Examining Differences in Feeding Strategies in *Tripneustes gratilla*, with a Focus on Management Implications

Directed Research (Biol 499)
in Marine Biology

University of Hawai'i at Manoa
December 2013

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ABSTRACT

Tripneustes gratilla, a common sea urchin in the tropical Pacific, is being investigated as a tool for bioremediation. In Kāneʻohe Bay, on the island of Oahu, Hawai'i, *T. gratilla* has been outplanted and is reducing abundance of the invasive *Kappaphycus spp.* algae complex. In this experiment, urchins outplanted onto Reef 44 were shown to have positive, significant growth rates between September 28 and November 16, 2013, with an average rate of 0.12 mm/day. Urchins were also shown to consume significantly less *Kappaphycus spp*. when the alga was hard to reach than when it was uncovered and easily accessible, simulating conditions on the reef. Both consumption rates were significantly above zero, however, indicating that *T. gratilla* is a good candidate for algal control in the area. Consumption rates did not vary significantly between small and large urchins in either the obscured or uncovered trials.

INTRODUCTION

Tripneustes gratilla is known as the collector urchin due to its tendency to hold algae and loose debris on its aboral surface (Brusca and Brusca, 1990). It has recently been demonstrated that the debris is used as a shield against ultraviolet radiation (Adams, 2001; Sigg et al., 2007). Aside from the collecting activity, *T. gratilla* differs from other sea urchins in that it grazes continuously, day and night (Stimson et al., 2007). *Tripneustes gratilla* has been shown to be an effective algal removal agent (Conklin and Smith, 2005; Cunha, 2006), and outplantings are currently underway on several reefs in Kāneʻohe Bay, Oahu, Hawai'i. Kāneʻohe Bay is one of only two areas in the world where the endangered, endemic coral *Montipora dilatata* has been found. Colonies have been tentatively identified on Maro Reef in the Northwestern Hawaiian Islands (NWHI), but positive identification is still unconfirmed (Forsman et al., 2010). *Montipora dilatata* is a National Oceanic and Atmospheric Administration (NOAA) Species of Concern (SOC) (NOAA, 2007), and is especially susceptible to slight environmental changes (Forsman et al., 2010).

Anthropogenic impacts from agriculture to urbanization to raw sewage discharge have altered the bay almost continuously for over 200 years (Hunter and Evans, 1995). In more contemporary times, *Dictyosphaeria cavernosa* has been the dominant macroalgae, but by 1983 its abundance had dropped dramatically. However, *Kappaphycus alvarezii* and *K. striatum*, first introduced in 1974, have since spread to most reefs in Kāneʻohe Bay (Rodgers and Cox, 1999). The same is true of *Gracilaria salicornia*, introduced in 1978(Russell and Balazs, 2009), and *Acanthophora spicifera*, introduced in 1950 (Russell and Balazs, 2009). These invasive algal species, notably *Kappaphycus spp.,* have been observed to overgrow many different coral species, and can harbor bacteria that reduce corals' immune systems and make them vulnerable to disease (NOAA, 2007).

Bioremediation efforts have been attempted on several reefs in Kāneʻohe Bay, using farmed *Tripneustes gratilla* as a grazer of macroalgae, but little data other than urchin abundance has been taken after these efforts. There is previous data, however, that indicates that *Kappaphycus spp*. is preferred by *T. gratilla* over several other macroalgae species (Stimson et al., 2007). *T. gratilla* has also been shown to maintain algal turf diversity, as it focuses on macroalgae and does not disrupt the smaller algae species (Harmelin-Vivien et al., 1992; Cunha, 2006).

This study was focused around the "poster colony" of *M. dilatata*, located on Reef 44 *Kappaphycus spp*. In July of 2013, 1400 small (<5cm test diameter) farmed *T. gratilla* from the Department of Land and Natural Resources of Hawai'i were released surrounding the poster colony, also known as Colony 12 (DePartee et al., 2011), in an effort to increase grazing pressure on *Kappaphycus spp.* and other algae in the area. *Kappaphycus spp.* has been shown to regenerate extremely fast from miniscule attachment points that human removal cannot access. Some urchins increase the relative size of their mouthparts when food is hard to access by slightly contracting the plates that make up their test (Levitan, 1991). This could allow urchins that have already depleted macroalgal cover to "finish it off" by accessing the small attachment points. As urchin consumption rates are positively related to body size, two different size classes of urchins were tested in order to fine-tune management approaches (Cunha, 2006).

It has also been reported that *Kappaphycus spp.* has highest growth rates in the summer and autumn months, so by conducting this experiment in the fall, the "worst case scenario" (highest algal growth rate) was tested (Bulboa and Paula, 2005). Historical data of percent cover indicate high amounts of *Kappaphycus spp.* surrounding Colony 12 (Ciarcia et al., 2010). *T. gratilla* has been shown to reduce macroalgae biomass in enclosures (Conklin and Smith; 2005, Cunha, 2006), and this study aimed to directly measure the amount of biomass consumed by individual urchins of varying size under different conditions.

There are concerns that as the urchins graze the macroalgae surrounding Colony 12 to low levels, they will move away from the site, which will allow the algae to grow again with continued detrimental effects. However, if the urchins are able to access smaller grazed thalli, they may linger in the same area long enough to reduce invasive algae beyond the point of recovery, or enough to be kept down by herbivorous fish after the urchins leave.

H_0: There will be no significant difference in the amount of algae eaten between the easily accessible and hard-to-reach food sources.

H_a: There will be a significant difference in the amount of algae eaten between the easily accessible and hard-to-reach food sources.

METHODS and MATERIALS

On Reef 44

In 2013, three trips were made out to Reef 44, on September 28, October 13, and November 16. Previous studies provided exact GPS coordinates of Colony 12 (Butcher et al., 2013), listed as N 21.4770, W -157.83168. Three teams with two snorkelers each searched within a 10m radius of Colony 12, collecting any *T. gratilla* that they could reach without excessively damaging the reef or urchin. Photos were taken of specific sites surrounding Colony 12 that exhibited *Kappaphycus spp*. nubs or large abundance, in order to monitor qualitatively how they changed over the course of the study. The collection continued for 45 minutes. One pillowcase full of *Kappaphycus spp*. weighing about 10 kg was also collected.

After the collection period, the teams met onboard the boat to measure test diameters of the collected urchins using calipers. The sizes and total counts were recorded. From these urchins, specimens were taken for further lab work. Two large (>8 cm test diameter) and 16 moderate (4-6cm test) urchins were brought back to the Hawai'i Institute of Marine Biology

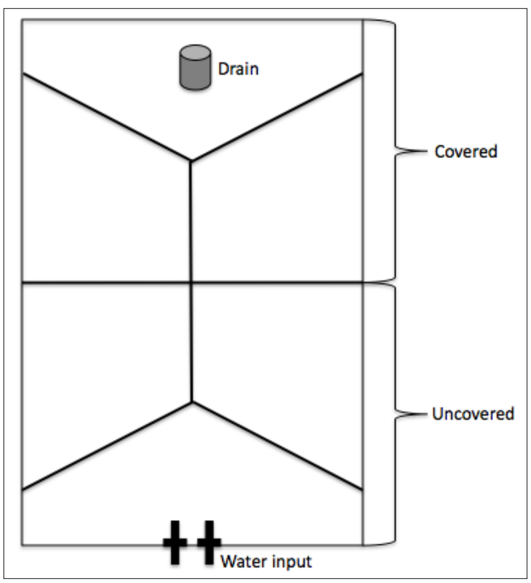
(HIMB). These urchins were kept together in a water table and fed a diet of *Kappaphycus spp.* (*ad libitum)* while awaiting experimental use. All remaining urchins were replaced back in the vicinity of Colony 12, with more placed on areas that appeared to need increased grazing activity. The *Kappaphycus spp*. was brought back to use as food for the lab animals.

In the lab

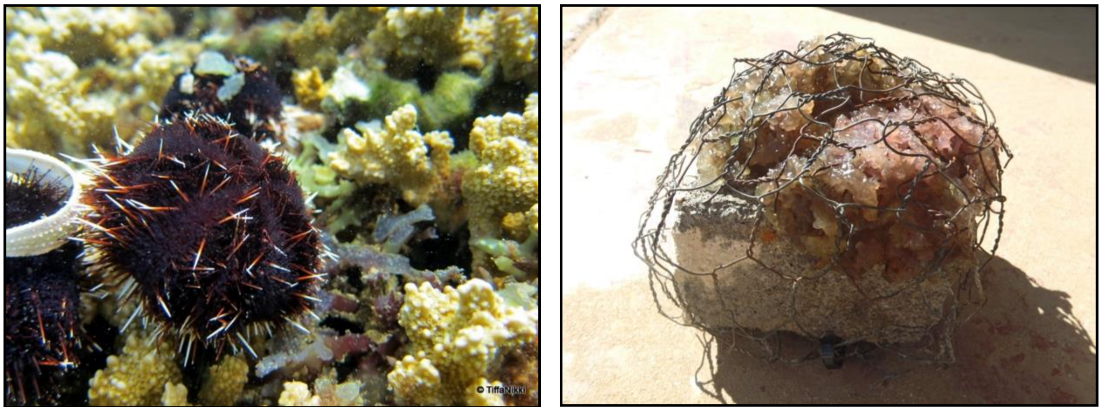
Experiments were conducted on a water table at HIMB, with chicken wire separating the table into six sections. The water tables were supplied with a continuous influx of ocean water. A separate water table housed the urchins that were not being tested along with *Kappaphycus spp*. The rate of flow of water was kept at an average of 6 L/min for both water tables, and temperature ranged between 24.5-27° C.

Half-sphere clumps of *Kappaphycus spp*. weighing between 100-200g were enclosed in a dome of chicken wire, in order to mimic the limited accessibility on the reef. All pieces of algae extending outside of the cage were cut to the same level as the chicken wire, leaving only enclosed algae as a food source. The chicken wire enclosed the algae and the apparatus was weighted down with half of a brick in order to hold it in place. The piece of algae was weighed wet before and after to determine lost biomass. Pieces of uncovered algae were placed in three of the sections, and the other three held obscured algae. Urchins' tests were measured, and they were then placed into their individual sections for four days. This process was repeated for both moderately large urchins, using a different urchin for each trial so as to alleviate individual effects, with a total of 26 trials. Each time the alga was left for 4 days before it was reweighed.

To compare feeding efficacy of hard-to-reach food sources, accessible food was also tested. Urchins were presented with pieces of loose *Kappaphycus spp*. that weighed between 100-200g. The alga was weighed wet before and after a 4-day soak period. These values were used as a control to compare against the hard-to-reach algae. Urchins were first presented with the easily accessible food, then the hard-to-reach food, in order to simulate natural conditions in which the food source transitions from one state to the next. This process was repeated in the same manner as the hard-to-reach food, resulting in 27 trials.



A schematic illustrating the experimental set up for the water table.



Images depicting T. gratilla *utilizing its collecting method, as well as attempting to access hard-to-reach food (left, courtesy of Nikki Gutlay). The other image depicts the obscured algae set up, with the cage held together by zip ties underneath the brick (right).*

RESULTS

A series of t-tests were performed to determine significance (IBM, 2011). The urchin round-ups gave varying abundances, but showed consistent and significant increase in test diameter over the study period (Fig. 1a). The average test diameter was 50.73 mm on Sept. 28, 53.56 mm on Oct. 13, and 56.74 mm on Nov. 16. This fits a polynomial trend line very closely (Fig. 1b, R=0.9999), indicating that there is faster growth earlier in the life history, a common phenomenon among urchins (Dafni, 1983) and other animals. Growth between Sept. 28 and Oct. 13 was 0.189 mm/day, whereas growth between Oct. 13 and Nov. 16 was 0.094 mm/day (Fig 1b.) An approximate 15 mm test diameter was used to estimate growth from the day of the outplanting (July 15) until Nov. 16.

The amount of algae consumed was determined to vary significantly between obscured and uncovered food sources (Fig. 2). *Tripneustes gratilla* consumed an average of 38.85 g of uncovered algae, and 19.85 g when it was obscured. However, there was no significant difference between consumption by urchin size classes in uncovered (Fig. 3, p=0.114) or obscured (Fig. 4, p=0.207) algal treatments, although larger size classes did eat more in both cases.

On Reef 44

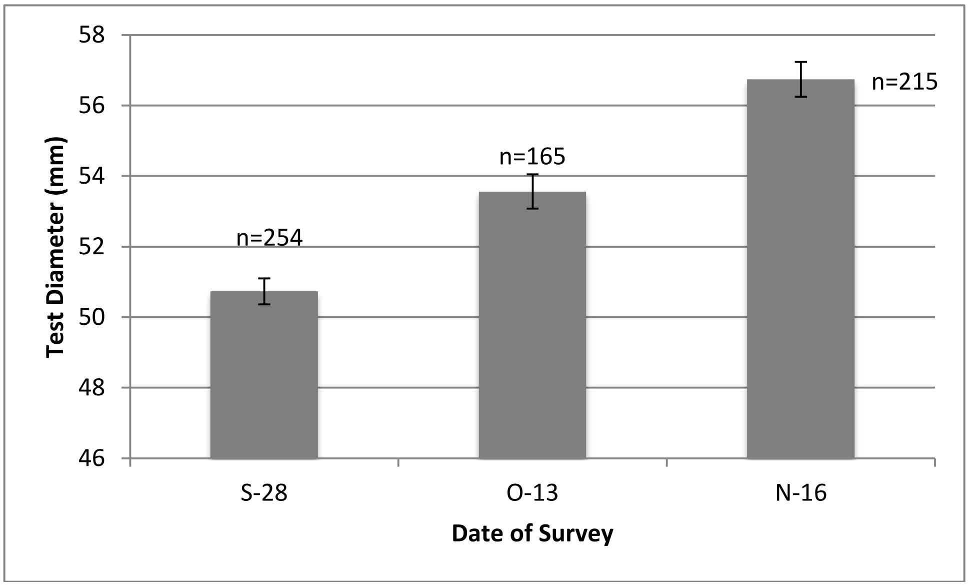


Fig. 1a. Average test diameters from consecutive survey dates, showing September 28, October 13, and November 16 (t-test, $p < 0.001$, mean ± st. error).

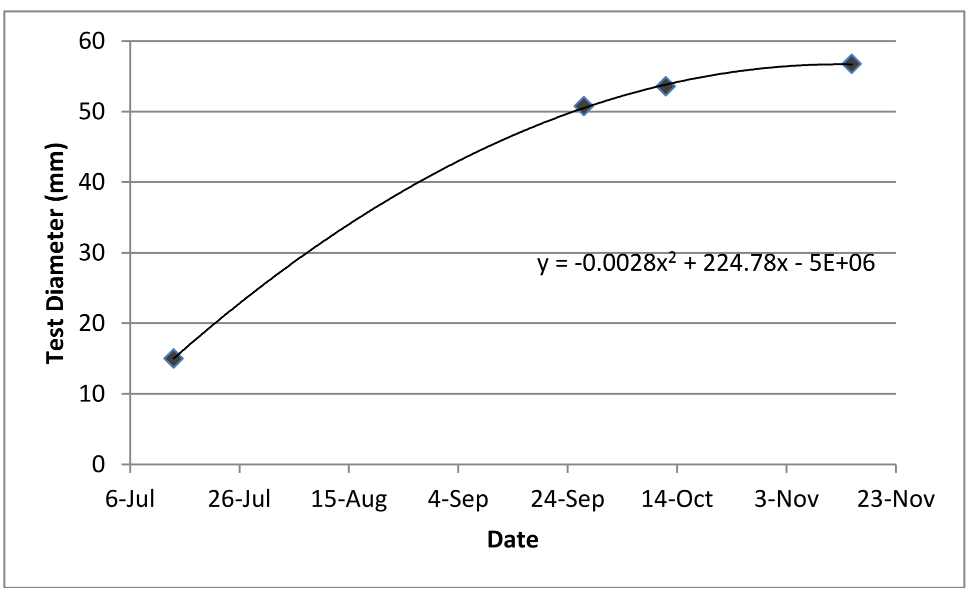


Fig. 1b. A projected growth curve using the estimated average starting size of outplanted urchins (R=0.9999).

In the lab

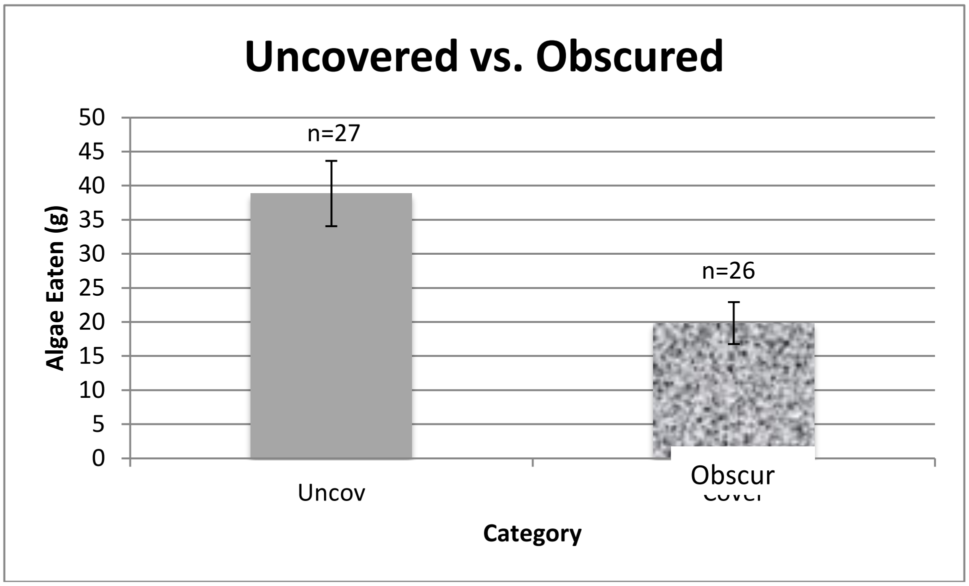


Fig. 2. Average algae consumption values for uncovered and obscured food supplies (t-test, p=0.0018, mean ± st. error).

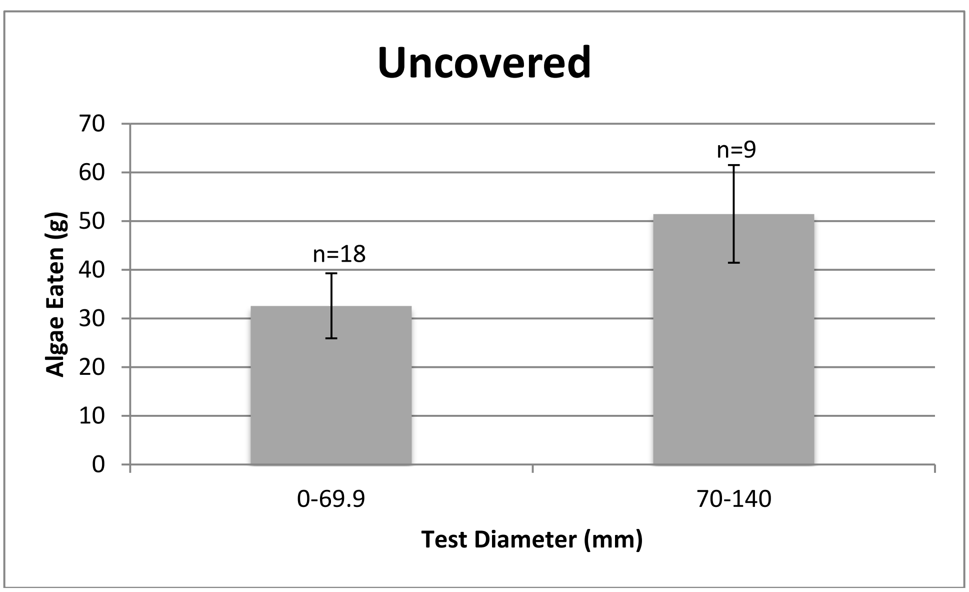


Fig. 3. Average uncovered algae eaten for two size classes, (t-test, p=0.114, mean ± st. error).

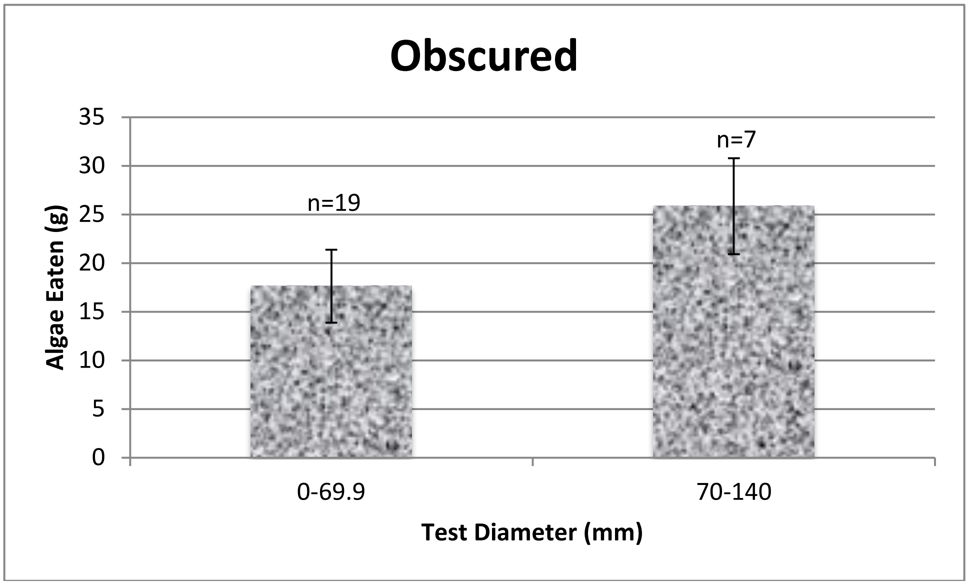


Fig. 4. Average obscured algae eaten for two size classes, (t-test, p=0.207, mean ± st. error).

CONCLUSION

The area surrounding Colony 12 on Reef 44 in Kāneʻohe Bay, Oahu, has seen a reduction in invasive algal cover following the outplanting of young *Tripneustes gratilla*. Qualitatively, the algae is not rebounding, and adjacent to Colony 12 there has been substantial loss of macroalgal cover. Quantitatively, the outplanted urchins are surviving and growing (Fig. 1a, 1b). Knowing that they are able to acclimate from lab conditions to the open reef environment is an important part of the success of *T. gratilla* in bioremediation. Larger urchins contain more gametes (Levitan et al., 1992), making them a valuable part of a possible repopulation of *T. gratilla* on the reefs in Kāneʻohe Bay. As urchin abundance increases, so too does reproductive success, increasing the chances of urchin population recovery (Levitan et al., 1992, Kalam et al., 2010).

When it comes to algae availability on the reef, *T. gratilla* appears to have some limitations in reaching algae that is obscured (Fig. 2). However, although urchins consumed significantly less algae when it was obscured, algae was still consumed, showing continued contribution to the reduction of algae on the reef. Also, because there were no significant differences between size classes with the same algae availability, large urchins do not appear to be impeded by larger body size (Fig. 3, Fig. 4). In fact, larger urchins ate more algae in both obscured and uncovered situations, but the differences between the size classes were insignificant. In terms of management, this indicates that if the larger urchins stay in the same area, they should be adequately effective at keeping down algae abundance. This would eliminate the need to continually replace urchins on reefs, although the urchins moving away from Colony 12 could potentially reverse this.

This experiment did have several limitations. First of all, metal chicken wire was used to simulate a coral reef, which is made of calcium carbonate and other minerals as well as animal tissue. This may or may not have an affect on the eating behavior of the urchins, but at the very least it may be possible for *T. gratilla* to bore through the coral with its sharp teeth. If this is the case, the urchins will be better able to access the algae, but will in turn affect the coral. The metal may also affect urchin behavior, as it may stimulate or repel feeding compared to uncovered algae. Another issue in the experiment was urchins behaving differently during different round ups. On October 13, many of the urchins seemed to be hiding in crevices that the surveyors were unable to access, leading to lower abundances and fewer urchins to measure.

Experimentally, there was some difficulty when it came to the obscured algae. Algae, especially *Kappaphycus spp*., do not have uniform shapes, which likely caused some differences in the amount of reachable algae between trials. This confounding factor was somewhat limited by repeated trials and use of the same cages for consecutive trials. The uncovered algae presented the potential problem of fragmentation resulting in lower weights. Before weighing, sections of the experimental water table were checked for pieces of *Kappaphycus spp*., and the largest piece found weighed only 0.40 g. The pieces were also fairly uncommon, with an average of 2 pieces per trial.

Overall, *T. gratilla* demonstrated its usefulness as an algae removal agent of both available and cryptic macroalgae. As the outplanted urchins continue to grow out on the reef, they consume more algae, and their chance of survival increases with size. *Tripneustes gratilla* has already been extremely effective at removing *Kappaphycus spp.* surrounding the *M. dilatata* colony in Kāneʻohe Bay, and will likely continue to reduce algal cover. Because the urchins are able to reach some of the less accessible food sources, it is more likely that they will remain in the proximity of Colony 12, preventing future algal domination and protecting the rare endemic coral.

For continued research into this topic, there are numerous parameters that remain unexplored. The first would be to conduct stomach content analyses of some of the urchins from Reef 44 and other reefs with *Kappaphycus spp.* to determine the exact diet of these urchins.

There is also interesting data to be gathered from continuing experiments outlined in this paper. More monitored urchin outplantings would be advisable, along with movement tracking and photoquadrat monitoring of areas where urchins are deployed. Tracking urchin movement over a long period could provide insight into whether there are daily migrations around the reef, and if larger urchins travel away from their original areas of deployment. One study found that *Tripneustes* move about 3.7 m per day on the reef flat (Tertschnig, 1989), with some individuals traveling much farther, having widespread effects on the reef. Additionally, photoquadrats could provide important *in situ* quantitative data, as well as easily illustrate the positive effects of the urchins for the public. Performing an experiment similar to this one with increased sample size and urchin size range would be useful to alleviate statistical errors and give a more well rounded view of the issue.

ACKNOWLEDGEMENTS

I would like to thank my P.I., Dr. Cynthia Hunter, for her help, instruction, and support on this project.

I would also like to thank Dr. Anuschka Faucci, Dr. Greta Aeby, Jonathon Blodgett, Lauren van Heukelem, Keisha Bahr, Tiffany-Nicole Gutlay, Taylor Massey, Tiara Stark, Rebecca Weible, and David Hoffman, along with the staff at HIMB, DLNR, the Super Sucker teams, and the University of Hawai'i at Manoa.

Special thanks to Celina Hayashi, for continuously loving and supporting me.

Budget for the project.

Item	*Price*	*Quantity*	*Subtotals*
Metal clippers	*7.99*	*2*	*15.98*
Chicken wire	*21.99*	*1*	*21.99*
Zip ties	*5.99*	*2*	*11.98*
Gloves	*5.29*	*3*	*15.87*
Rubber bands	*4.99*	*1*	*4.99*
Tubs	*9.99*	*1*	*9.99*
Pillowcases	*6.99*	*4*	*27.96*
			Total
			108.76

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