MEDICINAL PLANT EFFECTS ON THE REGENERATION AND RESPIRATION RATES OF BRITTLE STARS

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Abstract. Wound-healing medicinal plants have been used to increase skin regeneration rates in humans and rats by up to 80%. This study explored the extent of this treatment in a different class of deuterostomes, the echinoderms. It focused on understanding energy allocation of three species of brittle stars under four medicinal treatments by measuring arm regeneration rates and respiration rates. *Barringtonia asiatica* halted arm regeneration in *Ophiocoma scolopendrina* specimens entirely, while there was a 42% increase in average regeneration rates from the negative control group to the *Thespesia populnea* treatment group. Neither extended or acute medicinal plant exposure uniformly altered respiration rates, though there was a trend towards expanded energetic input in the *B. asiatica* and *Musa x paradisiaca* treatment groups in both respiration studies. Different wound–healing medicinal plants have varying effects on brittle star regeneration and respiration rates and may influence an organism's overall energy budget.

Key words: brittle stars; energy budget; Ophiocoma scolopendrina; *medicinal plants;* Thespesia populnea; Barringtonia asiatica; Aleurites moluccanus; Musa x paradisiaca; *regeneration;* Ophiocomella; Ophiomyxidae; *respiration; echinoderms; Moorea*

INTRODUCTION

The simplified Dynamic Energy Budget Theory has been an explanation for metabolic organization and energy allocation, stating that the food energy entering a single organism is equal to the energy allocated for the organism to do work (Sousa et al. 2010). One of these activities all individual organisms perform is growth. Growth includes cell enlargement, cell division, or regeneration of part of the organism (Cho et al. 2001). Ultimately, a portion of the energy from an organism's food intake would be allocated to its growth and regeneration. The aerobic energy resulting from food intake is directly related to the metabolic rate of the organism (Kleiber 1947). In addition, respiration rate measures the oxygen consumption of an organism per unit time per unit of body size and is thus related to the metabolic rate of the organism (Zeuthen 1953). Both refer to the aerobic conversion, through the electron transport chain, of an input source into the energy molecule adenosine triphosphate which the organism uses to do work (Cooper 2000).

Medicinal plants have been known to provide a wide array of uses including treating sickness, providing nutrients, and healing wounds. Wound-healing medicinal plants have been used for centuries to repair various skin abrasions, skin infections, skin pains, and other problems (Han 1998). By increasing the rate of wound healing, these medicinal plants have been known to reallocate a portion of the overall energy budget of a human in order to perform this activity faster. Historically, medicinal plant preparation was sacred and only spread through oral tradition (Petard 1972). Specifically, *Thespesia populnea, Barringtonia asiatica, Aleurites moluccanus,* and *Musa x paradisiaca* have been used in French Polynesia to soothe and repair skin in humans (Petard 1972; Murphy, personal communication).

These four medicinal plants have been studied extensively in chordates for their ability to expedite the healing process. *Thespesia populnea* was observed to increase the rate of wound healing and tensile strength in mice by 79.19% (Nagappa and Cheriyan 2001). Additionally, a single application of *Barringtonia asiatica* leaf extract to mice wounds resulted in full wound repair in half the time it took wounds of the same size with no treatment to heal (Musa 2011). *Musa* spp. sap has also been known to increase the rate of wound recovery in mice by thirty percent (Priosoeryanto et al. 2008). Lastly, *Aleurites moluccanus* nut oil was used to heal and treat wounds, cuts, and other skin ailments (Young et al. 2005).

Though the precise mechanisms of the healing processes of these four medicinal plants has not been studied, they increase the rate of epidermal regeneration in the class

Chordata. Deciphering whether this trend is also applied to Ophiuroidea, another class within the same clade of deuterostomes, will be explored. Though humans regenerate only one organ and ophiuroids also regenerate associated muscle, neural, exoskeletal, and somatic tissues, the treatments for both could be uniform (Dupont and Thorndyke 2006). This study aimed to develop medicinal plants in a broader scope, to expand respiratory and regenerative capacities, in order to fill this scientific gap.

Ophiocoma scolopendrina was specifically chosen as a study organism due to its observably fast and full regeneration, shared deuterostome phylogeny with humans, and previous use in modeling human stem cell development (Holm et al. 2008). Additionally, *Ophiocoma scolopendrina* was not known to readily autotomize, or release arms to escape stressful situations (Chinn 2006).

Brittle star regeneration is categorized into a mix of two of the three types of regeneration: morphallactic and epimorphic (Czarkwiani et al. 2013). Undifferentiated pluripotent cells are mixed with dedifferentiated myocytes to form a regenerative blastema structure, which in turn regenerates the brittle star arm (Biressi et al. 2010). Morphallactic regeneration was known to involve the repatterning of existing tissue with little additional growth, while epimorphic regeneration would regenerate an arm completely (Gilbert 2000). Together, complete regrowth of the brittle star arm would be achieved. This organism displayed strong characteristics for this study, previously regenerating at a relatively quick rate, recorded up to 3 mm/day (Chinn 2006). This species was found to be abundant in French Polynesia and does not burrow, providing easier collection techniques. Further development connecting treatments, biological processes, and experimentation between humans and brittle stars will help develop the extent of the latter in future medicine. *Ophiocomella* sp. was then also chosen for its small size and familial relation to *Ophiocoma scolopendrina* to be used in the respiration experiment (Devaney 1974).

This study aimed to survey the island of Moorea, French Polynesia to create a detailed catalog of echinoderms present in *Halimeda* spp. algae as well as to further experimentally develop *Ophiuroidea* brittle stars as a proxy for human medicine by addressing three questions: (1) Which genera of echinoderms live in *Halimeda* spp. algae and how can they be identified? (2) How do arm regeneration rates change with the application of medicinal plants to the site of amputation in *Ophiocoma scolopendrina* brittle stars? (3) How are respiration rates of *Ophiocomella* sp. and Ophiomyxidae sp. affected by medicinal plant application with extended medicinal exposure and acute medicinal exposure, respectively? It was hypothesized that, with the aid of medicinal plant extracts, *Ophiocoma scolopendrina* would experience faster arm regeneration rates. This also suggested that medicinally aided *Ophiocomella* sp. and Ophiomyxidae sp. would exhibit higher respiration rates than non-medicinally aided specimens.

METHODS

Study site

This study was conducted during the months of October and November 2015 on the island of Moorea, French Polynesia in the Society Islands (17°31'59.9"S, 149°49'59.9"W, Fig. 1).

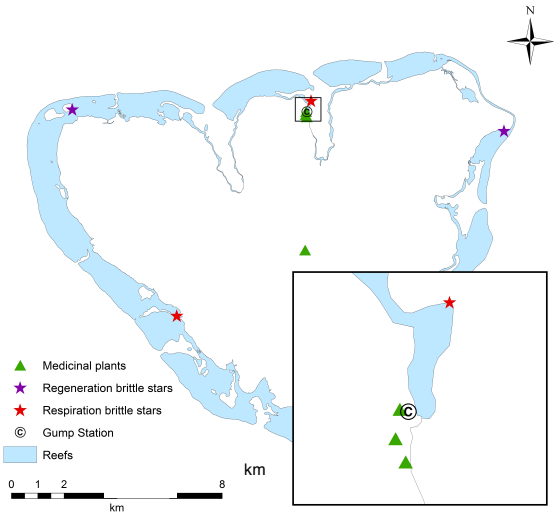

FIG. 1. Sites sampled in this study. Medicinal plants were collected at the same sites for all experiments.

Echinoderm Identification in Halimeda *spp.*

The Moorea *Halimeda* spp. echinoderm identification key and photographic guide was created as a result of collecting ten one gallon Ziploc bags of algae from the fringing reef of Cook's Bay and sorting through it for all echinoderms (Appendix A). The organisms were identified, photographed, and described.

The echinoderms were studied extensively to create the identification key.

Medicinal Plant Study Sites

Five wound healing medicinal plants were selected based on Hinano Murphy's recommendation for medicinal plant extract preparation: *Thespesia populnea, Barringtonia asiatica, Aleurites moluccanus, Musa x paradisiaca,* and *Cocos nucifera,* the latter of which is medicinally used in conjunction with *T. populnea* and *A. moluccanus* extracts (Appendix B-1; Murphy, personal communication). The collection sites were chosen based on proximity to the UC Berkeley Gump Research Station on Cook's Bay. *Thespesia populnea* and *B. asiatica* were found on the coast at Cook's Bay (17°29'30.9"S, 149°49'35.6"W). *Aleurites moluccanus* fruits were collected between the Belvedere and marae trails (17°32'11.0"S, 149°49'46.7"W). *Musa x paradisiaca* sap was collected adjacent to the Berkeley Gump station (17°29'25.76"S, 149°49'35.05"W). *Cocos nucifera* was collected between the Berkeley Gump Station and the Atitia Center (17°29'28.8"S, 149°49'36.6"W).

Medicinal Plant Collection Techniques and Extract Preparations

Plant extracts were prepared following the advice of a local medicinal healer, Hinano Murphy. Four milliliters of each extract were obtained and for the *T. populnea* and *B. asiatica* mixtures with *C. nucifera,* a 1:1 ratio was created.

Yellow sap from *T. populnea* was collected from green fruits on the tree. After 24 hours, 2 mL of the purified extract was separated out and placed into a 4 mL sterile vial. White *A. moluccanus* nuts, or candlenuts, were collected on the ground beneath the trees. Thirty candlenuts were collected, dried in a plant drier for 36 hours at 150°F, until the weights before and after drying no longer fluctuated. After the candlenuts cooled, the outer shell was cracked open, the inner almonds were collected, and the almonds were grinded in a plant blender. Using a 7″ by 7″ cheesecloth, the oil was extracted, allowed to settle for 24 hours, and 2 mL of purified extract was placed into a 4 mL sterile vial. *Cocos nucifera* oil was extracted from the coconut seed of a young tree. The seed was cut in half with a machete, the inner sponge was removed, and oil was extracted from the white remaining substance using cheesecloth. Two milliliters of *C. nucifera* oil were added to the *T. populnea* and *A. moluccanus* extractions to obtain the 1:1 ratio.

B. asiatica extract was obtained by collecting five green fruits from the tree, removing the inner seed, and blending the fruit in a plant blender for four minutes or until uniformly small pieces remained. The orange extract was squeezed through a 7″ by 7″ cheesecloth into a vial and after 24 hours the purified extract was placed into a four mL sterile vial. *Musa x paradisiaca* sap was obtained by sawing open a mature 8-15-foot banana tree trunk. After four minutes, the sap was squeezed out from the upper half of the tree and drained into a vial. The sap settled for 24 hours and the pure sap was removed from the top of the vial and was placed into a 4 mL sterile vial.

A caffeine solution was made to be used as a non-medicinal positive control because of its known properties to stimulate growth in echinoderms (Kindred 2009). Tahitian coffee was produced at a concentration of 100 micromoles using Tahitian Coffee and was placed in a 4 mL sterile vial.

Brittle Star Study Sites and Collection Techniques

For Experiment 1, two coastal sites were selected around the island of Moorea based on abundance of *Ophiocoma scolopendrina*: Motu Tiahura (17°29'17.4"S, 149°54'37.2"W) and Temae public beach (17°29'51.8"S, 149°45'22.6"W). The Motu Tiahura exhibited warm shallow water on the northwest side of the island and consisted of dispersed large (0.5-1.5 meter) rocks. *Ophiocoma scolopendrina* were found on the east side of the motu (Appendix B-2). Temae public beach was on the northeast side of the island with a similar environment. Thirty brittle stars were collected, twenty from the Motu Tiahura and ten from Temae.

The selected specimens were chosen based on a central disc size of 20-25 mm and intactness. Both site collections occurred in less than one meter of water, under rocks. Water temperatures at the Motu Tiahura, however, were slightly higher (1-2°C). *Ophiocoma scolopendrina* did not burrow, which allowed for capture by holding both sides of the central disc.

For Experiment 2, two different coastal sites were selected based on *Halimeda* spp. algal abundance because it was known to contain both *Ophiocomella* sp. and Ophiomyxidae sp.: the Haapiti Mangroves (17°33'34.51"S, 149°52'27.86"W) and the Fringing Reef of Cook's Bay (17°29'11.39"S, 149°49'30.21"W).

Ten one-gallon Ziploc bags were filled with *Halimeda* spp. cumulatively at these two sites, were brought back to the Gump Station, and were sorted until 20 *Ophiocomella* sp. and 20 Ophiomyxidae sp. were identified and collected (Appendix B-3).

Regeneration Experiment

Five specimens were placed in six different 30 cm diameter gardening buckets with two 14 cm^2 ceramic slabs each as cover for 48 hours to allow for acclimatization. The six experimental groups included treatments of: *T. populnea, B. asiatica, A. moluccanus, M. paradisiaca,* a positive control of caffeine, and a negative control of only seawater. Uniform seawater flow was provided to each of the buckets to continually replenish oxygen levels. Holes in the bottom of the gardening buckets allowed for flow out of the buckets and into the large flow tanks. Each brittle star was fed 2-5 3 mm pieces of detritus including dead Copepoda, Gastropoda, and Stomatopoda nightly between 9 and 11 PM. A detailed catalog of descriptions, photographs, and central disc area of each specimen in each group was created for uniform tracking and identification.

All 5 brittle stars from each group were removed from the flow tank and placed into an enclosed tub with seawater. The first brittle star was photographed with a ruler in the frame, one arm was removed with a scalpel five plates away from the central disc, and the brittle star was photographed again. Three drops of the designated medicinal extract were applied using a pipette topically to the cross section of the arm. The medicine was allowed to incubate in a dry tank for 1.5 minutes. The specimen was photographed again if autotomization occurred and was then replaced into its respective bucket.

Because the tubs had to be isolated from each other during medicinal application to avoid cross-contamination, the water in each tub was replaced every 30 minutes by draining the water and then allowing flow into the tub for 25 seconds to keep seawater volume uniform. Twelve hours after the time of application, a second set of the same medicinal plant extracts were applied, though the *T. populnea* group did not receive a second application due to excessive stress. Twenty-four hours after the initial tub isolation, each brittle star was rinsed and returned to the flow tank in its respective tub.

The arm regeneration progress in each brittle star was photographed with a ruler in the frame every other day for 22 days, with amputation on day 0. The regenerating arm length was measured using ImageJ to trace the arm from point of attachment to the edge of the arm (Rasband 1997). Data from day 12 to day 22 were used to calculate the average regeneration rates, standard deviations, standard errors, and confidence intervals (Appendix C-1).

Respiration Experiments

For the long duration medicinal plant treatment respiration study, the twenty *Ophiocomella* sp. were used due to their ability to fit in the respirometer and familial relation to *Ophiocoma scolopendrina*. The specimens were individualized based on the labels of the respirometer (A1, A2, B1, B2) and were allowed to acclimate to labeled 15 mL Falcon Tubes with 0.2 micrometer mesh sheets attached to the opening of the tube using a rubber band for 24 hours. The twenty brittle stars were then placed in the oxoplate and SensorDish Reader to obtain the respiration rates for sixteen minutes before any medicinal plant application (SDR version v38). The seawater added to the oxoplates was filtered through a 0.2 micrometer pipette filter to avoid aerobic bacteria. All twenty of the specimens were then weighed to normalize the respiration rates. Four specimens were exposed to each of the

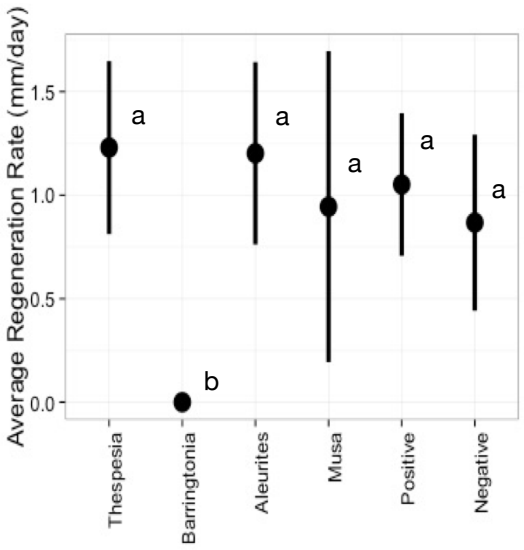

FIG. 2. Average arm regeneration rates of *Ophiocoma scolopendrina* with four medicinal plant treatments, a positive control group, and a negative control group with 95% confidence intervals.

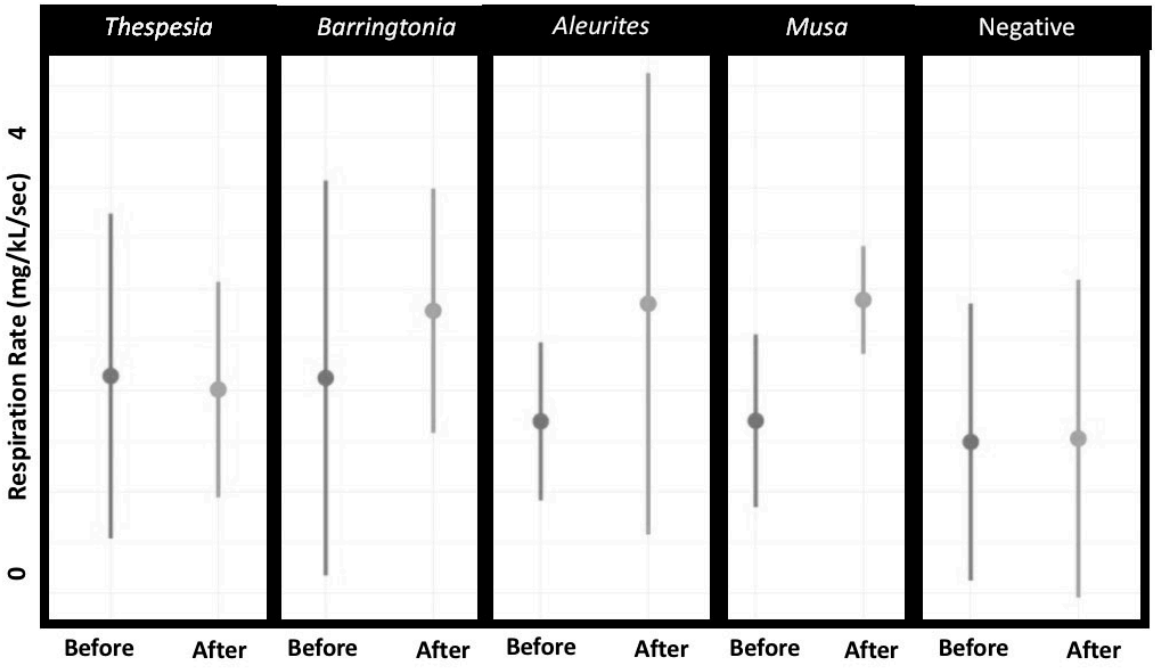

FIG. 3. Average respiration rates of *Ophiocomella* sp. before and after extended exposure medicinal plant treatments and a negative control treatment with 95% confidence intervals.

five different treatments: *T. populnea, B. asiatica, A. moluccanus, M. paradisiaca,* and a negative control group with no medicinal extract. During all respiration data collection sets, four wells were set as blanks, consisting of just sea water and no brittle stars. The medicinal extracts were applied in a 1:10 mL ratio of medicinal extract to seawater. After the Falcon Tubes were placed in the flow tank for 12 hours of medicinal incubation, the respiration rates of the specimens were recorded for sixteen minutes again. The respiration rates of the blanks during the first half of the experiment were averaged and subtracted from each individual specimen's respiration rate and the standard deviations, standard errors, and confidence intervals were calculated for each treatment group (Appendix C-2). The absolute value of the respirometer readings was obtained to demonstrate the specimens' oxygen consumption.

For the short duration medicinal plant treatment respiration study, the twenty Ophiomyxidae sp. were used. The same method was applied to the short duration study as was previously stated for the long term respiration study. The only difference was that the short term respiration study had an incubation period of one hour rather than 12. The respiration rates of the blanks were averaged and subtracted from each individual specimen's respiration rate and the standard deviations, standard errors, and confidence intervals were calculated (Appendix C-3).

Statistical Analyses

There were three sets of data that required statistical analyses in this study: the regeneration study, the long duration medicinal exposure respiration study, and the short duration medicinal exposure respiration study. In the regeneration study, no brittle star arm regeneration occurred until the 12th day and thus, the slopes of the arm lengths per day were calculated starting on Day 12 using the computational program R (R Development Core Team). The treatment group average rates from the regeneration study were analyzed using an Analysis of Variance and a Tukey's Honest Significant Difference posthoc test (Ambrose et al. 1995).

The next two respiration studies were manipulated to look at the first half of the sixteen-minute respirometer recordings because at those times the oxygen levels in the wells of the SensorDish Reader plates was greater than 80%. During all respiration data collection, four wells in the respirometer only contained seawater because at the conclusion of the sixteen minutes, the slopes of the oxygen levels in these blank wells were averaged and

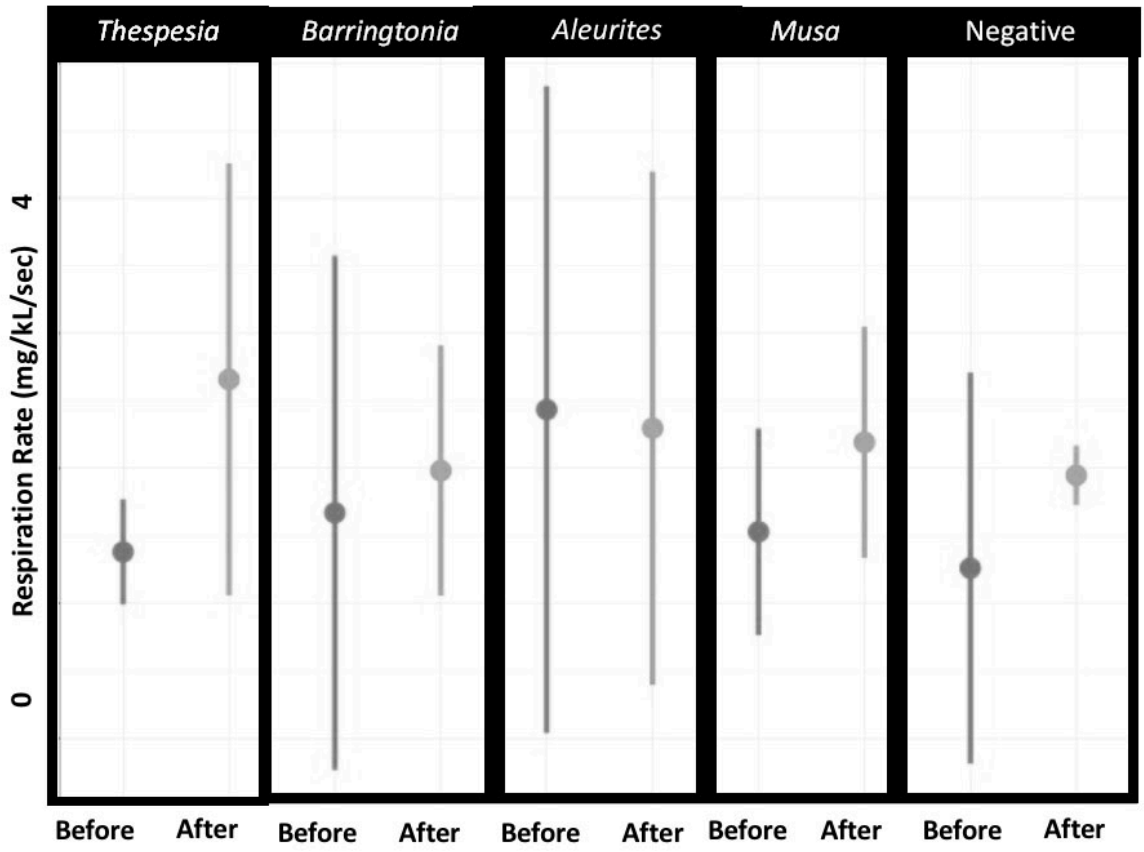

FIG. 4. Average respiration rates of Ophiomyxidae sp. before and after extended exposure medicinal plant treatments and a negative control treatment with 95% confidence intervals.

subtracted from each specimen's respiration rate to account for other processes occurring in the sea water. These respiration rates were then analyzed using a paired T-Test to compare each of the values before and after medicinal plant application (Ambrose et al. 1995).

RESULTS

Echinoderm Identification in Halimeda *spp.*

The Moorea *Halimeda* spp. echinoderm identification key and photographic guide detailed one genus of each of the following classes: Asteroidea, Holothuroidea, and Echinoidea (Appendix A; Horton 2015; Pomory 2007; Stöhr and O'Hara 2007). Six genera of Ophiuroidea were found and identified. Of these six, *Ophiocomella* sp. and *Ophiactis* sp. were the two genera of ophiuroids with six arms and divided by fission, while the other four genera had five arms. There was a prominence of *Ophiactis*, *Ophiocomella*, and Ophiomyxidae. The *Echinometra* sp., the two species of *Ophiocoma*, and the *Ophiarthrum elegans* found in the *Halimeda* spp. were juvenile forms of echinoderms that do not live in algae as mature individuals. The ophiuroids were the most prominent echinoderms found in the algae, followed by the asteroids, while the sea urchin and sea cucumber were least abundant.

Regeneration Experiment

Barringtonia asiatica halted regeneration entirely, compared to the 42% increase in average regeneration rate from the negative control group to the *T. populnea* treatment group (Fig. 2). With the 118% increase in the 95% confidence interval from the positive control group to the *M. paradisiaca* treatment group, *B. asiatica* still resulted in significantly lower average regeneration rates than all other treatment and control groups (ANOVA, Tukey's HSD posthoc test, $p<0.05$, alpha=0.05).

Respiration Experiments

Average respiration rates in *Ophiocomella* sp. in the *B. asiatica, A. moluccanus*, and *M. paradisiaca* increased from the before medicinal application average to the after medicinal application average by 160%, 60%, and 170%, respectively (Fig. 3). The average respiration rates of the *T. populnea* and negative control treatment groups remained constant before and after treatment (6% fluctuations in averages). With large 95% confidence intervals, no pairwise statistical significance was found between respiration rates before and after treatment (one-tailed paired t-test, $p>0.05$).

Average respiration rates of Ophiomyxidae sp. increased in the *T. populnea, B. asiatica, M. paradisiaca,* and negative control groups from 2-18%, but slightly decreased in the *A. moluccanus* treatment group's respiration by 33% (Fig. 4). The *T. populnea* treatment group before medicinal application and the negative control group after data had low variances, but all other groups had larger variances, and thus no correlations were statistically found (one-tailed, paired t-test, $p>0.05$).

DISCUSSION

While brittle stars and humans have recently experienced overlap in molecular studies, topical treatments can be more difficult to quantify as a result of many potential external variables (Dupont 2006). *Thespesia populnea* and *B. asiatica,* the two more common medicinal treatments for wound healing, were expected to have the greatest increase in regenerative rates following this overlap. Conversely, the *B. asiatica* treatment resulted in the lowest regeneration rates of all six groups while the *T. populnea* treatment resulted in the highest average regeneration rates.

Four days after the application of *B. asiatica,* three *Ophiocoma scolopendrina* specimens died, while the other two specimens continued to autotomize their arms. The mechanism of death and rupture of tissues while under uniform conditions with the other treatment groups is a clear indication that the extract itself directly resulted in the death of the specimens. *Barringtonia asiatica* was known to poison fish for centuries, however, this result on echinoderms had not been scientifically explored (Chakraborty et al. 1972). It is likely that the compounds present in this extract caused asphyxiation (Cannon et al. 2004).

T. populnea and *B. asiatica* treatments resulted in the most numerous voluntary autotomizations. These large number of autotomizations could have drastically delayed the regeneration rates because with more arms regenerating at one time, the organism is putting less energy into one arm and is instead distributing energy to multiple arms, resulting in slower bud formation rates (Ramsay et al. 2001). Other treatments did not have detrimental effects and the trend towards increased average regeneration rates with *T. populnea, M. paradisiaca,* and *A. moluccanus* treatments was promising. It is possible that the stressed specimens experienced skewed averages because this added internal factor could have caused an energy reallocation within the organism.

Additionally, brittle star arm regeneration was studied on Moorea in French Polynesia, and *Ophiocoma scolopendrina* previously exhibited much faster regeneration rates (Chinn 2006). Chinn's fifteen brittle stars had an average initial regrowth beginning on day nine, while this study did not exhibit regenerative bud formation until day 12. This difference should be further explored to determine the extent of external factors that may have caused this delay in growth over the past nine years.

The extended and acute exposure respiration studies did not demonstrate statistical significance. However, with a larger sample size it would be possible to see decreased respiration rates in the *B. asiatica* treatment group and increased respiration rates as a result of the other medicinal exposures. Though mechanism of action is unknown in echinoderms, *B. asiatica* was known to inhibit oxygen uptake through fish gills temporarily; the compound known to poison fish is called Ranuncoside VIII, a triterpenoid saponin (Cannon et al. 2004). This inhibits NADH-Q Reductase, prevents the mitochondria from using NADH, halts the electron transport chain and ATP production, and results in asphyxiation (Cannon et al. 2004). A larger sample size may have these trends due to the direct relationship between healing and respiration rates (Patterson et al. 1991). Following this trend, a decrease in respiration rate as a result of *B. asiatica* treatment could actually cause bacteria found in wounds to decrease their respiration rates; this would increase the amount of oxygen present in the tissues to expedite wound healing (Knighton et al. 1986).

The decrease in regeneration rates in the *B. asiatica* treated specimens and the varied trends with other medicinal treatments in both regeneration and respiration rates demonstrate that these medicinal plants have variable effects on brittle stars. No single medicinal plant treatment had consistent effects across all three studies and variance was high, leading to a couple conclusions: some specimens were positively affected by the medicinal plants while others were negatively affected, medicinal plants did increase both regeneration and respiration rates in up to two out of three of the studies, and there may have been other confounding factors to result in such high variances. Altered regeneration and respiration rates found in this experiment could be an indication of healing, stress, or surplus or lack of ATP production and thus a change in energy distribution. This complexity excites future discovery.

In the future, more studies should be conducted to determine why regenerative bud formation was delayed in the past nine years, how brittle star regeneration rates are affected by stress alone, the effects of equal viscosity medicinal plants on the respiration rates of different species of algal brittle stars, and the effect of medicinal secondary compound injection on arm regeneration rates of brittle stars.

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APPENDIX A

Written identification key and photographic guide to common marine invertebrates found in *Halimeda* sp. algae in Moorea, French Polynesia. This identification key is a product of intensive sampling of *Halimeda* sp. algae collected in the fringing reef of Cook's Bay and the Haapiti mangroves (Fig. 1). It includes three classes in the phylum Echinodermata: one species of Asteroidea, one species of Echinoidea sea urchin, one species of Holothurioidea sea cucumber, and seven distinct taxonomic groups of Ophiuroidea brittle stars. Each species contains a description of identification and detailed images. The echinoderms contain identifications based on observations under a stereo dissecting microscope and the imaging software (Leica Stereo Dissecting Microscope, SmartShooter Pro 3). The identification key is organized by trait and the photographic guide is organized alphabetically by genus. Additional identification information is listed in the photographic guide. All photographs were taken by the author.

Ophiuroid characteristics include:

1. Aboral disc: description (granules, skin, scales, spines), diameter
2. Oral disc: oral papillae, description (granules, skin, scales, spines)
3. Arm characteristics: number, length, spine description
4. Arm segments: shape of arm plates, number of spines per side of plate, number of podial scales, comparisons of proximal versus distal arm plate characteristics

Trait Displayed……New Trait Number

1a. Presence of arms ……... 2
1b. Absence of arms …… 9

2a. Arms fused to the central disc …… *Asterina* sp.
2b. Arms not fused to the central disc ……. 3

3a. Presence of five arms …… 4
3b. Presence of six arms ……. 7

4a. Presence of two oral papillae on top of the jaw ……..…… *Ophiocomella* cf. *sexradia*
4b. Presence of one tooth on top of the jaw …… 5

5a. Arm spines tightly wrap arm segment ……..…….. *Ophiactis* sp.
5b. Arm spines protrude laterally, perpendicular to the arm …….. *Ophiactis* cf. *savignyi*

6a. Presence of 3 papillae on each side of the jaw, distalmost papilla larger than other two…….
……..*Amphipholis squamata*
6b. Presence of 3-5 equal-sized papillae on each side of the jaw ……..…… 7

7a. Presence of translucent dental plate on top of the jaw; disc ornamented with granules and spines ……..…… Ophiomixydae sp.
7b. Absence of translucent dental plate on top of the jaw; disk ornamented with granules only ……..…… *Ophiocoma* sp.

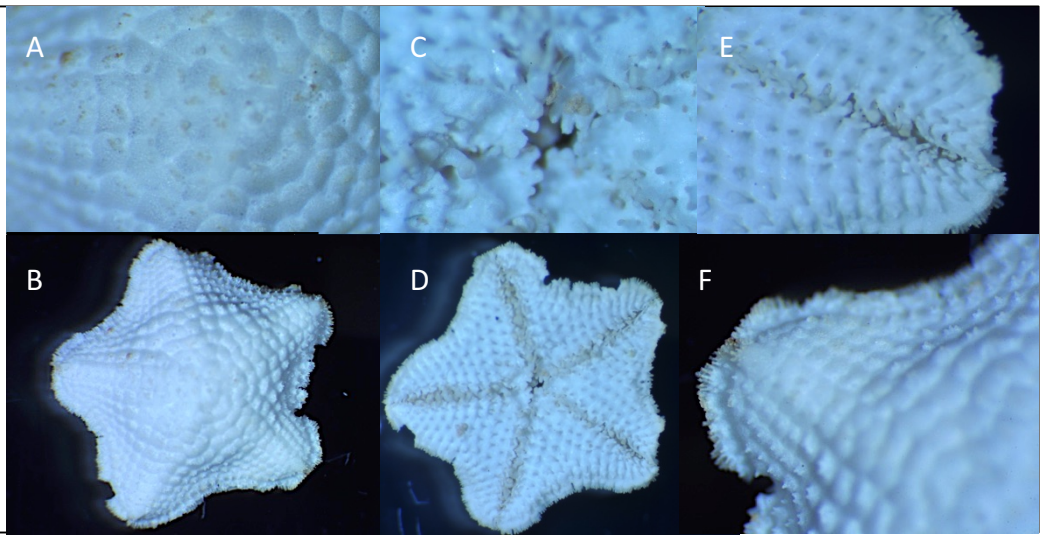
8a. Arms banded with red coloration on every 2-3 arm segments ……..……. *Ophiarthrum elegans*
8b. Arms brown or banded with brown coloration ……..…. *Ophiocoma* sp.

9a. Presence of spines …… *Echinometra mathaei*
9b. Absence of spines ……. Holothuroidea

Class Asteroidea

***Asterina* sp.**
(Emson and Crump 1979)
Identification—
-9-12 mm in diameter
-5 or 6 arms
-6-8 papilla
-Heart-shaped dorsal granules

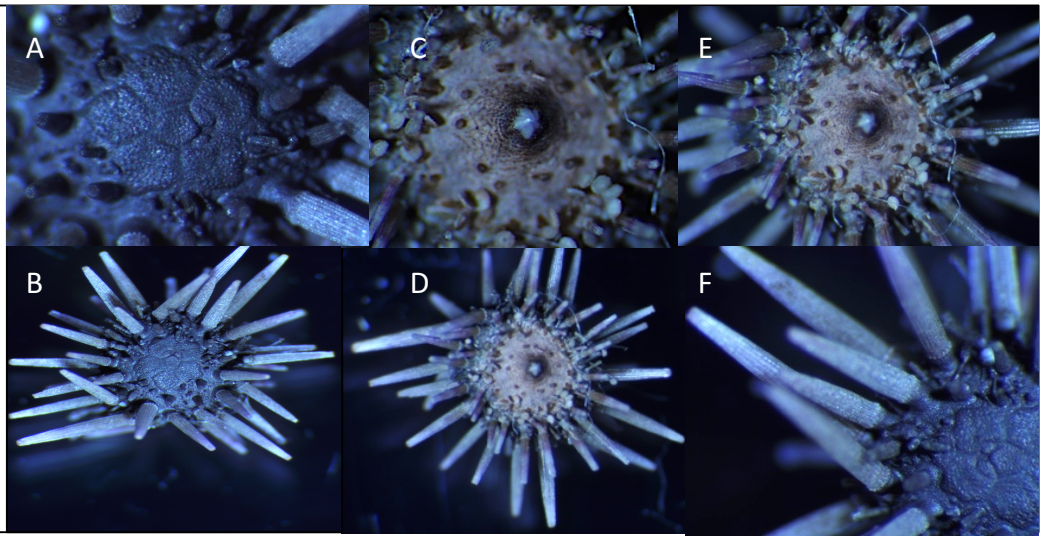
A) Zoomed dorsal
B) Dorsal view
C) Zoomed ventral
D) Ventral view
E) Ventral arm
F) Dorsal arm



Class Echinoidea

Echinometra mathaei
(personal observation)
Identification—
-Purple or green spines
-Flowered dorsal surface

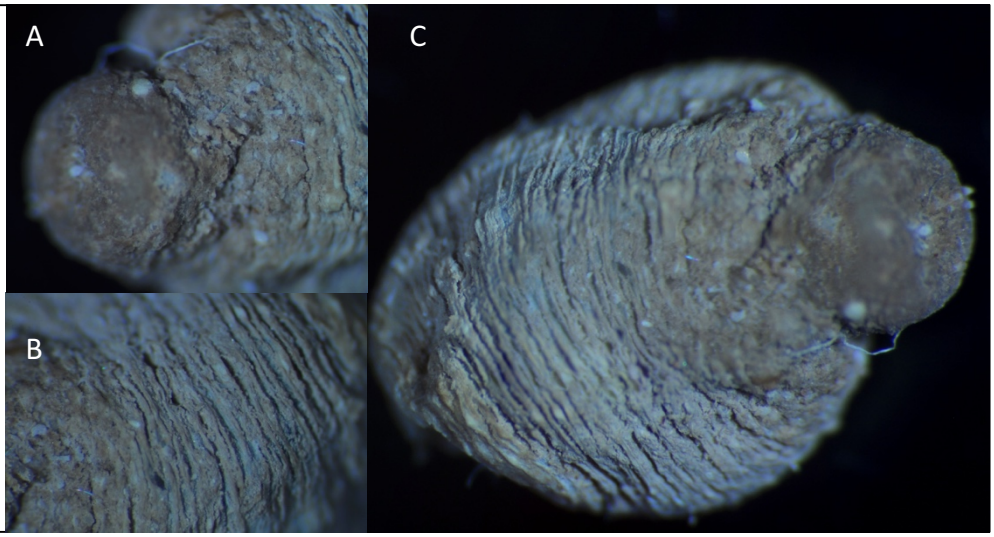
A) Zoomed dorsal
B) Dorsal view
C) Zoomed ventral
D) Ventral view
E) Spines and ventral
F) Zoomed spines



Class Holothurioidea

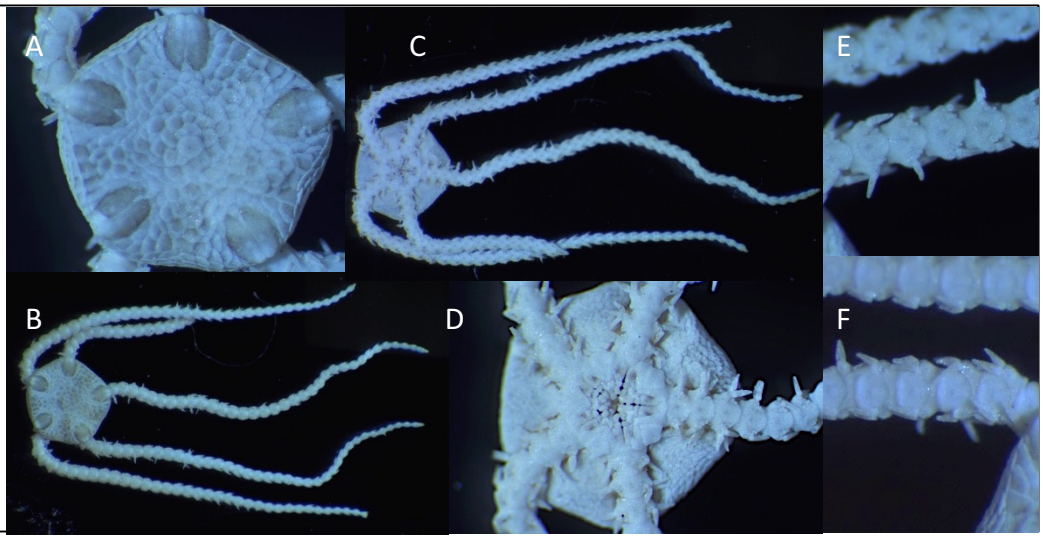
Holothurioidea
(genus and species unknown without cellular analysis)
(personal observation)
Identification—
-Presence of tube feet
-8.9 mm length
-4.8 mm width
-Tan colored

A) Zoomed anterior
B) Zoomed tube feet
C) Body



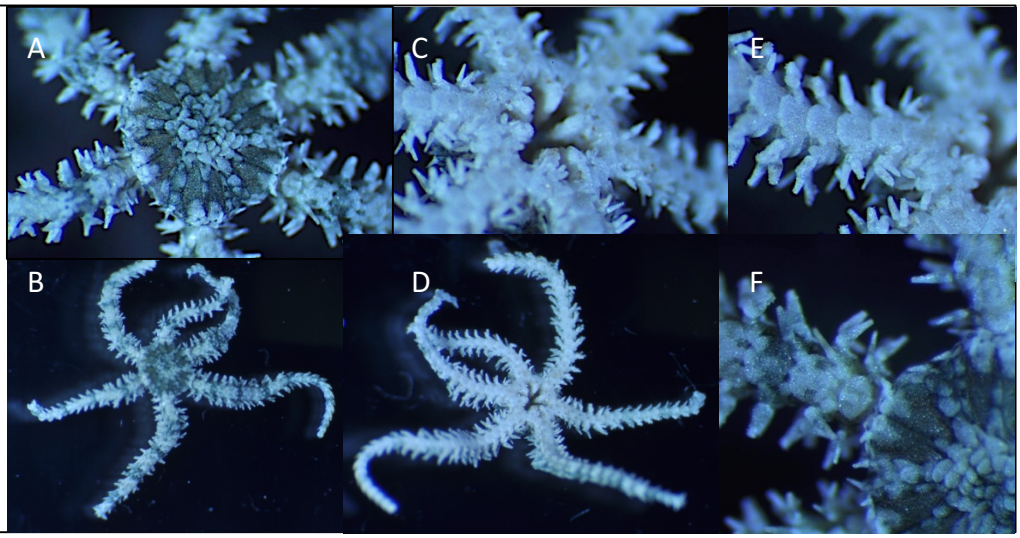
Class Ophiuroidea

Amphipholis squamata
(Pomory 2007)
Identification—
-5 arms
-5 mm disc diameter
-Arm 3-4x disc diameter
-separated plates
-2 podial scales
-6 oral papillae/jaw
A) Zoomed dorsal
B) Dorsal view
C) Ventral view
D) Zoomed ventral
E) Ventral arm
F) Dorsal arm



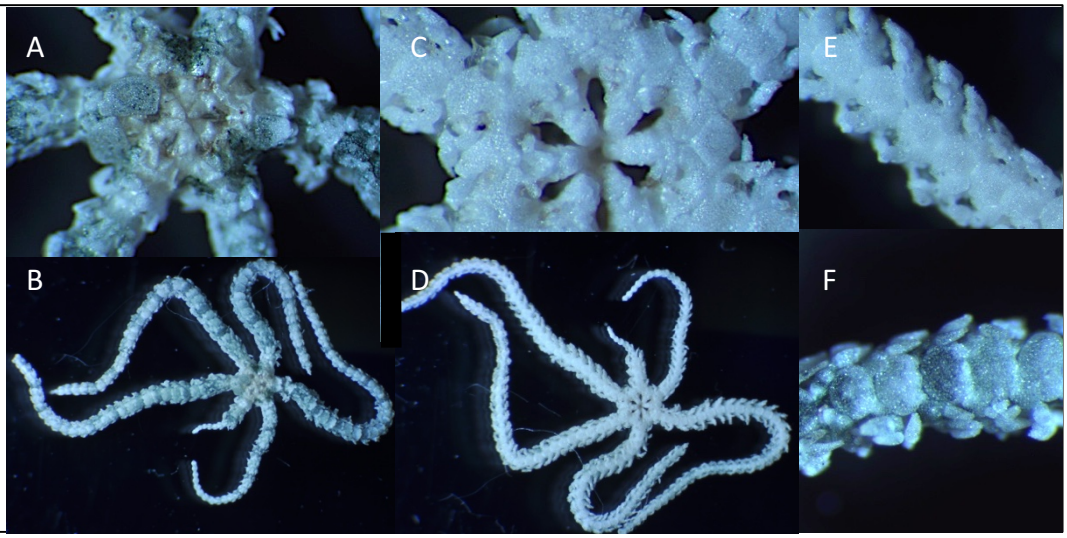
Ophiactis* cf. *savignyi
(Pomory 2007)
Identification—
-6 arms
-5-6 arm spines per side of plate
-4-6 oral papillae/ jaw
-Aboral scales
-Green-tan aboral disc

A) Zoomed dorsal
B) Dorsal view
C) Zoomed ventral
D) Ventral view
E) Ventral arm
F) Dorsal arm

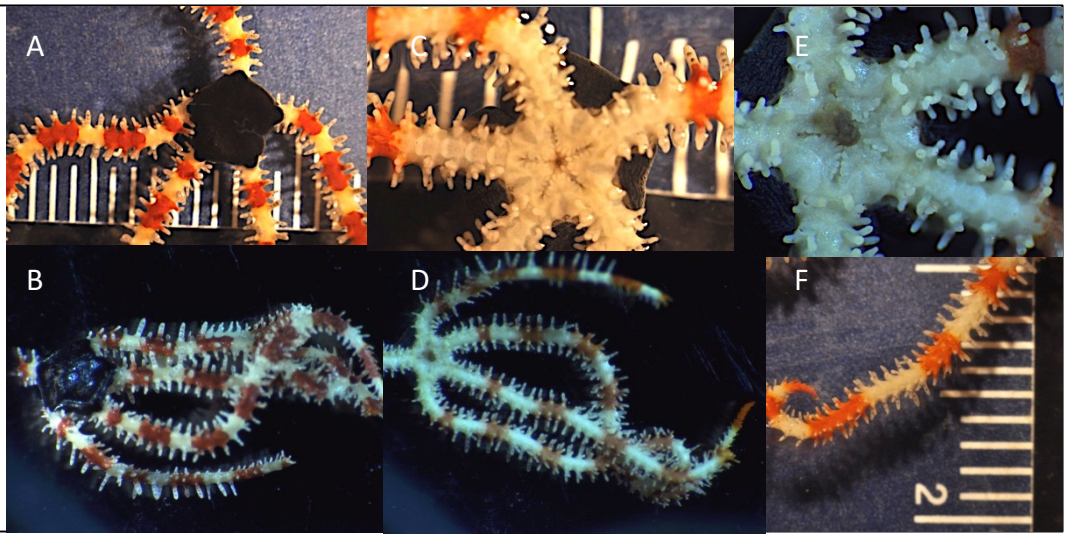


***Ophiactis* sp.**
(Pomory 2007)
Identification—
-6 arms
-Oral papillae at distal corners of mouth
-2-6 oral papillae/ jaw
-No aboral granules

A) Zoomed dorsal
B) Dorsal view
C) Zoomed ventral
D) Ventral view
E) Ventral arm
F) Dorsal arm

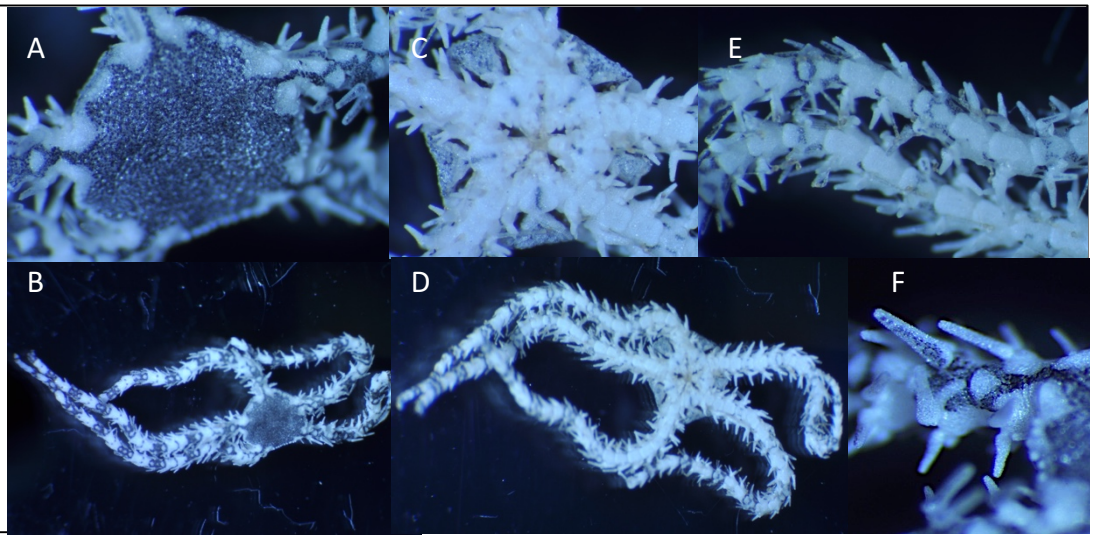


Ophiarthrum elegans
(Stöhr and O'Hara 2007)
Identification—
-5 arms with transverse red marks
-Naked disc
-Patterned arm spines
-Skin covered
A) Zoomed dorsal
B) Dorsal view
C) Zoomed ventral
D) Ventral view
E) Ventral arm
F) Dorsal arm



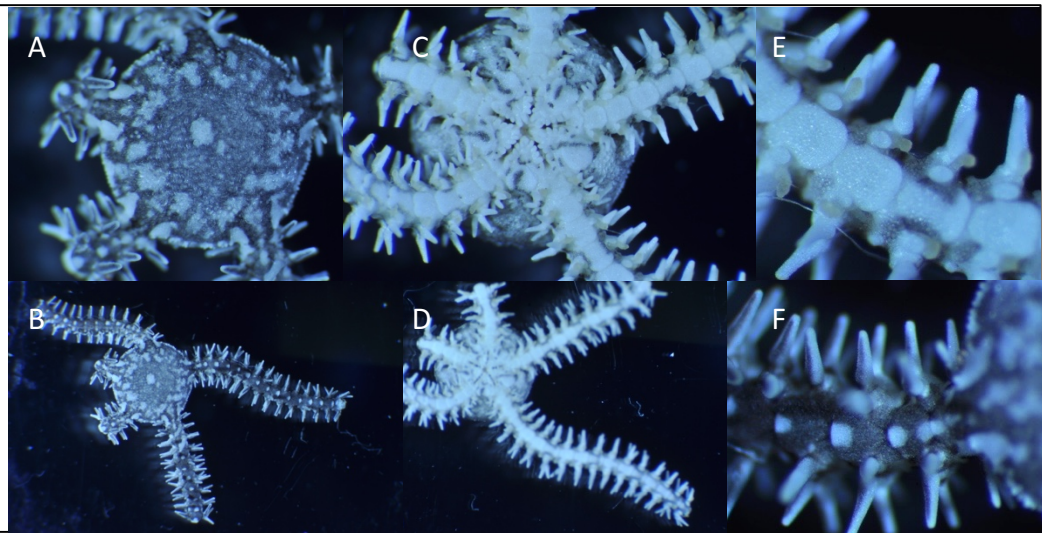
***Ophiocoma* sp.**
Color morph-1
(Pomory 2007)
Identification—
- 5 arms
-1-2 podial scales
-Oral and dental papillae present
-Granulated aboral disc

A) Zoomed dorsal
B) Dorsal view
C) Zoomed ventral
D) Ventral view
E) Ventral arm
F) Dorsal arm

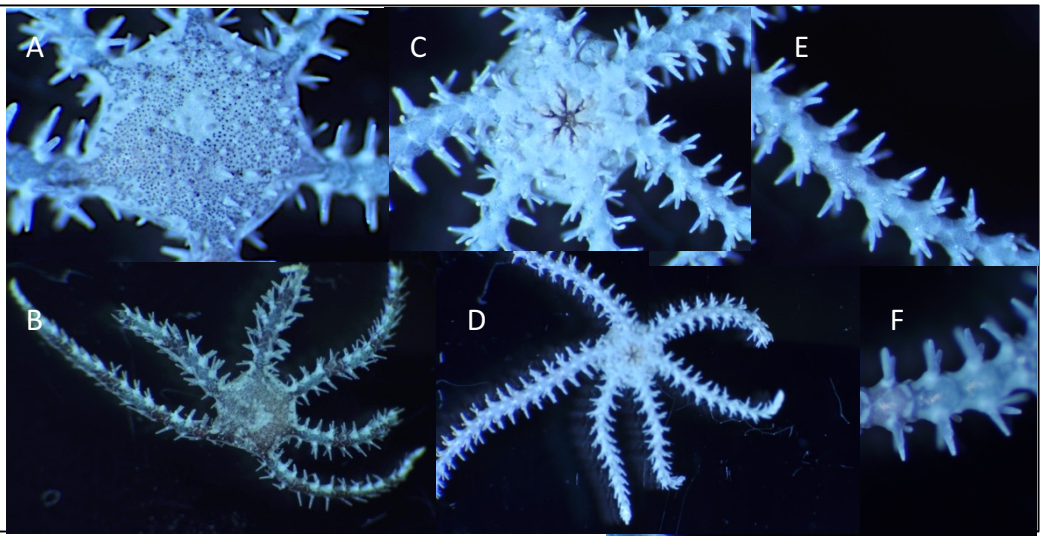


***Ophiocoma* sp.**
Color morph-2
(Pomory 2007)
Identification—
- 5 arms
-1-2 podial scales
-Oral and dental papillae present

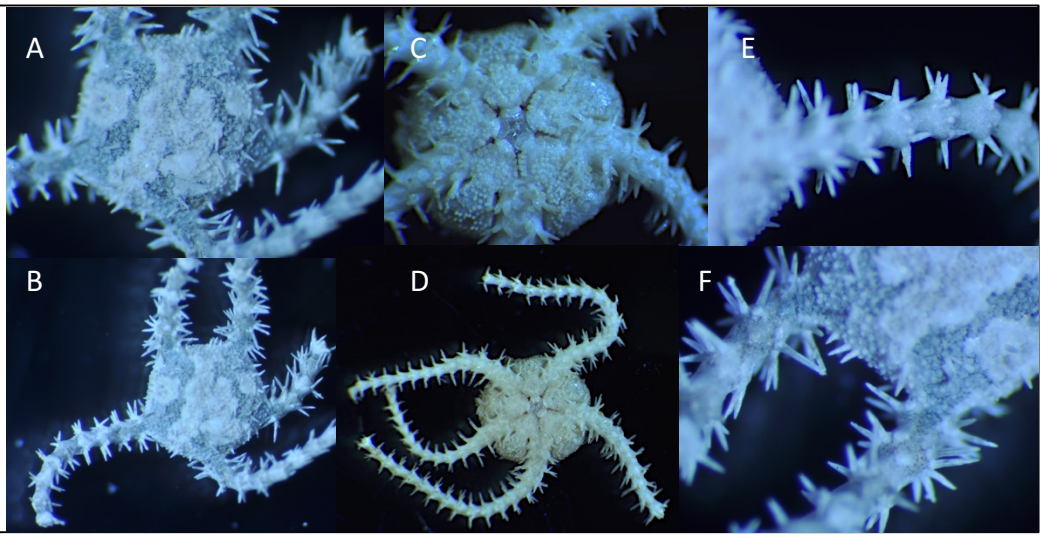
A) Zoomed dorsal
B) Dorsal view
C) Zoomed ventral
D) Ventral view
E) Ventral arm
F) Dorsal arm



Ophiocomella* cf. *sexradia
(Stöhr and O'Hara 2007)
Identification—
-6 arms
-Dark green disc
-4 mm disc diameter
-4 small arm spines
-Disc granules with some larger stumps
A) Zoomed dorsal
B) Dorsal view
C) Zoomed ventral
D) Ventral view
E) Ventral arm
F) Dorsal arm



Ophiomyxidae sp. (genus and species unknown)
(personal observation)
Identification—
-5 arms
-Aboral disc spines
-Granulated disc
-Translucent oral papillae
A) Zoomed dorsal
B) Dorsal view
C) Zoomed ventral
D) Ventral view
E) Ventral arm
F) Dorsal arm



APPENDIX B

Five species of medicinal plants and three species of brittle stars identified and used in this study. All photographs were taken by the author.

1) Medicinal Plants

Thespesia populnea

Barringtonia asiatica

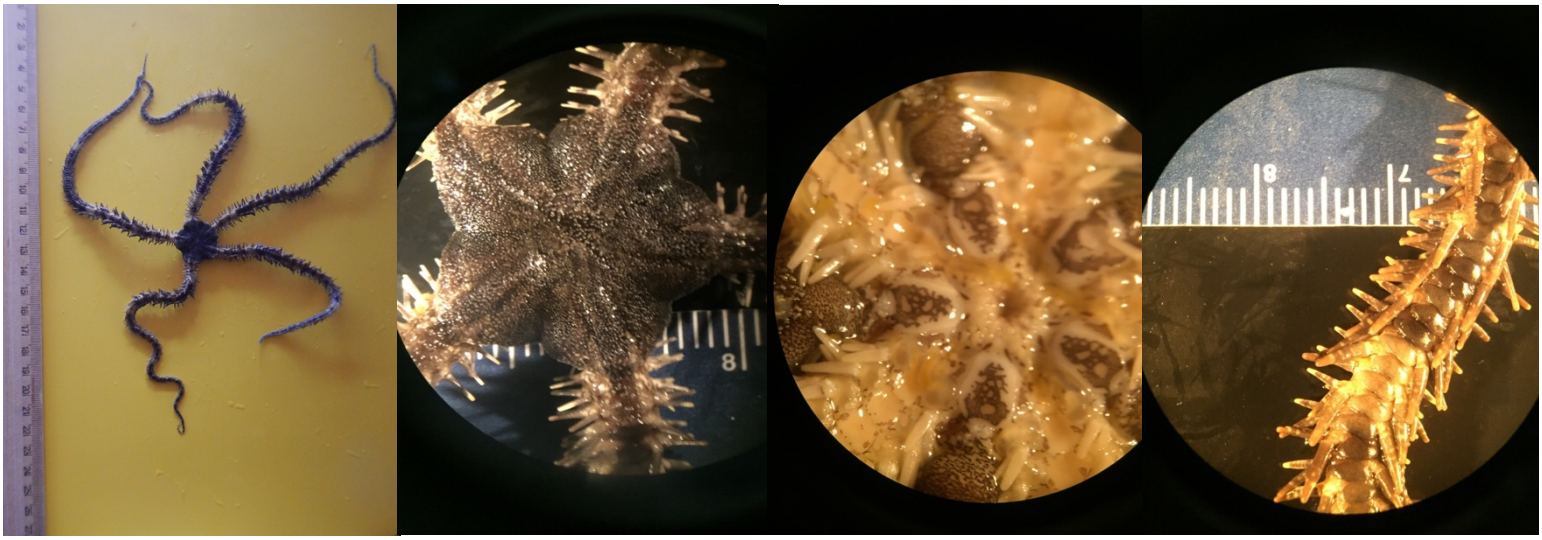
Aleurites moluccanus

Musa x paradisiaca

Cocos nucifera

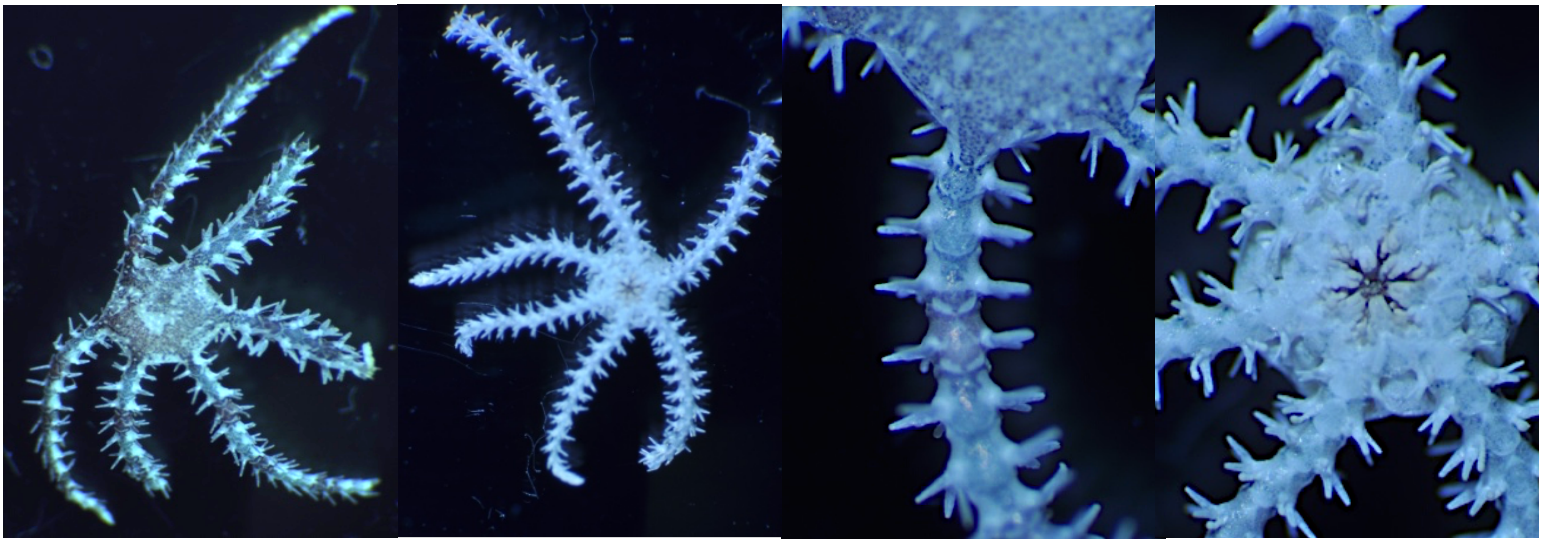
2) Regeneration Study

Ophiocoma scolopendrina

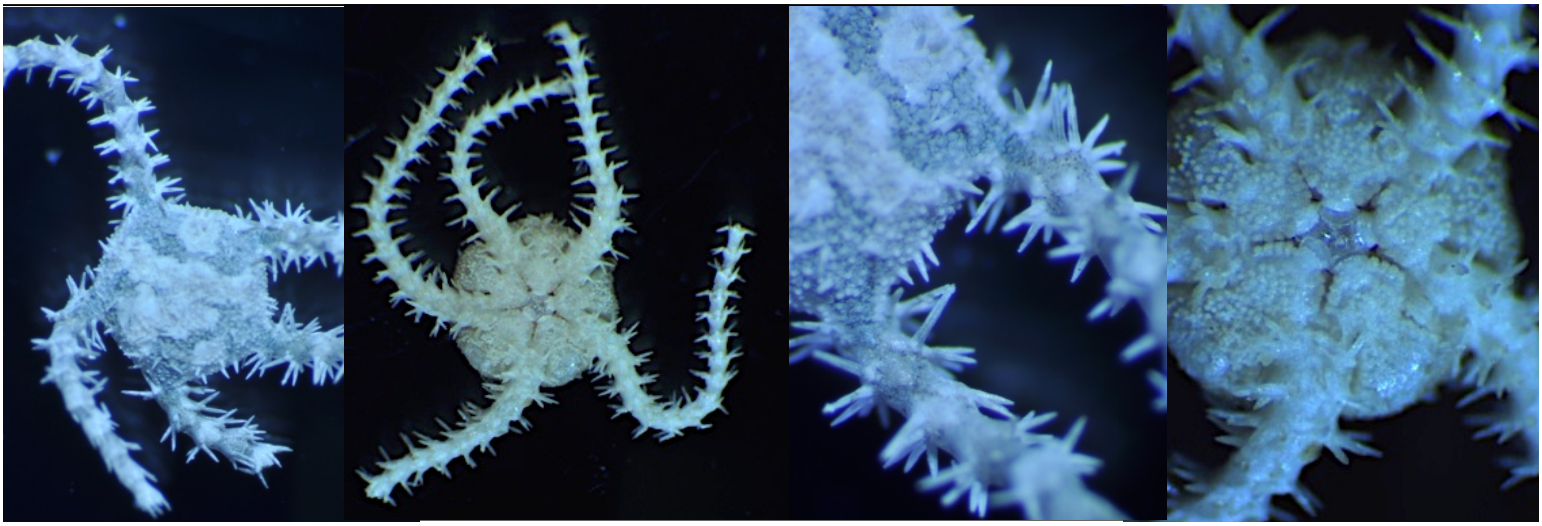


3) Respiration Study

Ophiocomella sp.



Ophiomyxidae sp.



APPENDIX C

Graphs and tables of the *Ophiocoma scolopendrina* arm regeneration data, *Ophiocomella* sp. extended duration respiration data, and Ophiomyxidae sp. acute duration respiration data.

1) Regeneration Data

Graph of arm length per day from day 0 to day 22 of *Ophiocoma scolopendrina* for each individual specimen.

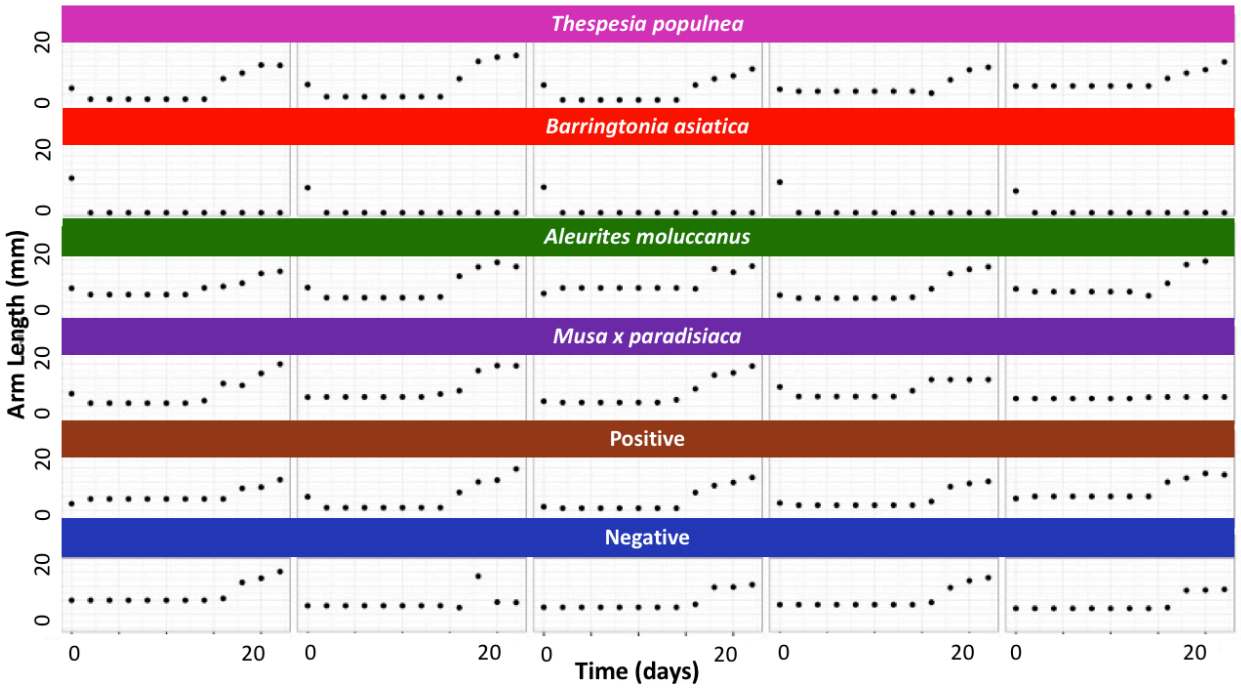


TABLE 1. Average regeneration rates, standard deviations, standard errors, and confidence intervals for six treatment groups of *Ophiocoma scolopendrina*. Each treatment group had five specimens.

Group	Treatment	Average Slope (mm/day)	Standard Deviation	Standard Error	Confidence Interval
1	*Thespesia populnea*	1.23	0.34	0.15	0.42
2	*Barringtonia asiatica*	0	0	0	0
3	*Aleurites moluccanus*	1.20	0.36	0.15	0.44
4	*Musa x paradisiaca*	0.94	0.61	0.27	0.75
5	Positive Control	1.05	0.28	0.12	0.34
6	Negative Control	0.87	0.34	0.15	0.43

2) Extended Duration Respiration Data—*Ophiocomella* sp.

Graph of oxygen level in the respirometer oxoplate wells from minute 0 to minute 8, the first half of the overall duration of the experiment, of *Ophiocomella* sp. for each individual specimen.

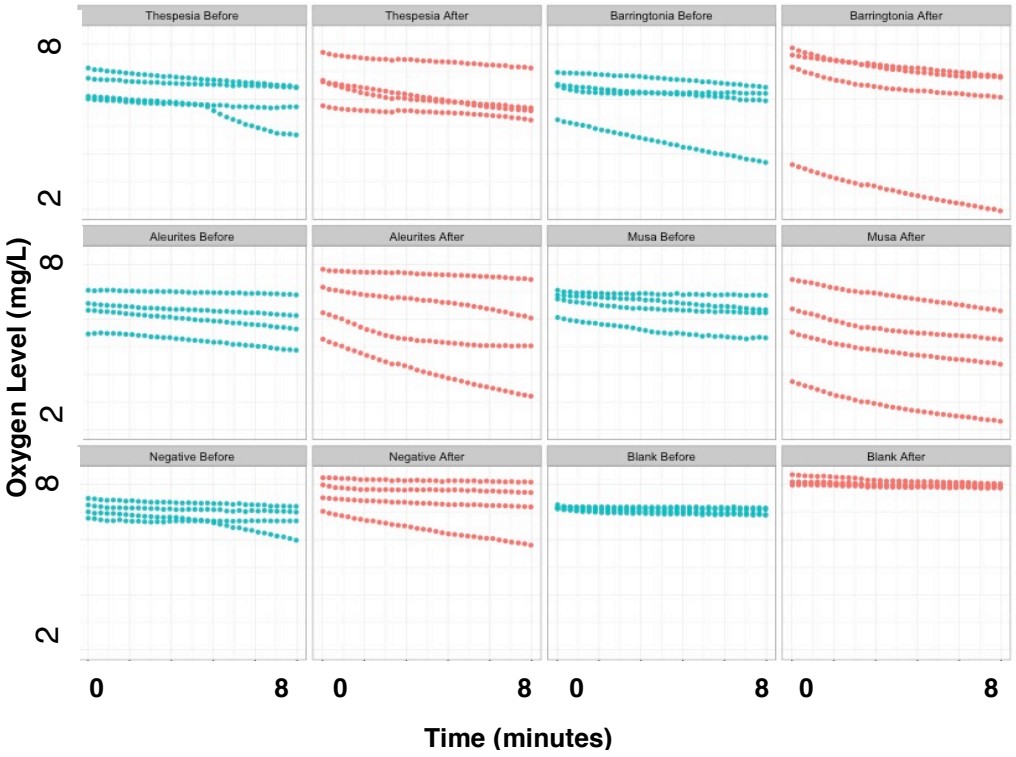


TABLE 2. Average respiration rates, standard deviations, standard errors, and confidence intervals for five treatment groups of *Ophiocomella* sp. before and after medicinal application. Each treatment group had four specimens.

Group	Treatment	Average Respiration Rate (mg/L/sec)	Standard Deviation	Standard Error	Confidence Interval
1 Before	*Thespesia populnea*	0.0011	0.0010	0.0005	0.0016
1 After	*Thespesia populnea*	0.0010	0.0007	0.0003	0.0010
2 Before	*Barringtonia asiatica*	0.0011	0.0012	0.0006	0.0019
2 After	*Barringtonia asiatica*	0.0018	0.0007	0.0004	0.0012
3 Before	*Aleurites moluccanus*	0.0007	0.0005	0.0002	0.0008
3 After	*Aleurites moluccanus*	0.0019	0.0014	0.0007	0.0023
4 Before	*Musa x paradisiaca*	0.0007	0.0005	0.0003	0.0008
4 After	*Musa x paradisiaca*	0.0019	0.0003	0.0002	0.0005
5 Before	Negative Control	0.0005	0.0008	0.0004	0.0014
5 After	Negative Control	0.0005	0.0010	0.0005	0.0016

3) **Acute Duration Respiration Data—**Ophiomyxidae sp.

Graph of oxygen level in the respirometer oxoplate wells from minute 0 to minute 8, the first half of the overall duration of the experiment, of Ophiomyxidae sp. for each individual specimen.

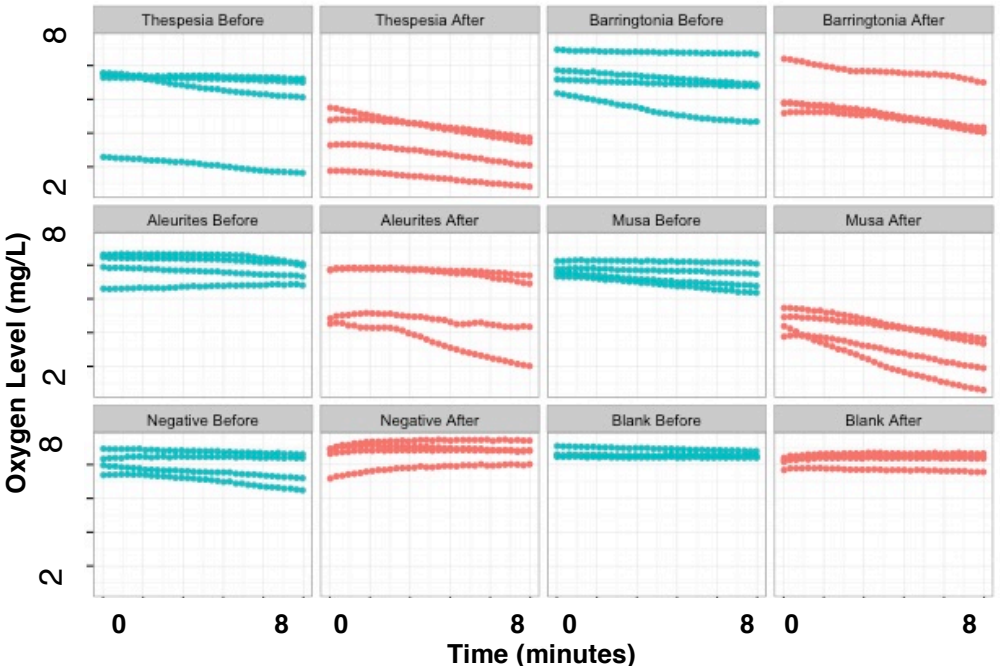


TABLE 3. Average respiration rates, standard deviations, standard errors, and confidence intervals for five treatment groups of Ophiomyxidae sp. before and after medicinal application. Each treatment group had four specimens.

Group	Treatment	Average Respiration Rate (mg/L/sec)	Standard Deviation	Standard Error	Confidence Interval
1 Before	*Thespesia populnea*	0.0006	0.0002	0.0001	0.0004
1 After	*Thespesia populnea*	0.0007	0.0010	0.0005	0.0016
2 Before	*Barringtonia asiatica*	0.0003	0.0002	0.0006	0.0019
2 After	*Barringtonia asiatica*	0.0000	0.0005	0.0003	0.0009
3 Before	*Aleurites moluccanus*	0.0004	0.0015	0.0008	0.0024
3 After	*Aleurites moluccanus*	0.0003	0.0012	0.0006	0.0019
4 Before	*Musa x paradisiaca*	0.0005	0.0005	0.0002	0.0008
4 After	*Musa x paradisiaca*	0.0002	0.0005	0.0003	0.0009
5 Before	Negative Control	0.0007	0.0009	0.0005	0.0014
5 After	Negative Control	0.0000	0.0001	0.0000	0.0002