EFFICACY OF *BARRINGTONIA ASIATICA* AS CONTROL FOR CORALLIVOROUS SEASTARS, *ACANTHASTER PLANCI* AND *CULCITA NOVAEGUINEAE*

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Abstract. Outbreaks of corallivorous seastars pose one of the most impactful natural threats to coral reef communities throughout the Indo-Pacific. *Acanthaster planci* are well known for their cyclical population patterns and widespread destruction of coral cover in the Great Barrier Reef and many Pacific islands. The less destructive *Culcita novaeguinaea* is not known to overpopulate, but shares *A. planci* living and feeding preferences. Current control methods for *A. planci* are strongly limited by costs and materials required to execute them in the remote locations outbreaks often occur. To investigate a natural and more easily accessible control alternative, this study assesses the efficacy of *Barringtonia asiatica*, a traditional Polynesian "fish poison" tree, against *A. planci* and *C. novaeguineae*, as well as its potential secondary effects on the environment. This study aimed to analyze the differences in mortality rates between two different seastar species injected with *B. asiatica*, as well as *C. novaeguineae* injected with different doses. Although *B. asiatica* did successfully cause 100% mortality within 22 hours in both *A. planci* and *C. novaeguineae*, no statistically significant difference in mortality rates was found between any of the treatments. Analysis comparing seastar size and time of death was also conducted and found no significant correlation. Furthermore, no visible negative secondary effects of the process to surrounding marine life were found. The results of this study suggest *B. asiatica* to be a promising control alternative for corallivorous seastars, however, further studies are needed to more thoroughly investigate the substance's secondary effects on the surrounding marine community.

Key words: corallivores; seastars; Barringtonia asiatica*; fish poison tree;* Acanthaster planci*;* Culcita novaeguineae*; crown of thorns seastar; cushion star; population control; biocontrol; Moorea, French Polynesia*

INTRODUCTION

Global climate change is currently the greatest natural threat to our coral reefs (Moutardier et al., 2015). Coral reef ecosystems around the world are experiencing increased rates of imbalance and degradation induced by increased concentration of greenhouse gases. Warmer ocean temperatures and increased carbon dioxide absorption leading to acidification are prompting more frequent mass coral bleaching events and are synergistically deteriorating our marine environments (Osborne et al., 2011). This continues to pose an increasing threat to the health of marine resources and coastal communities worldwide. Human intervention and conservation efforts are absolutely necessary to preserve our coral reef communities and mitigate the effects of human-induced climate change.

Population outbreaks of the crown-of-thorns seastar, *Acanthaster planci*, are recognized, along with climate change and tropical cyclones, as one of the most impactful natural disturbances to coral reef ecosystems throughout the Indo-Pacific (Rivera-Poseda et al., 2013; Leray et al., 2012). *Acanthaster planci* are exclusive scalenterinian corallivores that in low densities promote marine biodiversity, but in high, may significantly damage coral reef systems and threaten the food security and economic value they provide (Kayal et al., 2012). In the Great Barrier Reef alone, 42% of the observed coral loss between 1995 and 2009 was attributed to *A. planci* overpopulation (De'ath et al., 2012). *Culcita novaeguineae*, or the cushion star, is much less destructive and shares mainly the same living and feeding preferences as *A. planci*. Both are asteroid seastars in the Valvatida order. *Acanthaster planci* have an average feeding rate of 289 cm^{2} of coral per day (Keesing & Lucas, 1992), while *Culcita novaeguineae* average 28 cm^{2} per day (Glynn & Krupp, 1986). Although no known population outbreaks of *C. noviguineae* have been reported, they do feed on live coral and may pose a threat should a relevant ecological imbalance arise.

Recent years have seen an increase in the frequency and severity of *A. planci* population outbreaks. The exact parameters constituting *A. planci* presence as an "outbreak" vary, although many agree that it may be defined as: 14 starfish per

1000m^2, 40 starfish per 20 min swim, or 100 starfish per 10 min tow survey (Reichelt et al, 1990). Outbreaks are popularly hypothesized to be a result of increased anthropogenic disturbance and nutrient input into the ocean, which encourages *A. planci* larval development and survival (Reichelt et al, 1990; Grossman, 2014). Overfishing of the triton's trumpet (*Charonia tritonis*) and humphead wrasse (*Cheilinus undulatus*), the few natural predators of *A. planci*, also contribute to their overpopulation. These events decrease the overall resiliency and integrity of our coral reef ecosystems, making control methods integral to environmental conservation efforts.

Acanthaster planci induced destruction has prompted human intervention internationally from both scientific and government agencies alike (Bell, 2008). Since the 1960's, various chemicals have been investigated for use as control methods for *A. planci*; the most popular of which currently being ox bile injections (Rivera-Posada et al., 2013). However, such methods are strongly limited by costs of required personnel training, chemical production, and shipping. Sodium bisulphate, another popular chemical control, is estimated to cost $35 USD per seastar (Moutardier et al., 2015).

Barringtonia asiatica, or the fish poison tree, is native to many islands in the Indo-Pacific and is commonly known for its ichthyotoxic properties. All parts of the plant contain the toxin saponin, although the plant's seed is most often used traditionally throughout the pacific to stun or kill fish (Ravikumar et al, 2015). No studies have been conducted assessing the toxicity of *B. asiatica* on seastars, although it presents a promising natural and easily accessible control alternative.

In Moorea , *A. planci* densities were observed to be unusually high in 2002, and were studied on a large-scale by Kayal et al. between 2006 and 2010. The study showed the slow moving migration of *A. planci*, and may be used to synthesize their population dynamics and feeding patterns. In the study, the highest densities of *A. planci* were firstly observed the Northwestern edges of the island in 2006 and slowly progressed east, and then southeast by 2010. The surveys showed *A. planci* preferred to inhabit the outermost slopes and passes of Moorea's barrier reefs, but slowly moved to shallower locations in search of coral as overall *A. planci* densities increased. Bell, (2008) and Park 2008, also studied *A. planci* on Moorea. Bell's study found there to be no difference in the feeding preferences of cushion stars in the presence of crown-of-thorns. Park's study found there to be no significant difference in *A. planci* densities within Marine Protected Areas of Moorea. Both studies contributed to our overall comprehension of *A. planci* presence and behavior in Moorea.

Numerous relevant studies of *C. novaeguineae* in Moorea have also been conducted, mostly concerning their distribution and feeding patterns, as well as their symbiotic relationship with shrimp. Such studies have found *C. novaeguineae* to be common throughout both the flats and slopes of Moorea's fringing and back reefs (Yokley, 2016; Roberge, 2000; . They also found *C. novaeguineae* to have a feeding preference of *Pocillopora sp.* (Bell, 2008).

The overall purpose of this study was to investigate the effect of *B. asiatiatica* injections on *A. planci* and *C. novaeguineae*. This study compared the effect of B. asiatica on the different sedifferent doses of the plant extract as well as any potential secondary effects the process may have on surrounding marine organisms. I evaluated the plant's potential as an easily accessible, effective, and natural alternative control method. Considering the many limiting factors of current controls, and the known toxicity of *B. asiatica* against fish, the plant extract could very well revolutionize crown-of-thorns population management and significantly assist in coral reef conservation and restoration efforts around the world.

METHODS

Study and Collection Sites

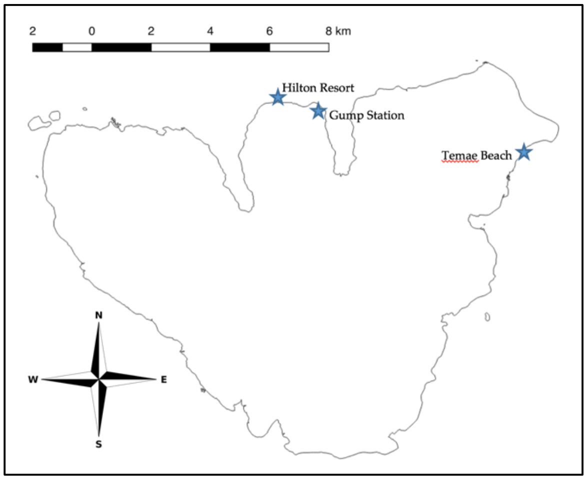


Figure 1. Map of Mo'orea, French Polynesia. Courtesy of the Geospatial Innovation Fcaility, University of California, Berkeley.

This study was conducted on the island of Mo'orea, in French Polynesia. All seastars were collected between October and November of 2017 (as labeled in **figure 1**). Six *A. planci* were collected from different areas around Mo'orea: two from the beach of the Hilton Mo'orea Lagoon Resort & Spa

(17°29'06.0"S 149°50'42.0"W), one from the back reef just North of UC Berkeley's Richard B. Gump Research Station (17°29′21.7″S 149°49′33″W), two from the fringing reef between the Hilton and Gump Station, and one from the back reef of Temae Public Beach (17°29'57.25"S, 149°45'46.26"W). All *C. novaeguineae* were collected from the fringing reef just North of Gump Station.

Study Organisms

Acanthaster planci females have tens of millions of eggs that are fertilized externally in seawater. Planktonic larval development then occurs for about 2 weeks, then the larvae settle and metamorphose. Juvenile seastars live benthically and feed on algae up until about 6 months post settlement, when they began solely feeding on coral. These seastars reach sexual maturity at approximately 2 years of age (15cm in diameter). Adult *A. planci* are red/brown in color and have a central disk with 5-25 radiating arms (Kayal et al, 2012). Its entire aboral surface is covered by 10-20 cm long, sharp venomous spines, making handling and manual removal difficult. *Acanthaster planci* are known to live a maximum of 8 years (Reichelt et al, 1990). *Culcita novaeguineae* have only 5 short arms with spines no more than 2 cm long and follow the same life cycle. Juvenile *C. novaeguineae* have a much more distinctive star shape, but as adults, the tissue between its arms grow together, giving it a more "inflated", pentagonal appearance (Glynn & Krupp, 1985). In both species of seastars, size is about the only indicator of age, although unreliable as the seastars do not change dramatically in size past maturity. Being that *A. planci* age was not easily distinguishable, the exact ages of seastars used in this study were unknown, however, all were adults.

Seastar handling

Seastars were brought back to Gump Station and stored outdoors in large flow tanks with circulating seawater. *Acanthaster planci* were stored until a desired minimum sample size was reached and fed *Acropora sp.* about once a week before experiments began. *Culcita novaeguineae* were tested within 48 hours and not fed. All seastars were separated for all experiments (one seastar per flow tank) and given at least 24 hours to acclimate to lab settings before being tested.

Experimental design

A longitudinal study in which each individual seastar was treated as its own control was conducted due to the unavailability of *A. planci*. After a minimum 24 hour acclimation period, each seastar was injected with a sham injection of seawater of either 60mL or 30mL, corresponding with their experimental treatment dosage. Seawater was the diluting substance used for *B. asiatica*. The control treatment was conducted to ensure seastar death was not a result of injection procedure or seawater alone. Each seastar was then observed for 24 hours following sham injection and given another 24 hours to recover before being injected with their respective experimental treatments. There were three experimental treatment groups: *A. planci* injected with 6omL of *B. asiatica* solution, *C. novaeguineae* injected with 60mL, and *C. novaeguineae* injected with 30mL. All seastars were injected using a 20 gauge needle between 1700 and 1900. Each seastar was haphazardly injected 3 times in the central disk with 10 or 20mL per injection, for a total of either 30mL or 60mL per seastar. Mortality of each seastar was recorded every 2 hours for 24 hours only, to not allow time for regeneration and recovery, following *B. asiatica* injections.

B. Asiatica *preparation and injection*

Mature, green *B. asiatica* seeds were collected from Paopao Valley and the western coastline of Cook's Bay. The seed casings were cut off and seeds were chopped into smaller pieces and grinded using a Ninja® blender. 500g of *B. asiatica* grinds were combined with 1000mL of seawater, and then blended together and strained through cheesecloth for a more uniform solution of 0.5g/mL. This process was repeated for all treatment groups and *B. asiatica* solutions were made and used fresh each of the three time. One seastar was spared to use as an unpoisoned control for another component of this project. Seastars were then left alone and monitored for a period of 14 hours.

Statistical analyses

All statistical tests were conducted using RStudio (Version1.0.153, 2009-2017 RStudio, Inc.) Differences in mortality rates between the two seastar species, as well as between *C. novaeguineae* injected with different doses of *B. asiatica*, were analyzed using Welch Two Sample t-tests, with alpha set equal to 0.05. The relationships between seastar size and time to death were analyzed using regression and analysis of variance tests (ANOVA).

Secondary effects of B. asiatica *on the community*

B. asiatica is a known fish poison (Ravikumar et al, 2015), and although their effects may be favorable when injected into *A. planci* or *C.*

novaeguineae, they may negatively affect surrounding marine life. To investigate the potential negative secondary effects of *B. asiatica* injections, all seastars were left in their flow tanks 24 hours after injecting them, 5 sea urchins of a variety of species (*Echinometra mathaei, Echinothrix calamaris, Diadema savignyi,* and *Tripneustes gratilla*) were haphazardly placed in each tank. A different set of urchins was used each time for a total of 90 urchins used in this study. Urchin mortality was observed for 5 days following seastar injections. For control, each treatment group had one seastar spared (3 total) and left out of water for 24 hours to allow an unpoisoned, “natural” death.

RESULTS

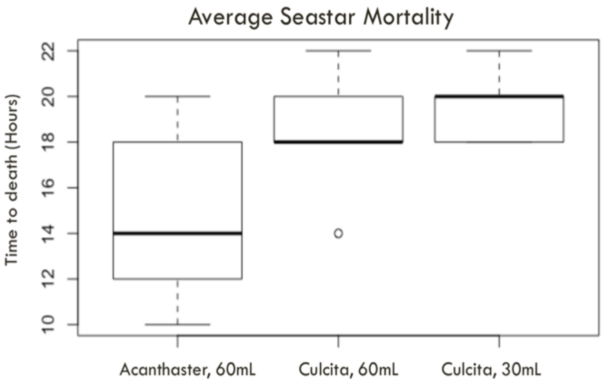


Figure 2. Average seastar mortality per treatment.

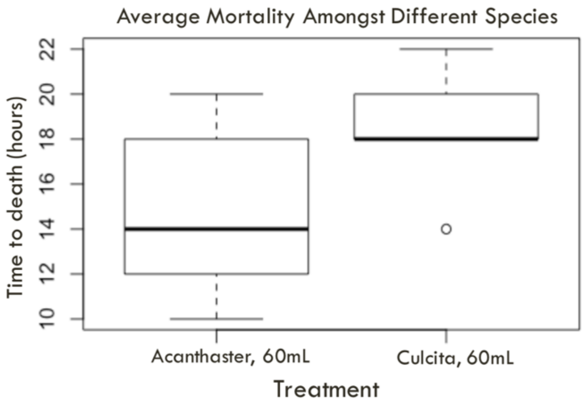


Figure 3. Average mortality amongst *C. novaeguineae* injected with 30mL and 60 mL.

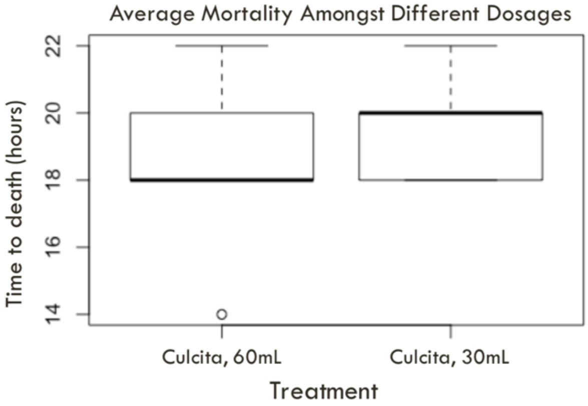
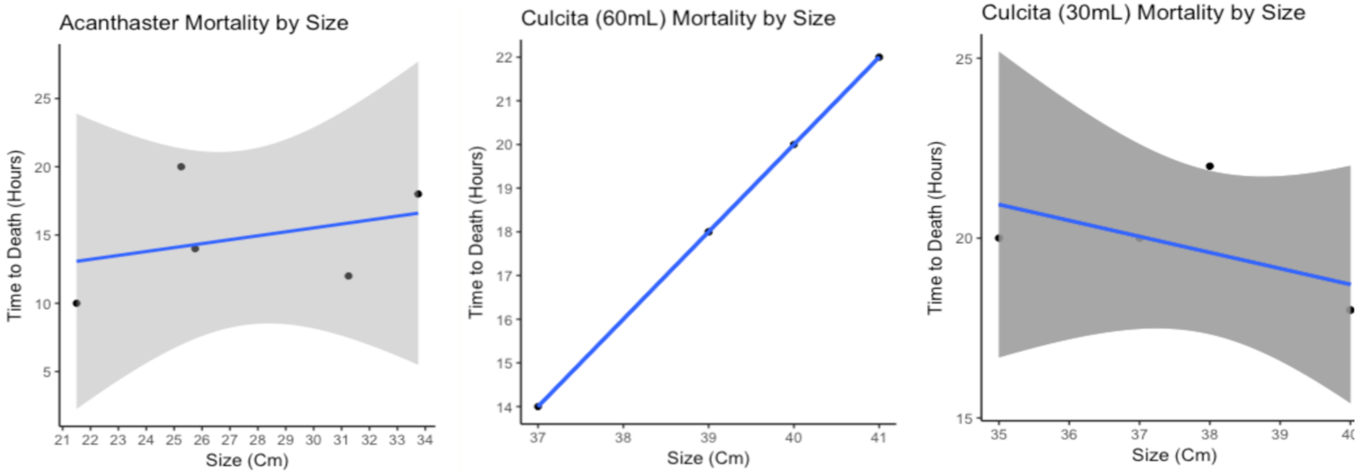


Figure 4. Average mortality amongst *A. planci and C. novaeguineae.*



Figures 5-7. Linear regressions showing correlations of respective seastar species size and time to death, per treatment

Average seastar mortality

Average seastar mortality within each treatment group (**figure 2**) was achieved for: *A. planci* at 14.6 hours, *C. novaeguineae* (60mL) at 18.4 hours, and *C. novaeguineae* (30mL) at 19.2 hours.

Different species & different dosages

Welch Two Sample t-tests showed no significant difference in mortality between the two different species, *A. planci* and *C. novaeguineae* (**figure 3**, t = -1.71, df = 7.40, p-value > 0.05), as well as no significant difference between mortality rates of the two *C. novaeguineae* test groups injected with different dosages, 30mL and 60mL, of *B. asiatica* (**figure 4**, t = -0.048, df = 7.50, p-value > 0.05).

Seastar size & time to death

Linear regressions (**figures 5-7**) showed no significant correlations between seastar size and time to death amongst any of the three experimental treatment groups: *A. planci* treated with 60mL ($F_{1,3}$ = 0.41, p-value > 0.05), *C. novaeguineae* treated with 60mL (p-value > 0.05, $F_{1,3}$ = 0.75) and *C. novaeguineae* treated with 30mL ($F_{1,3}$ = 0.06, p-value > 0.05).

Secondary effects

After 5 days, there was no mortality amongst any of the 90 urchins placed in the tanks after the seastars had been injected with *B. asiatica* solution.

DISCUSSION

Although results did not show any statistically significant relationships, *B. asiatica* was indeed successful in causing 100% mortality of all seastars in all treatments. There was no significant difference in mortality between the different species. Amongst *C. novaeguineae*, there was no significant difference in mortality between the 30mL and 60mL treatment, which might suggest that for practical application, a 30mL treatment might be sufficient. Both *A. planci* and *C. novaeguineae* reached 100% mortality within 22 hours of being injected with *B. asiatica* solution. Also, injected seastars did not appear to have any negative affects on surrounding marine life. However, I would suggest more research to look into this more in-depth and assess the potential long term environmental impacts of this procedure.

CONCLUSION

For the conservation of our marine resources and restoration of coral communities affected by *A. planci* overpopulation, more studies such as this one are needed to find affordable, if not free, and natural solutions to this problem. Current methods are effective, although very limited and not always feasible with the costs associated with obtaining the materials needed. More studies on *C. novaeguineae* feeding rates and patterns are also needed to more accurately assess their affect on our marine environments.

ACKNOWLEDGEMENTS

I give my sincerest thanks and appreciation to the University of California and Richard B. Gump Station for continuing to offer this unique undergraduate learning experience. It has undoubtedly been the highlight of my undergraduate career. Being born and raised in American Samoa, I cannot express enough how thankful I am to have had this rare opportunity to study what I love in a place so much like home, surrounded by a culture so much like my own.

I thank our entire teaching team for the time they've invested, not just in this class, but in my future. Thank you all for believing in my abilities and project, often times more than I did myself. A big, heartfelt thank you to my classmates, whom this project would've been impossible without, for helping collect my organisms and dispose of them days after they've died (R.I.P. Queen Yonce and sorry, Sophie). Shout out especially to Sky, Elana, Brent, Sarah, Madelief, Annie, Chris, Liz and Aaron! Last but not least, thank you to the Gump Staff, especially Miriama, Jacques and Tony for all their assistance in setting up my aquaria. Fa'afetai tele lava and mauruuru roa!

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