THE REPELLING EFFECT OF GOLDEN APPLE SNAIL *(Pomacea canaliculata)* EGG EXTRACT ON ALGAE

Glenn Anjo C. Casiano[1], Hermes Paul T. Catalayban[2], Christine M. Melliza[3], Reneir Mhart M. Parreño[4], and Vincent L. Vecendario[5]

[5]John B. Lacson Foundation Maritime University-Arevalo
Sto. Niño Sur, Arevalo, Iloilo City, Philippines
e-mail: vince.weak09@gmail.com

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Abstract. There is an ongoing search for an alternative way to repel algae that is environmentally friendly, thus, this study was conducted. This study aimed to determine the repelling effect of golden apple snail (Pomacea canaliculata) egg extract on algae. Pure natural extract of golden apple snail eggs was taken and mixed with ordinary paint in different concentrations. Five metal sheets with three replicates for a total of 15 metal sheets in each block were prepared and painted with treatments. The metal sheets were distributed randomly in each of the three blocks and were exposed to the sea at an average depth of two meters for four days. Randomized complete block design (RCBD) was utilized as a research design of the study. Prior the final experiment, there were 6 treatments that undergone toxicity testing namely Treatment A - 80% (200 ml) golden apple snail egg extract and 20% (50 ml) ordinary paint, Treatment B – 50% (125 ml) golden apple snail egg extract and 50% (125 ml) ordinary paint, Treatment C – 20% (50 ml) golden apple snail egg extract and 80% (200 ml) ordinary paint, Treatment D – 100% (250 ml) antifouling paint 1 (positive control), Treatment E – 100% (250 ml) antifouling paint 2 (positive control), and Treatment F – 100% (250 ml) ordinary paint (negative control). Since the tested Treatment B was toxic because it had a result of 100% mortality rate on algae when tested during toxicity testing, it was removed. Thus, Treatment C was named to Treatment B and so forth to make it continuous. After the toxicity testing and final experiment, Treatment A had the least mean weight of 0.10 g of attached algae while Treatment E (100% ordinary paint) had the greatest mean weight of 0.94 g of attached algae. Block 2 (Villa Beach) had the least mean weight of attached algae on metal sheets after four days of sea exposure with a mean weight of 0.20 g for descriptive data analysis. For inferential data analysis, there were significant differences in the mean weight of attached algae after applying with various treatments and blocks for four days. Result showed that Treatment A, Treatment B, and Treatment D were the best treatments while Block 2 had the least mean weight of attached algae. It is recommended to use Treatment A (80% golden apple snail egg and 20% ordinary paint) because it had the least mean weight of attached algae and it has the presence of ovorubin and PcPV2 which were toxins found on golden apple snail eggs (Dreon, Frassa, Ceolín, Ituarte, Qiu, Sun, Fernández, and Heras, 2013). These two toxins might be the reason for the repelling effect of *P. canaliculata* to algae. The conceptualization of this study is based on a maritime issue specifically biofouling on ships.

1 INTRODUCTION

Fouling is the unwanted growth of organisms on ship's hull or any man-made structure submerged at sea (IMO, 2003). Fouling on ships results to reduction of speed. As a result, an increase of fuel consumption is needed for ships to travel to its destination thus more money is needed. This effect is due to the resistance of the hull's movement through the water (US Naval Institute, 1952).

For centuries, it became a problem in the maritime industry, but during 1960s the organotin compound tributyltin (TBT) was used to coat ship's hull to act as an antifouling system. However, during the 1970s and 1980s, it was discovered that TBT is toxic for the marine organisms such as shellfish. Thus, in 1990 the International Maritime Organization (IMO) adopted a resolution which eliminate antifouling paints containing TBT and in January 2008 the complete prohibition was accomplished (IMO, 2003). As a result of ecological impact of TBT, there is a continuous search for antifouling compounds that are economically and environmentally friendly. The search of finding an antifouling material is to utilize a readily available and non-toxic material such as the eggs of a pest specifically the golden apple snail (Gopikrishnan, Radhakrishnan, Pazhanimurugan, Shanmugasundaram, and Balagurunathan, 2015).

Golden apple snail or *Pomacea canaliculata* is an invasive snail species from South America introduced to Asian countries including the Philippines to fight protein deficiency (International Rice Research Institute, 2017). But due to the loss of its commercial use, it was neglected in which it spread and became one of the major rice pests in the Philippines. During spawning season, the females crawl out of the water and lay 25-500 eggs which hatch in 10 to 15 days (New Pest Advisory Group, 1998). Its eggs contain a biochemical defense which includes ovorubin which have anti-digestive and anti-nutritive properties and PcPV2 neurotoxin (Dreon et al., 2013).

There are more than 4000 species of fouling organisms, mostly found near sea inlets and shallow waters due to the availability of nutrients. These organisms may also be introduced to other places by ship hulls when the attached species release larvae and leave them at new different ports (Corrosionpedia, 2018). Algae are aquatic organisms that have the ability to conduct the process which uses sunlight to create food from carbon dioxide and water or also known as photosynthesis (Vidyasagar, 2016). Algae can settle and develop on a broad range of surfaces including both natural and artificial or man-made structures. They are fast settlers and can displace numerous species in the competition for food, space and resources (Hellio, Lebret, and Thabard, 2009).

This study was conceptualized because of the following reasons: First, golden apple snail egg is readily available. Second, fouling can result to the deformation of ship's hull which lessens the ship's speed and increases the fuel consumption, and lastly, after the ecological effects of TBT, there is a worldwide continuous search for economically and environment-friendly antifouling materials (New Pest Advisory Group, 1998; US Naval Institute, 1952; Gopikrishnan et al., 2015).

This study was conducted in the coastal areas of Iloilo from Oton Beach, Oton, Iloilo passing Villa Beach, Villa, Iloilo City to Molo Beach, Molo, Iloilo City. The basis for blocking are due to high density of population and presence of commercial establishments along the beaches. The coastal waters of Iloilo are within the 200 MPN/100 ml standard for fecal coliform concentration. This means that the coastal waters of Iloilo are rich in coliform which could be considered nutrient to algae (Ambient Water Quality Report Annual, 2015).

In this study, mixtures of two different concentrations of ordinary paint and golden apple snail egg extract were the experimental treatments. Two different commercial brands of antifouling paints and an ordinary paint were the control treatments. Both groups are the independent variables. The weight of attached algae (g) on the metal sheets in a span of four days was the dependent variable. The mixtures were applied in the metal sheets which were exposed in the beaches of Oton, Iloilo (Block 1), Villa, Iloilo City (Block 2), and Molo, Iloilo City (Block 3) if it is effective in the prevention of the attachment of algae.

This study aimed to determine the repelling effect of golden apple snail (*P. canaliculata*) egg extract on algae after four days of exposure to seawater. Specifically, this study aimed to answer the following questions: 1. What is the toxicity level of golden apple snail (*P. canaliculata*) egg extract on algae and crabs? 2. What is the mean weight (g) of attached algae after applying with various treatments after four days of exposure to seawater? 3. What is the mean weight (g) of attached algae among blocks after four days of exposure to seawater? 4. Is there a significant difference in the mean weight (g) of attached algae after applying with various treatments for four days of exposure to seawater? 5. Is there a significant difference in the mean weight (g) of attached algae among blocks for four days of exposure to seawater?

2 MATERIALS AND METHODS

There were five treatments with three replicates that were used in this experiment: Treatment A - 80% (200 ml) golden apple snail egg extract and 20% (50 ml) ordinary paint, Treatment B – 20% (50 ml) golden apple snail egg extract and 80% (200 ml) ordinary paint, Treatment C – 100% (250 ml) antifouling paint 1 (positive control), Treatment D – 100% (250 ml) antifouling paint 2 (positive control), and Treatment E – 100% (250 ml) ordinary paint (negative control). Prior the final experiment, there were 6 treatments that undergone Treatment A - 80% (200 ml) golden apple snail egg extract and 20% (50 ml) ordinary paint, Treatment B – 50% (125 ml) golden apple snail egg extract and 50% (125 ml) ordinary

paint, Treatment C – 20% (50 ml) golden apple snail egg extract and 80% (200 ml) ordinary paint, Treatment D – 100% (250 ml) antifouling paint 1 (positive control), Treatment E – 100% (250 ml) antifouling paint 2 (positive control), and Treatment F – 100% (250 ml) ordinary paint (negative control). Since the tested Treatment B was toxic, it was removed. Thus, Treatment C was named to Treatment B and so forth to make it continuous. These treatments were applied to metal sheets with corresponding concentrations and were exposed across Oton, Iloilo (Block 1), Villa, Iloilo City (Block 2) and Molo, Iloilo City (Block 3) Beaches, respectively at an average depth of two meters.

The following materials were used for the conduct of this experiment: 45 metal sheets (9 inches x 8 inches), paper tape, scissors, sand paper, mortar and pestle, beakers, golden apple snail egg taken from Poblacion South, Oton, Iloilo, commercial antifouling paints, bamboos, containers, ordinary paint (enamel), analytical weighing scale, scraper, wires, rope, pliers, floater, knife, and paint brush.

3 DATA COLLECTION

3.1 Extracting golden apple snail egg and conducting the toxicity test

Golden apple snail egg (taken from Poblacion South, Oton, Iloilo) was used for the experiment. In extracting golden apple snail egg, the researchers brought the snail eggs to a laboratory, and the researchers conducted an extraction with the assistance of the laboratory personnel. The extracted golden apple snail eggs were used in determining its toxicity level at Southeast Asian Fisheries Development Center/Aquaculture Department (SEAFDEC/AQD), Tigbauan, Iloilo, on algae (*Enteromorpha clathrata Roth Greville*) and crabs (*Scylla serrata*) (OECD Guideline for Testing of Chemicals, 1984).

3.2 Preparing the metal sheets, formulating the pain concentration and trial-and-error set-ups

Forty-five metal sheets with dimension of 9 inches x 8 inches were used. Four holes were placed on each edges of the metal sheets and were sanded down so that paint will adhere to the sheets efficiently. The golden apple snail egg extract was mixed with oil-based paint (enamel). The concentrations of the mixtures were based on the treatments. Trial-and-error set-ups were conducted for the purpose of reducing mistakes during the proper procedure of experiment and also to master the process.

3.3 Preparing and retrieving the experimental set-ups

The 45 metal sheets were coated with different concentrations of mixtures. Using fishbowl method, random numbers were picked for the arrangement for each block. The set-up was composed of 15 experimental units with five treatments with three replicates for each

block. Randomized complete block design (RCBD) was utilized. The plates were labeled from A1 to A3, B1 to B3, C1 to C3, D1 to D3, and E1 to E3 for each block. The letters A to E were picked randomly to determine the arrangement of treatments on each block. Next, the metal sheets were hung with the use of a wire that was inserted into the holes on the edges of the sheets. The set-up was made of bamboos that were put together forming a frame secured with wires on corners and this acted as the base for the sheets to be hung. When the set-up was exposed into the water, empty containers served as floaters that acted as a marking guide for locating the set-up. After four days of exposure, the metal sheets were retrieved. The metal sheets which contain the needed results were preserved for the next process.

3.4 Determining the mean weight (g) of algae

The retrieved metal sheets were scraped individually by the researchers with the use of a scraper. The scraped algae were weighed separately and collected data were compared to determine the most effective treatment. Figure 2 shows the randomization of treatments with three replicates for each block.

4 DATA ANALYSIS

On the toxicity test of algae, it was measured by using percentage. For the descriptive data analysis, algae were counted in each treatment using mean and standard deviation as statistical tools and for inferential data analysis, randomized complete block design (RCBD) was used set at .05 level of significance.

For the Post hoc tests, the means of the treatments and blocks were compared using Scheffe test set at .05 level of significance.

For the toxicity test, LD50 or Lethal Dose 50 was used with the following scheme:

> 50% - Toxic
< 50% - Non-toxic

5 RESULTS AND DISCUSSION

5.1 Descriptive Data Analysis

5.1.1 Toxicity test results

Tables 1 and 2 shows the results of toxicity test on algae and crabs. Treatment B, a metal sheet with concentrations of 50% extracted golden apple snail eggs and 50% ordinary paint that was tested on crabs showed 20% mortality. A non-toxic description for Treatment B on crabs for the result was less than 50%.

On algae, it yielded a 100% mortality rate on both replicates and was described as toxic. All descriptions were based on LC50 protocol. Because of this, it was removed. See below.

5.1.2 Mean weight (g) of attached algae among treatments and among blocks

Table 3 shows the weight of attached algae (g) in averages of each replicates of each treatments and blocks. Treatment A (80% golden apple snail egg extract and 20% ordinary paint) having the mean weight of 0.10 g of attached algae and has the lowest among the treatments. Treatment E (100% ordinary paint), which is the negative control, having a mean weight of 0.94 g of attached algae and has the highest mean weight (g) among treatments.

Block 1 (Oton Beach) and Block 3 (Molo Beach) have the most attached algae on metal sheets after four days of sea exposure with the same mean weight of 0.42 g. Block II (Villa Beach) has the least attached algae with a mean weight of 0.20 g. See below.

5.2 Inferential Data Analysis

5.2.1 Significant difference in the mean weight (g) of attached algae on treatments and blocks

Table 4 shows that *F* computed for treatments, which is 4.97 is greater than *F* tabulated, which is 4.76. Thus, there is a significant difference in the mean weight (g) of attached algae among treatments and at least one has a different mean weight of attached algae, $F_{tab} > F_{comp}$.

On the other hand, *F* computed for blocks, is 8.20, is greater than *F* tabulated, which is 5.14. This means that there is a significant difference in the mean weight of attached algae among the blocks, $F_{tab} > F_{comp}$. See below.

Mean weights of each treatment are compared using Scheffe test as shown in Table 5. All the treatments are not significantly different from each other but only Treatment A (80% golden apple snail egg extract and 20% ordinary paint) and Treatment C (100% antifouling paint 1) are significantly different.

Thus, Treatment A, Treatment B and Treatment D are the best treatments having the least mean weights of attached algae.

Due to the effects of the compound tributyltin or TBT which is an ingredient on antifouling paints, there is still an ongoing search for alternatives of antifouling paint (Gopikrishnan et al., 2015). The presence of ovorubin which has an anti-nutritive and anti-digestive property and PcPV2 which is the only reported genetically encoded toxin found inside an egg were utilized as an alternative ingredient to antifouling paints (Dreon, Frassa, Ceolín, Ituarte, Qiu, Sun, Fernández, and Heras, 2013).

Mean weights of each block are compared using Scheffe test as shown in Table 6. Blocks 1 (Oton Beach) and 2 (Villa Beach) and Blocks 1 (Oton Beach) and 3 (Molo Beach) are not significantly different from each other but Block 2 (Villa Beach) is significantly different from Block 3 (Molo Beach).

Thus, Block 1 and Block 2 have the least mean weight of attached algae. According to Brunn (2012), algae quickly respond to changes like change in pH level, change in amount of sunlight, and change in salinity. The ratio of nitrogen and phosphorous in the body of water caused by water pollution, helps the growth and development of algae. That might be the reason why Blocks 1 & 2 have the least mean weight (g) of attached algae.

6 CONCLUSIONS

Based on the results, the following are concluded: Treatment B (50% golden apple snail egg extract and 50% ordinary paint) is toxic because LD50 protocol stated that if mortality rate was greater than 50%, the treatment used was toxic because it had a result of 100% mortality rate; Treatment A (80% golden apple snail egg extract and 20% ordinary paint), Treatment B (20% golden apple snail egg extract and 80% ordinary paint), and Treatment D (antifouling paint 2) were the best treatments since they had the least mean weights (g) of attached algae after four days of exposure to seawater. PcPV2 and ovorubin as toxin and protein present in golden apple snail egg has the anti-nutritive and anti-digestive property according to Dreon, Frassa, Ceolín, Ituarte, Qiu, Sun, Fernández, and Heras (2013) and were utilized as an alternative ingredient to antifouling paints that is used as an antifouling ingredient to repel the attachment of algae; and Block 2 (Villa Beach) and Block 1 (Oton Beach) were the best blocks since they have the least mean weights (g) of attached algae due to less presence of household in the location. According to Brunn (2012), algae rapidly react to changes like change in pH level, change in amount of sunlight, and change in salinity. The ratio of phosphorous and nitrogen in the body of water caused by pollution in the water, helps the growth and development of algae. That might be the reason why Block 2 has the least mean weight (g) of attached algae.

Table 1. Toxicity Result of Golden Apple Snail (*P.canaliculata*) Egg Extract on Algae (*E.clathrata Roth Greville*)

Treatments	Weight (g)		Ave.Wt. (g)	Initial Wt. (g)	Growth (g)	Total Wt. (g)	Mortality Rate (%)	Remarks
	Wet	Dry						
A1	50.53	47.27	48.90	10.11	1.37	11.48	0	NT
A2	51.26	47.71	49.49	10.21	1.64	11.85	0	NT
B1	-	-	-	10.23	0.00	0	100	T
B2	-	-	-	10.04	0.00	0	100	T
C1	53.94	52.56	53.25	10.18	0.92	11.10	0	NT
C2	54.99	53.25	54.12	10.29	1.16	11.45	0	NT
D1	52.78	50.84	51.81	10.31	1.10	11.41	0	NT
D2	51.82	49.82	50.82	10.01	1.08	11.09	0	NT
E1	53.77	50.98	52.38	10.09	1.27	11.36	0	NT
E2	53.27	50.16	51.72	10.18	0.74	10.92	0	NT
F1	52.3	50.73	51.52	10.07	1.91	11.98	0	NT
F2	51.54	49.79	50.67	10.11	0.01	10.12	0	NT

Table 2. Toxicity Result of Golden Apple Snail (*P. canaliculata*) Egg Extract on Crabs (*S. serrata*)

Treatments	No. of Crabs (N=5)		Mortality Rate (%)	Remarks
	Dead	Alive		
A1	0	5	0	NT
A2	0	5	0	NT
B1	1	4	20	NT
B2	0	5	0	NT
C1	0	5	0	NT
C2	0	5	0	NT
D1	0	5	0	NT
D2	0	5	0	NT
E1	0	5	0	NT
E2	0	5	0	NT
F1	0	5	0	NT
F2	0	5	0	NT

Note. NT = Non-toxic and T = toxic. Mortality rate for LD50: > 50% - Toxic and <50% - Non-toxic. Treatments A1 and A2 - 80% (200 ml) golden apple snail egg extract and 20% (50 ml) ordinary paint, Treatments B1 and B2 – 50% (125 ml) golden apple snail egg extract and 50% (125 ml) ordinary paint Treatments C1 and C2 – 20% (50 ml) golden apple snail egg extract and 80% (200 ml) ordinary paint, Treatments D1 and D2 – 100% (250 ml) antifouling paint 1 (positive control), Treatments E1 and E2 – 100% (250 ml) antifouling paint 2 (positive control), and Treatments F1 and F2 – 100% (250 ml) ordinary paint (negative control).

Table 3. Mean Weight (g) of Attached Algae After Four Days of Exposure to Seawater

	Treatments							
Blocks	A	B	C	D	E	Total	Mean Wt. (g)	SD
1	0.12	0.14	0.29	0.19	1.37	2.12	0.42	0.55
2	0.07	0.08	0.17	0.07	0.60	1.00	0.20	0.24
3	0.10	0.24	0.49	0.41	0.85	2.09	0.42	0.35
Total	0.29	0.46	0.95	0.68	2.83			
Mean Wt. (g)	0.10	0.15	0.32	0.23	0.94			
SD	0.05	0.09	0.17	0.18	0.55			

Note. Treatment A - 80% (200 ml) golden apple snail egg extract and 20% (50 ml) ordinary paint, Treatment B – 20% (50 ml) golden apple snail egg extract and 80% (200 ml) ordinary paint, Treatment C – 100% (250 ml) antifouling paint 1 (positive control), Treatment D – 100% (250 ml) antifouling paint 2 (positive control), and Treatment E – 100% (250 ml) ordinary paint (negative control). For blocks, Block 1 – Oton Beach, Iloilo Block 2 –Villa Beach, Iloilo City and Block 3 –Molo Beach, Iloilo City.

Table 4. Results of Randomized Complete Block Design (RCBD)

Sources of Variation	df	SS	MS	F_{comp}	F_{tab}
Treatments	3	0.08	0.03	4.97*	4.76
Blocks	2	0.09	0.05	8.20*	5.14
Error	6	0.03	0.01		
Total	11	0.20			

Note. Asterisk (*) means significant at .05 level of probability.

Table 5. Comparison of Treatment Means Using Scheffe Test

Treatments	Mean Wt. (g) of Attached Algae
A	0.10^{a}
B	0.15^{ab}
C	0.32^{b}
D	0.23^{ab}

Table 6. Comparison of Block Means Using Scheffe Test

Block	Mean Wt. (g) of Attached Algae
1	0.42^{ab}
2	0.20^{a}
3	0.42^{b}

Note. Block 1 – Oton Beach, Iloilo; Block 2 –Villa Beach, Iloilo City; and Block 3 –Molo Beach, Iloilo City.

7 RECOMMENDATIONS

Based on the findings and conclusions, the following are recommended: use of treatment A (80% golden apple snail egg extract and 20% ordinary paint) as an antifouling agent against algae; further studies should be conducted to validate the results; other species of snail eggs to be used as an additive to paints; the egg extract must be tested to other fouling organisms; and more arrays of marine organisms must be tested in toxicity test.

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