *Pyconopodia helianthoides*, *Pisaster ochraceus*, *Pteraster tesselatus,* and *Henricia pumila sp. nov*. . Species were collected from several intertidal areas around San Juan Island. These included: Eagle Cove, Roche Harbor, Snug Harbor, Argyle Creek, and Friday Harbor. Sea stars were stored in a sea table with well aerated, circulating seawater. Larger species such as *Pisaster ochreceous* and *Pycnopodia helianthoides* were stored in separate tanks from smaller species as well as each other. Individuals were acclimated to aquarium conditions for at least 2 days after being collected from the field.

Experimental design:

Studies were conducted in two different tanks. One tank was 102 cm in diameter and filled with sand collected from the beach at Eagle Cove so that the bottom of the tank was covered by 2.5- 3 cm sand. This tank was filled with 17.5 cm deep of water so that sea stars were completely immersed during their righting response. The second tank was a 128.4 cm long by 60.2 cm wide sea table that served as a flat surface for the experiment. This sea table was filled with water so that it was 18.4 cm deep and any additional animals were removed to allow for sufficient space. Both tanks were cleaned before the experiments. After sand was placed in the first tank, the sand was allowed to settle and was smoothed out to allow for a uniform surface to conduct the experimental trials.

Sea stars were removed from the sea table where they were stored in and placed in the experimental tank with the aboral side on the substratum away from obstacles such as the side of the tank and flowing water. A timer then recorded the time it took for them to right themselves. Righting was not considered completed until all arms were touching the substratum and were uncrossed. As many as four individuals were tested simultaneously to allow for efficient data collection, but only if the tank was large enough to allow for sufficient space so that no individual touched another during the righting response.

Once righting was completed, arm-to-arm length was measured using a meter stick and recorded. Sea stars were then put into a separate tank to distinguish them from the individuals that had not yet conducted the experimental trial. Trials were run with a one-day gap in between to minimize fatigue from repeated righting behavior. The same procedure was used in observing the righting response in both tanks. The same individuals were used in both trials.

In addition to testing the righting response time in different substrata, the determination of a leading arm that might initiate the response was also observed. The madreporite, which is located on the aboral side, was used to designate which arm initiated the sea star righting response. (Figure 1) (Polls and Gonor 1975; Jennings 1907). An arm was considered to be initiating the response when it had begun to turn over using the tube feet on the tip of the arm. In some cases multiple arms were used simultaneously.

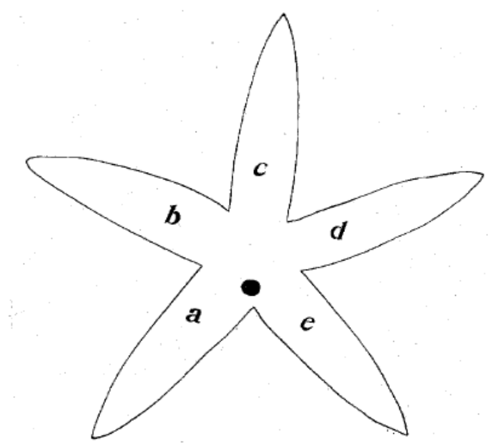

Figure 1. Asteroid arms labeled to determine the dominant arm. Arms are labeled rotating clockwise, using the madreporite as an aboral landmark (Jennings 1907).

Data analysis:

Data was analyzed using several different statistical tests. To eliminate noise within the data set, species were group in families. All four *Henricia* species are in the family Echinasteridae. *Pisaster ochraceous, Pycnopodia helianthoides*, and *L. hexactis* are in the family Asteriidae. *Pteraster tesselatus* is in the family Pterasteridae. Linear regressions were used to determine the correlation between size and time on each substratum in each family. Chi squared was used to test the significance in which arm a sea star used to initiate the righting response. T-tests were used to determine the significance between the response time on table and sand within a family. A one-way ANOVA was used to test the significance of the righting times on the different substrates between families.

Results:

Overall it was more difficult for individuals to right themselves in sand (mean= 420 s, SE =44.97) as opposed to on a sea table (mean= 395 s, SE= 33.62) (Figure 2). Some individuals failed to completely right themselves in sand, which was signified by inactivity or stalled in the flipping process for 30 to 45 mins. This included all except one *Pteraster tesselatus* individual, one *Pisaster ochraceous*, and one *Henricia pumiila sp.*.

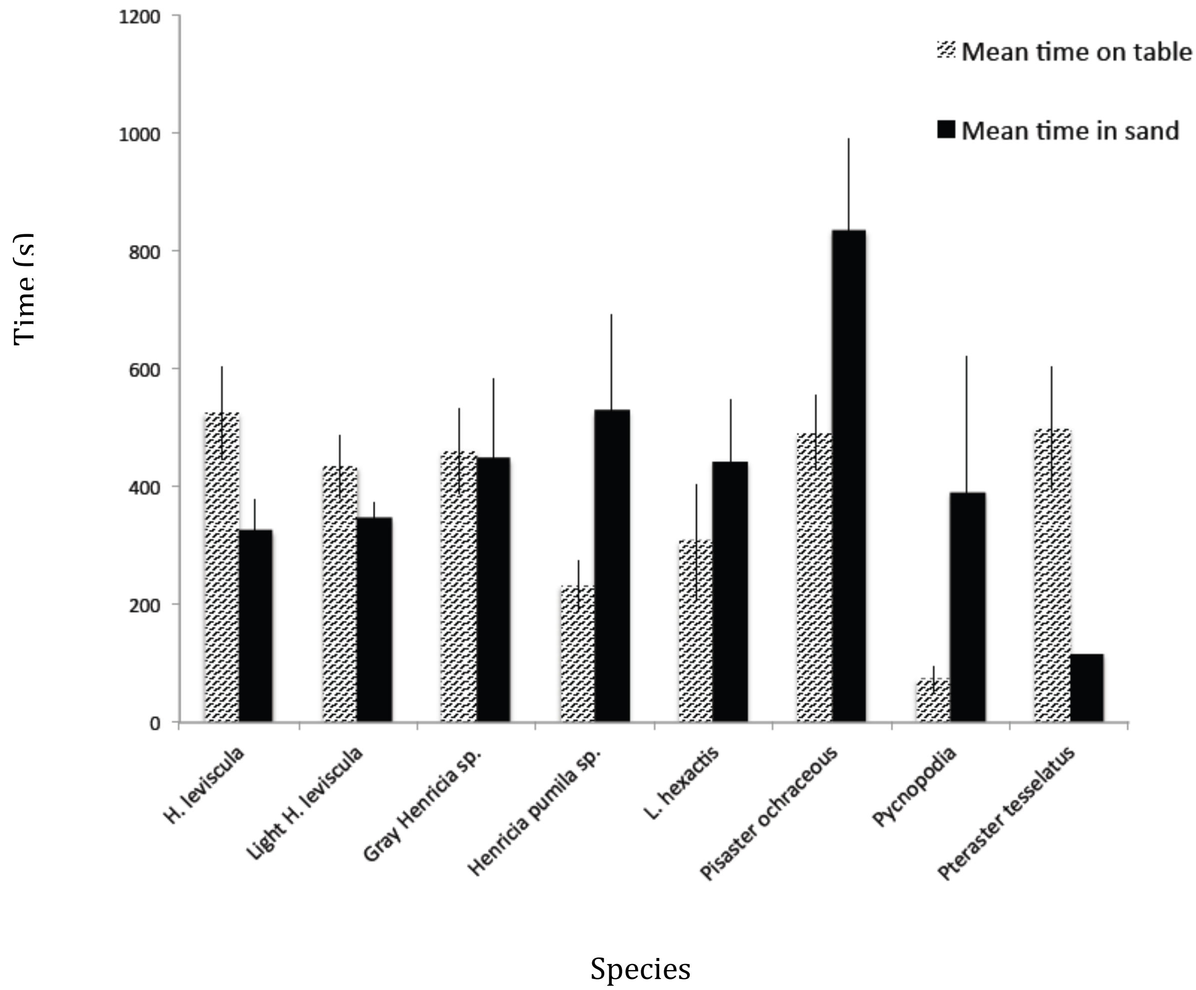

Figure 2. The average time it took for each species to right itself in sand versus on a sea table. Three of the four *Henricia* species performed better on sand than on the table. All other species performed better on the table than on sand. One of the species, *Pteraster tesselatus*, failed to right itself on sand with the exception of one individual.

Echinasteridae showed no significant difference of the righting response time in sand versus in a table (p= 0.627) (Figure 3). The time it took for Asteriidae species to right themselves in sand was significantly different (p= 0.04) than it took for them to right themselves on a table. Since members of Pterasteridae, with one exception, were unable to right themselves on sand a statistical test could not be performed to determine significance. After 45 minutes Pterasteridae individuals showed no progress in righting themselves and were removed from the experimental trial.

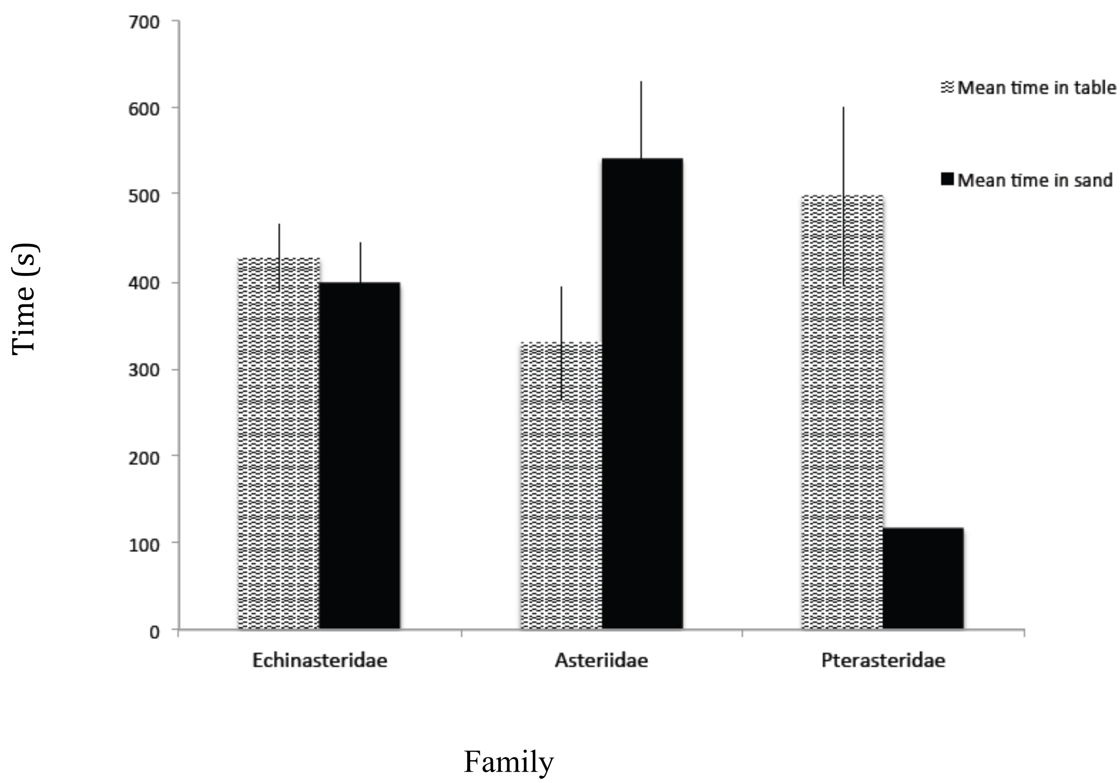

Figure 3. The average times it took for each family to right themselves in sand versus on a sea table. The times it took for Echinasteridae and Asteriidae to right themselves on the table was significantly different (p=0.013). Error bars are standard error.

There was a significant difference between the righting response of asteroid species on a table comparing all three families (p=0.025). When performing this test the ANOVA mentioned that only one of the pairwise comparisons was significant. To determine which pair of families was significant to each other a t-test was performed on each family pair. The only pair that showed a significant result was the response time on a table between the families Echinasteridae and Asteriidae ($p= 0.013$). All other pairwise comparisons of the sea star response time on a table showed no significance; Pterasteridae and Asteriidae ($p=0.096$) and Echinasteridae and Pterasteridae ($p= 0.470$). The righting time of sea star species in the family Asteriidae and Echinasteridae while in sand showed an insignificant difference to each other ($p= 0.516$).

After a linear regression was conducted, two comparisons of individual size versus time showed a significant difference. There was a positive correlation between size and the righting time of Echinasteridae species on a sea table ($p= 0.008$) (Figure 4). Other comparisons were not significantly correlated. The response time on a table for both families Pterasteridae ($p= 0.772$) and Asteriidae ($p= 0.414$) were not significant. There was no significant correlation between size and righting time on sand for all families Echinasteridae ($p=0.480$) and Asteriidae ($p=0.06$) (Figure 5).

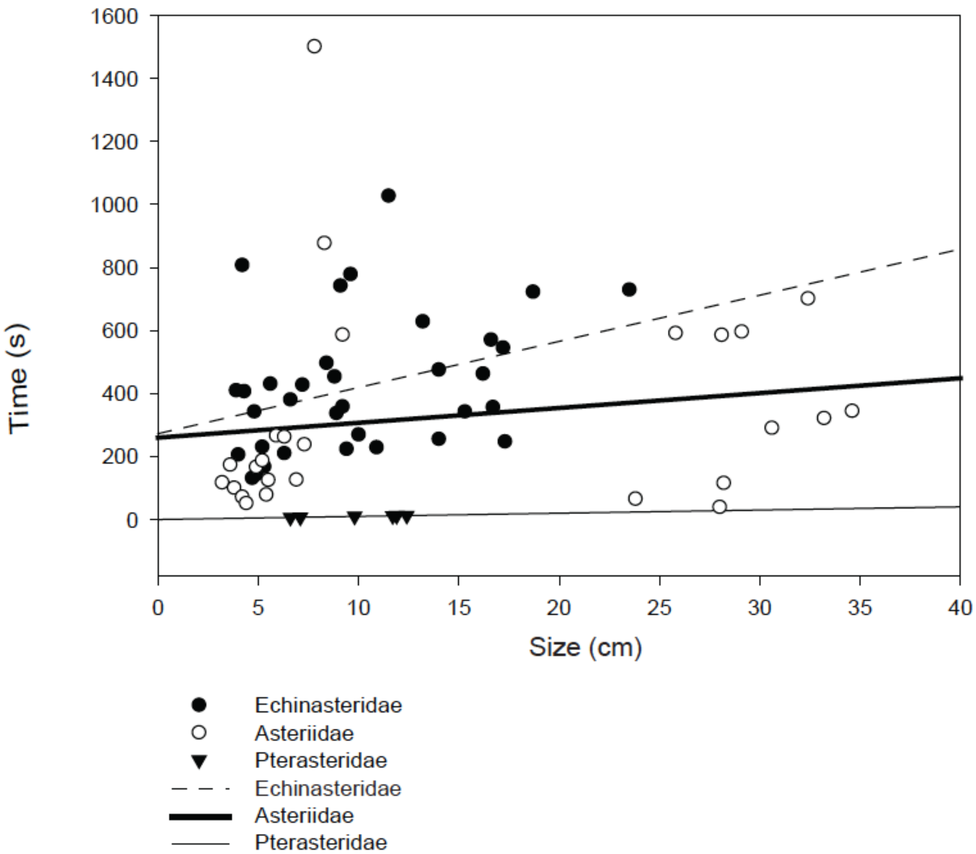

Figure 4. The correlation between size and the righting response time on a sea table as shown for all three families. Echinasteridae showed a significantly positive correlation between response time on a table and size ($p = 0.008$).

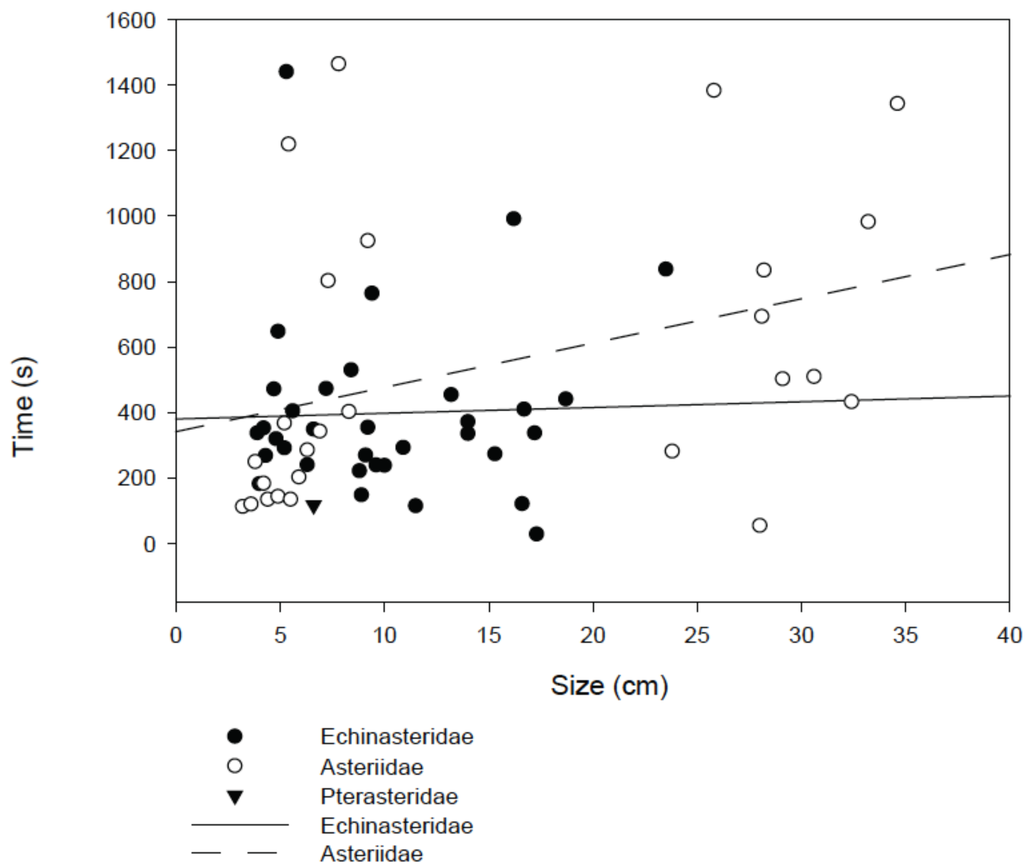

Figure 5. The correlations between individual size and the time it took that individual to right itself while in sand. Only one family showed a significantly positive trend, Asteriidae (p= 0.006).

The arm that was used to initiate the righting response in each species showed no significant trend when tested with a chi squared test for the individual species (Table 1). This was true for both response times on sand and on the table.

Table 1. Chi squared and p-values for the determination of a leading arm that initiated sea star righting response. There was no significance between species. *Pycnopodia helianthoides* was not measured because of the high number of arms making it difficult to determine which arm was used first. *Pteraster tesselatus* was excluded from this analysis because this species doesn't have an easily visible madreporite that could be used as a landmark to designate the initiating arm.

Species	**Table X2**	**Table P-Value**	**Sand X2**	**Table X2**
H. leviscula	8.55	0.073	2.409	0.66
Light H. leviscula	6	0.199	2.389	0.66
Gray Henricia sp.	1.93	0.75	3.167	0.53
Henricia pumila sp.	3.71	0.45	4.5	0.34
L. hexactis	4.25	0.51	6.499	0.26
Pisaster ochraceous	3.36	0.5	1.214	0.875

Discussion:

Tube feet adhesion is needed for a species to right itself. Sea stars will begin the righting response by turning the tip of one arm over and using their tube feet to grip to the ground allowing the individual to step ahead so that the podia helps to pull one arm over and the others slowly follow (Jennings 1907). However, the ability for an asteroid species to right themselves in sand took longer overall than it took for them to right themselves on a sea table, which points to the possibility that tube feet aren't the main factor in determining if a righting response will be successful or not. Another possibility that allows for a species to be successfully righted could be muscle control and the ability to bend their arms so that a righting response can be initiated. An additional factor could be differences in morphology, especially arm length and having arms that are long enough from the central disk to allow the arms to be flexible and to bend over. This reasoning is hinted at by examining the unsuccessful righting behavior *Pteraster tesselatus* in sand. This species has short stout arms and an arm to disk ratio of only 1.1 to 1.9 compared to other species that was able to completely right themselves in sand such as *Henricia leviscula* that have an arm to disk ratio of 3 to 7 (Lambert 2000). When *Pteraster tesselatus* was placed upside down in sand its initial reaction was to curl the tips of its arms underneath itself. It then tried to puff up its body to push off the substratum as opposed to using its tube feet to adhere to the sand. Their tube feet were unable to adhere to the sand causing most of the individuals to fail in completing the righting response. The one individual that was successful was the smallest specimen and succeeded in puffing its body enough to push itself off of the sand rather than using its tube feet. So it seems that podia adhesion to the substratum, arms flexibility and size, along with good muscle control is responsible for a successful righting response in any substrate.

The other two families, Echinasteridae and Asteriidae had different outcomes to the time it took to right themselves in sand versus on a sea table. Echinasteridae showed no significant difference in righting success time between sand and table. In fact, when examining the graph of the individual species it looks like most of the *Henricia* species, except for *Henricia pumila sp.,* had a lower response time in sand as opposed to on a table. This could be due to their long slender arms and high arm to disc ratio which allows for greater flexibility of the *Henricia* species overall. According to Lambert

(2000) *H. leviscula* seems to have a characteristic righting response where the individual attempts to right itself by raising its central disc, pushes its arms on the substrate, and then uses its tube feet allows the some arms to step ahead while the others fold over the central disc. This response points to great flexibility in the species due to its long slender arms. *Henricia pumila sp.* has arms that are short and stout with short spines as opposed to the other *Henricia* species (Eernisse et al. 2010). The difference between the arms of the *Henricia* species could be the reason why *Henricia pumila sp*. took longer to right itself in sand as opposed to on the table. The species lacked the flexibility of the other *Henricia* species due to its shorter and wider arms. Overall *Henricia pumila sp* had a different righting response than the *Henricia spp*. where *Henricia pumila sp*. would fold itself over so that it was folded in half as opposed to somersaulting like the other species. Overall Echinasteridae contains species that were more constrained in size, with similar responses and morphologies than Asteriidae did which contributed to the different outcomes and significance.

Asteriidae did show significance in the response time in sand versus on a table. This family contains a wide variety of species with varying arm numbers*, L. hexactis, Pycnopodia helianthoides*, and Pisaster *ochraceous*. Some of these species had individuals that responded considerable fast on both substrates such as *L. hexactis* and *Pycnopodia helianthoides*, while others such as *Pisaster ochraceous* showed a slower response. The greater arm length in two of these three species is most likely correlated to having a faster response time. More arms on an individual means that there is a greater amount of tube feet that the sea star can use to adhere to the surface since each asteroid species in general has 2 rows of tube feet per arm (Migita et al. 2005). However, this was not always the case and while some individuals of the species with a greater amount of arms than the other species did considerably well in righting themselves, other individuals took much longer on sand than on the table. This signifies that there is not a trend in species, but rather responses that are determined by the individuals themselves in correlation with its biology.

There was no correlation between righting response time and size when species were placed in sand. Since the species in the family Asteriidae had such a wide variety of size ranges, it seems that the righting response of the smaller individuals were somewhat cancelled out by the righting response of the larger individuals causing insignificance for the response on the sand. This shows that species in general had difficulty righting themselves on sand compared to on a sea table. There was however a significant correlation between size and time for one family, Echinasteriidae, on the sea table, showing that as size increases the righting response time increases. This seems counterintuitive because sea stars with longer arms have more tube feet, which should allow for a greater amount of adhesion to the surface. However, it seems that smaller individuals are more flexible and therefore able to right themselves more quickly than the larger individuals. When comparing two species with the same amount of arms but different overall sizes, *Henricia spp* and *Pisaster ochraceous*, the larger of the two species, *Pisaster ochraceous*, showed a longer response time in sand compared to *Henricia sp*., demonstrating the trend that an increase in size increases the righting response time. *Pisaster ochraceous* also has stiffer and stouter arms than *Henricia spp* showing the importance of flexibility in the success of an individual righting itself. This could also be because larger individuals have an increased mass than smaller individuals causing there to be a greater weight for them to successfully flip over. Unfortunately weight was not measured is this study so it cannot be determined if it's a factor or not.

Comparing the righting response times on the table of the families Echinasteridae and Asteriidae, produced significant results. Asteriidae consists of the larger species in the study and therefore had an increase in time for the righting response to be completed compared to the species in Echinasteridae that has a small range of morphologies within the family. This signifies that species with similar morphologies have a similar reaction to being placed with their aboral surface on the substratum.

There is also a potential metabolic factor in the time it takes for an individual to right itself successfully. Most of the specimens that I used in my study were starved before the trials were completed, however one individual of *L. hexactis* was observed to be feeding on the first day of the trials and had to be excluded from that day's trial, but was used two days later. The time that it took for this individual to right itself on both substrates (262 s on table, 285 s on sand) was not greatly different from the other *L. hexactis* individuals despite the fact that the other individuals were starved. This could be an interesting potential study to determine how metabolism plays a role in the righting behavior.

Similar to other studies (Polls and Gonor 1975) no preference in which arm was used to initiate the righting response was found. There was an equal likelihood that an individual would use any one of its arms to lead the righting response. This contradicts previous results that sea stars have a similar response when righting themselves indicating bilateral tendencies (Ji et al. 2012). Ji et al (2012) found that one asteroid species, *Asterias amurensis*, showed a similar righting behavior between the individuals in the study. The righting behavior was shown to act on a bilateral plane of symmetry allowing the authors to conclude that sea stars behave as bilaterians (Ji et al 2012). My study conflicts with this result. None of the eight species in my study behaved similarly to one another and didn't have a characteristic species response to being turned over on their aboral side. Some individuals behaved similar to each other, but this could be between individuals of the same species and also individuals of different species. My results were more similar to a similar study by Polls and Gonor (1975) that showed that *Henricia leviuscula* and *L. aequalis* showed no preference to a specific righting behavior or leading arm that was used to initiate the righting response. Species were equally likely to choose any of their arms to start the righting process. Both my study and the study by Polls and Gonor (1975) points toward asteroids behaving like radial animals rather than bilaterians. Other previous studies have examined the preferred direction of locomotion in asteroid species (Smith 2008; Cole 1913). Normally a sea star will move by leading with one of its five arms but can switch to lead with another arm when encountering an obstacle or predator which demonstrates their pentaradial symmetry (Smith 2008). Both of these studies found that sea star species didn't have a preference to which arm would start crawling first (Cole 1913; Smith 2008).

Since no other studies have examined the righting response on different substrates I am unable to compare my findings to previous experiments. However, many studies have examined the righting response in sea stars that are placed under stressful conditions and can be used to interpret the overall sea star righting response. One of these studies used lower salinity levels than its natural habitat to stress *Pisaster ochraceous* and found that there was no significance in the results but there was a trend towards an increase in righting time with an increase in stress (Held and Harley 2009). This indicates that as a sea star species is stressed it becomes less able to right itself signaling the decreasing health of the species. Another study examined the difference in the righting response times when sea stars were placed in varying temperatures (Kleitman 1941). At temperatures that were lower than 10 ° C, no righting response was initiated (Kleitman 1941). As temperature increased to 11 to 15 ° C a righting response was slowly initiated and time increased as the temperature increased (Kleitman 1941).

This study has shown that most sea stars are able to successfully right themselves when placed on substrata with smaller grain size than where there are usually found, on rocks, in their natural habitat. It shows that tube feet aren't the only mechanism used when an asteroid species is able to successfully right itself. To further demonstrate this idea, another study should be conducted which removes the tube feet of sea stars and observes their righting response. By removing their tube feet the dominant mechanism that sea stars use to right themselves would be observed.

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