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HABITAT DISTRIBUTION AND COMPARISON OF BRITTLE STAR (ECHINODERMATA: OPHIUROIDEA) ARM REGENERATION ON MOOREA, FRENCH POLYNESIA

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Abstract. Autotomy and regeneration are widespread in many groups of invertebrates and vertebrates, such as annelids, crustaceans, amphibians, and reptiles. Regeneration is common in all classes of Echinodermata and prevalent in ophiuroid brittle stars. Moorea, French Polynesia was surveyed for species of brittle stars living on coastal areas of the island in different habitats. Ophiuroid populations were sampled in habitats such as a mangrove marsh, a sandy beach with coral rubble and a jetty with coral rubble and conglomerate coral to determine percentages with regenerating arms. *Macrophiothix longipeda* (Lamarck 1816) from the mangrove marsh and two populations of *Ophiocoma scolopendrina* (Lamarck 1816) from the beach and jetty were studied to determine if there were differences in experimental rates of arm regeneration after induced autotomization. Each habitat was colonized by distict ophiuriod assemblages and had different percentages of regenerating individuals; *M. longipeda* was found to be regenerating multiple arms simultaneously and had the highest rate of regeneration. Regeneration rates differed by species; mostly likely influenced by habitat, ecology and biology of each species.

Key words: echinoderm, ophiuroid, brittle star, autotomy, regeneration, intact arms, regenerating arms, Macrophiothrix longipeda*,* Ophiocoma scolopendrina

INTRODUCTION

Autotomy is an effective strategy evolved by many invertebrates and vertebrates to avoid lethal predation. Autotomy results in the loss of biomass, which likely affects an individual's energy metabolism and allocation during regeneration of the lost tissue. Species that differ in their ecology, physiology, and phylogeny may autotomize and regenerate at different rates and in response to different stimuli such as predation, environmental factors such as water flow, asexual reproduction (fission), or conspecific interactions.

Loss of biomass can be caused by physical perturbations (Ball et al., 1967; Woodley, 1980; Tilmant et al., 1994); interspecific and intraspecific fighting (Berzin and Caldwell, 1983; Harris, 1989; Smith and Hines, 1991); and partial predation (Vlas, 1979a; Turner et al., 1982; Bowmer and Keegan, 1983; Clavier, 1984; Woodin, 1984; Bergman et al., 1988). In sublethal predation the affected individual regenerates the missing structure, thereby escaping death (Pomory and Lawrence, 2001). Regeneration after injury allows the individual to survive and contribute reproductively to the population. However, regeneration requires additional energy in addition to normal energetic processes. Pomory and Lawrence (2001) suggest that regeneration can affect the fitness of an individual by using energy that would otherwise be distributed to basal energetics, reproduction, and growth.

Many marine organisms, particularly echinoderms, autotomize appendages. Regeneration occurs in all five classes of Echinodermata to replace external and internal organs like arms, appendages (spines and pedicellariae), viscera (digestive tube, gonads) (Carnevali and Bonasoro, 2001). Carnevali and Bonasoro (2001) observed that regeneration is frequent in crinoids and ophiuroids as both classes have long, fragile arms that are often amputated voluntarily or via trauma followed by total regrowth of the lost structures. Regeneration is so common that individuals assessed in nature usually have regenerating arms at many different growth stages. Additionally, lost body fragments can live for a period of time after being separated, and can sometimes undergo independent partial or total regeneration (Carnevali and Bonasoro, 2001). Also, some asteroids, ophiuroids, and holothuroids asexually reproduce via fission. Adult individuals split into two or three parts, regenerating all parts into complete, independent individuals (Emson and Wilkie, 1980). The commonality of regeneration

across the phylum suggests that regeneration is an essential aspect of their life-cycle (Carnevali and Bonasoro, 2001).

Brittle stars are capable of losing arms or parts of arms, and sometimes the aboral portion of the central disk and viscera to predation, autotomy, and asexual reproduction (Wilkie, 1978). Wilkie (1978) observed that a significant proportion (20-100%) of the ophiuroid population may be regenerating lost structures. Emson and Wilkie (1980) provided evidence that a majority of noneuryalid ophiuroids (i.e. brittle stars) have the ability to autotomize their arms at any intersegment. Arm breakage is common and may be caused by waves, intraspecific confrontations and predations (Emson and Wilkie, 1980). It is considered an adaptation in which the sacrificed structure allows for the survival of the individual, even though the amount of energy for regeneration is considerable (Bowmer and Keegan, 1983). The family Amphiuridae can release the whole aboral disc, which holds the stomach, gonads, and genital bursae (Emson and Wilkie, 1980). *Ophiocomina nigra* drops arm spines, most likely a form of opportunistic self-detachment (Wilkie, unpub. obs.). Studies involving many species suggest that sublethal damage is a heavy burden and there is prominent variation between conspecific populations and within populations over time (Sides, 1987; Skold and Rosenberg, 1996).

The primary goals of this study were to describe the brittle star diversity and distribution in Moorea and determine if arm regeneration rates differ between species from various habitats and with different ecologies. Specifically the objectives were: (1) describe locations where brittle star species were observed to provide useful ecological context; (2) measure the relative abundance of these species in different habitats; (3) assess the proportion of the populations with fully intact arms; and, (4) measure and compare the rate of regeneration of two species, *Macrophiothrix longipeda* (Lamarck 1816) and *Ophiocoma scolopendrina* (Lamarck 1816), collected in different habitats. *Macrophiothrix longipeda* I expected species richness to be different at each location due to environmental factors and ecologies of each species. Percent of individuals with fully intact arms were expected to differ by species and location because of the specifics of each habitat and characteristics of each species, such as feeding mode and escape response to predators. Rate of arm regeneration was anticipated to differ between *O. scolopendrina* and *M. longipeda* dependent on ecology and physiology, as with rate of autotomization.

METHODS AND MATERIALS

Site descriptions

Moorea (17° 30' S, 149° 50' W) is a high volcanic island of the Society Archipelago, French Polynesia, in the Pacific Ocean. Four coastal study sites around Moorea were assessed from September to November, 2006 for brittle stars (Fig. 1).

The marsh (17° 33.382' S, 149° 52.457' W) near Haapiti is an area lined by mangrove trees on the banks. This location was assessed via snorkeling in < 2 m of water by turning over rocks and coral rubble, and digging into the sand.

Motu Tiahura is located off the northwest tip of Moorea (17° 29.241' S, 149° 54.632' W). The survey location was characterized by a sandy beach with shallow water and dispersed coral rubble and rocks on the eastern side of the island. Brittle stars were assessed by turning over rocks and rubble.

The Vaipahu barrier reef crest (17° 28.562' S, 149° 49.299' W) located outside of Cook's Bay, North of the Richard B. Gump Research Station, on the northeastern side of the island. It was characterized by several species of coral and coral rubble. Various collections of brittle stars took place in September and October via snorkeling, collecting coral rubble, and allowing the ophiuroids to drop out of the rubble onto the water table due to stress and lack of water.

The jetty (17° 29.110' S, 149° 49.893' W) between Opunohu and Cook's Bay, beyond the Vaipahu barrier reef crest was assessed on two separate days for brittle stars. The jetty consisted of concrete and rock rubble protruding 30 m from the beach into the ocean. Brittle stars were observed under rocks and in crevices.
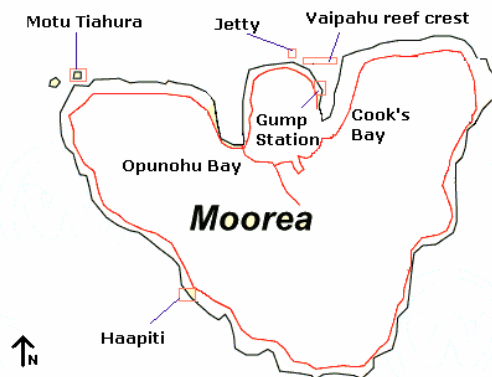

Fig. 1. Map of Moorea, with study sites boxed.

Brittle stars with all intact arms were collected at the four sites to assess differences in species abundance, diversity between different locations, and to observe arm regeneration in the laboratory. However, the ophiuroids from the mangrove marsh were mostly not fully intact and already in the process of regenerating arms.

Brittle stars from the mangrove marsh in Haapiti were collected via snorkeling and turning over various sizes of rocks and coral rubble. Ophiuroids were handled gently as they were fragile, often losing arms or parts or arms when contacted. Five individuals of *M. longipeda* were collected 23 September, 2006 and 18 individuals of the same species were collected 3 October, 2006. Also on 11 October, 2006 a single *Ophiocoma erinaceus*? Muller and Troshel 1842 individual was collected under coral rubble.

Ophiuroids on Motu Tiahura were collected by turning over rocks and coral rubble in shallow water < 1m deep. *O. scolopendrina* was the only species found. Eleven individuals were collected on 25 September, 2006 and seven individuals were collected on 9 October, 2006.

Collections at the barrier reef crest were more haphazard, as a majority of the rubble brought back to the Gump Station was collected for potential octopus dens. Brittle stars were collected only after they emerged from the rubble because of lack of water. Therefore, a majority of the animals collected died and thus were not included in the arm regeneration study.

Two species of brittle stars were observed and one was collected at the jetty. Ten *O. scolopendrina* were collected on 8 October, 2006 and 14 individuals were collected on 18 October, 2006 under rock and within crevices of coral and conglomerate rock.

Natural history

The marsh, motu and jetty habitats were surveyed to determine distribution of species around Moorea, population density, percent of the population with arms fully intact, and percent regenerating. However, the barrier reef crest was not surveyed because all the ophiuroids were hiding in rocks and coral crevices. Therefore, it was impossible to determine these parameters in the field.

The habitats were surveyed for 1.5 hours in an area 29 m by 24 m. An equal and thorough search was conducted by turning over all rocks and rubble that were large enough to provide shelter for ophiuroids and small enough for one person to turn over. Every individual was counted and all were visually assessed as having all complete arms or as incomplete because the arms were not fully intact, and how many of those arms were regenerating. Percents of fully intact individuals and those regenerating arms were calculated.

Experimental design

Individuals captured to assess arm regeneration in situ were housed in individual containers with 2 mm mesh to allow for constant water flow and aeration. Each individual was provided with a thin layer of sand and coral rubble for shelter. They were fed a small piece of turkey meat every other day and fresh sand was provided once a week. The sea water temperature ranged from 26-29 ° C.

Fifteen *O. scolopendrina* from Motu Tiahura, 19 *M. longipeda* from the marsh and 18 *O. scolopendrina* individuals from the Vaipahu jetty served as study animals for the regeneration experiment. Three individuals from the motu, four from the marsh and seven from the jetty served as controls. A majority of the *M. longipeda* ophiuroids from the marsh were in the process of regenerating arms; therefore, they were simultaneously regenerating arm A (which was experimentally autotomized) as well as the other arms.

On 19 October, 2006, designated day 0, each of the study individuals were pinched mid-arm to induce autotomize of the arm opposite the madreporite, designated arm A. The animal was provoked so that it would voluntarily release its arm or part of its arm under these artificial sublethal predatory conditions. The number of segments away that the arm was autotomized from the provoked point, and the time elapsed between provocation and release were recorded. Central disc diameter measurements were taken then and also again on day 25, the last day of measurement to establish an average body size proxy. Measurements of growth were taken every other day with vernier calipers, beginning on day 2 and lasting until day 25.

Three other species from the Vaipahu reef crest were collected, though not in significant numbers. However, they were assessed similarly for arm regeneration rates. Two regenerating individuals and one control of *Ophiocoma sp.*, and two regenerating *Ophiarthrum elegans* Peters 1851 were supplemented into the experiment on 23 October, 2006. One *Ophiocoma sp.* individual was already missing a tip of an arm that had yet to

begin regenerating, which was used for the regeneration data. The other individual was induced to autotmize arm A. One of the *O. elegans* individual was already missing the tip of an arm, which was designated A and measured. The other individual was regenerating two missing tips; both were measured for regeneration. One ophiodermatid brittle star was added to the experiment on 27 October, 2006, which was provoked to autotmize part of its arm.

Controls were handled similarly to the study individuals; however, they were not induced to autotomize and left with fully intact arms. Arm A was measured every other day beginning on day 2 to assess for baseline growth. All *M. longipeda* individuals were simultaneously regenerating multiple arms; therefore, regeneration data was also collected for the other four arms.

Statistical analyses

The means of the disc diameters, individual rates of regeneration, and total regeneration over the duration of the experiment for the marsh, motu and jetty ophiuroids were compared via a one-way ANOVA (or Kruskal-Wallis for non-normally distributed data) analysis using JMP IN 5.1 (SAS Institute 2004), with an $\alpha < 0.05$. ANOVA analysis was also used to compare the time elapsed between provocation and autotomization, and the number of segments away the ophiuroid autotomized from the disturbed point between the marsh, motu and jetty individuals. Additionally, *M. longipeda* was regenerating multiple arms in addition to the experimental regenerating arm. Therefore, ANOVA analysis was performed to determine differences in rate of regeneration among the other four arms. Tukey-Kramer pair tests, with the Bonferroni correction for multiple pair tests, were also performed to assess which species were significantly different from each other. Individual regeneration was normalized for body size by standardizing over disc diameter.

RESULTS

Natural history

One dominant ophiuroid species was found at each of the marsh, motu and jetty locations. *M. longipeda* was found under large rocks and coral rubble at the mangrove marsh. When collecting, *M. longipeda* would burrow into the coral rubble and sand to escape. Often times, only arms were recovered. Arms were readily lost while collecting; therefore, arms were most likely broken

by handling prior to the beginning of the regeneration experiment. *Ophiocoma scolopendrina* was the only species living at Motu Tiahura. Individuals were found living under rock and coral rubble in shallow water. Several species of brittle stars were collected within the coral rubble: an ophiodermatid species; *Ophiocoma brevipes*? Peters 1851*; Ophiarthrum elegans* Peters 1851; *Ophiocoma sp*.; *Ophiocomella sexradia* (Duncan 1887); and, another small 6-armed species. Individuals emerged from the rubble due to stress when exposed to the dry environment for an extended period of time; therefore, they died due to extreme stress. *O. scolopendrina* was also the major species living at the Vaipahu jetty. However, one *M. longipeda* individual was observed under a rock and subsequently burrowed into the sand.

The jetty habitat had the highest abundance of ophiuroids, followed by the mangrove marsh and Motu Tiahura (Table 1). The brittle stars at the motu had the highest population percentage of regenerating arms. The highest population percentage of intact arms was found at the motu (Table 3).

LOC	N	NI	NR	N Unk
M.T.	40	16	23	1
M.M	28	3	15	10
V.J.	126	34	56	36

Table 1. Abundance, number of individuals with all intact arms and regenerating arms. LOC = Location; M.T. = Motu Tiahura; M.M = Mangrove marsh, Haapiti; V.J. = Vaipahu jetty; N = total number of individuals; NI = number of individuals with all intact arms; NR = number of individuals with regenerating arms; N Unk = unknown regeneration.

Individuals observed to be regenerating one or two arms were prominent at the jetty and motu, whereas only one individual from the mangrove marsh was regenerating either one or two arms (Table 2). Ophiuroids regenerating three arms were observed at a higher population percentage at the marsh, followed by the motu and jetty (Table 2). Few individuals from Motu Tiahura or the Vaipahu jetty were found to be regenerating four or five arms. However, the majority of the population sampled at the mangrove marsh was regenerating four or five appendages (Table 2).

LOC	I R	2 R	3 R	4 R	5 R
M.T.	12	7	3	1	0
M.M	1	1	4	4	5
V.J.	32	10	11	2	1

Table 2. Number of individuals regenerating one or more arms. Locations are the same as Table 1. 1R = regenerating one arm; 2R = regenerating 2 arms; 3R = regenerating 3 arms; 4R = regenerating 4 arms; 5R = regenerating 5 arms

LOC	% I	% R	% UNK
M.T.	40%	58%	2%
M.M	11%	53%	36%
V.J.	27%	44%	29%

Table 3. Population percentages of intact, regenerating and unknown individuals. Locations are the same as Tables 1 & 2. % I = percent of the population with all intact arms; % R = percent regenerating; % UNK = percent with unknown regeneration

O. sexradia and the other species of 6-armed brittle star were very small, disc diameters ranged from 2-5mm. After a few days of captivity, they were observed to either be cannibalizing each other, or dividing by fission. Small ophiuroids commonly reproduce asexually by autotomizing half of their body, or part of the visceral disc.

Arm regeneration experiment

Average regeneration of the autotomized arm per day and the total length regenerated over the duration of the experiment significantly differed by species and their habitat, $p < .0001$ (Table 4). The average rate of arm regeneration per day was significantly different between *M. longipeda* and both *O. scolopendrina* populations. Similarly, the total regeneration was significantly different between *M. longipeda* and *O. scolopendrina*, but the jetty and motu populations were not significantly different. *M. longipeda* had the highest regeneration rate, followed by *O. scolopendrina* (Fig. 2).
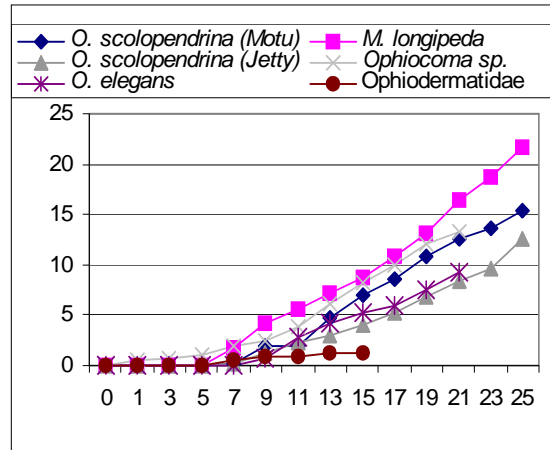

Fig. 2. Total rate of arm regeneration (mm) for the duration of the experiment for all species.

The average regeneration rate of the three ophiuroid groups had a strong positive relationship ($R^2 = 0.64$) for the duration of the experiment, implying that the regeneration rate increased with each subsequent day (Fig. 3).
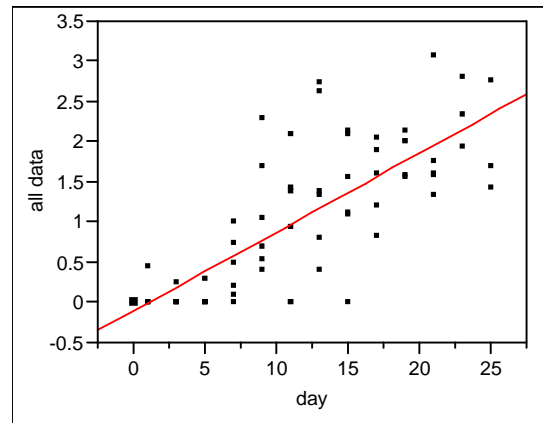

Fig. 3. Average regeneration rate (mm) of motu, marsh and jetty ophiuroids over the duration of the experiment.

Average body size, or disc diameter, was significantly different within the three groups of ophiuroids, $p < .0001$ (Table 4). Furthermore, disc diameter was significantly varied between the ophiuroids from the marsh, motu and jetty habitats (Tukey-Kramer test). As disc diameter increased, arm regeneration decreased, as shown by Fig. 4. The average regeneration rates of all the individuals were weakly, negatively correlated to disc diameter ($R^2 = 0.08$). Similarly, the total regeneration of all individuals was also weakly, negatively correlated to disc diameter, $R^2 = 0.11$, as seen in Fig. 5.
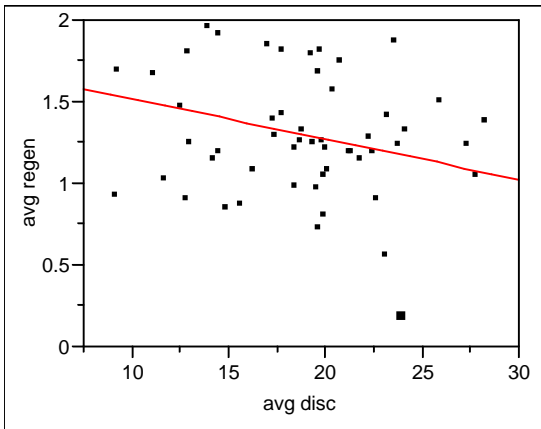
Fig. 4. Average regeneration rate (mm) in relation to disc size (mm) for motu, marsh and jetty ophiuroids.

Fig. 6. Average time elapsed (s) between provocation and autotomization for motu, marsh and jetty populations. M.L. = *M. longipeda*; O.S. = *O. scolopendrina*.
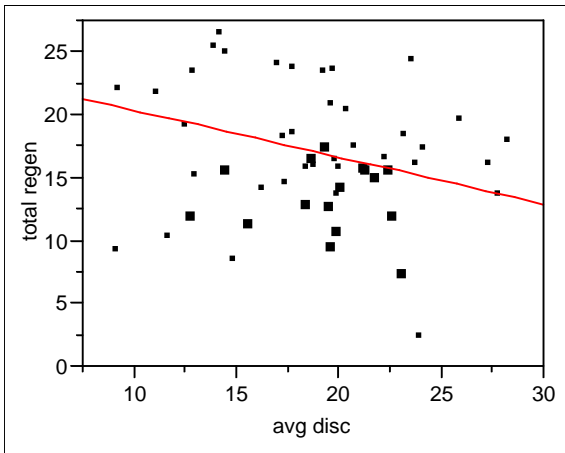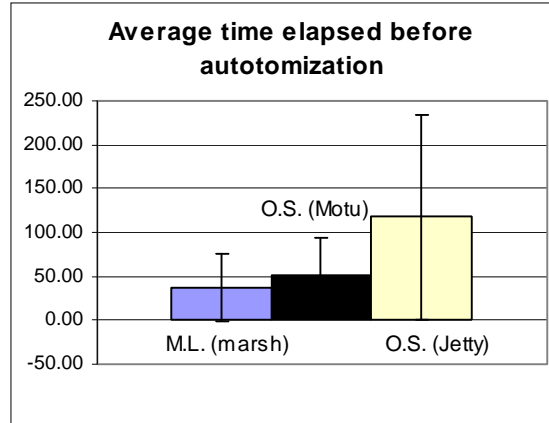

Fig. 5. Total regenerated growth (mm) in relation to disc size (mm) for motu, marsh and jetty ophiuroids.

The time elapsed between provocation and autotomization of the arm was also significantly different among the marsh, motu and jetty brittle stars (Table 4). Specifically, there were significant differences between the times it took for the jetty ophiuroids to release their arms compared to those from the motu or mangrove marsh. However, there was no significant difference between brittle stars from Motu Tiahura or the marsh in the time leading up to autotomization. *M. longipeda* had the shortest elapsed time before releasing the arm (Fig. 6).

The number of segments away from the point of disturbance where brittle stars autotomize is thought to be directly related to type of stress it experiences. The same force and effort was applied to each ophiuroid to provoke autotomization. There were no significant differences between the marsh, motu, or jetty populations in terms of how many segments away from the point of stress it released it arm, $p > 0.2000$ (Table 4).

M. longipeda was simultaneously regenerating multiple arms while also regenerating arm A. There were significant differences in the rates of regeneration of arms B-E, $p > 0.04$ using a one-way Kruskal-Wallis test. However the rates between pairings of each arm using the Tukey-Kramer test showed no significant differences between arm B-E and no difference when paired with the regeneration rate of arm A (Table 4).

The average baseline growth of the intact arm A in the controls was higher in comparison to the average regeneration rate of experimental organisms in the three groups of ophiuroids (Fig. 7).
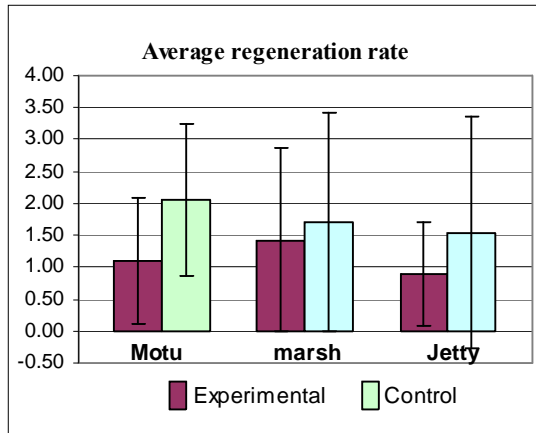
Fig. 7. Average regeneration rate (mm) of study and control individuals

The numbers of ophiuroids collected from the Vaipahu barrier reef crest were not significantly adequate for statistical analyses. However, the preliminary data showed that *Ophiocoma sp.* had the highest total regeneration and rate of regeneration among the species from the reef, followed by *O. elegans* and the ophiodermatid brittle star (Fig. 2 and 8).

Data	Test	p-value	T-K test
Avg disc D	ANOVA	<0.0001*	*M.L.* A
			O.S.† B
			*O.S.*Φ C
T disturb. and auto.	Kruskal-Wallace	>0.0071*	*M.L.* A
			O.S.† A
			*O.S.*Φ B
# seg away auto.	Kruskal-Wallace	>0.2000	*M.L.* A
			O.S.† A
			*O.S.*Φ A
Avg R/day	ANOVA	<0.0001*	*M.L.* A
			O.S.† B
			*O.S.*Φ B
Tot. R	ANOVA	<0.0001*	*M.L.* A
			O.S.† B
			*O.S.*Φ B
M. L. avg R	Kruskal-Wallace	>0.0432*	no arms sig. different

Table 4. Summary of ANOVA and Kruskal-Wallis tests for difference between groups and Tukey-Kramer tests for differences between pairs. * = significant. D = diameter; T = time; auto = autotomization; R = regeneration; *M.L.* = *M. longipeda*; *O.S.* = *O. scolopendrina*; † = motu; Φ = jetty
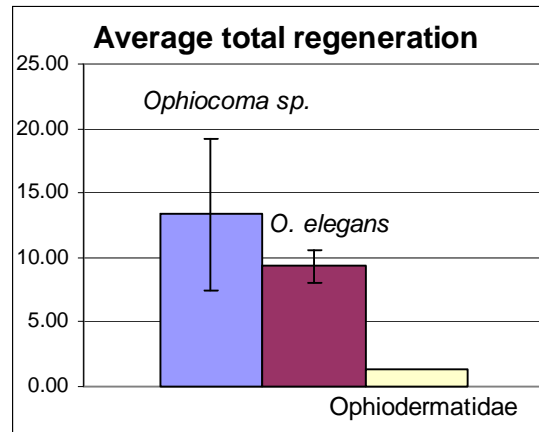

Fig. 8. Average total regeneration (mm) for reef crest species.

DISCUSSION

Natural history

Unique brittle star assemblages were observed in the mangrove marsh in Haapiti, the intertidal area of Motu Tiahura, Vaipahu jetty, and Vaipahu barrier reef crest. Some species, such as *M. longipeda* were only found in one location, suggesting habitat preference. Other species, *scolopendrina*, *O. elegans*, *Ophiocoma sp.*, *O. brevipes*?, *O. erinaceus*?, *O. sexradia* and the ophiodermatid, occurred at different habitats. Distinct characteristics of these habitats, such as water flow, substrate, nutrient resources, and predation most likely influenced the abundance and distribution of these species in Moorea and the motu. For example, *M.* longipeda, only found in the fine sediment at the marsh, probably utilized this habitat because it is a deposit-feeder (pers. obs.) and the fine sand grains had higher nutrient value.

Abundance and density in each habitat could also be mediated by interspecific and intraspecific factors unique to each location, such as predation and territoriality. Hydrodynamics could mediate where ophiuroids were located and what nutrients were available.

The occurrence of *O. scolopendrina* at Motu Tiahura and the jetty could be attributed to the locations having similar habitat. Although the brittle stars at the motu were found under rocks and rubble on the sandy substrate and those at the jetty buried within conglomerate rock and rubble, both

locations were very shallow and the water was calm. *O. scolopendrina* is a suspension feeder, waving its arms in the water column, and a deposit-feeder, like many other ophiuroids (Oak and Scheibling, 2006). The hydrodynamics at the motu and jetty could have been similar, carrying similar nutrients in the water column. The flow rate in combination with the shallow water could have been more damaging to ophiuroids with longer, more fragile arms, for example *M. longipeda*, but benign those with more robust appendages, such as *O. scolopendrina*.

Varying proportions of the population with intact and regenerating arms between habitats could have been influenced by many factors. Burrowing ophiuroids tended to lose arms more readily than epibenthic species (Emson and Wilkie, 1980). *M. longipeda* was observed to be regenerating multiple arms simultaneously. This species was also observed burrowing into rubble and sand. *O. scolopendrina* was not regenerating many arms simultaneously and was observed under rocks or in crevices, and not burrowing. These observations supported Emson and Wilkie's idea that burrowing species more readily lost arms.

Predation and abundance of predators could have affected the number of individuals experiencing sublethal predation and autotomizing appendages. Palatability could have also influenced the portion of the population that is regenerating appendages (Soong et al., 1997).

Behavior such as feeding could correlate to how much exposure a species experiences. Feeding modes could be dependent on habitat and water flow. Therefore, a species that suspension feeds by waving its arms into the water column was more exposed to predators than a species that deposit fed under coral rubble. *O. scolopendrina* is a suspension feeder and deposit feeder and *M. longipeda* is predominantly a deposit feeder. However, *M. longipeda* more readily autotomized. Arms were not as important in deposit feeding as they were in suspension feeding; therefore, releasing arms might not hinder *M. longipeda* feeding.

Arm regeneration experiment

Total and rate of arm regeneration differed by species. *M. longipeda* had a higher rate and total regeneration, while *O. scolopendrina* from the motu and jetty were comparable, as expected. These differences could be attributed to the ecology of each species.

M. longipeda could have had a higher rate and total regeneration because it lost arms more readily. This species' arms autotomized frequently while in the field and had the fastest time between stimulus to autotomization in the laboratory. Arms might not be vital to its ecology; therefore it more readily lost arms to predators or disturbances. Since it lost arms more often and with less severe stress than *O. scolopendrina*, it could be compensating by regenerating at a faster rate; thereby growing a longer new appendage for the duration of the experiment. *M. longipeda* was slower moving than *O. scolopendrina* and burrowed into the substrate as a defense (pers. obs.). Therefore, its best defense against predation could be autotomization. It may have evolved to more readily release it arms to a predator because it could not out-run a predator. In order for the species to maintain its fitness, it also evolved to regenerate that tissue more rapidly. Also, because arms might not be vital in burrowing, arms are often lost as a way to confuse predators and prevent them from burrowing after the brittle star.

O. scolopendrina less readily lost arms and also had a lower total and rate of regeneration. In the field, *O. scolopendrina* was observed to predominately suspension feed. This mode of feeding could largely depend on having intact arms. Therefore, *O. scolopendrina* was more reluctant to autotomize arms. Also, since arms were not lost as readily, fast regeneration would not be as important, as regeneration did not seem to be as prevalent compared to *M. longipeda*. *O. scolopendrina* was also observed to crawl away very rapidly from a predator. Arms were used in locomotion; therefore, they were important in escaping predators and not as easily released (Woodley, 1975).

Rate of regeneration is thought to be dependent on temperature or seasonality and gonad development (Pomroy and Lawrence, 2001). Decreased or no regeneration was observed in other studies during times of colder temperatures (Stancyk et al., 1994; Dobson, 1988). Temperature could be refuted as a cause of the differences in rates of regeneration between species for the duration of this experiment, as the ambient water temperatures were constant between species' enclosures.

Regeneration is also likely food dependent (Skold and Rosenberg, 1996; Stancyk et al., 1994). If there were sufficient nutrients, the brittle stars would not have to use stored reserves to regenerate. Feeding was constant between species; therefore, food availability was most likely not the reason for the differences in regeneration between species. However, dominant feeding modes differed between species. For example, *O. scolopendrina*

was often observed suspension feeding, while *M. longipeda* was predominantly observed to deposit-feed.

Simultaneous regeneration of multiple arms also affected the rate at which growth occurs (Soong et al., 1997). Arms were designated according to their position to the madreporite. Therefore, position differentiation would lead to functional differentiation among the arms. Some arms could be designated for feeding while others are used for gripping the substrate. The feeding arms could be more important to the brittle stars' fitness; therefore they grew back faster. Regeneration could also have been affected my neighboring arms. *M. longipeda* was regenerating multiple arms at the same time at different stages, including the forced autotomization of arm A. There were no significant differences between the growth rates of any of the arms. It was possible that *M. longipeda* did not have differentiation between the five arms. If they all served the same or equal functions, there would be no benefit to regenerate one faster than the others. Also, when multiple arms are regenerating, more energy is required. Therefore, it is logical that the more arms a brittle star is regenerating, the slower the growth will be, due to increased energy expenditure. However, Zeleny (1903) stated that when multiple arms were regenerating, the energy put into replacement of the lost appendages was greatly increased. Accordingly, the more arms regenerating, the greater is the rate of regeneration of each arm.

Regeneration rate increased over time in all species. This observation could be attributed to the undifferentiated stem cells. After the initial healing of the autotomized area, new undifferentiated cells divided and regenerated. As more and more stem cells were formed by division of the generation before, regeneration increased exponentially (Carnevali and Bonasoro, 2001).

Disc diameter was significantly differed between the groups of ophiuroids. The biology and physiology of the *M. longipeda* could be different than that of *O. scolopendrina* in terms of size. Also, because there was a disc size disparity among the groups, regeneration rates were normalized for body size.

Regeneration rate decreased with body size when marsh, motu and jetty ophiuroids were analyzed. This correlation suggested that smaller individuals had a greater regeneration rate. This could be an adaptation for survival. Smaller individuals might be pressured to grow large faster in order to compete. Or, because smaller individuals did not have as much biomass to sustain, more energy was put into regeneration. This relationship was contrary to other studies. Zeleny (1903) concluded that there was a relationship between the size of the brittle star and the rate of arm regeneration. The maximum rate was achieved by medium sized individuals and lower for both small and large individuals. Manner et al. (1960) stated that size had no effect on regeneration in salamanders. There was no relationship within each species of brittle star.

Autotomization occured when the arm separated from any intersegmental joint proximal to the stressed area (Wilkie, 1978). No significant differences were observed in the number of segments away autotmization occurred from the point of disturbance for the marsh, motu or jetty ophiuroids. Autotomization was likely dependent on the type of stress. Since the provocation of autotomization was constant throughout the populations, it could explain why there was no difference between them. Therefore, this characteristic might not have been species specific or dependent on habitat within these selected populations. However, other brittle stars like amphiurids, tended to autotomize significantly farther away from the stimulus (Wilkie, 1978).

As expected, baseline growth in the controls was greater than the regeneration rate of the autotomized arm for all the groups of brittle stars. The control animals did not undergo the stress of losing an arm nor have to recuperate. They had all five functioning appendages and did not have to put in extra energy or siphon energy from other processes to regenerate an arm.

CONCLUSION

This study collected nine different species from four locations in Moorea and its motu. Undoubtedly they were more ophiuroids inhabiting the island within the surveyed habitats and those not in this survey.

In the field, different species of brittle stars commonly had between 20-100% of the population with regenerating individuals (Wilkie, 1978). The *M. longipeda* population at the mangrove marsh in Haapiti and the *O. scolopendrina* population at Motu Tiahura had over half of the individuals regenerating at least one arm. *O. scolopendrina* at Vaipahu jetty was found to have 44% of the population regenerating at least one arm. *M. longipeda* was commonly regenerating multiple arms, mostly with 4 or 5 regenerating simultaneously. Multiple regenerating arms were less common in both *O. scolopendrina* populations. The differences in regenerating proportions of each

population were likely due to habitat characteristics and ecology of each species.

The experimental regeneration study yielded data that supported the hypothesis that regeneration rates differ between species. *M. longipeda* had a faster regeneration rate and a greater total regeneration than *O. scolopendrina* from both Motu Tiahura and the Vaipahu jetty. However, the two populations of *O. scolopendrina* did not have significantly different regeneration rates.

Differences in regeneration rate and length of regeneration were most likely due to ecological, physiological and biological factors of each species. Habitat, feeding mode, predation, available nutrient resources, body size, and the simultaneous regeneration of multiple arms have been suggested as influences on regeneration.

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