The effects of autotomy and regeneration on the locomotion and behavior or brittle stars (Echinodermata: Ophiuroidea) of Moorea, French Polynesia

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Autotomy and regeneration of body parts is a defense mechanism that a multitude of taxa have evolved in order to escape predation. While both autotomy and regeneration are very commonly observed traits in brittle stars, little has been studied about them in relation to many aspects of their ecology, including its effects on their locomotion and behavior. This study compared the tendency to autotomize across brittle star taxa by way of a field survey and quantified the effects of autotomy on the locomotion and behavioral ecology of genus *Ophiocoma.* This was done by taking and analyzing videos the movements of eighteen individuals over the course of four weeks, comparing locomotive and behavioral changes over the course of the autotomization and regenerative process. Of the three genera of large epibenthic brittle star found, there was no one genus that seemed more likely to autotomize than another. When examining the effects of autotomy on *Ophiocoma*, there were very few differences among any of the sampling periods or treatments. Any changes occurred immediately after autotomy, but did not persist for more than that one sampling period, and yielded results comparable to those of pre-autotomy one week into regeneration. This may imply that *Ophiocoma*—and brittle stars as a whole—are extremely well adapted to autotomy as a defense strategy, more so than many other taxa who also employ autotomy as a defense mechanism.

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THE EFFECTS OF AUTOTOMY AND REGENERATION ON THE LOCOMOTION AND BEHAVIOR OF BRITTLE STARS (ECHINODERMATA: OPHIUROIDEA) OF MOOREA, FRENCH POLYNESIA

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Abstract. Autotomy and regeneration of body parts is a defense mechanism that a multitude of taxa have evolved in order to escape predation. While both autotomy and regeneration are very commonly observed traits in brittle stars, little has been studied about them in relation to many aspects of their ecology, including its effects on their locomotion and behavior. This study compared the tendency to autotomize across brittle star taxa by way of a field survey and quantified the effects of autotomy on the locomotion and behavioral ecology of genus *Ophiocoma.* This was done by taking and analyzing videos the movements of eighteen individuals over the course of four weeks, comparing locomotive and behavioral changes over the course of the autotomization and regenerative process. Of the three genera of large epibenthic brittle star found, there was no one genus that seemed more likely to autotomize than another. When examining the effects of autotomy on *Ophiocoma*, there were very few differences among any of the sampling periods or treatments. Any changes occurred immediately after autotomy, but did not persist for more than that one sampling period, and yielded results comparable to those of pre-autotomy one week into regeneration. This may imply that *Ophiocoma*—and brittle stars as a whole—are extremely well adapted to autotomy as a defense strategy, more so than many other taxa who also employ autotomy as a defense mechanism.

INTRODUCTION

The act of predation, both lethal and sub-lethal, is considered one of the major processes that drives adaptive evolution [1, 2]. Organisms have various strategies for avoiding predation; these

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include physical defenses such as spines, camouflage and cryptic coloration, and the manufacture of bodily toxins [2]. One complex strategy of predator avoidance involves sacrificing biomass in order to avoid lethal predation. This phenomenon referred to as 'autotomy'. Autotomy is observed in diverse variety taxa, including lizards [3, 4], arthropods [5, 6], and echinoderms [7, 8, 9]. After autotomy, individuals usually regenerate the lost or injured body part, which allows the organism to continue to survive after predation and contribute to the reproductive population [10]. Despite the short term benefits of autotomy regarding immediate increased chance of survival, autotomy and regeneration can affect aspects of overall fitness. These include feeding capacity [11], behavior [12], reproductive output [13], growth [14], and locomotion [6, 15]. Loss of a locomotive structure can significantly impair running or swimming ability [16], ability to forage or hunt for food [17], and even escape from future predation attempts [18]. Studies also suggest that there are energetic costs associated with regeneration as well. Redistributed energy allocation due to regeneration may hinder growth as well as locomotive ability [7, 8].

Autotomization and regeneration occurs in all five classes of echinoderms. All echinoderms have a high capacity for regeneration, as they can replace both internal organs such as digestive structures and gonads as well as external structures such as arms and spines [19]. Members of the class Ophiuroidea, known as the basket and brittle stars, are well known for their capabilities for autotomy and regeneration concerning their long, fragile arms. It has been widely observed in the literature that brittle star arms are often autotomized either voluntarily or via trauma, which is then followed by total regrowth of the lost structures [7, 20]. Many individuals surveyed in nature either have lost or are re-growing arms, which suggests that brittle stars may not only be highly adapted to autotomy as a defense mechanism, but it is also an essential part of their ecology [19].

While the process of regeneration on its own has been highly studied in brittle stars due to their impressive regeneration time, there is actually very little literature examining the effects of autotomy and regeneration on other specific aspects of brittle star ecology, especially locomotion and escape behavior. Brittle star movement and locomotion is complex. Unlike other echinoderms, they do not entirely rely their tube feet for locomotion. Instead, they rely on the physical movement of their long, multijointed limbs to pull themselves over the substrate [21]. Even though brittle stars have perfect pentaradial symmetry, they exhibit distinct bilateral motion with noted coordination among each of their limbs despite their lack of a central "brain" to control their movements [22]. Brittle stars exhibit two distinct locomotor modes—"rowing" and "reverse rowing" [22, 23, 24]. "Rowing" involves four arms being used to propel the brittle star along the substrate with the fifth arm pointed in the direction of motion either passively or for sensory purposes [22]. "Reverse-rowing" is similar, but with the passive/sensory arm trailing behind [22]. Brittle stars use their pentaradial symmetry to their advantage by having the ability to change direction extremely efficiently. They turn not by physically moving their oral disk, but by simply changing which arm is the leading or tailing arm [22]. Consequently, there is seemingly no preference in leading or trailing arms in intact individuals, fixed limb identities, or presence of an anatomical anterior (despite other radially symmetrical organisms such as jellyfish moving along a fixed axis of motion [25]).

The mechanism for arm coordination in brittle stars is unclear [22]. Brittle star arms have chemo-, photo-, and mechanoreceptors on each of their arms, allowing them to perceive and react to local stimuli [20, 26]. The only existing previous study that quantitatively examines brittle star locomotive behavior and coordination only examined intact individuals of a single species [22]. Loss of sensory and mobility structures can also lead to a loss in coordination, and cause organisms to favor lost or weakened structures [27, 28].

The overall goal of this study is to examine autotomy in brittle stars on an ecological level. Specifically, I examined whether autotomy is an avoidance strategy used at a similar magnitude across a number of brittle star taxa, along with quantifying the effects autotomy and regeneration have on their movement and behavior. To achieve this goal, I asked these questions: 1) Are some taxa of brittle stars more prone to autotomy than others in the field? 2) Do brittle stars of species *Ophiocoma* (the most easily found large epibenthic brittle star in the waters of Moorea; observation, 2015) have a preference for rowing or reverse rowing and does autotomy and/or regeneration affect these preferences? 3) Is there a difference in the frequency *Ophiocoma* use an autotomized arm to lead or tail at different points in the regeneration process? 4) Does autotomy or regeneration affect movement speed? And lastly, 5) does autotomy or regeneration affect coordination or other aspects of their behavioral ecology? I hypothesize that 1) no one genus of brittle star will be more prone to autotomy in the field than another, and the percentage of autotomized or regenerating arms will be the same among taxa; 2) *Ophiocoma* will have a preference for reverse rowing and autotomy/the process of regeneration will affect this preference and individuals will reverse row more; 3) brittle stars will prefer to lead and tail with non-autotomized or regenerating arms; 4) brittle stars with autotomized or regenerating arms will be will be on average slower than when they were intact, and will become slower further into the regeneration process; and 5) brittle stars will be less coordinated post autotomy and will switch direction and locomotive mode more than when intact, due to decreased ability to freely change direction and move efficiently. Also, sheltering behavior will be altered due to decreased ability to change direction and sense the shelter.

METHODS

Collection and field study sites

Moorea (latitude 17 30’ S, longitude 149 50’ W) is the second largest of the windward group of islands of the Society Islands, French Polynesia. Moorea is surrounded by a barrier reef with a lagoon between the reef and shore, with five reef islands made of cemented conglomerate and coral rubble that are locally known as motus. Three sites around Moorea were chosen as field survey and collection sites that were spatially and ecologically distinct from one another and found in the past to have high densities of brittle stars: Motu Temae, Motu Tiahura, and the mangroves at Ha’apiti, [29].

Temae (17.497° S, 149.759° W) is the largest of Moorea’s motus, located off its northeastern shore (Fig 1). Unlike the other motus, Temae is connected to the main island due to the fact that the lagoon was filled in upon the construction of an airport in the mid-1900’s. The majority of the motu has been converted into a public beach, but there is an area just before the beginning of the conglomerate platform in the intertidal area with a high amount of coral rubble where brittle stars take shelter. The substrate there is primarily composed of very coarse coral sediment and exposed bedrock with some sand, and the water is often less than 10cm deep with a high current just a meter or two further from shore.

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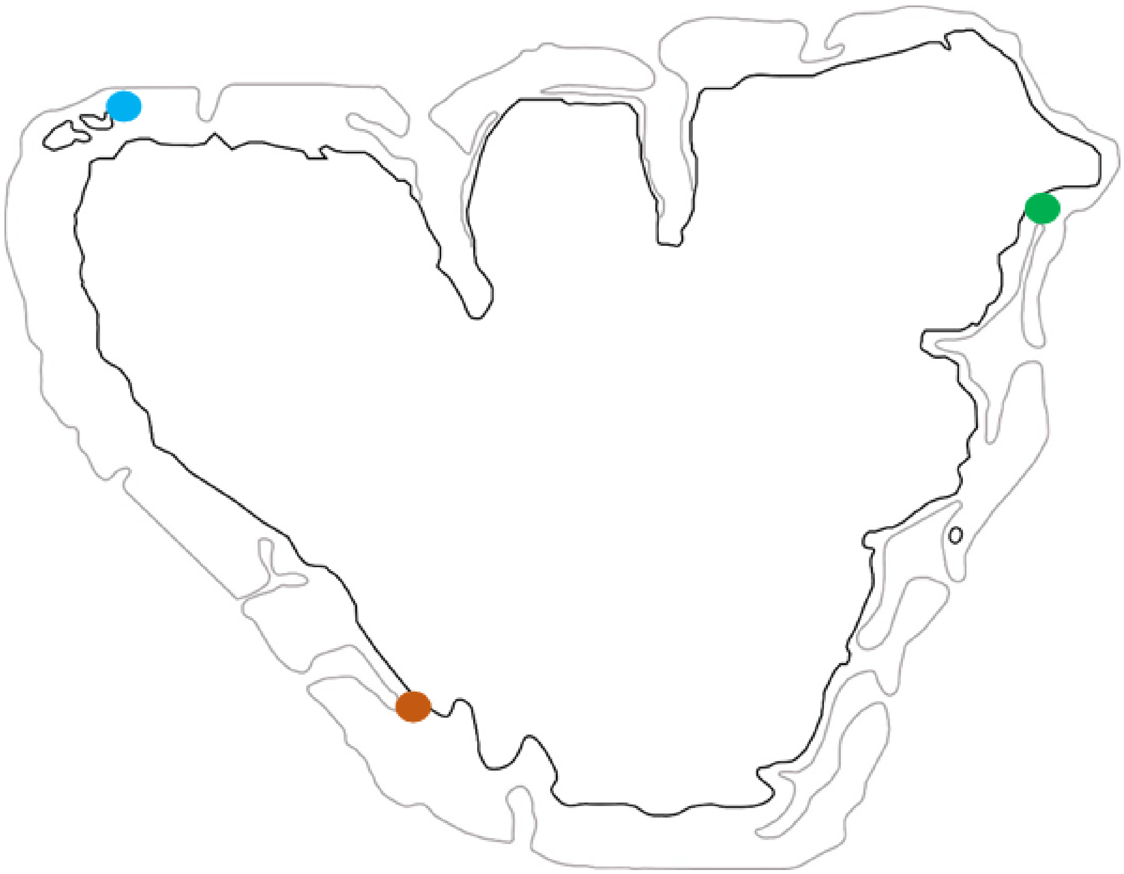

FIG. 1. Sites sampled in this study known to have ophiuroids. Green is Temae, blue is Tiahura, and orange is the Ha'apiti mangrove.

Tiahura (17.847°S, 149.910° W) is Moorea's third largest motu. It is physically separated from its northeastern tip by a dredged boat channel and only accessible by kayak. Brittle stars are mainly found on the eastern side of the motu just below the intertidal zone and where there is very little current. The vast majority of the substrate is composed of medium-fine coral sand and the water is always less than a meter deep. Large pieces of coral rubble have been deposited along the shore of the motu about 0.5 - 1.5 meters from the intertidal zone, where brittle stars take shelter.

Ha'apiti (17.5627° S, 149.871° W) is located on the southwestern side of the island and is one of the main localities of the invasive mangrove *Rhizophora stylosa* on Moorea. The sediment here is a mix of very silty terrestrial-derived sediments and coarser coral sand. There are fewer rocks here than at either Temae or Tiahura, and there is a very strong current present at this site. Water depth can vary depending on the time of day, from more than a meter to less than 0.5m depending on the tides.

Field survey

A field survey was taken at the three study sites to obtain a baseline of the diversity of the brittle stars of Moorea and to investigate how frequency of autotomy and regeneration may vary among brittle star taxa. A study site at each of the locations was established where physical conditions such as substrate type, current intensity, and water depth were relatively constant. Then, starting at one end of the established site, rocks were flipped over while either walking or snorkeling along the shore until 10 rocks were found with brittle stars taking shelter under them. These individuals were then collected, identified (or taken back to the lab for identification), the diameter of their oral disks measured, and the degree of autotomy or regeneration of their limbs assessed (i.e., how many arms were missing or regenerating). They were then photographed and released back to where they had been found. This research was undertaken as part of the UC Berkeley Biology and Geomorphology of Tropical Islands field course in association with the UC Berkeley Gump Station and adheres to all university rules and regulations according to the appropriate field permit.

Lab Study

Nineteen intact individuals of similar size (with oral disk diameters ranging from 1.5-2cm) of the genus Ophiocoma were collected from Temae and Tiahura and brought back to the lab. They were stored in a glass aquarium with flow-through seawater and shelters made with stacked ceramic tiles. Each individual was given a code name and photographed for later reference.

The behaviors and movements of each individual were studied over the course of four weeks. A separate arena measuring 64cmx32cmx10cm was constructed out of a clear plastic bin in order to perform movement trials, with a long ruler acting as a vertical scale and a horizontal scale drawn onto the bottom of the arena at regular intervals along the length of it.

Trials took place in a well-lit area where each individual was placed under an opaque plastic cup in the middle of the arena order to simulate finding shelter under a rock in the wild. The cup was then lifted and the responses of each individual recorded with a waterproof digital camera (while making sure that a shadow was not cast over the arena) until it found the shelter constructed out of ceramic tiles at one end of the arena, or until five minutes had passed. This provided a baseline estimate of locomotion ability for each individual at each point in time during the regeneration process: Pre-autotomy (W0), immediately post-autotomy (W1), a week into arm regeneration (W2), and then two weeks of regeneration (W3). This provided a baseline estimate for the locomotion ability at each stage of the regeneration process (i.e. pre-autotomy, immediately post autotomy, beginning of soft tissue regeneration, beginning of hard skeletal regeneration).

After the first week, the arm of each individual just to the right of the madreporite was amputated 20 arm segments from the oral disk in order to standardize the degree of autotomy among individuals, which was based on an estimate of average degree of autotomy of individuals in the field. Trials were conducted immediately after amputation, and then at two weeks into the regeneration process. Videos and data were collected for each week, with five replicate trials for each week done over the course of two days.

Videos were analyzed by examining the behaviors and movements of each individual and recording the distance traveled, the directionality of the escape response, locomotor mode, the leading or trailing arm (designated A1-5, A1 being the amputated arm), and movement speed, along with noting any other significant behaviors such as which arm is the sheltering arm (the arm that first comes into contact and finds the shelter) and noting the number of locomotive and direction switches as a proxy to quantify coordination.

Statistical Analyses

Differences in percentage of autotomized or regenerating arms among genera were examined for significance using ANOVA. Differences among percent distance traveled rowing, reverse rowing, using the amputated arm (A1) to lead or trail, and speed among the weeks in order to examine how autotomy affects the use of arms was examined for significance using a repeated measures ANOVA with a series of post-hoc pairwise t-tests. Differences in number of switches in leading/tailing arms and locomotive mode among different points in time were also examined for significance using a repeated measures ANOVA with a series of post-hoc pairwise t-tests.

RESULTS

Field Survey

Three genera representing two families of large epibenthic brittle stars were found among the three field sites: *Ophiarthrum*, *Macrophiothrix*, and *Ophiocoma. Ophiocoma* dominated both Motu Tiahura and Temae public beach, while *Macrophiothrix* was the only genus of brittle star found at the Ha'apiti mangroves. *Ophiarthrum* was only found at Temae public beach. *Ophiocoma* had the highest percentage of autotomized and regenerating arms in the field, with an average of 32.5% (SD +/- 2.0) of arms either autotomized or regenerating (Fig 2).

Macrophiothrix and *Ophiarthrum* both had an average of 13% (SD +/- 10.0) and 10% (SD +/- 10.0) of autotomized or regenerating arms in the field respectively. However, despite this apparent trend, these differences are not statistically significant.

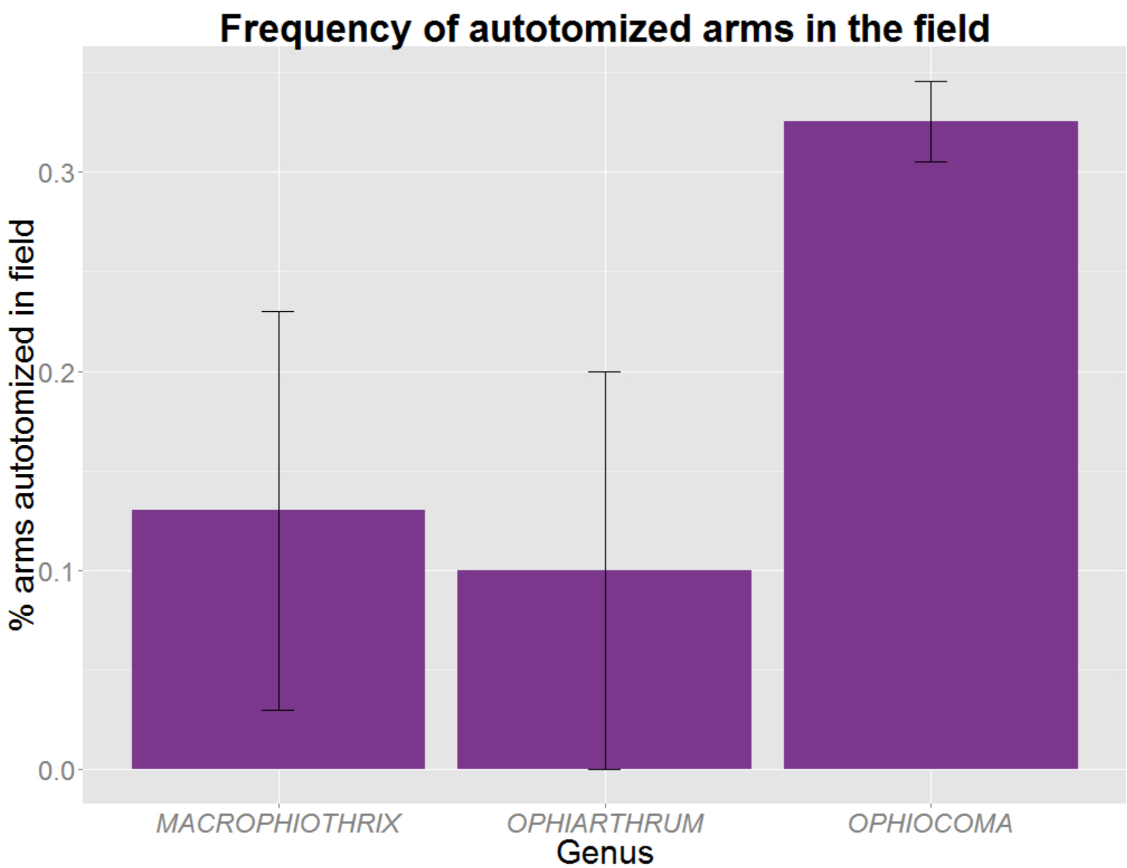

FIG. 2. Bar plot comparing frequency of autotomized arms among three genera, *Macrophiothrix, Ophiarthrum,* and *Ophiocoma,* in the field.

Locomotive Mode Preference

There was an overall preference for rowing over reverse rowing ($p<0.001$) within all of the time points. Rowing accounted for 62%-73% of the entire distance traveled by all individuals (Fig 3). Reverse rowing, on the other hand, accounted for only 27%-38% of the percent total distance traveled. However, while the differences in the percent distance rowing versus reverse-

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rowing were all significant within each sampling period, these values did not differ over time among each sampling period. Rowing was consistently the preferred locomotive mode, with no significant difference in the percent distance traveled among each week.

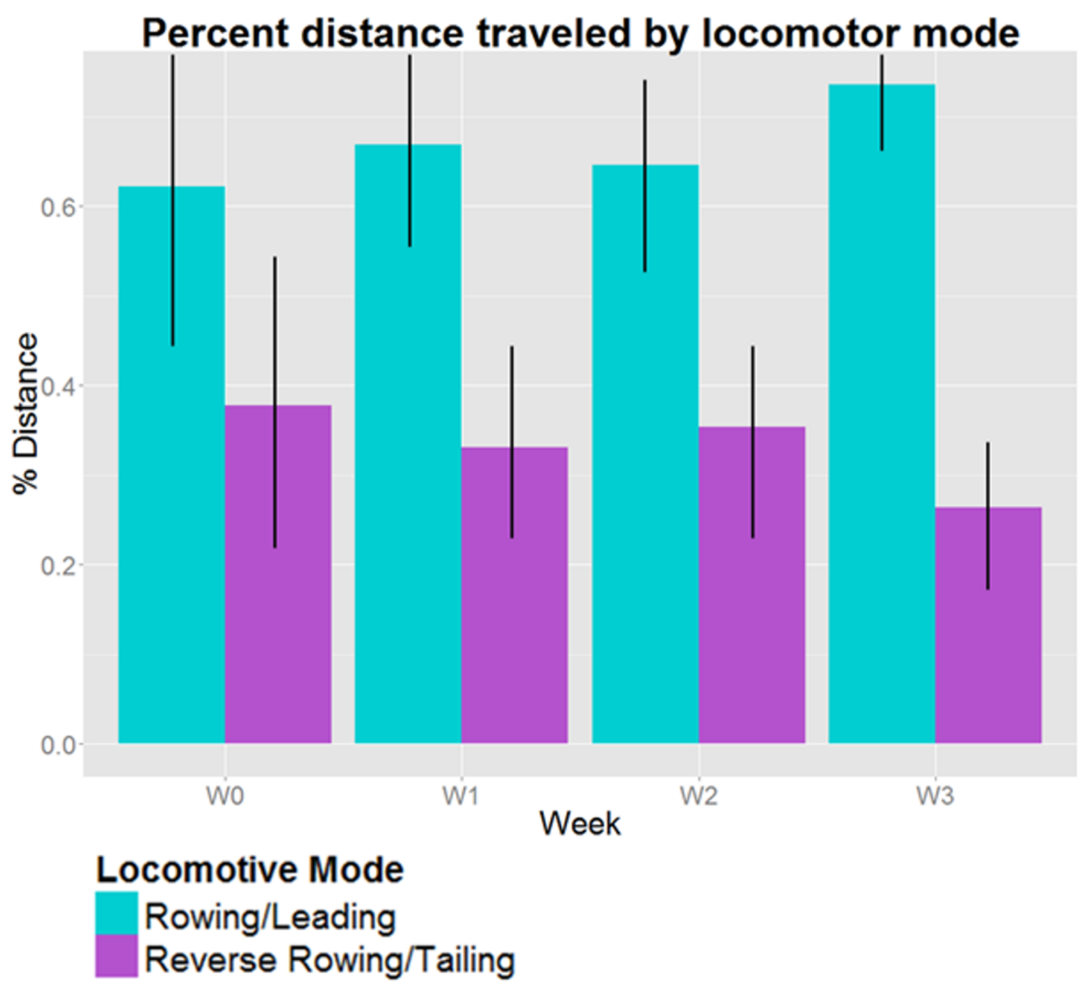

FIG. 3. Bar plot comparing distance spent rowing and reverse rowing. There is an overall preference for rowing over reverse rowing, with no change concerning this among each sampling period.

Autotomized Arm vs Non-Autotomized Arm Preference

Overall, brittle stars used A1 less to lead or tail after autotomy. Pre-autotomy, brittle stars used A1 as a leading or trailing arm an average of 19.12% (SD +/- 10.86) of the distance they traveled. Immediately following autotomy, individuals tended to use the autotomized arm less, using it as a leading or tailing arm over a mean 13.04% (SD +/- 9.07) of distance traveled in that time point. In the first week of regeneration, individuals used the autotomized arm to lead/tail almost slightly less than immediately post-autotomy, using A1 as a leading or tailing arm a mean 12.11% (SD +/- 7.72) of the distance traveled. In the second week of regeneration, individuals used the autotomized arm to lead or tail almost the same amount as they did immediately post autotomy, with A1 leading/tailing 13.36% (SD +/- 9.67) of the distance traveled. However, the differences between these means were not significant at the $p<0.05$ level for any of the sampling periods (Fig 4).

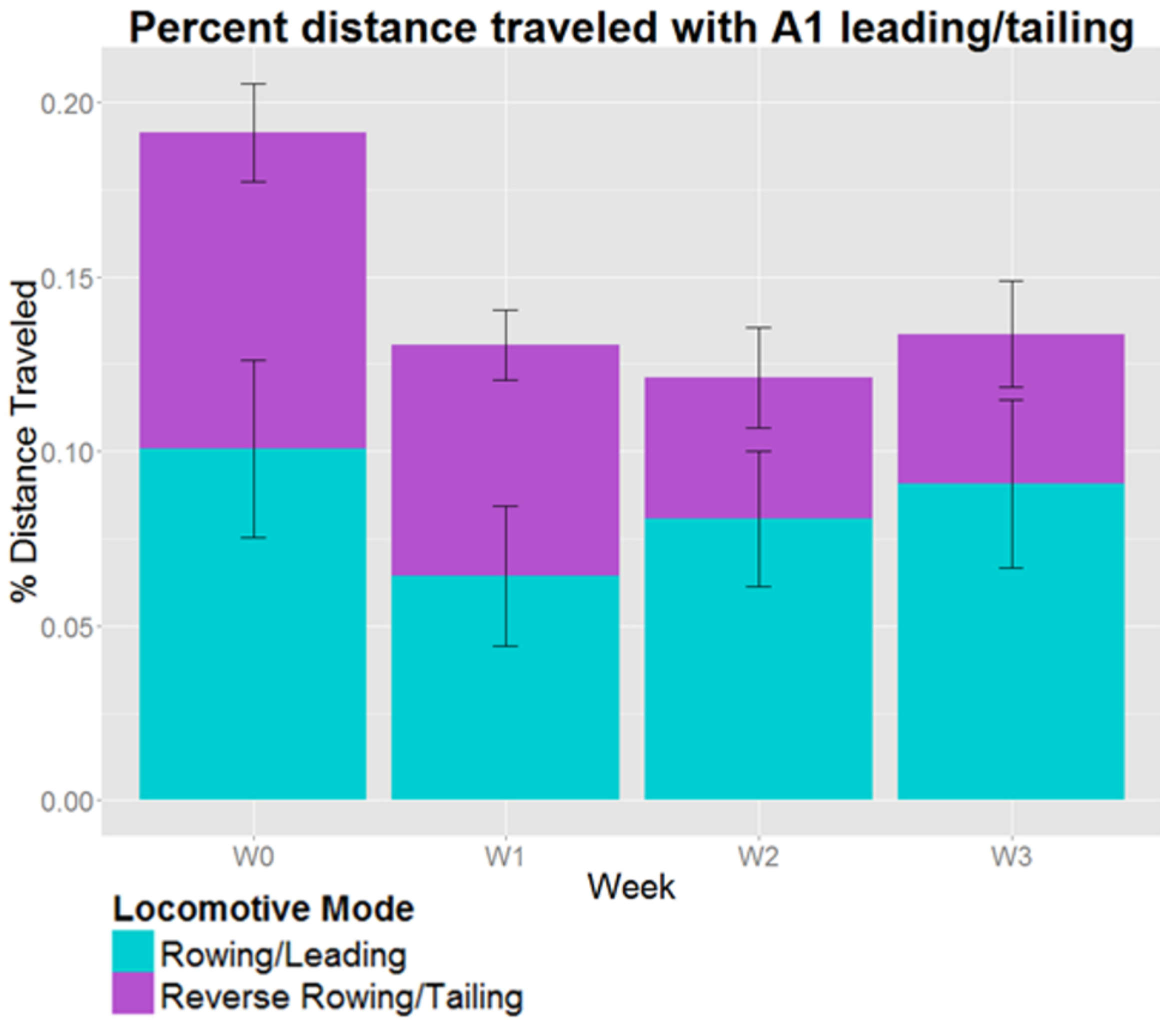

FIG. 4. Bar plot comparing frequency autotomized arm is used to lead or tail among the weeks. It is used the most to lead/tail in W0, and there is no overall preference to let it lead or tail.

However, the breakdown of rowing versus reverse rowing with A1 leading or tailing did not reflect the overall preference for rowing over reverse rowing. While generally brittle stars led with A1 rather than tail with it, these differences within each week proved not to be statistically significant. There was only one instance where the autotomized arm tailed for a higher percentage of distance than it led, which was the sampling period immediately post autotomy. It

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tailed for 6.69% (SD +/- 4.25) of the distance and led for 6.43% (SD +/- 8.53) of the distance; even then, the difference is only very slight (Figure 4).

Locomotive Speed

There was a difference in average speed in brittle stars among the sampling periods. Pre-autotomy, the average speed of the brittle stars was 0.937cm/sec (SD +/- 0.260). Immediately after the autotomization of A1, average speed significantly increased to 1.120cm/sec (SD +/- 0.156), where it then decreased again the next week to 0.915cm/sec (SD +/- 0.160) and stayed below 1cm/sec the second week of regeneration at 0.984cm/sec (SD +/- 0.119). However, while W1 was faster than either W0, W2, or W3 ($p<0.005$), the average locomotive speed of the brittle stars at W0, W2, and W3 were not significantly different from each other, which suggests a boost in speed immediately post autotomy (Fig 5).

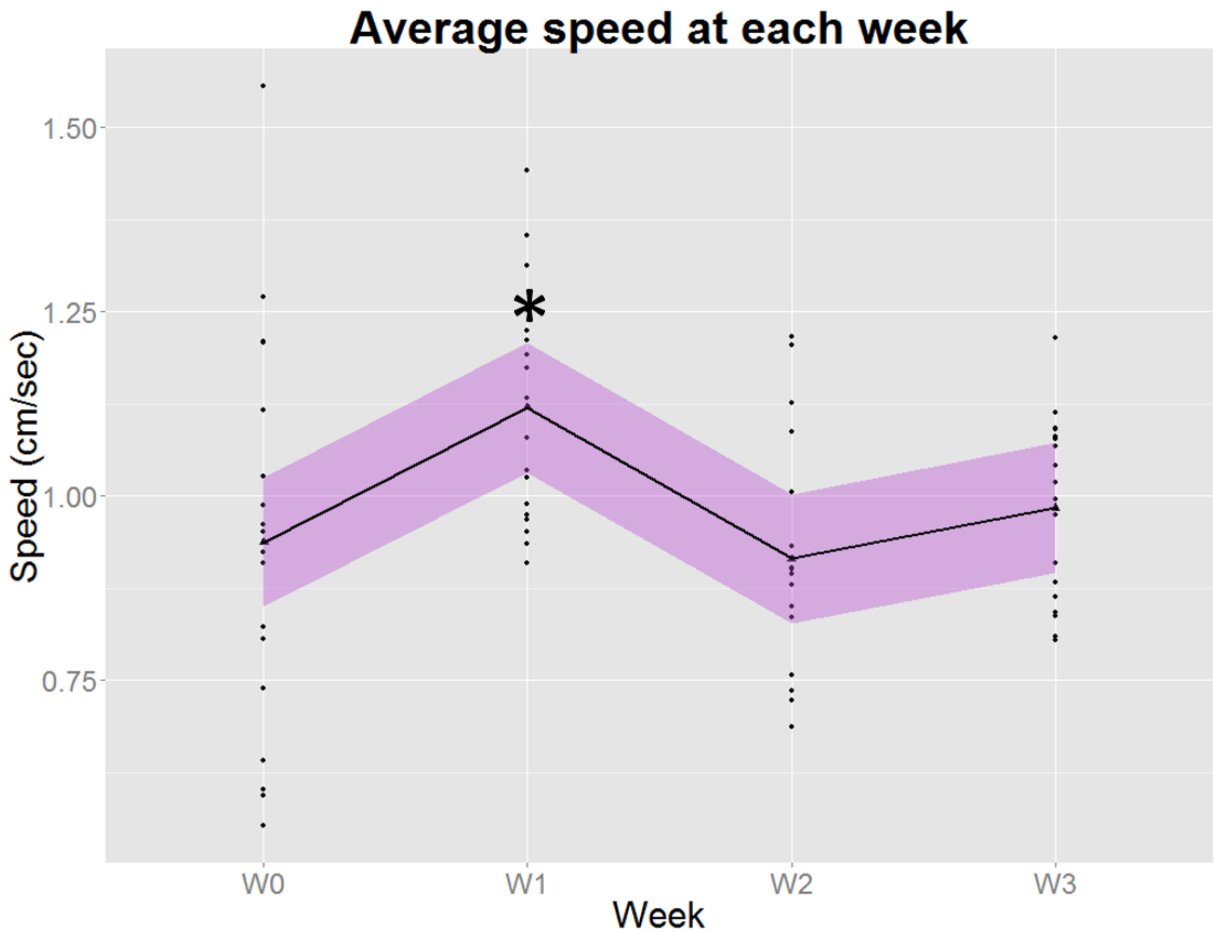

FIG. 5. Line graph showing change in speed over the weeks. There was a rise in speed immediately after autotomy, which then decreased to a speed similar to W0. (*Denotes significance)

Coordination and Sheltering Behavior

Brittle stars were overall less coordinated immediately post-autotomy ($p<0.0001$), but then recovered back to pre-autotomized levels the week afterward and onwards (Fig 6). Pre-autotomy, there was an average of 3.54 (SD +/- 1.21) locomotive switches. In the two weeks of regeneration (W2 and W3), coordination decreased slightly with an increase of locomotive switches to 4.64 (SD +/- 1.48) and 4.40 (SD +/- 1.09) on average respectively. However, there was no statistically significant difference in these means among these weeks. The week immediately post-autotomy (W1), however, the average number of locomotive switches was 6.93 (SD +/- 1.78).

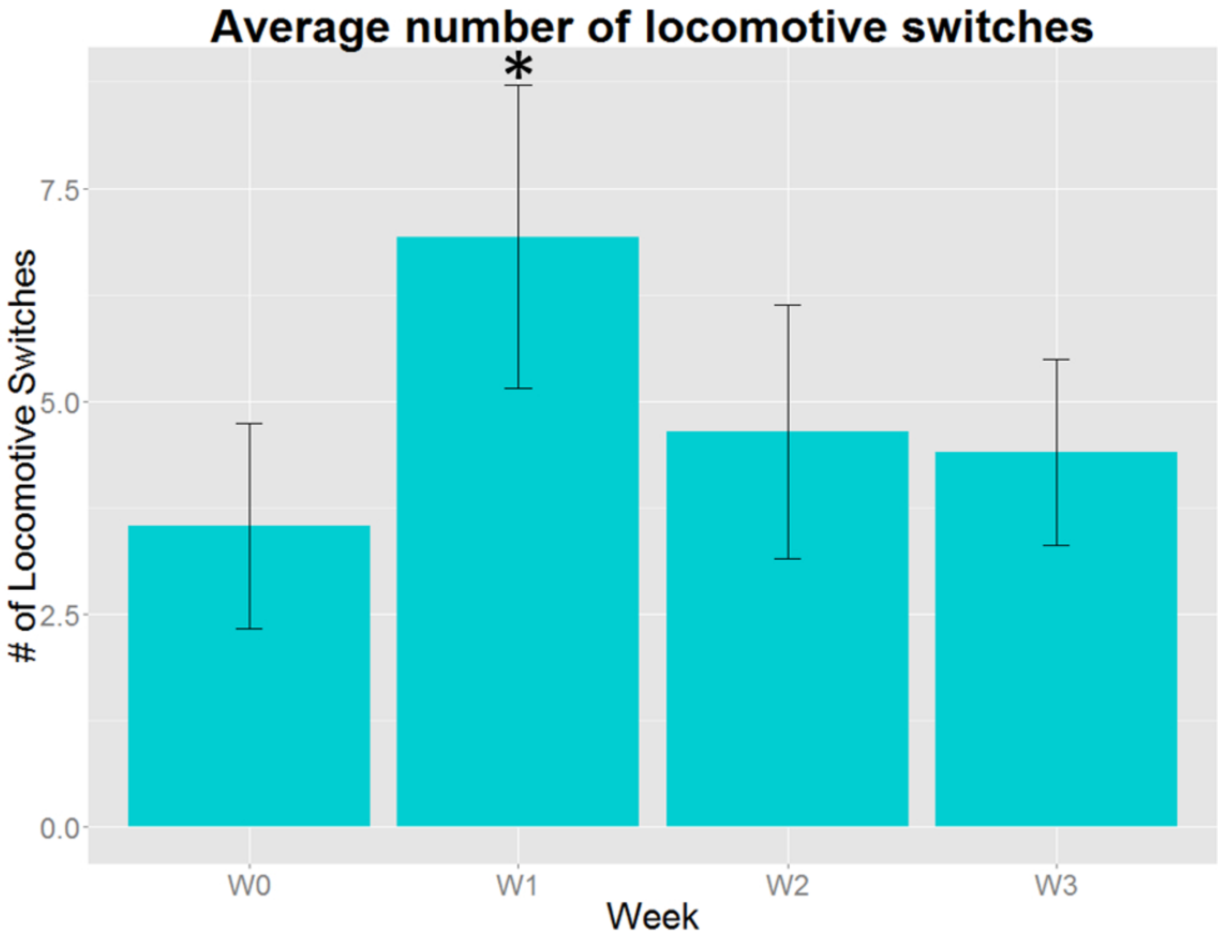

FIG. 6. Bar plot comparing relative coordination among the weeks. Brittle stars were less coordinated in W1, but quickly recovered in W2, which continued to W3. (*Denotes significance)

Sheltering behavior changed after autotomy ($p<0.0001$). Before autotomy, A1 was the sheltering arm an average of 16.11% (SD +/- 15.32) of the time, while immediately post-autotomy A1 only found the shelter 2.22% (SD +/- 9.16) of the time, and never found the shelter in any of the sampling periods afterward (Fig 7).

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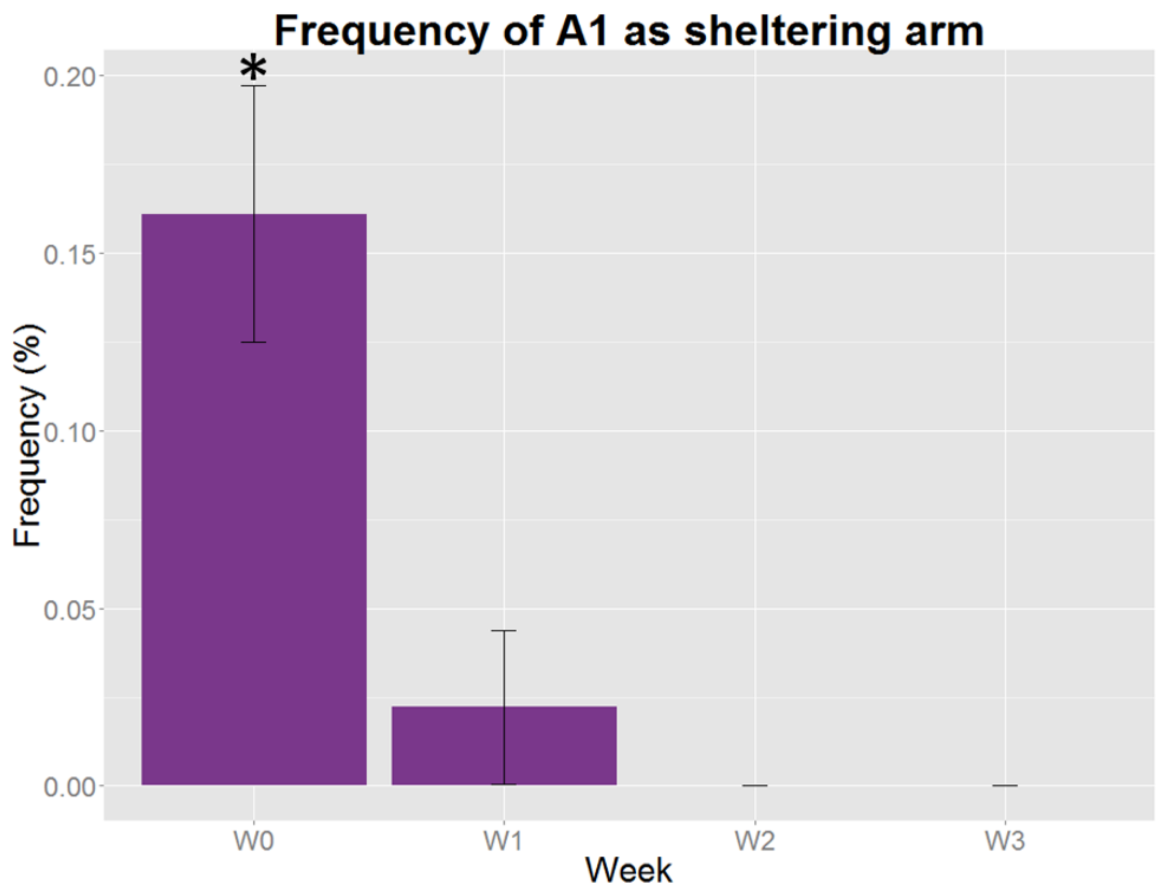

FIG. 7. Bar plot comparing frequency that A1 found shelter among the weeks. A1 rarely finds shelter after it is autotomized.

DISCUSSION

Field Study

Most comparative studies focusing on autotomy only examine its role among broader taxa such as phylum and class; consequently there is very little literature comparing the use of autotomy within ophiuroids as a group. When I compared the frequency of autotomy among three different genera of brittle stars found at different spots around Moorea, I found there may be a reason for this. I was able to reject the null hypothesis by finding that there was not one or more genera with a higher frequency of autotomy in the field.

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Of the three genera of large epibenthic brittle stars that were found—*Macrophiothrix, Ophiarthrum,* and *Ophiocoma*—the percentage of autotomized or regenerating arms did not differ significantly. These three genera also represent two different families. *Ophiarthrum* and *Ophiocoma* both belong to the family Ophiocomidae, while *Macrophiothrix* belongs to the family Ophiotrichidae. Both of these families belong to the suborder Ophiurinia, but the fact that they do not differ in propensity for autotomy even among families may suggest a relatively equal adaptation to this strategy among a broad taxa of brittle stars. Anecdotal evidence of brittle stars from a wide range of taxa regularly autotomizing or regenerating in the field from a variety of sources also seem to support this [7, 30].

However, my confidence in this result is reduced slightly due to the disparity in number of individuals found of each genera. By far the most abundant group was *Ophiocoma,* while only a few individuals of both *Macrophiothrix* and *Ophiarthrum* were able to be recovered and assessed for autotomy. While I standardized my data by averaging the percentage of autotomized arms found at each location for each sampling period, the fact of the matter is that I had much less data for both *Macrophiothrix* and *Ophiarthrum* than *Ophiocoma*, which could potentially skew results. I am also hesitant to make a broad general statement such as, "all brittle stars autotomize at roughly equal frequency," due to the fact that each of the groups of brittle stars found were more or less morphologically similar and inhabited similar niches. I only focused on large epibenthic brittle stars under rocks; there are a multitude of much smaller brittle stars on rocks and in algae (observation, 2015) that may have different survival strategies and do not rely entirely on autotomization as their main mode of predator avoidance.

Locomotive Mode and Arm Preference

The individuals of genus *Ophiocoma* exhibited an overwhelming preference for rowing over reverse-rowing as their locomotive mode of choice, rejecting the first half of the null hypothesis that there would be no difference in frequency of rowing versus reverse-rowing. This corroborates an earlier study that quantified an analyzed brittle star movement, which also produced results that supported brittle stars having a preference for rowing over reverse-rowing [22]. However, these preferences did not change post-autotomy or at any point during the regeneration process, which may suggest that neither autotomy nor the act of regeneration affect locomotion in this genus at one of its basest levels.

Similarly, there was actually no significant differences in the mean distance traveled using it as a leading or tailing arm. This leaves me unable to reject the null hypothesis and suggests that there truly is, in general among *Ophiocoma* as a whole, no preferential treatment for arms as leading or tailing arms whether they are autotomized or intact. This is also in line with Astley's (2012) earlier study of brittle star movement, despite the fact that all of the individuals in that study were intact. This also may imply that overall directionality (aka the ability to move in any direction) is in no way hindered by the fact that the individual has an autotomized or regenerating arm. I am fairly confident in this result due to the fact that the power analysis I ran suggested I had more than enough trials to support my hypothesis if it was true.

While autotomy did not affect locomotive or arm preference in general, this is not reflected in the proportion of distance spent with the autotomized arm leading versus the autotomized arm tailing. There was actually no real difference between time spent with A1 as the tailing arm and A1 as the leading arm. Immediately post-autotomy, the time spent doing either were actually roughly the same, with slightly more distance covered with A1 tailing rather than leading. This may actually coincide with an overall decrease in coordination immediately after autotomy. The lack of preference for A1 to lead or tail post autotomy also imply that individuals use the autotomized arm to tail more than usual, due to it perhaps being a more defensive arm position.

Locomotive Speed

Some past studies into the effects of autotomy note a marked decrease in speed post autotomy, especially if the autotomized body part is a main locomotive structure [16, 32]. I hypothesized that speed would change post autotomy, and I was able to find evidence for this and reject my null hypothesis. However, it was not in the way that I had originally predicted. I predicted that speed would decrease post autotomy and then continue to decrease further into the regeneration process due to the energetic toll regeneration takes on the individual. However, the only significant change in speed was immediately post autotomy. The brittle stars actually became faster than before, but then went back to pre-autotomy speeds for the two sampling periods afterward.

This burst of speed may actually be part of an escape response that is included with autotomy due to predation. In a previous study examining the escape speed of two different species of *Ophiocoma,* this burst of speed immediately after autotomy was not observed [33]. However, other studies examining autotomy in different taxa indicate that autotomy occurs when the risk of predation is higher than the cost of fleeing [34]. So all of the organism's energy at that moment may be dedicated to fleeing and finding shelter, which may translate to a burst of speed. This phenomenon occurs in taxa such as lizards and arthropods [3], and may also be true of brittle stars as well.

There is no significant difference among locomotive speeds at pre-autotomy and the two sampling periods into the regeneration process. This lack of speed decrease may imply that the energetic costs of regeneration do not affect locomotive speed. Which, considering that autotomy and regeneration is such a regular part of brittle star ecology, may imply an adaptation for energetic redistribution that does not negatively affect their ability to escape future predation events.

Coordination and Sheltering Behavior

One cost of limb autotomy in many taxa is decreased coordination upon loss of a locomotive structure [28, 35]. However, coordination in brittle stars was found to decrease significantly only immediately after arm autotomy, to then return roughly the same as it was pre-autotomy after a week into regeneration. This may indicate that individuals become disoriented after an autotomization event or tend to "scramble" (as denoted by the higher number of locomotive switches) away from the direction of threat as fast as possible in order to find shelter.

Compared to immediately after autotomy, brittle stars pre-autotomy and the two weeks into regeneration tended to be more efficient with their movements, switching leading/tailing arm and locomotive mode only where they needed to make a change of direction. Immediately post-autotomy, brittle stars would lose their economy of movement, switching leading arm or locomotive mode despite still attempting to move in the same net direction. There tended to be a lot of slippage of the oral disk when it came to rowing with the autotomized arm, which may have incited some of those locomotive switches. In W1, and even somewhat in W2 and W3, there were a number of times where other limbs had to compensate for the autotomized arm when that arm was being employed as a rowing arm. It may be that the autotomized arm cannot reach as far across the substrate and cannot generate as much force as other arms because of the smaller surface area or simply the amount of tube feet in contact with the substrate. One week post-autotomy saw the regeneration of the distal tube feet, and the second week post-autotomy there was evidence of an increase in autotomized arm length. Increased surface area and tube feet may be a factor as to why the brittle stars seem to be more stabilized at those time points.

Autotomy had a drastic effect on sheltering behavior. The way a brittle star would find shelter is an arm would come into contact with a space or entrance to the constructed shelter where it was significantly shaded from the sun. It would then change direction of motion and that arm would lead as it either rowed or reverse rowed into the opening. A1 was almost never the sheltering arm after it was autotomized. Oftentimes the edge of the arm would brush up against the shelter entrance, but was not long enough to actually enter the shelter. However, since there is seemingly no preference for use of non-autotomized arms over autotomized arms to lead or tail, the ability for a brittle star to travel in the direction of the autotomized arm should not be hindered. It may instead indicate an advantage of longer arms, that an individual with longer arms may have a larger sensory radius, which is helpful in finding shelter to escape predators.

Conclusions

Overall, *Ophiocoma* seem to be incredibly well adapted to autotomy as a defense strategy. Autotomy and regeneration seem to have minimal effect on their locomotive ability. Their ability to move in any direction was not hindered upon arm autotomy, and overall speed was not changed negatively either. There was decreased coordination immediately post-autotomy, but this may be compensated by the boost in speed they also experience at the same time. There is evidence of a loss of sensory ability due to the autotomized arm not being able to find shelter. However, that may only be a function of sheer arm length. Brittle stars have no central brain; signals come from receptors on arms that determine direction and mode of movement [21]. With decreased arm length they may have a decreased ability to sense things further away from their oral disk. Autotomy does not seem to have any effect on *Ophiocoma's* ability to otherwise process environmental signals.

Because there was no evidence for any one taxa of brittle star (genus or family) to autotomize arms more frequently than another, these results may be applicable to a broad range of brittle star taxa other than just *Ophiocoma.* In which case, brittle stars as a whole—or in the very least, *Ophiocoma*—do not seem to suffer some of the drawbacks of autotomy that other taxa do. Geckos [31], spiders [16], and crickets [35] often suffer from a significant decrease in coordination and running speed post autotomy. In brittle stars, autotomy does not seem to even affect their basic locomotive preference. Brittle stars may be some of the organisms best well adapted to autotomy as a defense strategy of any taxa. There seem to be very few negative effects from autotomy on apparent future survival, especially in terms of ability to escape from future predation attempts.

This study is limited for a variety of reasons. First, there are many more taxa of brittle stars than those just found under the rocks in Moorea. To remedy this, this experiment could be repeated on different taxa of brittle stars from different suborders or families in order to gain a broader scope of how brittle stars have adapted to autotomy as a whole. Second, I only examined arm preference as a function of autotomized arms versus non-autotomized arms. While previous literature supports that as a general rule brittle stars do not have any sort of arm preference, I did not examine each brittle star for individual preferences. A future study could examine how autotomy affects the preferences in individual stars. Third, this study only looked at the effects of single arm autotomy on brittle stars. Many brittle stars out in the field had two or more autotomized or regenerating arms, sometimes even all five. The effects of autotomy on brittle star locomotion could be further examined by looking at multiple autotomy rather than only single autotomy. Fourth, I only looked at the physical effects of autotomy on locomotion; it did not account for autotomy's energetic effects or effects on other aspects of their behavior. Previous studies have found that regeneration of multiple arms has effects on gonad production and ability to forage for food [8]. Future studies could focus on the energetic effects of autotomy and regeneration on brittle star behavior or respiration rates.

Despite its limitations, this study provides a deeper examination of aspects of brittle star ecology that is the subject of very little literature. There is only one other quantitative analysis of brittle star locomotion, which serves as the basis for most of the research presented here. Even more generally, this study provides evidence that when adapted as a defense strategy, autotomy and regeneration of body parts can be an extremely effective way to avoid predation without significantly affecting long-term survival. In the words of Monty Python, "Tis but a scratch," when it comes to brittle stars.

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Literature Cited

1. Vermeij JG. Unsuccessful predation and evolution. Am Nat. 1982 Dec; 120(6):701-720.

2. Arbuckle K, Speed MP. 2015. Antipredator defenses predict diversification rates. Proc Nat Acad Sci U S A. 2015 Sep; 112(44):13597–13602.

3. Daniels CB. Running: An escape strategy enhanced by autotomy. Herpetologica. Jun 1982; 39(2):162-165.

4. Samia DSM, Blumstein DT, Stankowich T, Cooper WE. Fifty years of chasing lizards: new insights advance optimal escape theory. Biol Rev Camb Philos Soc. 2016 May; 91(2):349-366.

5. Maginnis TL, Isikbay M, Degerstedt SG, Luethke TJ, Ortman M. 2015. The effects of cheliped autotomy and regeneration on aggression in purple shore crabs (*Hemigrapsus nudus)*. Mar Freshw Behav Physiol. 2015 May; 48(4):241-251.

6. Dominguez M, Escalante I, Carrasco-Rueda F, Figuerola-Hernandez CE, Ayup MM, Umana MN, Ramos D, Gonzalez-Zamora A, Brizuela C, Delgado W, Pacheco-Esquivel J. Losing legs and walking hard: effects of autotomy and different substrates in the locomotion of harvestmen in genus *Prionostemma.* J Arachnol. 2016 Apr; 44:76-82.

7. Lawrence JM, Vasquez J. The effect of sublethal predation on the biology of echinoderms. Oceanologica Acta. 1996; 19(3-4):431-440.

8. Pomory C, Lawrence J. Arm regeneration in the field in *Ophiocoma echinata* (Echinodermata: Ophiuroidea): effects on body composition and its potential role in a reef food web. Mar Biol. 2001 Oct; 139(4):661-670.

9. Rubilar T, Meretta PE, Cledon M. Regeneration rate after fission in the fissiparous sea star *Allostichaster capensis* (Asteroidea). Int J Trop Biol. 2015 Jun; 63(2):321-328.

10. McVean A. Autotomy. Comp Biochem Physiol A Mol Integr Physiol. 1975 Jul; 51(3):497-498

11. Diaz-Guisado D, Gaymer CF, Brokordt KB, Lawrence JM. Autotomy reduces feeding, energy storage and growth of the sea star Stichaster striatus. J Exp Mar Bio Ecol. 2006 Oct; 338(1):73-80.

12. Blackwell PRY, Christy JH, Telford SR, Jennions MD, Passmore NI. Dishonest signalling in a fiddler crab. Proc Biol Sci. 2000 Apr; 267(1444):719-724.

13. Nichols D. Reproductive seasonality of the comatulid crinoid *Antedon bifida* (Pennant) from the English Channel. Philos Trans R Soc Lond B. 1994 Jan; 343(1304):113-134.

14. Barrios JV, Gaymer CF, Vasquez JA, Brokordt KB. Effect of the degree of autotomy on feeding, growth, and reproductive capacity in the multi-armed sea star *Heliaster helianthus.* J Exp Mar Bio Ecol**.** 2008 Jun; 361(1):21-27.

15. Henry GJ. Factors affecting the survival of the brittlestar *Ophioderma brevispina.* Ypsilanti: Eastern Michigan University; 1993.

16. Cooper WE, Perez-Mellado V, Vitt LJ. 2004. Ease and effectiveness of costly autotomy vary with predation intensity among lizard populations. J Zool Soc Lond. 2004 Mar; 262(3):243–55.

17. Smith LD, Hines AH. The effect of cheliped loss on blue crab *Callinectes sapidus Rathburn* foraging rate on soft-shelled clams *Mya arenaria*. J Exp Mar Biol Ecol. 1991 Oct; 151(2):245–56.

18. Dial BE, Fitzpatrick LC. Predator escape success in tailed versus tailless *Scincella lateralis.* Anim Behav. 1984 Feb; 32(1):301–2.

19. Carnevali MDC, Bonasoro F. Introduction to the biology of regeneration in Echinoderms. Microsc Res Tech. 2001 Dec; 55(6):365-368.

20. Lawrence JM. Energetic Costs of Loss and Regeneration of Arms in Stellate Echinoderms. Integr Comp Biol. 2010 Oct; 50(4):506-514.

21. Lawrence JM. A Functional Biology of Echinoderms. Baltimore: The Johns Hopkins University Press; 1987.

22. Astley HC. 2012. Getting around when you're round: quantitative analysis of locomotion of the blunt-spined brittle star, *Ophiocoma echinata*. J Exp Biol. 2012 May; 215(11):1923-1929.

23. Glaser OC. Movement and problem solving in *Ophiura brevispina*. J Exp Zool. 1907 Jun; 4(2):203-220.

24. Arshavskii YI, Kashin SM, Litvinova NM, Orlavskii GN, Feldman AG. 1976. Types of locomotion in ophuirans. Neurophysiology. 1976 Sep; 8(5):398-404.

25. Beklemishev WN. Principles of Comparative Anatomy of Invertebrates. Chicago: University of Chicago Press; 1969.

26. Moore A, Cobb JLS. Neurophysiological studies in photic responses in *Ophiura ophiura*. Comp Biochem Physiol A Mol Integr Physiol. 1985; 80(1):11-16.

27. Ortego F, Bowers WS. Induction of autotomy in the American bird grasshopper *Schistocera americana* (Drury) by the ecsydone agonist RH-5849 and investigation of its mode of action. Experentia. 1996 Jan; 52(1):42-50.

28. Gillis GB, Bonvini LA, Irschick DJ. Losing stability: tail loss and jumping in the arboreal lizard *Anolis carolinensis*. J Exp Biol. 2009 Mar; 212(5):604-609.

29. Chinn S. Habitat distribution and comparison of brittle star (Echinodermata: Ophiuroidea) arm regeneration on Moorea, French Polynesia. Biology and Geomorphology of Tropical Islands. 2006 Dec; 14:38-52.

30. Fleming PA, Muller D, Bateman PW. Leave it all behind: a taxonomic perspective of autotomy in invertebrates. Biol Rev Camb Philos Soc. 2007 Aug; 82(3):481-507.

31. Ballinger RE, Nietfeldt JW, Krupa JJ. An experimental analysis of the role of the tail in attaining high running speed in *Cnemidophorus sexlineatus* (Reptilia: Squamata: Lacertilia). Herpetologica. 1979 Jun; 35(2):114-116

32. Guffey C. Leg autotomy and its potential fitness costs for two species of harvestmen (*Arachnida opiliones*). J Arachnol. 1997 Dec; 26(3):296-302.

33. Price JM, Cassel LR, Gordon MJ, Phillips RS, Reinsel KA, Welch JM. Effect of arm loss on escape speed of two epibenthic brittle stars. BIOS. 2014; 85(4):187-191.

34. Dominguez-Lopez ME, Ortega-Leon AM, Zamora-Abrego GJ. Tail autotomy effects on the escape behavior of the lizard *Gonatodes albogularis* (Squamata: Sphaerodactylidae) from Cordoba, Colombia. Rev Chil Hist Nat. 2015 Jan; 88:1.

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35. Fleming PA, Bateman PW. Just drop it and run: the effect of limb autotomy on running distance and locomotion energetics of field crickets (*Gryllus bimaculatus*). J Exp Biol. 2007 Apr; 210(8):1446-1454.