TOXICITY OF *BARRINGTONIA ASIATICA* ON *LITTORARIA COCCINEA* AND SIZE AND WEIGHT CORRELATION TO TOXICITY RESISTANCE

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Abstract. *Barringtonia asiatica* is a widely used plant for treating several illnesses and for holding ichthyotoxic properties on fish. However, this fishing practice may hold adverse effects on other unintended target organisms besides fish. In this, *Littoraria coccinea* was used as a proxy for similar organisms that may be unintendedly affected. A toxicity test was concluded to best visualize the effect of *B. asiatica* on *L. coccinea* based on differing amounts of the toxin. Using preliminary data to figure out the correct concentrations of the powder, I created these amounts: 0.01g, 0.02g, 0.04g, 0.08g, and 0.16 g. The toxin was administered to the gastropods once they were placed in containers containing 20ml of the diluted toxin. Each concentration of the toxin had 10 individual containers with one gastropod in each of them totaling to 60 gastropods. Gastropods were observed over a 96-hour period with a 12-hour analysis in between. I recorded the number of dead and live gastropods and derived an LC50 per time period. I also wanted to see if there was a positive correlation to the size and weight of gastropods and its resistance to a given amount of the *B. asiatica* toxin. Thus, the LC50 from the 48-hour toxicity test, 0.01/20 g/ml, was used to observe the mortality rate of gastropods. Gastropod lengths and weights were a random assortment taken from their natural environment. The same 96-hour analysis was applied onto this test with a 12-hour observation in between. The results from the data analysis stated that there was a positive correlation between size and weight to different concentrations of the toxin. Further, mortality rate is dependent upon the amount of exposure and the amount of *B. asiatica* powder used. In this, we can assume that the *B. asiatica* toxins can kill *L. coccinea* and other phylogenetically similar organisms under situations with reduced waves and currents.

Key words: *Spermatophyta*; Barringtonia asiatica; *size correlation;* Littoraria coccinea*; weight correlation; toxicity test; Moorea; French Polynesia*

INTRODUCTION

Throughout time, different parts of the world have used various methods for fishing. Various types of prey are caught utilizing plants that can poison fish or in other words, the plants are ichthyotoxic (Ravikumar et al. 2015). Most fish die within minutes to an hour depending on the type of fish or the level of toxicity of the fish poison plant (Howes 1930). In all over the South Pacific this form of fishing is mostly practiced on low tide shores, ponds, or bodies of water where there is reduced movement. There are 11 other stupificants that are used for the same purpose as *B. asiatica* like *Derris trifoliata, Pittosporum* and *Tephrosia purperia*. These plants can be easily found in Fiji, New Caledonia, Papua New Guinea, and the Loyalty Islands. Ways of cultivating and using fish poison plants vary upon the type of fish

poison plant with, for example, roots being collected from a plant named *Derris*, kernels collected from *Cerbera*, and stems and leaves collected from *Euphrobia*. For *Barringotnia* and another plant like *Berbera*, seeds are cooked (Barrau 1955). In French Polynesia; however, there are a number of species of the genus *Barringtonia* used to stupefy fish (Howes 1930). *B. asiatica* is one of the sixty-nine species of Barringtonia in the world (Morrison et al. 1994). *B. asiatica*, whose taxonomy is of the Kingdom Plantae, Phylum Tracheophyta, Class Magnoliopsida, Order Lecythidales, and Family Lecythidaceae can be mostly found within terrestrial environments (World Conservation Monitoring Centre. 1998)

Originally brought from early Polynesian settlers to the islands, *B. asiatica* is one of the most extremely widespread species along with *B. racemosa* and *B. acutangula.* They naturally disperse through streams or near the sea. *B. asiatica* and *B. racemosa* extend from the Pacific islands to East Africa while *B. acutangular* disperse from India to Australia (Prance 2012). Thus, *B. asiatica* has a prominent role in the cultural traditions of many countries and this old fishing method may be practiced to this day. Although, through interviews with the local people of Moorea, most stick to modern fishing methods since the *B. asiatica* fishing method is banned in French Polynesia (pers. comm.).

Traditionally, the seed of the *Barringtonia* trees, also called 'vutu' by many indigenous Polynesian peoples, is mainly used to kill fish but can also be used for toys and games within the Pacific Islands. Commonly made into marbles, tops, lagging pieces, and small balls (Morrison et al. 1994). *B. asiatica's* usage has been carried widely throughout Madagascar, the Nicobari islands of India, Celebes, Australia, Cocos (keeling) islands, Kenya, Singapore, Sri Lanka, Taiwan, Province of China, Philippines, the Mariana islands, Fiji, New Britain, Solomon Islands, Vietnam, Queensland, American Samoa, and most of French Polynesia (World Conservation Monitoring Centre. 1998, Ravikumar et al. 2015). The fruit of the tree which are lantern shaped in form are usually collected as immature or mature fruits, they are also considered to resemble a mythical human heart according to Polynesians, the outer layers are stripped off like those from a coconut, the seeds are then grated using the thorny stump near the *B. asiatica* tree called the *kunial* according to the Nicobari tribes. Polynesians also used the same methods of collection and fishing practice as the Nicobari tribes (Ravikumar et al. 2015, Tetiaroa Society). This pulp is then collected and taken to rocky, intertidal low areas near the beach where it is spread within a concentrated area believed to contain many fish (Ravikumar et al. 2015). This dispersal is contained in a 20 to 20-meter area and the entire seed averages to about 34 mg. 34 mg of an average seed was deciphered by weighing the seed I collected after grinding it at the lab that I was preoccupying at the Gump station. A local at Moorea's Atitia Center named Francis stated that this amount is usually used for a village while a family would need only a quarter of the seed (pers. Comm.). On another note, 1-1.5 kg of the seed pulp has been used before for larger villages (Ravikumar et al. 2015).

Barringtonia asiatica contains a triterpene ester saponin that is known to cause hypoglycemia and disrupt protein digestion in various marine organism (Francis et al. 2002, Rumampuk et al. 2010). These compounds, however, do not fully poison the fish making it ideal for fishermen to use and eat. Human consumption of different parts of the *B. asiatica* tree can have different effects. Seed consumption can kill intestinal worms, coughs, ring worms, influenza and bronchitis. Fruit juice is used to treat scabies. Leaves are used as food, herb, spice, sore joints, and back aches, hernia, rheumatism, and diarrhea. The stem and boiled bark is used for stomach aches, tumors, epilepsy, constipation, toothaches, and tuberculosis (Ragasa et al. 2011, Pauku 2006). The triterpene saponins found in *B. asiatica* are also highly effective as antibacterial and antifungal agents. Such

antifungal and antibacterial activity is owed to the leaves on *B. asiatica* which have been found to contain the compounds germanicol caffeoyl ester and camelliagenone. (Elevitch 2006, Umaru et al. 2018).

Further, the compounds that create this activity are ranuncoside VII and oleanane glycoside. It has been found that ranuncoside VII compound is mostly responsible for the ichthyotic activity (Burton et al. 2003). Thus, I would like to see if the saponins found in the seed of the *B. asiatica* will have any adverse effects to the closest and testable marine life living in an intertidal area where mobility or escape from the fishing practice is limited. So far, the direct use of *B. asiatica* to assess the lethality on marine organisms besides fish has been documented on *artemia salina* or brine shrimp, tobacco cutworms, mollusks, crown of thorns star fish (*Ancathaster planci*) and protozoans like the *Plasmodium falciparum, Giarda Trophozites, Leshmania* species (Mojica and Micor 2007, Drabble 2017, Chaieb and Tayeb 2009, Mentor R. Hamidi et al. 2014, Francis et al. 2002). Knowing that *B. asiatica* has been tested on a micro and macroscopic level, I wish to study organisms that are exposed to this fishing practice. However, the tolerance to toxic chemicals by different marine invertebrates cannot be fully studied due to limitations, restrictions, and time constraints.

Thus, I have decided to study the effects of *Barringtonia asiatica* on *Littoraria coccinea* and observe their mortality rate once exposed to the toxin. I also wanted to see if a smaller dosage of the *B. asiatica* toxin would be needed based on the smaller size of *L. coccinea* in comparison to large fish which are the targeted organisms (Roy 1996). In this, my study site hosted an abundant population of *L. coccinea*. Having this abundancy would be beneficial to my study considering that my experiment deals with mortality rates. Further, with the use of phylogenetic information for *L. coccinea*, implementation of the data resulting from the experiment may be transferred to the organisms found within the *Littoraria* family and onto other organisms related to this family due to their morphological similarities. The species with the highest positive autocorrelation to my organism feature similar trait values like morphological similarities. In this, organisms similar to *L. coconiea*, may have similar susceptibility to the toxin (Guénard et al. 2011). There are five orthograstropoda clades that may be affected by *B. asiatica* (i.e. *patellogastropoda, neritomorpha, vetigastropoda, caenogastropoda, and heterobranchea*). Alternatively, specific organisms that would be morphologically similar to the *L. coccinea* gastropod community would be limpets, snails, slugs, and sea snail whom are also found in these intertidal areas where the traditional fishing is practiced (Zapata et al. 2014). This would also encompass the Christmas tree worms and other Mollusca found in Moorea. In short, such organisms would be susceptible to high concentrations of the *B. asiatica* toxin. My first goal is to see at what dosage will the organisms die and then use a general toxicity test to figure out the LC50 from the test. The LD50 or LC50 is defined as the toxin that kills half of the test subjects being observed (Government of Canada, Canadian Centre for Occupational Health and Safety). I will collect varied amount of size lengths and mass to see if these factors have a role in the timing of death for the gastropods. The significance within these results will be used to further prove that size holds a significant part on the resistance of organisms no matter the species and that toxicity relative to the size of the organism will vary (Gorth et al. 2011). A Logistic Regression analysis will be used for the Toxicity test experiment since the deaths will be followed in separate sequential time periods whereas the data collected from size and mass will be tested for normal distribution using Normality Test and may be placed in a T test if they are normally distributed. Most studies have used the HPLC system to create a correct solvent concentration and identify the correct compounds within that solvent for the toxicity

test (Burton et al. 2003). Due to the limitations in the lab, alternative methods were used.

METHODS

Study site

My research and my field site was located at the UC Berkeley Gump Research Station on the island of Moorea, French Polynesia. I collected my study organism, *Littoraria coccinea*, on the rocky outcrops near the station's shoreline. Collection of the gastropods was done using two sieves that could close the gastropods from escaping.

Preparation for the Toxicity Test

To test the response of *L. coccinea* to *B. asiatica* toxicity, I first collected *B. asiatica* seeds from the UC Berkeley Gump Research Station. Seeds collected were both mature (brown and hardened) and immature (green). Seeds were brought into the lab, where they were pulverized and grinded in a blender. The whole seed was used as that is the common practice of traditional fishers that use this amount for an entire village according to personnel from Moorea's Attia Center (pers. comm.), a center known for the cultural knowledge sustained by the locals of Moorea.

The seed powder was then sifted through 1 mm sieve onto a 250 μm sieve. The powder was then collected from the 250μm sieve. The total powder collected from one mature seed at the lab was 23.445g. This is not the average mass but taken from a collected seed only once.

I created a concentration gradient to identify the LD50 based on my results of preliminary trials. The final amounts used for each group was 0.1g, 0.2g, 0.4g, 0.8g, and 1.6g under a stock solution of 100 ml for each amount of the toxic seed powder which was then administered as 20 ml for 10 containers for each concentration group. 10 individual gastropods placed in each individual container for 5 groups including the controls totaled to 60 *L. coccinea* gastropods for the toxicity test.

Experimental Design for the Toxicity Test

The experiment was separated into 7 stations which were the controls, the 0.1/100 g/ml stock solution group, 0.2g/100 g/ml stock solution group, 0.4/100 g/ml stock solution group, 0.8/100 g/ml stock solution group, and the 1.6/100 g/ml stock solution group. All cups were filled with a 20 ml solution from the stock solution for each group of the powdered toxin creating: 0.01g, 0.02g, 0.04g, 0.08g, and 0.16g of the diluted toxin per container. *L. coccinea* was administered the poison once placed in the cups. All snails were observed over a 96-hour period. Specifically, the snails were checked every 12 hours until the 96-hour experiment was completed. At each time, the number of dead and alive gastropods was recorded.

Mortality Assessment

The snails were observed for mortality by dropping two 0.5 drops of sea water into the mouth of their shells for reaction using a pipet. Two 0.5 drops of sea water were used as to not completely dilute the toxin that has been absorbed by their bodies since that may disturb the objectivity within the test. In other words, this is a form of poison control within the experiment.

Toxicity Test Statistical Analysis

Data was entered to Excel and converted into the mean corrected % mortality using Abbott's formula (Corrected % Mortality = ((%alive control - %alive treated)/(%alive control)) * 100). Corrected % Mortality is the LD50 result, the % alive control is the percentage of the mean of the control group, the % alive treated is the percentage of the mean of each experimental group which was given different concentrated levels of the *B. asiatica* poison. The data would be inputted

into each part of the equation and then divided by the %alive control. This would all then be multiplied by 100 to get a percentage. After obtaining each result using this formula per group, each mean corrected % formula of each group would be highlighted as the Y-value and the X-value would be the concentration groups including the controls (0) which would then be plotted on a graph. On Excel, a chart element of a logarithmic trendline was added onto each graph. This logarithmic trendline would then obtain the regression equation which would help retrieve the LC50. The equation was then calculated through algebraic means in order to figure out the LC50. An equation that would be obtained for example, would be "*y=#(x)+#*" (Miller et al. 2010). All charts for both the Toxicity test and the size and weight correlation test were tested for normality on the PAST software.

Preparation for Size and Weight Correlation Test

Once the LD50 was determined, I used the associated concentration groups that killed half of the population, .01 g/ml, and used this concentration in a follow up experiment to explore the influence of size on mortality. In particular, I exposed 20 *L. coccinea* representing a range of sizes from the largest encountered to the smallest encountered. The gastropods were measured in mm length and weighed on a scale (g). In my study, 5.439g was weighed for the 0.01g concentration, 5.776g for the 0.02g, 5.161 for 0.04g, 5.53g for 0.08g, and 5.799 for 0.16g. Thus, the total mass for all the gastropods was 5.541g in my study. The experiment contained 20 separate cups with 1 stock solution of .02/200 g/ml which was diluted into .01/20 g/ml per cup. The gastropods were then placed into each cup and observed over a 60-hour period, including observations every 12 hours. Ten different *L. coccinea* were picked at random for each group for the size and weight correlation test.

Size/ Weight Correlation Statistical Analysis

After the data was collected, I used a T-test since my data came out normal according to different normality tests per time interval using the PAST software. The T-test utilized had Two-Samples assuming unequal variances. A P-value was derived which described whether the null hypothesis could be accepted or not.

RESULTS

Toxicity Test Results

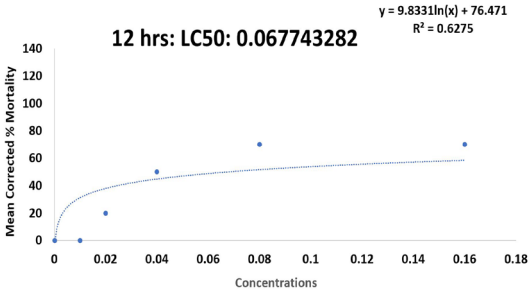

FIG. 1. All of the gastropods survived at .01/20 g/ml and .02/20 g/ml. Mortality increased for the other concentrations.

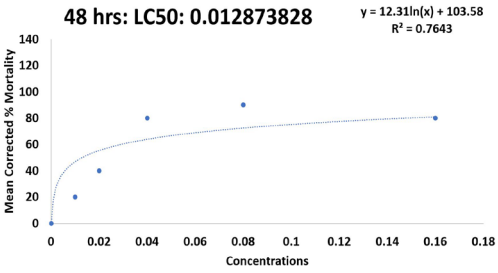

FIG. 2. Half of the gastropods die and the LC50 will be utilized for the size test.

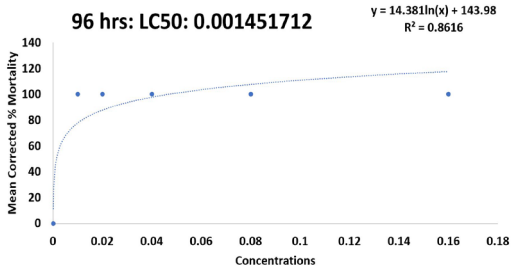

FIG. 3. The lowest LC50 derived from this test shows overexposure to toxicity as a larger factor to mortality rate.

Statistical Results

Normality tests stated that the population of snails under study came out as normally distributed according to the Shapiro-Wilk test, the Anderson-Darling test, and the Jarque-Bera test. I ran these tests per each time interval of the experiment. For the size correlation tests, the same normality tests were tested for the group under study and the P-value indicated that the null hypothesis, that being that there is no correlation between the death of the snails and higher concentration of the toxic seed powder over time, should not be rejected (P-value > .05). However, this doesn't disprove that my data is statistically significant.

Mortality Assessment

Lethargy and death seemed to be almost immediate with it taking about 30 minutes per snail. Fizzing or frothing from the mouth of the shells were documented as a lead to the death of the snail.

Toxicity Test Explanation

The pattern highlighted as time continues forward through the 96-hour period is that the LC50 begins to lower in its dosage of the *B. asiatica* toxin. In 12 hours, lower amounts of the B. asiatica powder causes less mortality especially at the start of the test where exposure is initiated (Fig. 1). This particular trend is seen throughout the 96-hour period but is more distinguishable within this time frame. Afterwards by 48 hours to 96 hours, snails increase in mortality due to the amount of exposure to the toxin and the amount of the diluted toxic seed powder (FIG. 2 and FIG. 3) Thus, the amount of the diluted toxic seed powder is a prevalent factor to mortality rate and that overexposure decreases the LC50.

Size and Weight Correlation Results

FIG. 4. At 12 hours, the average length with a 90% confidence interval of live snails was 14.53 mm and 12.4 mm of dead snails.

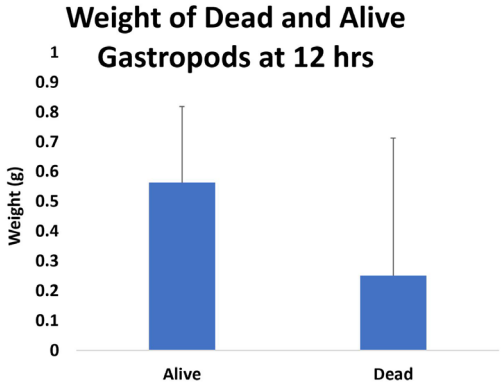

FIG. 5. The average weight of live gastropods was 0.56 g and the average dead was 0.25 g.

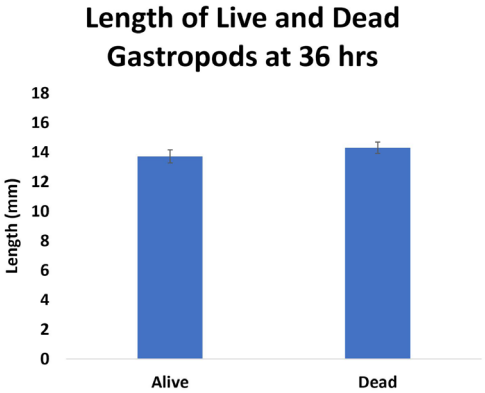

FIG. 6. At 36 hours, the average length of live gastropods was 13.7 mm and the average dead was 14.5 mm.

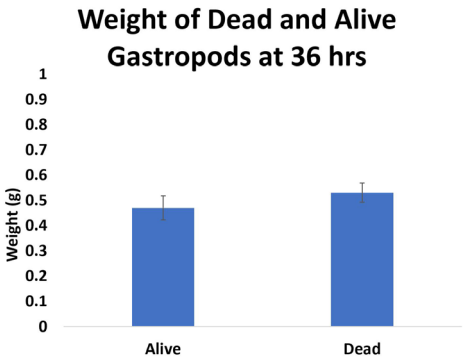

FIG. 7. The average weight of live gastropods was 0.47 g and the average dead was 0.53 g.

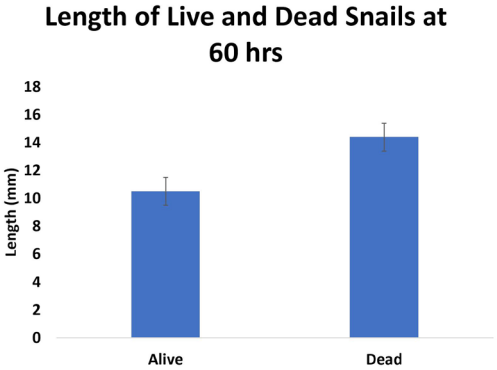

FIG. 8. At 60 hours, the average length of live gastropods was 10.5 mm and the average length of dead gastropods was 14.38 mm.

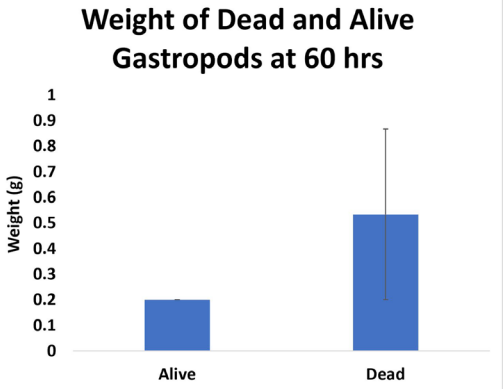

FIG. 9. The average weight of live gastropods was 0.2 g and the average weight of dead gastropods was 0.53 g.

Size and Weight Correlation Statistical Results

Normality tests performed for the size and weight correlation test suggest that the populations observed and studied were normally distributed and that only one normality test, Anderson-Darling, states that it isn't. Due to this, I decided to do a T-test of two samples assuming unequal variances because two normality tests, Shapiro-Wilk and Jarque-Bera, state that the populations studied are normal.

Size and Weight Correlation Explanation

At 12 hours, there are more gastropods alive with the average being 14.53 mm and 0.56 g (Fig. 4 and Fig. 5). During the middle of the test, 36 hours, two more gastropods have died; the average alive is 13. 7 mm and 0.47 g (Fig. 6 and Fig. 7). This leads to the 48-hour time interval in which 0.01/20 g/ml of the *B. asiatica* dilution killed half of the gastropod population at 48 hours until the 96 hours mark whereby all organisms had a mean corrected % mortality of 100%. Thus, 0.01/20 g/ml was implemented. After 60 hours, results using the T-test could not be achieved since two data points, two live snails, could no longer be calculated into the whole (Fig. 8 and Fig. 9).

DISCUSSION

The LC50 from my toxicity test ranged from 0.001 to 0.07 g/ml based on the time period of the test. Concentration of the *B. asiatica* toxin represented reduced as time passed stating the importance of long-time exposure for these types of tests. This may lead into the idea that higher concentrations of the toxin in a smaller duration of time would result in different data. This would also lead to the question of the amount of intoxication needed for *L. coccinea* to die within this shorter time period. In my study, at 12 hours, the LC50 of the gastropod population die within 0.067/20 g/ml (Fig. 1). At 48 hours, the LC50 is 0.013/20 g/ml (Fig. 2) and at 96 hours, it is 0.0014/20 g/ml (Fig. 3). From these calculations, the LC50 decreases through time, implying that there would be less doses of the toxin needed to kill half of the population

since the group is highly susceptible to mortality as further exposure continues.

Research done on the Marquises archipelago can help us understand the speed of the currents found in Moorea with the maximum velocity being 10 cm s^{-1} (Rougerie and Rancher 1994). The average speed indicated here would state that at a relatively short time period, all of the gastropods would be fine within these situations. However, if fishing is practiced in stagnant water, it can create adverse effects within this population and within other species niches that are morphologically similar to *L. coccinea* (Ravikumar et al. 2015). Since this fishing practice is mainly used to kill fish, the time used to kill fish will have to apply for these populations of marine invertebrates. Taken from a study, *B. asiatica* took 23 and 17 minutes at 150 to 200 ppm to kill the *Ambasis commersonii* which was the smallest fish of the study. If the smallest fish took this amount of time to surface and if fishermen were satisfied with this result, 17 to 23 minutes of a regular dose of 23.445 g of powder from a mature seed (which was the average seed of one *B. asiatica* fruit weighed at my lab) would kill all of the *L. coccinea* in stagnant waters under this area of concentration. At my study, mortality increased as the concentration of *B. asiatica* increased which is reflected by the results found in a study testing *Artemia salina* (brine shrimp). Mortality also increased significantly up to the last time period of their paper which is also reflected in my study (Mojica and Micor 2007).

B. asiatica has also been administered to terrestrial organisms such as the *Spodoptera litura* (tobacco cutworm) and a general toxicity test was also devised showing the same trends as in my study. Using the taro leaf dipping method, in which drops of the toxin were applied to leaves and ingested by *S. litura*, both time and different dosages was correlated to mortality. Higher doses of the toxin and longer exposure to the toxin increased mortality (Dono et al. 2012).

Further, in relation to the size correlation test, Roy studied different sizes of fish under different concentrations of the toxin based on their size while also recording their time in which half of the studied population died. *Ambasis commersonii* (69 mm) was given 200 ppm of *B. asiatica* and its LC50 arose around 23 and 17 min, *Tilapia Mossumbica's* (42 mm) was also given 200 ppm of the *B. asiatica* and its LC50 was in 40 min, and *Megalops cypinoides* (largest fish) was given 139 ppm; half of the population died at 145 mm. In this, the higher dosage within these time frames needed for fish to surface, average time being 40 min and higher would kill a community of orthogastropoda even if the dosage levels are at ppm (Roy 1996). This is reflected within my size correlation test in which gastropods will die despite their size overtime. This observation can also be seen in a paper testing fluoride intoxication in varying size of fish namely rainbow trout, carp, and goldfish. Although there are various factors that may contribute to the different reactions to fluoride in the wild, it has been concluded that larger fish succumb to the toxin last meaning that larger fish are more resistant to the toxin (Sigler and Neuhold 1972). Further, fish gathered from the Madison river showed that there is a positive correlation between intoxication per fish size and fluoride concentration (Sigler and Neuhold 1972). Another paper that analyzed this relationship studied the correlation between body surface area and intoxication from various doses of Melphalan on dogs with tumors. Although they saw a higher correlation of weight to toxicity than body surface area, they saw that weight was significantly correlated to hematological toxicity from Melphalan dose as seen here in their logistic regression analysis (Page et al. 1988).

Overall, *B. asiatica* was able to kill the invertebrate species *L. coccinea* through the smallest concentration possible in the lab, .01/20 g/ml. This is the smallest concentration in grams possible since other studies have used ppm as their main units (Mojica and

Micor 2007). The time period in which the organisms were observed to be half dead was at 48 hours in which the LC50 derived was 0.01 g/ml and was applied as a proxy for the size and weight correlation tests (Fig. 2). Size and weight may not be necessarily correlated to toxicity resistance as seen with the larger P-values conducted through T-tests. However, PASS stated that the population was normally distributed, and through this, we can conclude that the small sample size may be a factor to rejecting the null hypothesis.

Through the trends or patterns seen through the figures, we can conclude that size and weight play a major factor in those that are alive especially at the 12-hour mark. Within the 12-hour period of both the size and weight correlation test, there are many more *L. coccinea* individuals alive at this point with a few already dead, those being 11 mm in length, 13 mm, and one outlier being 16mm (Fig. 4). This same analysis is duplicated in the weight correlation test in which individuals were weighed at: 0.2 g and 0.4 g (Fig. 5). It would be noteworthy to state that the 13 mm snail was weighed at 0.2 g meaning that it was significant in my test to have both weight and size analyzed in my experiment since both units may deal with different factors such as shell length being longer but fragile in comparison to other organisms of the same population.

CONCLUSION

Through interviews with the locals at Moorea, most people stick to modern fishing practices since the fishing practice of using *B. asiatica* is banned; however, in other parts of the world, this type of fishing practice is still used which is why this study is considered useful (World Conservation Monitoring Centre 1998, Ravikumar et al. 2015). Further, it would be particularly interesting to see if whether timing and length plays a larger factor in resistance to the toxicity of an organism rather than varying types of concentration levels provided for varying sizes/mass of the organism.

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APPENDIX A

TOXICITY NORMALITY TESTS

	A	B
N	6	6
Shapiro-Wilk W	0.8543	0.9054
p(normal)	0.1704	0.4068
Anderson-Darling A	0.4465	0.2981
p(normal)	0.1756	0.4631
p(Monte Carlo)	0.1898	0.5018
Jarque-Bera JB	1.136	0.5945
p(normal)	0.5666	0.7429
p(Monte Carlo)	0.0974	0.4587

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12 hours

	A	B
N	6	6
Shapiro-Wilk W	0.8543	0.8543
p(normal)	0.1704	0.1704
Anderson-Darling A	0.4465	0.4465
p(normal)	0.1756	0.1756
p(Monte Carlo)	0.1898	0.1898
Jarque-Bera JB	1.136	1.136
p(normal)	0.5666	0.5666
p(Monte Carlo)	0.0974	0.0974

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24 hours

	A	B
N	6	6
Shapiro-Wilk W	0.8543	0.8813
p(normal)	0.1704	0.2749
Anderson-Darling A	0.4465	0.3839
p(normal)	0.1756	0.2671
p(Monte Carlo)	0.1881	0.2902
Jarque-Bera JB	1.136	0.6984
p(normal)	0.5666	0.7053
p(Monte Carlo)	0.0947	0.3328

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36 hours

	A	B
N	6	6
Shapiro-Wilk W	0.8543	0.8753
p(normal)	0.1704	0.2482
Anderson-Darling A	0.4465	0.3717
p(normal)	0.1756	0.2894
p(Monte Carlo)	0.1952	0.3136
Jarque-Bera JB	1.136	0.7744
p(normal)	0.5666	0.679
p(Monte Carlo)	0.0894	0.2401

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48 hours

	A	B
N	6	6
Shapiro-Wilk W	0.8543	0.7551
p(normal)	0.1704	0.02234
Anderson-Darling A	0.4465	0.6948
p(normal)	0.1756	0.03325
p(Monte Carlo)	0.1899	0.0315
Jarque-Bera JB	1.136	1.758
p(normal)	0.5666	0.4151
p(Monte Carlo)	0.0963	0.036

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60 hours

	A	B
N	6	6
Shapiro-Wilk W	0.8543	0.7937
p(normal)	0.1704	0.05153
Anderson-Darling A	0.4465	0.5968
p(normal)	0.1756	0.0644
p(Monte Carlo)	0.1898	0.0639
Jarque-Bera JB	1.136	1.124
p(normal)	0.5666	0.5701
p(Monte Carlo)	0.0974	0.093

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72 hours

	A	B
N	6	6
Shapiro-Wilk W	0.8543	0.5571
p(normal)	0.1704	0.0001344
Anderson-Darling A	0.4465	1.355
p(normal)	0.1756	0.000392
p(Monte Carlo)	0.1881	0.0001
Jarque-Bera JB	1.136	3.382
p(normal)	0.5666	0.1843
p(Monte Carlo)	0.0947	0.0001

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84 hours

	A	B
N	6	6
Shapiro-Wilk W	0.8543	0.4961
p(normal)	0.1704	2.073E-05
Anderson-Darling A	0.4465	1.599
p(normal)	0.1756	7.649E-05
p(Monte Carlo)	0.1899	0.0001
Jarque-Bera JB	1.136	3.56
p(normal)	0.5666	0.1686
p(Monte Carlo)	0.0963	0.0001

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96 hours

FIG. 1. Normality test came out normal until the 60-hour mark where the mortality rate increased.

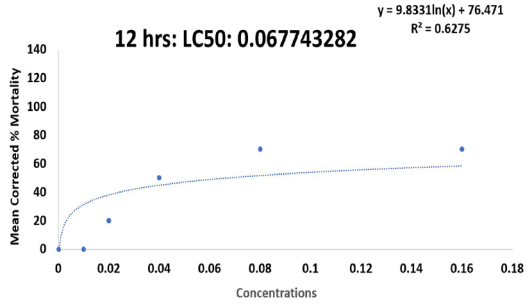

FIG. 1. All of the gastropods survived at .01/20 g/ml and .02/20 g/ml. Mortality increased from .04/20 g/ml, .08/20 g/ml, and 0.16/20 g/ml.

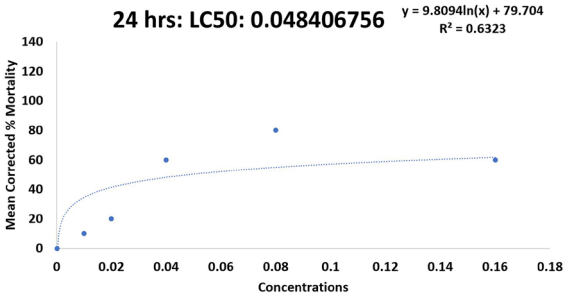

FIG. 2. There is one death at .01/20 g/ml and there is a further decline of live snails.

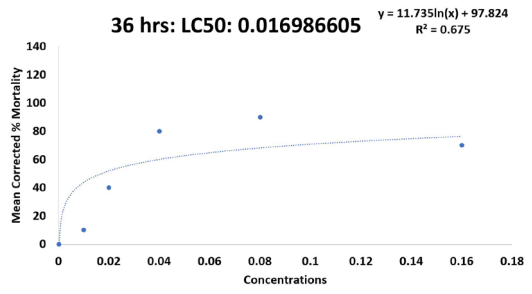

FIG. 3. There is a continuing increase in mortality with 0.16/20 g/ml holding the highest from this point and onwards.

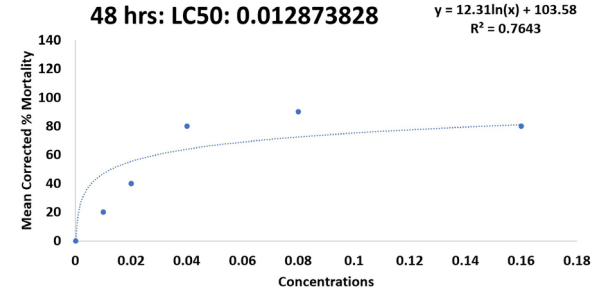

FIG. 4. Half of the gastropods observably die by this time period. This LC50 will be used by for the size and weight correlation test.

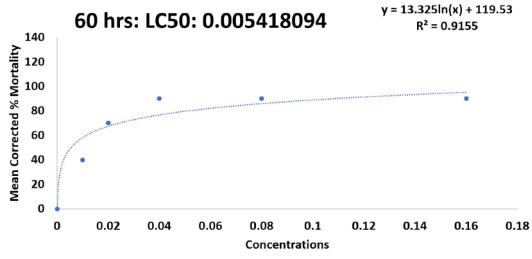

FIG. 5. Mortality increases exponentially at 0.02/20 g/ml.

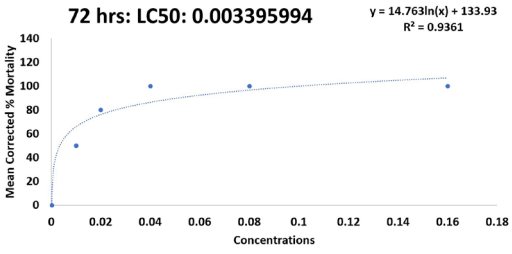

FIG. 6. Mortality continues to increase in all of the concentrations.

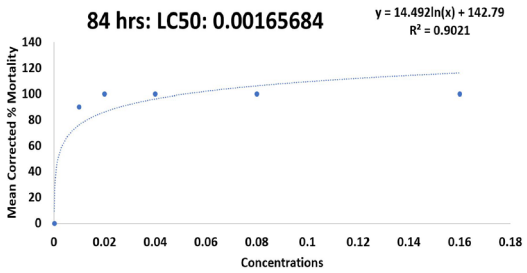

FIG. 7. All gastropods have died but one at 0.01/20 g/ml.

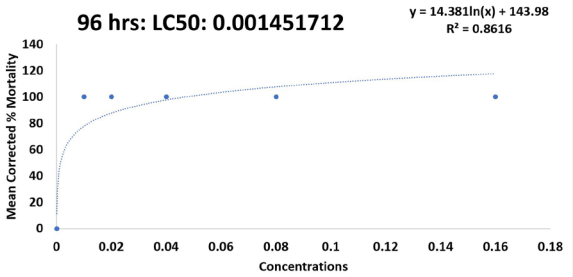

FIG. 8. The lowest LC50 derived from this test shows overexposure to toxicity as a larger factor to mortality rate.

SIZE AND WEIGHT NORMALITY TESTS

	All
N	20
Shapiro-Wilk W	0.9049
p(normal)	0.05101
Anderson-Darling A	0.7574
p(normal)	0.04055
p(Monte Carlo)	0.0417
Jarque-Bera JB	1.353
p(normal)	0.5085
p(Monte Carlo)	0.261

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FIG. 1. Normality test here states that the population of gastropods studied was normally distributed according to two tests: Shapira-Wilk and Jarque-Bera.

SIZE AND WEIGHT CORRELATION TEST RESULTS

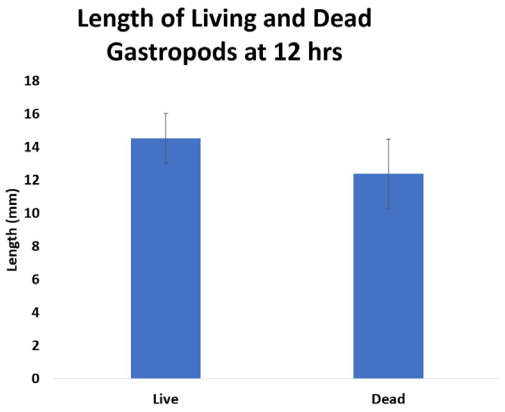

FIG. 1. 1. At 12 hours, the average length with a 90% confidence interval of live snails was 14.53 mm and 12.4 mm of dead
snails.

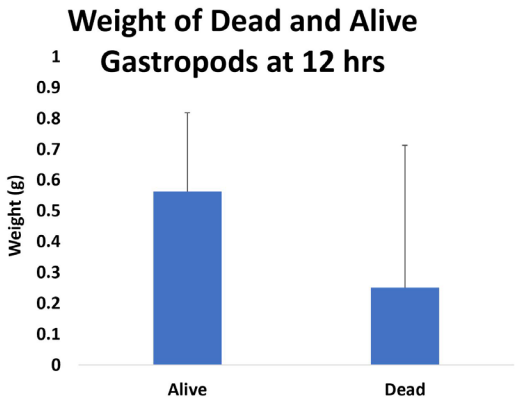

FIG 1. 2. The average weight of live gastropods was 0.56 g and the average dead was 0.25 g.

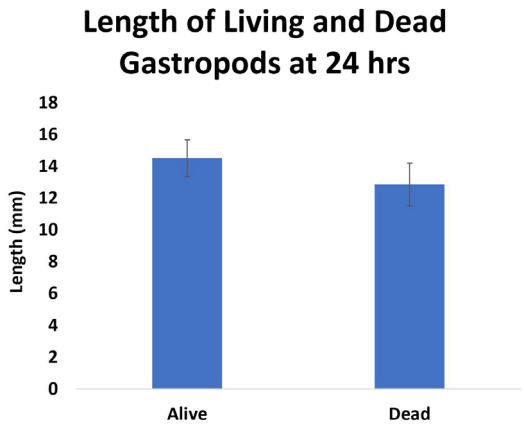

FIG. 2. 1. The average alive are 14.5 mm and the average dead are 12.83 mm.

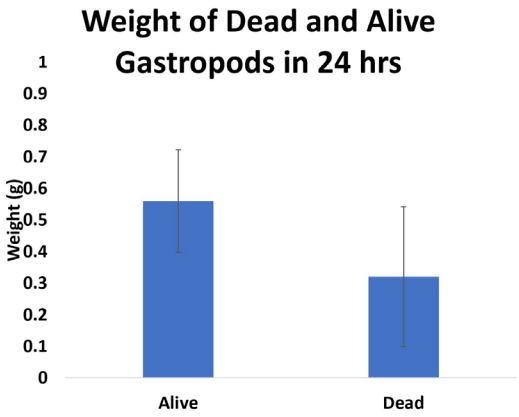

FIG. 2. 2. The average alive are 0.56 g and the average dead are 0.32 g.

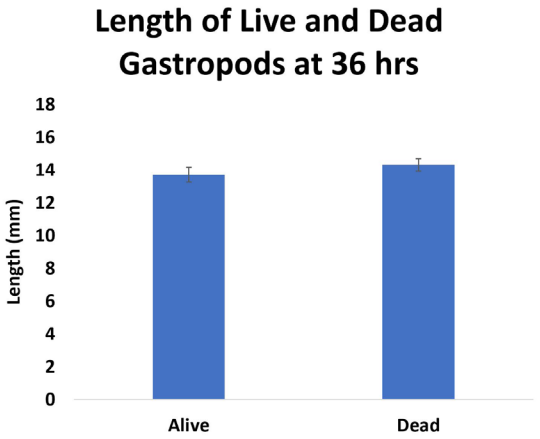

FIG. 3. 1. The average alive are 13.7

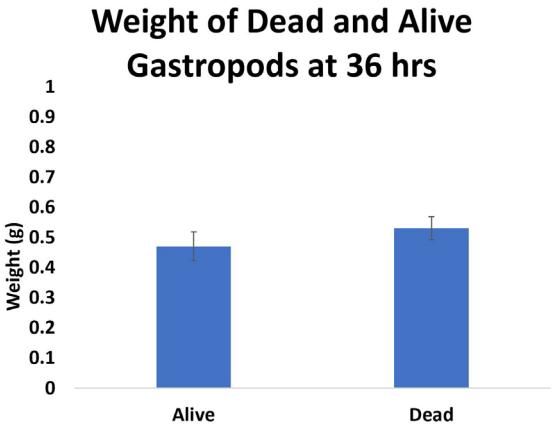

FIG. 3. 2. The average alive are 0.47 g and the average dead are 0.53 g.

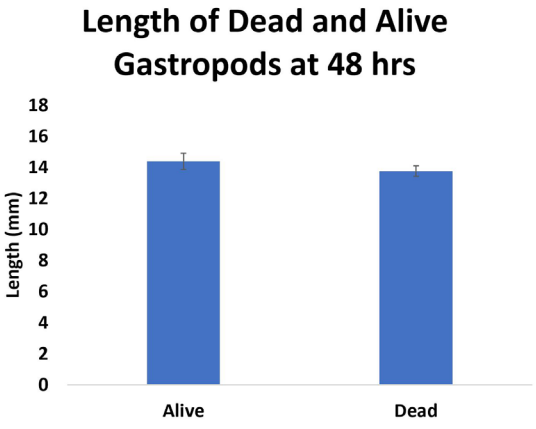

FIG. 4. 1. The average alive are 14.4 mm and the average dead are 13.8 mm.

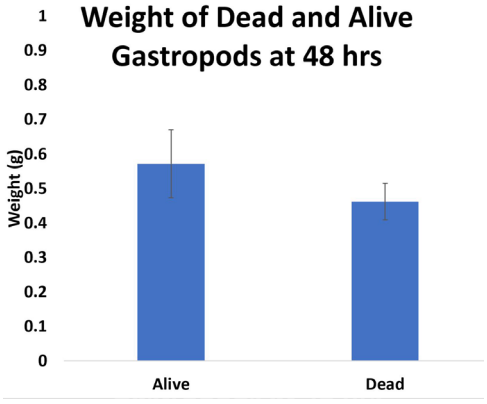

FIG. 4. 2. The average alive are 0.57 g and the average dead are 0.46 g.

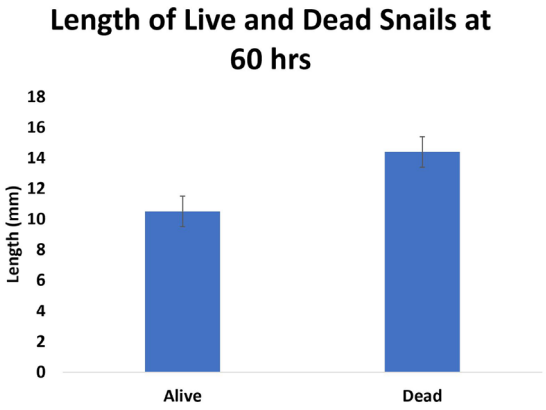

FIG. 5. 1. The average alive are 10.5 mm and the average dead are 14.4 mm.

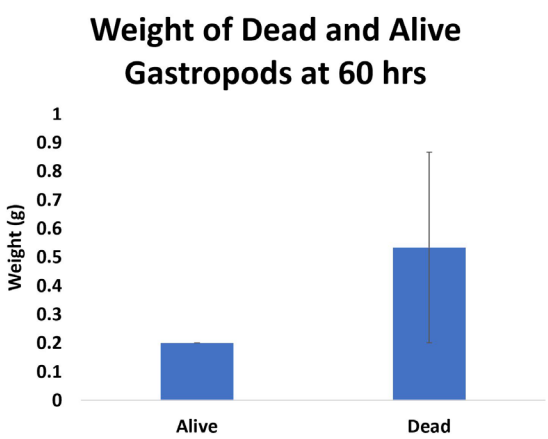

FIG. 5. 2. The average alive are 0.2 g and the average dead are 0.53 g.