ADAPTIVE REGENERATION OF OPHICOMA SCOLOPENDRINA (ECHINODERMATA: OPHIUROIDEA) UNDER TWO FEEDING TREATMENTS IN MOOREA, FRENCH POLYNESIA

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Abstract. Regeneration is common amongst all classes of echinoderms and is easily studied in the laboratory with abundant and tractable ophiuroid specimens. However, ophiuroid regeneration rates, both between species and within the same species, can exhibit uncommonly high variation even under identical laboratory conditions. Eighty-four *Ophiocoma scolopendrina* (Lamarck 1816), a brittle star which extends its arms out into the water column to suspension feed, were collected from the upper-intertidal zone of rocky beaches on the island of Moorea, French Polynesia for laboratory regeneration experiments. To determine the effects of position of autotomy and feeding level on regenerated length and differentiation of new biomass, two treatments were performed: an amputation treatment either 5 mm from the central disk or 30 mm from the distal tip; and a feeding treatment of high-food or low-food. Additionally, a field survey was conducted to assess predation pressure and autotomy of *O. scolopendrina* in upper-intertidal zones. This study demonstrated that *O. scolopendrina* are well adapted to the high levels of sub-lethal predation at the arm tips by quickly resuming the ability to detect predators via photo and chemoreception. The results of this investigation can help to better inform calculations of energy cycling in ecosystems and has potential applications for further research on the connections between ophiuroid life-history and predation pressure on variable regeneration rates.

Key words: ophiuroid; brittle star; echinoderm; regeneration; intact arm; regenerating arm autotomy; *Ophiocoma scolopendrina*; *Amphiura filiformis*

INTRODUCTION

Autotomy and regeneration after sub-lethal disturbance has long been a topic of scientific inquiry (Dinsmore 1991, Wilkie 2001, Sides 1987, DuPont and Thorndyke 2006, Sköld & Rosenberg1996, Bowmer and Keegan 1983), occurring across many phyla including: annelids, arthropods, cnidarians, echinoderms, and molluscs (Pomory and Lawrence 1999). This unique developmental process involves cell turnover and tissue repair, replacement of lost structures after self-induced or traumatic mutilations, and even complete regrowth of whole individuals from body fragments (Candia-Carnevali 2006). The potential for regeneration is expressed to the greatest extent in the five classes of echinoderms (Candia-Carnevali et al. 2007), which provide accessible models for the study of regeneration.

The brittle stars (Ophiuroidea), even more so than most echinoderms, are famous for their regenerative capacity (Bowmer and Keegan 1983). Ophiuroids from the Arctic to the Antarctic have a significant proportion of individuals regenerating at least one arm throughout the year (Lawrence and Vasquez 1996). This continual regeneration requires energy beyond that needed for the processes of maintenance, growth, and reproduction (Pomory and Lawrence 1999). Changes in behavior, reproductive success, and susceptibility to predation can all result from this energetic cost (Woodin 1984, Smith 1992).

*Ophiocoma scolopendrina* (Lamarck 1816) is a dominant species on shallow, intertidal reef flats throughout the tropical Pacific, Japan, and the Red Sea (Tamura & Tsuchiya 2010). *O. scolopendrina* represents an important link between decomposers, primary producers, and higher level predators by removing bacteria and macroalgae particles from reef ecosystems (Tamura and Tsuchiya 2010; James and Pearse 1969; Oak and Schiebling 2006). This species exhibits a unique combination of feeding behaviors, with suspension feeding (arms extended in the water column) being the primary method
However, *O. scolopendrina* also engages in deposit feeding and surface film feeding (associated with neuston and detrital particles in seafoam during high tide), as well as irregular scavenging (Oak and Schiebling 2006; Tamura and Tsuchiya 2010).

Like most ophiuroids, *O. scolopendrina* is highly vulnerable to predation, mostly by visual predators such as crabs, shrimp, and predatory fish (Hendler 1984). In fact, predation, not storms or other physical disturbances, is the main cause of arm autotomy and regeneration in tropical ophiuroids (Sköld and Rosenberg 1996). Therefore, many brittle stars including *O. scolopendrina* expose only one to two arms at a time when capturing food (Aronson 1991; Munday 1993; Oak and Schiebling 2006). On Moorea, over half of the individuals in *O. scolopendrina* populations have been observed to be regenerating at least one arm (Chinn 2006).

This shallow water species provides a tractable model for studying regeneration in tropical reef ophiuroids. Regeneration rate in this species has already been investigated on Moorea (Chinn 2006). As Chinn 2006 found, regeneration often produces unexpectedly high variability, even among individuals under identical laboratory conditions (Salzwedel 1974, D'Andrea et al. 1996, Nilsson and Sköld 1996). Building off of the findings of Dupont and Thorndyke 2006, I hypothesize that the variability in *O. scolopendrina* can be explained by the location of the point of autotomization. Since suspension feeding is the primary mode of food capture for *O. scolopendrina*, it is important to have arms functional for feeding as soon as possible after sub-lethal predation. Thus, if autotomy occurs close to the oral disc, *O. scolopendrina* may sacrifice differentiation for the quick regrowth of tissue needed to resume feeding, allocating energy to biomass regrowth only. However, if autotomy occurs at the distal tip of the arm, the star may allocate energy into differentiation of the cells of the re-grown tip, restoring chemo and photoreception functionality (DuPont and Thorndyke 2006).

In order to test this hypothesis, I investigated the role of location of autotomization relative to the oral disk and its effects on regeneration rate and differentiation in *O. scolopendrina*. Additionally, I investigated the effect of food availability on regeneration rate and arm differentiation, hypothesizing that a starvation treatment will hinder both regeneration and differentiation.

To complement my experimental data, a field survey of two locations with high densities of *O. scolopendrina* was conducted to observe the frequency and location of autotomization in a typical community of *O. scolopendrina*. This was the first study to investigate the role of feeding level and the location of autotomy on regeneration rates of a tropical ophiuroid species, and thus adds to a current body of ophiuroid knowledge that is decidedly Euro-centric.

### METHODS

#### Study site

Moorea, French Polynesia (17° 30’ S, 149° 50’ W) is a 1.5 million year old high volcanic island of the Society Archipelago.
located in the southern Pacific Ocean (Fig. 1). The Pihaena jetty (17° 29.110’S, 149° 49.893’W) is located between Cook’s and Opunohu Bay (Fig. 1). The jetty consists of concrete and rock rubble extending 30m from the beach into the ocean. Additionally, the middle section of the jetty has decayed faster than the two ends, exposing some coral conglomerate. Motu Tiahura is located off the northwest tip of Moorea (17° 29.241’S, 149° 54.632’W). A similar white sand beach with dispersed coral rubble and rocks in shallow water characterizes the eastern side of the motu where the field survey took place.

Regeneration Survey

*O. scolopendrina* is restricted to upper intertidal zones in water < 1m deep (Chartock 1983). During the months of October and November 2012, one coastal site on the island and an outlying motu were assessed for *Ophiocoma scolopendrina* density, regeneration frequency, and location of recent arm autotomy. Likely brittle star habitat was visually assessed to determine search area, and an 18 x 2m transect was outlined. All rocks within the 2 meters of the shoreline or jetty in the transect area were overturned to reveal any ophiuroids. For each *O. scolopendrina* collected, the oral disk was measured and the number of arms regenerating was noted. Arm regeneration was easy to distinguish upon visual inspection, with a distinct contrast in the width and color of the arm where autotomy and regeneration was occurring. Arms that showed a recent break with no visible regeneration were not counted and were treated as if they had automized during the course of collection. Additionally, the location of autotomy (proximal, mid-arm, or distal tip) was also recorded. Ophiuroids that were inaccessible in that they could not be collected without damaging arms were counted, but disk length and autotomy location was not recorded.

The total number of arms regenerating was calculated as the sum of the total number of arms observed regenerating divided by the total number of arms at the site (5 arms per star times the number of stars).

Laboratory Manipulations

On September 25th and 26th 2012, 42 intact brittle stars were collected from the Pihaena jetty and surrounding shoreline. Stars were housed in two 55 x 40 cm glass aquariums, with a 19cm depth of flowing seawater pumped directly from the lagoon surrounding the Gump Station. Water temperature ranged from 27-29˚C for the duration of the experiment. Each tank of brittle stars was provided with a 1.5 cm layer of sediment harvested from the jetty area as well as plenty of rocks for cover. All stars were allowed sixteen days to acclimate to the tank environment before experiments were undertaken.

The glass sides of the tank and the standpipe were scrubbed once a week to remove any algae growth and biofilm accumulation. Sediment was changed every two weeks (lining up with the bi-monthly feeding of the low food treatment) to reduce the possibility of obfuscating treatment effects via food addition in the new sediment. To control for variation between the two aquariums, the *O. scolopendrina* in each of the two feeding treatments were swapped between the tanks every two weeks, corresponding with the addition of new sediment.

To help mitigate the effects of possible pseudoreplication at the feeding treatment level, the experiment was doubled at week 2 for a total of four tanks with a total n of 84. Individuals in the second cohort were treated exactly the same as those in the first, and were followed for the final three weeks. Data was handled as outlined in the results section.
**Experiment 1: Laboratory amputations**

Two amputation treatments were performed on intact individuals on October 12, 2012, designated as Day 0. Central disk length was measured and weight pre limb-loss was recorded. All 84 stars, with disk diameters ranging from 17-27 mm, were immersed in a 3.5% MgCl$_2$ anesthetic solution for 8-10 minutes until no movement was detected. Then, experimental amputation was attained by gently pressing a scalpel blade across a natural inter-vertebral autotomy plane (Dupont and Thorndyke 2006). Three amputation treatments were applied to 14 stars each (7 per tank): amputation 5mm from the central disk, amputation 30 mm from the distal tip, and a control group with no amputations. In ophiuroids, each arm possesses equal regenerative capacity (DuPont and Thorndyke 2006), thus different arms were cut (in relation to the madreporite) on different brittlestars for identification purposes.

Regenerated length (RL, see Table 1 for abbreviations) of the amputated arm was
measured (under anesthesia) and recorded every four days using a dissecting microscope and a Fisher® scientific ruler. Every week, the differentiation of regenerates was assessed under a dissecting microscope and photographs were taken (Fig. 3). Around 2-3 weeks after amputation it was possible to divide the regenerated length into two distinct parts (Fig. 4): the proximal differentiated length contacting fully developed ossicles, podia, and spines; and the remaining distal portion with poorly developed segmentation and undefined podia and spines. The proportion of the regenerated length that was completely differentiated (0 if undifferentiated, 100 if fully differentiated) was divided into the total RL to create a DI out of 100 (Table 1). According to DuPont and Thorndyke 2006, DI is a good indicator of the timing of neuropeptide expression and recovery of the physiological nervous system.

Experiment 2: Feeding effects

Two feeding treatments were investigated: a “high food” treatment in which the treatment group was fed every day, and a “low food” treatment group, which was fed every two weeks. For each feeding, 2.1 g of Tetra® fish food was provided: 0.1 g per individual (Pomory and Lawrence 1999), or an average of 1.89 kJ d⁻¹ for the high food treatment and 0.135 kJ d⁻¹ for the low food treatment. The circulating natural seawater coming from the lagoon adjacent to Gump Station undoubtedly provided additional nutrient input to the two tanks, and individuals were switched every two weeks to compensate for any variation in water flow between the two tanks. RL was measured every four days and DI was assessed every week, as outlined previously in Experiment 1.

Statistical Analysis

Since the same individuals were being measured at evenly spaced periods over time, a two-way repeated measures MANOVA was used to investigate the effect of time as well as the two treatment effects: feeding level and amputation treatment, on the two dependent variables: RL and DI. This created four tests of interest: RL by amputation treatment (Fig. 6), RL by feeding treatment (Fig. 5) DI by amputation treatment, (Fig. 8) and DI by feeding treatment (Fig. 7). All tests were analyzed with an α<0.05. To test for the sphericity of variance, Mauchly’s sphericity of variance test (1940) was used. A Greenhouse-Geisser correction factor (1959), considered the most conservative correction for violations of sphericity, was applied to any data that failed Mauchly’s test. Since there were three amputation treatments, a Tukey-Kramer post-hoc analysis was used to distinguish the source of significantly different results after the repeated measures MANOVA. All statistical tests were run using JMP® 10 software (SAS Institute Inc. 2012).

RESULTS

Regeneration Field Survey

Motu Tiahura and the Pihaena Jetty, two sites with large populations of Ophiocoma scolopendrina, had similar distributions of both total arm regeneration rates and the location of arm autotomy (Fig. 2). In total, 89.5% of O. scolopendrina observed at Motu Tiahura showed evidence for regeneration (n=84), while the number was 88.9% at the Pihaena Jetty (n=109). The number of arms regenerating (out of all arms total at each site) was 42.3% at Motu Tiahura and 43% at the Pihaena Jetty.

To establish background data and to investigate potential differences in the position of arm autotomy in the field, the number of arms regenerating proximal to the
disk, mid-arm, and at the distal tip was compared. At both sites, the dominant location of arm autotomy was at the tip, followed by mid-arm and the central disk (Fig 2). The distribution was similar across sites.

**Experiment 1: Laboratory Amputation**

A repeated measures MANOVA was conducted that examined the effects of amputation position on regenerated length (RL; see Fig X. for abbreviations) over the duration of the experiment. Because the data violated Mauchly’s sphericity of variance test (p<0.001) a Greenhouse-Geisser correction (1959) was applied. There was a significant difference (F (2, 25) = 12.44, P<0.001) between the mean regenerated lengths of the three cutting treatments: 5mm CD, 30mm DT, and control (Fig 6). *O. scolopendrina*, who had undergone the 30mm DT cutting treatment had the highest mean RL, 8.79 mm at the end of 32 days, contrary to the initial hypothesis that the 5mm CD treatment would produce the highest RL. The 5mm CD cutting treatment produced the second highest mean RL (5.77 mm) with the control group growing the slowest (2.5 mm), as expected. In other words, the group amputated at 30 mm DT had an on average 34% longer regenerates than the
5mm amputation treatment. A Tukey-Kramer HSD revealed that the mean regenerated lengths for all three cutting treatments were significantly different from one another (P<0.05). The repeated measures estimate also revealed that there was a significant effect of time (F(7,19)=16.97, p<0.001) on RL. No significant interaction term was encountered between cutting treatments and feeding level, the treatment applied in experiment 2.

The same repeated measures MANOVA was performed to assess the effects of amputation position on differentiation of the regenerated arm (Fig. 8). The DI was significantly higher for the 30mm cutting treatment (F(1,19)=9.49, P<0.007) than under the 5mm CD treatment (a DI of 78 versus 67), as expected. The Greenhouse-Geisser correction factor was applied. There was also a significant effect of time of DI (F(7,19)=16.96, P<0.001). No significant interaction term was observed between cutting treatment and feeding level (F(2,25)=2.32, P=0.12).

**Experiment 2: Feeding effects**

As in Experiment 1, a repeated measures MANOVA was performed, this time analyzing the effect of feeding level on RL. *O. scolopendrina* had significantly longer regenerates under a low-food treatment (7.62 mm) than under a high-food treatment (3.74 mm) with the Greenhouse-Geisser correction applied (Fig. 5, F(1, 25)=11.98, P=0.0019). There was a significant effect of time (F(7,19)=16.97, p<0.001) as seen in Experiment 1.

Again, a repeated measures MANOVA was carried out to determine the effect of feeding level on differentiation of the regenerate (Fig. 7). The regenerated portion of *O. scolopendrina* arms under the low food treatment (DI of 80.7) had a higher DI every week than regenerates under a high-food treatment with an average DI of 63.4 (F(1, 19)=12.02, P=0.003). There was also a significant effect of time (F(4,16)=9.57, P<0.001).

**DISCUSSION**

**Regeneration Field Survey**

The results of the field survey were startlingly similar across the two transect locations at Pihaena jetty and Motu Tiahura (Fig.2). The finding that approximately 43% of the total *O. scolopendrina* arms are regenerating at any given location suggests the existence of a high predation pressure. As expected, many common ophiuroid predators such as fish, crustaceans, and other echinoderms (Stewart 1996, Emson and Wilkie 1980, Sides 1987, Duineveld and Van Noort 1986) were observed in the upper intertidal zone favored by *Ophiocoma scolopendrina*, including an abundance of small crustaceans and juvenile wrasse (pers. obs.).

Furthermore, the incidence of *O. scolopendrina* regenerating one or more arms, between 85-90% at each site, is higher than a previous survey conducted on Moorea (Chinn 2006). However, this rate is consistent with other surveys of ophiuroid regeneration (DuPont and Thorndyke 2006, Bowmer and Keegan 1983, Clements et al 1984, Sides 1987, Stewart 1996, Sullivan 1988) where as little as 5% of the population was observed to be intact (Clements et. al. 1994, Duinevald and Van Noort 1986).
The location of arm autotomy was also similar across the three O. scolopendrina habitats, with approximately 70% of regenerating arms autotomizing at the tip, 20% at mid-arm, and around 10% close to the oral disk (Fig. 2). The ability of ophiuroids to autotomize between vertebral ossicles at any point along their arm (Stöhr 2012), as well as O. scolopendrina’s role as a rubble-dwelling suspension feeder may explain this pattern. For example O. scolopendrina tends to expose one to three arms at a time when feeding, with the central disk well hidden under rubble or in crevices (Oak and Schiebling 2006). Thus, grazing fish and crustaceans have easiest access to arm tips suspended the furthest into the water column. In fact, ophiuroid tissue is a common finding in gut content analyses of tropical wrasses and other grazing fish (Morton et al 2008, Wainwright 1988). The dominance of tip regeneration undoubtedly affects the energetic cost of regeneration that O. scolopendrina undergoes, as compared to other ophiuroid species of differing life histories.

To put the numbers in perspective, there were 545 arms at the Pihaena Jetty, (~15 arms m-2) sweeping the water column at any given time, removing bacteria, algae, and dinoflagellates from the detritus pathway and turning it in to material that can be used by secondary and tertiary consumers without the loss of the ophiuroid itself. The results of this field survey indicate high-predation pressure in this small ecosystem, therefore energy as ophiuroid biomass is constantly being turned over and transferred. Thus this tissue loss may mean an overlooked, renewable source of secondary production in soft-bottom, benthic habitats (Clements et al 1994). Since calculations of standing biomass and production do not often include estimates of regenerated echinoderm tissue, researchers may be omitting an important source of energy for fish, shrimp, and crabs, with repercussions on the accuracy of energy transfer all the way up the trophic ladder.

In addition, the prevalence of tip regeneration suggests that the transfer of biomass across trophic levels from detrital material to higher-level consumers is affected by the location of arm autotomy, with more biomass being transferred through ophiuroid species vulnerable to full-arm removal (i.e. benthic burrowers) than in upper-intertidal zones dominated by species such as O. scolopendrina. Only a few studies (De Vlas 1979, O’Connor et al. 1986, Pomory and Lawrence 2001) have attempted to estimate the amount of energy from regenerated biomass available to predators, and this knowledge is non-existent in the upper-intertidal zone of the tropics.

Experiment 1: Laboratory Amputations

Significant differences were found among the amputation treatments, with arms cut close to the distal tip both regenerating and differentiating faster than arms amputated close to the central disk and in the control group. This indicates that the position of arm amputation can explain significant variability in observed regeneration rates, both in the field and in the lab.

The results of this experiment contradict the only other published study on this topic and the basis for my hypotheses. In DuPont and Thordyke’s 2006 study on A. filiformis, they found that ophiuroid arms regenerate faster when amputated close to the disk. Presumably this was because ophiuroids can partition energy use between growth and functionality (differentiation) based on location of autotomy, and since a short arm is useless for food capture, the energy is allocated solely towards recovery of the length of the arm. However, I propose that because ophiuroids possess this unique ability to partition energy during the regeneration process (Fielman et al. 1991) that O. scolopendrina has evolved a mechanism to mobilize energy more efficiently to its distal tips for regrowth and differentiation.

Predation is the major selective pressure in coastal tropical ecosystems (Johannes 1978), and from the field survey we are able to conclude that O. scolopendrina faces a high incidence of sub-lethal predation at its distal tips. Thus, it is entirely possible that O. scolopendrina has developed a mechanism to mobilize resources, most likely via the nervous system, to recoup both length and differentiation at the tips. Amphiurid ophiuroids, such as A. filiformis (the study organism of Dupont and Thordyke) have higher frequencies and faster rates of arm regeneration, suggesting that they are better adapted for the regeneration process. Lending credence to the evolutionary explanation, Pomory and Lawrence (1999) suggested that habitat and predator environments may not influence only behavioral changes as previously though, but physiological ones as well.
The most likely location of evolutionary change due to the selective pressures of predation is the ophiuroid nervous system. The nervous system serves as the source of many cells in the regeneration process as well as the initial promoter of regeneration (Candia-Carnevali and Bonsaro 2004). Neural secreted factors have been hypothesized to control the rate of differentiation during regeneration (Candia-Carnevali and Bonsaro 2004), thus these factors may play a key role in O. scolopendrina’s ability to quickly regenerate and differentiate their distal tips. Additionally, the distal tip’s role in detecting predation via photo and chemoreception (Rosenberg and Lundberg 2004) further underscores the need for quick regrowth and full functionality under high-predation conditions, but the mechanism for this process remains unknown.

Experiment 2: Feeding effects

Significant differences were also found when the relationship between feeding level and RL and DI were analyzed. Again, contrary to my hypotheses, the laboratory experiments revealed that O. scolopendrina under a low-food treatment had significantly higher RLs and DIs than those under a high-food treatment. However, unlike Experiment 1, these findings do not contradict the established literature. Pomory and Lawrence (1999) found that under starvation conditions, the regeneration of another Ophiocoma species, O. echinata, also increased. These findings support Fielman et al. (1991) who contended that under limited energy conditions, ophiuroids would devote energy to regeneration in order to increase the capability to capture food.

Pomory and Lawrence (1999) also showed that O. echinata under starvation treatments demonstrated a decrease in gonad and stomach material, which presumably was broken down and incorporated in the processes of regeneration and differentiation. However, Amphirurid ophiuroids cannot rely on reserves from the stomach and gonads since they often autotomize their entire disk, necessitating the use of energy reserves from the arms. This suggests that the mechanisms of ophiuroid tissue break down and reallocation is not fully understood and further emphasizes the physiological differences that are present between temperate Amphirurid ophiuroids and their tropical Ophiocoma counterparts.

Conclusion

This study attempted to explain some of the variation in ophiuroid regeneration by testing the effects of amputation position and feeding level. However, the results of this study contradict the nascent existing literature, further complicating a problem that has dogged ophiuroid researchers for decades. Future research focusing on the effects of predation history on regeneration rate and differentiation patterns is necessary to fully understand possible evolutionary adaptations to specific predation pressures. Additionally, as the repeated measures MANOVA showed, both the slope of RL and the DI changed over the duration of the five-week experiment. However, in a study on another Ophiocoma species Sullivan (1998) estimated that it would take 315 days to regenerate one full arm. Therefore long-term studies are needed to capture any patterns in regeneration and differentiation that occur after the five-week mark.

Understanding regeneration in echinoderms is vital to the proper quantification of energy transfer dynamics in tropical reef ecosystems. Since ophiuroids such as O. scolopendrina are an often overlooked renewable source of secondary production in coastal and pelagic ecosystems, studies that can quantify biomass available to predators via regeneration estimates can help better inform marine managers. Additionally, further understanding of the migratory stem cells (coelomocytes and amoebocytes) in the radial water canal of ophiuroids and how the neurally secreted factors, which control the activation and differentiation of regenerated tissue, evolve under the different predation pressures highlighted in this study has potential application for the manipulation and activation of human stem cells.

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