Improving the Management of Invasive Golden Apple Snails (*Pomacea canaliculata*) in the Singapore Quarry

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ABSTRACT

The invasion of the golden apple snail (*Pomacea canaliculata*) into Asia has the potential to cause serious ecological damage to healthy aquatic ecosystems. The two main current control methods used, molluscides and manual removal, are ineffective and promising enhancements have not yet been widely tested. The snail's impact on macrophytes in Singapore has also not been established. Thus, my study objectives were: (1) estimate the impact of the golden apple snail on the macrophytes in the Singapore Quarry; (2) investigate new control methods based on the snails' habitat distribution and organic attractants (boiled and fresh papaya leaves, newsprint). I used a gut content analysis to evaluate the diet of the snails, quadrats and transects to characterize the snails' distribution, and an experiment to test the effectiveness of the attractants. I found that the snails were indeed feeding preferentially on macrophytes, that there were significantly more snails in warmer areas of the Quarry (water temperature > 33.34°C), and that boiled papaya leaves was the most effective attractant, especially in the short run (< 2 hours). Thus, my study justifies urgent control of the golden apple snail in the Quarry and suggests that current manual removal efforts can be enhanced by placing boiled papaya leaves in warmer areas of the Quarry to concentrate the snails. As a case study of a tropical quarry lake, it also confirms that conclusions from the literature about the golden apple snail diet and susceptibility to attractants, but not their distribution, can be generalized to diverse habitats.

KEYWORDS

invasion ecology, habitat distribution, organic attractants, gut content analysis, temperature

INTRODUCTION

The invasion of the golden apple snail (*Pomacea canaliculata*) into Asia has the potential to cause serious ecological damage to healthy aquatic ecosystems. Originally from Argentina, the snails are voracious herbivores and can reduce native species richness by outcompeting other herbivorous macroinvertebrates (Fang et al. 2010). In Singapore, invasive populations of the snail have been established in natural freshwater bodies since at least 1993 (Ng et al. 1993). In Southeast Asia, golden apple snail invasions have also been documented to cause clear-water wetlands dominated by macrophytes, or macroscopic aquatic plants, to transform into turbid, phytoplankton-dominated systems (Carlsson et al. 2004). This transformation is problematic as it reduces biodiversity and the availability of ecosystem services like nutrient cycling (Carlsson et al. 2004). The destructive potential of the golden apple snail is also compounded by its life history traits, which are typical for that of a successful invasive species (Sakai et al. 2001); it is a food generalist (Lach et al. 2000), has high fecundity (Teo 2004), and matures rapidly under tropical conditions (Teo 2004). However, despite the threat posed by the golden apple snail, there has been little progress in developing a control method that is efficient, economical and environmentally-friendly.

Today, the two main methods that farmers and managers use to control the golden apple snail are applying molluscides and manually removing the snails; neither method is ideal, and although promising enhancements exist, they have not been widely tested (Joshi 2005). The main problem with existing control methods is that molluscides pollute the environment and are expensive, while manual removal of the snails via handpicking is very inefficient (Litsinger and Estano 1993). Furthermore, methods that could enhance the efficiency of handpicking have only been tested under very limited conditions (Joshi 2005). One promising possibility is targeting handpicking efforts based on the microhabitat distribution of the snail, i.e. the distribution of the snail within a single habitat (Crowl and Schnell 1990). Microhabitat studies conducted in streams have found that golden apple snails cluster in shallower areas (Seuffert and Martin 2010a) and are more active as temperatures rise (Seuffert et al. 2010b). However, such studies have only been conducted in Argentina. To further target handpicking efforts, organic attractants can also be used to attract the snails to a more central location. Attractants like fresh papaya leaves and newsprint appear to be effective, but have only ever been tested in rice fields in the Philippines (Joshi 2001).

Also, boiled attractants have never been tested, but could be even more effective since the snails feed preferentially on macrophytes with low physical and chemical defenses (Qiu & Kwong 2009). Thus, understanding the ecology of the golden apple snail in terms of its microhabitat distribution and investigating the efficacy of various attractants in different habitats is important in enhancing the effectiveness of the manual control method.

In the case of the Singapore Quarry, additional diet studies are also needed to establish the need for control of the golden apple snail, because its impact on the macrophyte populations in the Quarry has not yet been established. Feeding experiments (e.g., Lach et al. 2000, Carlsson and Lacoursiere 2005) and a stomach content analysis (Kwong et al. 2009) conducted in other tropical aquatic habitats in Asia have found that the snails feed preferentially on macrophytes. Thus, local park managers from the Singapore National Parks Board (NParks) are concerned that the snail will destroy macrophyte populations in the Quarry (Ramakrishnan s/o R. Kolandavelu 2011, personal communication). Yet, they have not personally observed this damage (Koa Tian Leng 2013, personal communication) and no formal studies have been performed on the diet of the snail in the local context. Hence, managers are still unsure if intensive control of the golden apple snail is actually necessary. Moreover, NParks currently uses only handpicking to control the golden apple snail and avoids using molluscides to ensure that no other wildlife is harmed (Koa Tian Leng 2013, personal communication). However, these handpicking efforts have not been very successful due to a lack of manpower (Koa Tian Leng 2013, personal communication). For a more scientifically-rigorous and efficient control protocol to be developed in Singapore, the diet and distribution of local golden apple snails and the efficacy of attractants under local conditions must be investigated.

In my study, I will investigate these three factors in the context of the Singapore Quarry to determine if there is an urgent need for control of the golden apple snail in the Quarry and how current handpicking efforts can be made more efficient. First, in order to estimate the snails' impact on the macrophytes in the Quarry, I will ask whether they feed preferentially on these macrophytes or on other food sources like green algae. I hypothesize that macrophytes and organic detritus will dominate the snails' diet (Kwong et al. 2009). Second, I will ask whether and how the golden apple snail distribution within the Quarry can be predicted by abiotic factors like water depth and water temperature. I hypothesize that snail density will be positively correlated with water temperature and negatively correlated with water depth. Lastly, I will ask if organic attractants like fresh papaya leaves, boiled papaya leaves and newsprint are effective in attracting golden apple snails under the conditions at the Singapore Quarry. I will test this by conducting an experiment at the Quarry and observing the number of snails attracted to each substance. I hypothesize that all three attractants will be more effective than the control in attracting the snails. I also hypothesize that the boiled papaya leaves will be the most effective attractant (Qiu & Kwong 2009).

METHODS

Study system

The site where I conducted all my field work was the Singapore Quarry (1°21”N, 103°46”E), a freshwater quarry lake located about 10km north of the Singapore Central Business District (Figure 1).

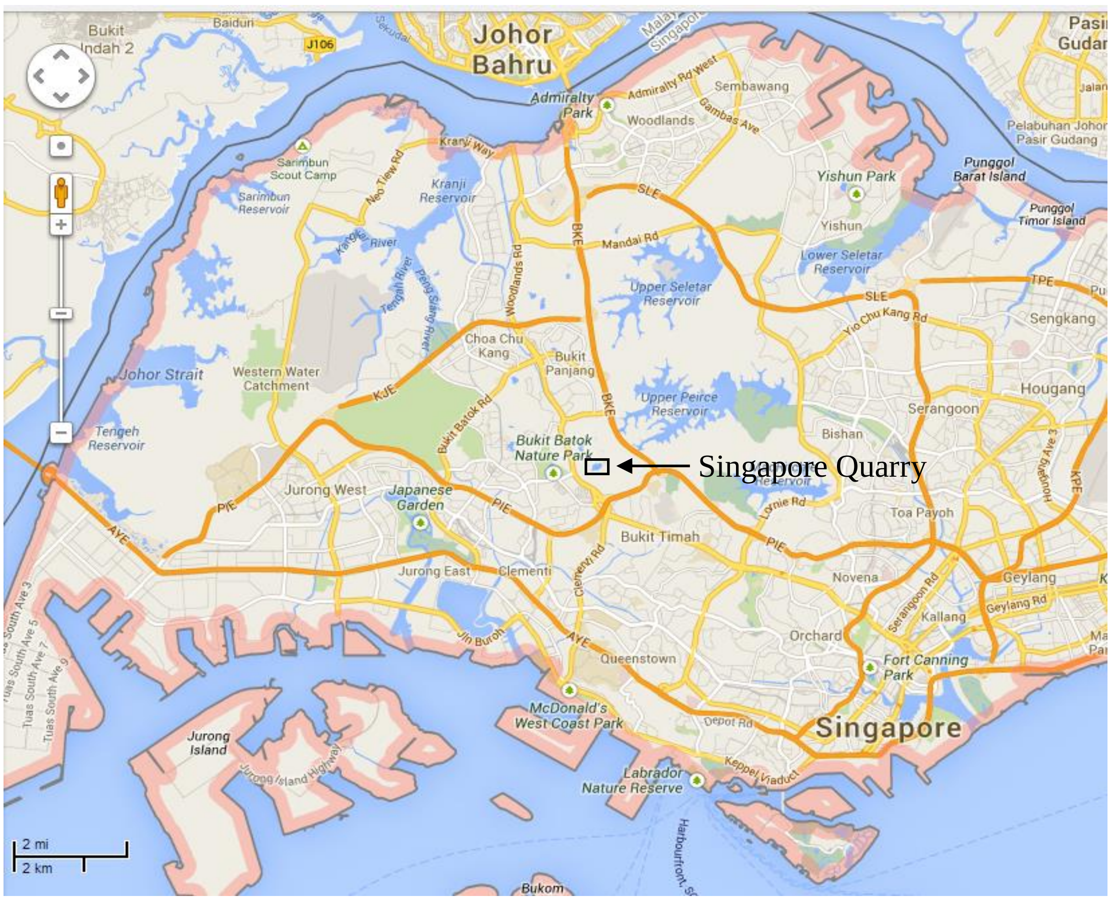


Figure 1. Map of Singapore with location of the Singapore Quarry highlighted.

I chose this site as it is currently the only location in Singapore where the National Parks Board (NParks) has implemented formal golden apple snail control efforts. The Quarry is an unused granite quarry that became filled with rainwater. NParks built shallow islands and planted cattails and reeds in parts of the lake to attract migratory birds (Ramakrishnan s/o R. Kolandavelu 2011, personal communication). It is roughly oval in shape with a maximum length of 350m and a maximum width of 190m. The shallow region where I worked (defined as depth < 0.5m) occupies a rectangular area of about 160m x 6m along the northwestern bank (Figure 2).

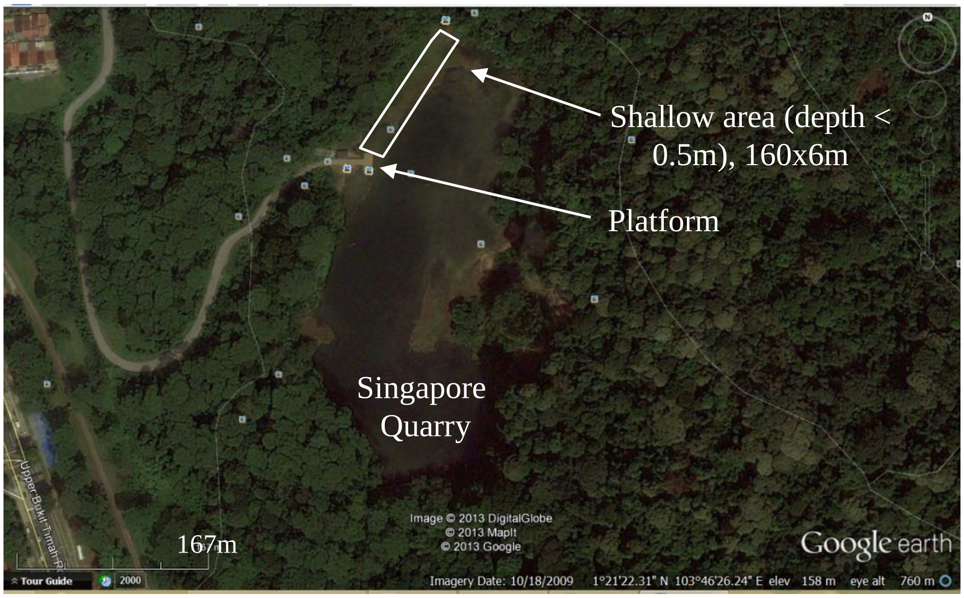


Figure 2. Annotated Google Earth image of the Singapore Quarry.

Field work was carried out every weekday from early June to end-August 2013. However, due to safety reasons, I stopped work during heavy rain and thunderstorms. This occurred an average of about once per week.

Data collection methods

Gut content analysis

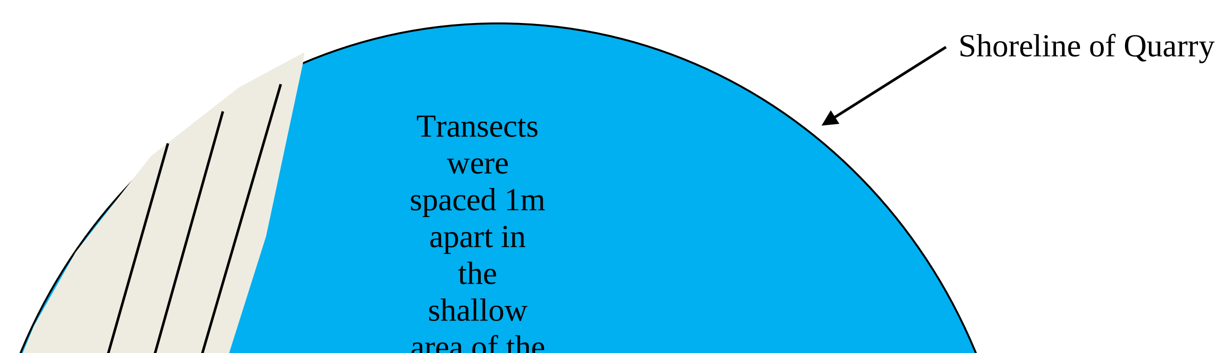
To estimate the diet composition of the snails, I conducted a gut content analysis modeled after that described by Kwong et al. (2009). First, I collected a grab sample containing 24 snails from the Singapore Quarry in August 2013. Upon collection, I measured shell width, length and height (Teo 2004) of each snail using calipers. I recorded the lengths to the nearest millimeter. Next, I cracked the shell of each individual by knocking it against a hard surface and preserved each specimen in 10% formalin. Subsequently, in the laboratory, I dissected each specimen and dispersed the stomach contents in 10ml of water.

To quantify the stomach contents of each snail, I used a grid count. I transferred 1ml of the stomach content mixture into a Sedgewick-Rafter counting cell and examined it at 100x magnification under a compound microscope (Olympus CX22). Then, I chose 100 of the 1,000 grid cells randomly for examination (5 columns chosen randomly by a number generator x 20 rows). I used a local guidebook to classify the stomach contents into six categories: macrophytes, cyanobacteria, diatoms, green algae, invertebrate parts and amorphous detritus (Tin et al. 2011). Then, for each cell, I recorded whether each food category was present (1 or 0). I repeated this process five times for each stomach, giving a total of 500 grid cells examined per stomach. Then, I calculated the relative abundance in percent of each food item in that individual. Lastly, I used these individual scores to calculate an overall average and standard deviation for the relative abundance of each food item.

Microhabitat Distribution Survey

To reduce the likelihood that my conclusions would be invalidated by undetected confounding variables, I measured a wide range of abiotic and biotic factors. I included water temperature, water depth and the presence/absence of shade because I hypothesized they would be the most significant predictors of distribution. However, I also measured other possible confounding variables like dissolved oxygen concentration (Seuffert and Martin 2013), distance to nearest emergent macrophyte (Seuffert and Martin 2013), salinity and water pH.

To estimate the magnitude of these variables and quantify the corresponding golden apple snail density throughout the shallow region of the Quarry, I used transects and quadrats. First, I marked out four parallel transects stretching across the shallow area of the lake (depth < 0.5m). They were located 1m apart and of variable length as the shoreline was not straight (Figure 3).



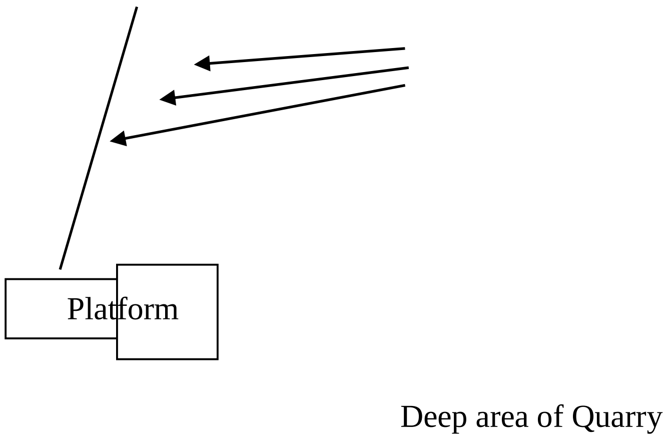


Figure 3: Layout of transects within the Singapore Quarry

I placed 0.5m x 0.5m quadrats along each transect at randomly generated distances, making sure each one was a minimum of 2m and a maximum of 5m away from the next quadrat. For each quadrat, I removed all the snails in the top 2cm of the sediment using a sieve, recorded the number of golden apple snails present, and calculated the golden apple snail density in each quadrat. Then, I recorded whether the quadrat was completely in the sun or at least partially shaded. Next, I used a tape measure with a precision of 0.1cm to determine the water depth at the center of each quadrat, and the distance from the center of the quadrat to the nearest emergent macrophyte. I defined nearest emergent macrophyte as the nearest plant leaf or stem that broke the water surface. Finally, I used a multi-parameter water quality meter (YSI 556) to measure the water temperature, pH, salinity and dissolved oxygen concentration at the center of each quadrat. In total, I collected data for 91 quadrats.

Organic Attractants Experiment

To investigate the ability of my three proposed organic attractants to attract golden apple snails to a central location, I compared them to a control in an experiment modeled after that described by Joshi and de la Cruz (2001). All replicates of the experiment were performed in regions of the Singapore Quarry where the water depth was less than 0.1m. For each replicate, I placed a 6cm^2 piece of each organic attractant on the sediment surface and weighted each attractant down with rocks of approximately the same size. The three organic attractants used were fresh papaya leaves, boiled papaya leaves and newsprint. The control was represented by a circular piece of string which encompassed a surface area of 6cm^2 and was also weighted down with a rock. I located the attractants and the control the same distance away from the shore and 0.5m away from each other (Figure 4). I also randomized the positions of each treatment for each replicate.

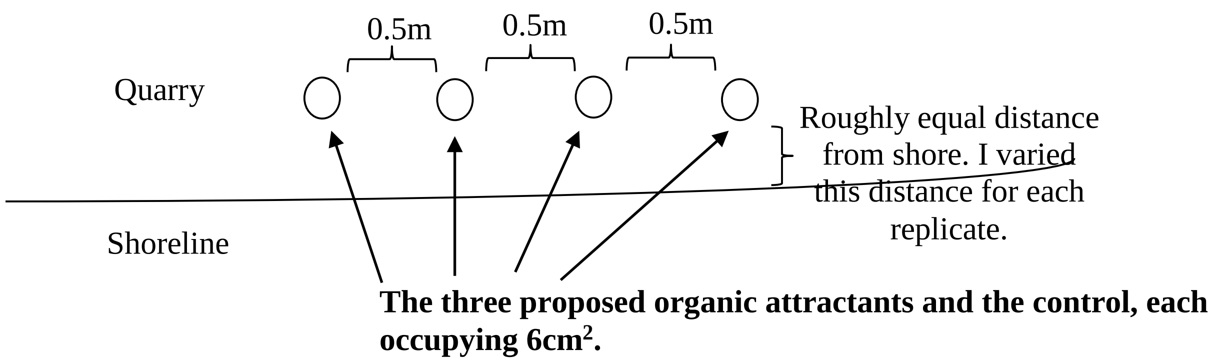


Figure 4. Layout of transects within the Singapore Quarry.

To estimate how attractive each treatment was, I counted the number of snails in contact with each attractant and touching/within the control circle. I recorded this figure at 10-minute intervals for the first hour, then once at the two-hour mark, and finally once at the 24-hour mark. In total, I conducted 30 replicates of the experiment over five weeks from early July to mid-August.

To improve the internal validity of the experiment, I randomized some possible confounding variables and standardized others. First, I varied the start time of the replicates, albeit only between 10am and 4pm, for safety and logistical reasons. I also varied the location of the replicates within the area of the Quarry where the water depth was less than 10cm. In addition, I randomized the relative positions of the attractants and control between replicates using a random number generator. To ensure standardization, I made sure that all the papaya leaves were collected from papaya plants within 12 hours of the start of each experiment. I also boiled the leaves within 2 hours of the start of each experiment and always used newsprint from the day's edition of *The Straits Times* newspaper.

Statistical analysis methods

To test my hypothesis that macrophytes make up a significantly larger proportion of the golden apple snail diet as compared to other types of food, I used a Kruskal-Wallis H test to compare the average relative abundance of each food item type in the snails' gut contents. Because the Kruskal-Wallis H test indicated that there was a significant difference in the means ($p < 0.001$), I then used the Mann-Whitney U test to conduct pairwise comparisons of the food types. For the Mann-Whitney U tests, I calculated an adjusted p-value based on Holm's method (Holm 1979) to control the familywise error rate. I used the statistical program R (R Core Team 2013) to perform these and all my other statistical tests. I considered differences significant when the p-value was less than 0.05. I chose to use the Kruskal-Wallis H and Mann-Whitney U tests as I was comparing multiple means and I determined based on visual examination of the gut contents data that they did not follow a normal distribution.

To visualize the distribution of the golden apple snails in the Quarry, I created histograms showing the frequency of occupied quadrats along the gradients of each of the habitat variables I measured. I chose to include only occupied quadrats in the histograms since this gives the conditions under which golden apple snails were actually found. Then, by observing the histograms, I determined whether the distribution of golden apple snails for each variable was roughly normal or skewed to the left or right. I also calculated the median and mean (± standard deviation) value of each variable at which the golden apple snails were found.

To test my hypothesis that the distribution of the golden apple snails is best explained by water depth, water temperature, and the presence/absence of shade, I ran a recursive partitioning analysis using the "party" package in R (Torsten et al. 2006). I chose to use recursive partitioning analysis because it is tolerant of non-normal and non-linear data. The "party" package uses a recursive partitioning algorithm to select the habitat variables which significantly affect the golden apple snail distribution ($p < 0.05$) and rank them in order of effect. Next, it identifies the best "splitting point" along the range of each significant habitat variable such that the number of snails found above the splitting point is significantly different from the number found under the splitting point ($p < 0.05$) (Torsten et al. 2006). Finally, the package presents all this information in a graphical conditional inference tree. After observing the tree, I also ran a correlation analysis to check if any of the habitat variables that were picked as significant were significantly correlated with the other habitat variables ($p < 0.05$). I again used an adjusted p-value based on Holm's method to control the familywise error rate. Habitat variables that were not picked as significant but which were correlated with significant variables might be important to test in future studies.

Lastly, to evaluate the effectiveness of each organic attractant in the experiment and determine if this effectiveness varied with time, I created a mathematical model that included linear terms for time and each treatment, as well as a quadratic term for time:

$$Y \sim aTime + bTime^2 + cF + dB + eN$$

Y refers to the number of snails attracted while a, b, c, d, and e represent the numerical coefficients for each variable in the equation. F, B, and N are dummy variables that represent fresh papaya leaves, boiled papaya leaves, and newspaper respectively. This means, for example, I coded fresh papaya leaves as F=1, B=0, N=0. I coded the control as F=0, B=0, N=0. I used R to determine the values of each of the coefficients in the model and whether any of them were significant ($p < 0.05$). If they were significant, I concluded that the corresponding variable significantly affected the number of snails attracted.

I chose to include a quadratic term for time in my model based on the shape of the graphs I obtained after plotting number of snails attracted versus time for each attractant (Figure 9). The curve for the boiled papaya leaves followed that of a quadratic graph as it peaked at 2 hours before declining again. Although the curves for the fresh papaya leaves and newsprint were still rising at the time point when I ended my experiments (24 hours), I expect that their attractiveness will eventually peak and then decline thereafter, resulting in a quadratic shape curve. As they are organic materials, they will eventually be consumed or broken down by the organisms in the Quarry, which means that their effectiveness must eventually decline.

RESULTS

Gut content data and Kruskal-Wallis analysis

Amorphous detritus made up the largest proportion of the snails' diet, followed by macrophytes, dinoflagellates, green algae, cyanobacteria, and invertebrate parts respectively. On average, amorphous detritus was 35.96 ± 12.48 % of the gut contents. Macrophytes made up 22.42 ± 4.17 %, dinoflagellates 18.07 ± 6.85 %, green algae 16.82 ± 4.53 %, and cyanobacteria 6.68 ± 4.88 %. Invertebrates made up a negligible part of the gut contents (0.04 ± 0.07 %) (Figure 5).

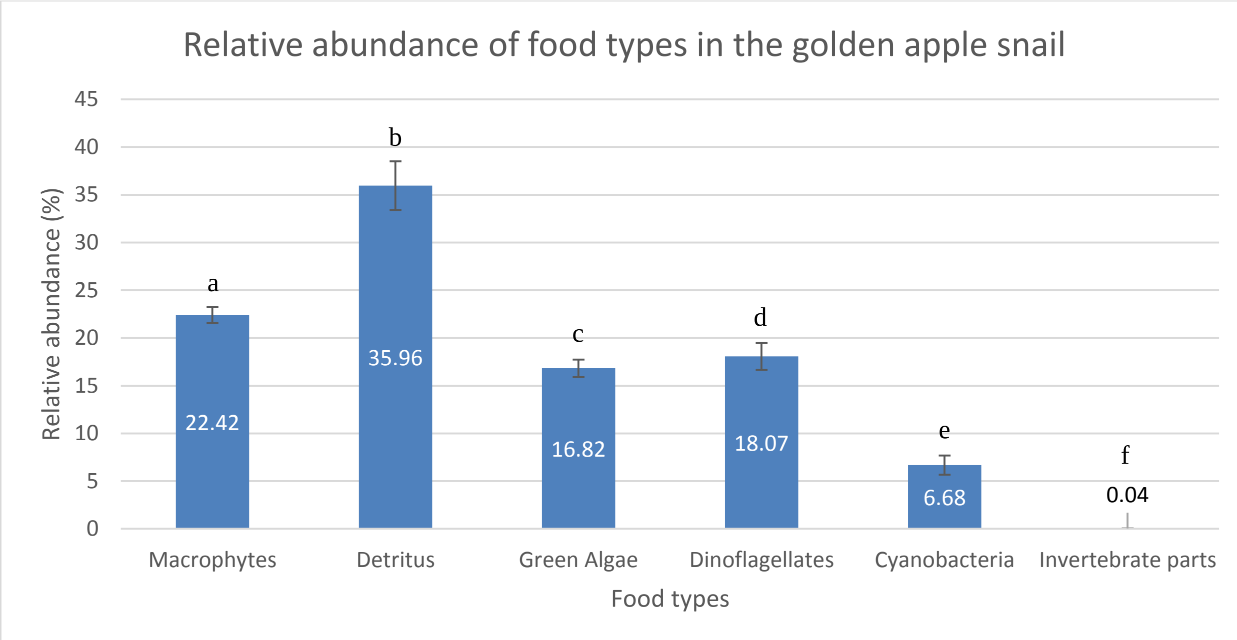


Figure 5. Relative abundance (%) of gut contents of the golden apple snail. Numbers inside the columns indicate the average relative abundance for each food type and error bars indicate the standard error. Letters above the column indicate statistical significance – columns with the same letter are not significantly different from each other. In this case, all the pairwise combinations were significantly different from each other.

The Kruskal-Wallis H test confirmed that the relative abundances of the food types were significantly different from each other ($\chi^2(5, N = 3600) = 2720.01$, $p < 2.2e\text{-}16$). A Mann-Whitney U test indicated that the relative abundance of macrophytes and detritus differed significantly from each other ($W = 346696.5$, $p < 2.2e\text{-}16$). The relative abundance of both macrophytes and detritus was also significantly higher (p-value < 0.001) than the relative abundances of the green algae, dinoflagellates, cyanobacteria, and invertebrate parts. The Mann-Whitney U test for all the other possible pairwise combinations also showed that they were all significantly different from each other ($p < 0.001$) (Table 1). This confirms that amorphous detritus was the most abundant food type followed by macrophytes, dinoflagellates, green algae, cyanobacteria, and invertebrate parts respectively (Figure 5).

Table 1. P-values from the Mann-Whitney U test comparing the average relative abundance of each food item type in a pairwise fashion. P-values are adjusted using Holm's method. All the p-values are statistically significant (p < 0.05).

	Macrophytes	**Detritus**	**Green algae**	**Dinoflagellates**	**Cyanobacteria**	**Invertebrates**
Macrophytes		< 2.2e-16	< 2.2e-16	8.861e-07	< 2.2e-16	< 2.2e-16
Detritus	< 2.2e-16		< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16

Green algae	< 2.2e-16	< 2.2e-16		5.156e-06	< 2.2e-16	< 2.2e-16
Dinoflagellates	8.861e-07	< 2.2e-16	5.156e-06		< 2.2e-16	< 2.2e-16
Cyanobacteria	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16		< 2.2e-16
Invertebrates	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	

Habitat distribution data and recursive partitioning

The overall density of golden apple snails was low and right-skewed (Figure 6). The median number of snails per quadrat was 1 and the mean number was 1.516 ± 2.286 (n=91).

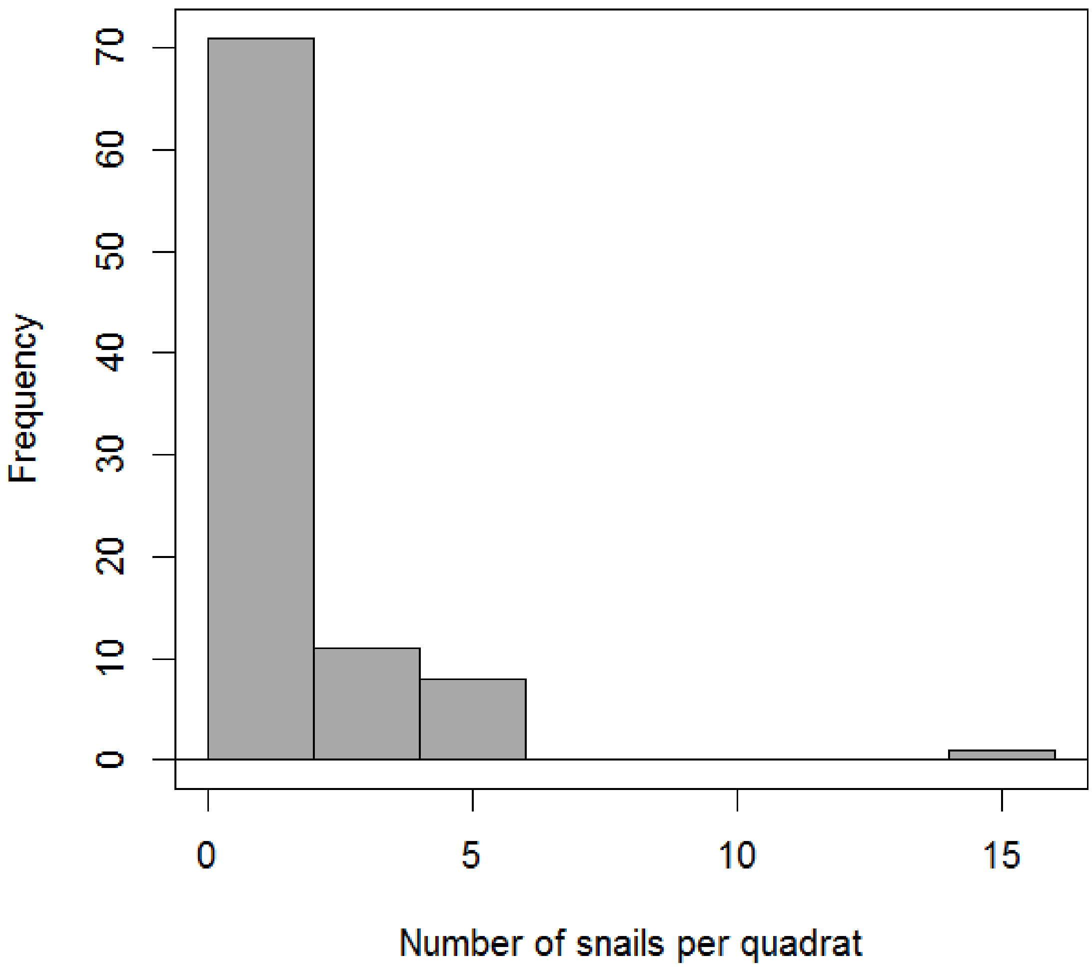


Figure 6. Histogram showing frequency of quadrats containing a particular number of golden apple snails. Median = 1, mean ± standard deviation = 1.516 ± 2.286, n = 91.

The distribution of occupied quadrats (snail density > 0, n =46) was right-skewed for water depth (Figure 7b) and distance to nearest macrophyte Figure 7c). That is, more occupied quadrats were found at shallower depths and closer to macrophytes. The median depth at which snails were found was 7.00 cm and the median distance from macrophytes was 21.00 cm. The rest of the habitat variables resulted in approximately normal distributions (Figure 7a, d-f). The mean temperature at which snails were found was 32.31 ± 1.78°C, the mean dissolved oxygen level 7.78 ± 1.44 mg/l, mean pH 10.31 ± 0.756, and mean salinity 0.137 ± 0.0136 ppt.

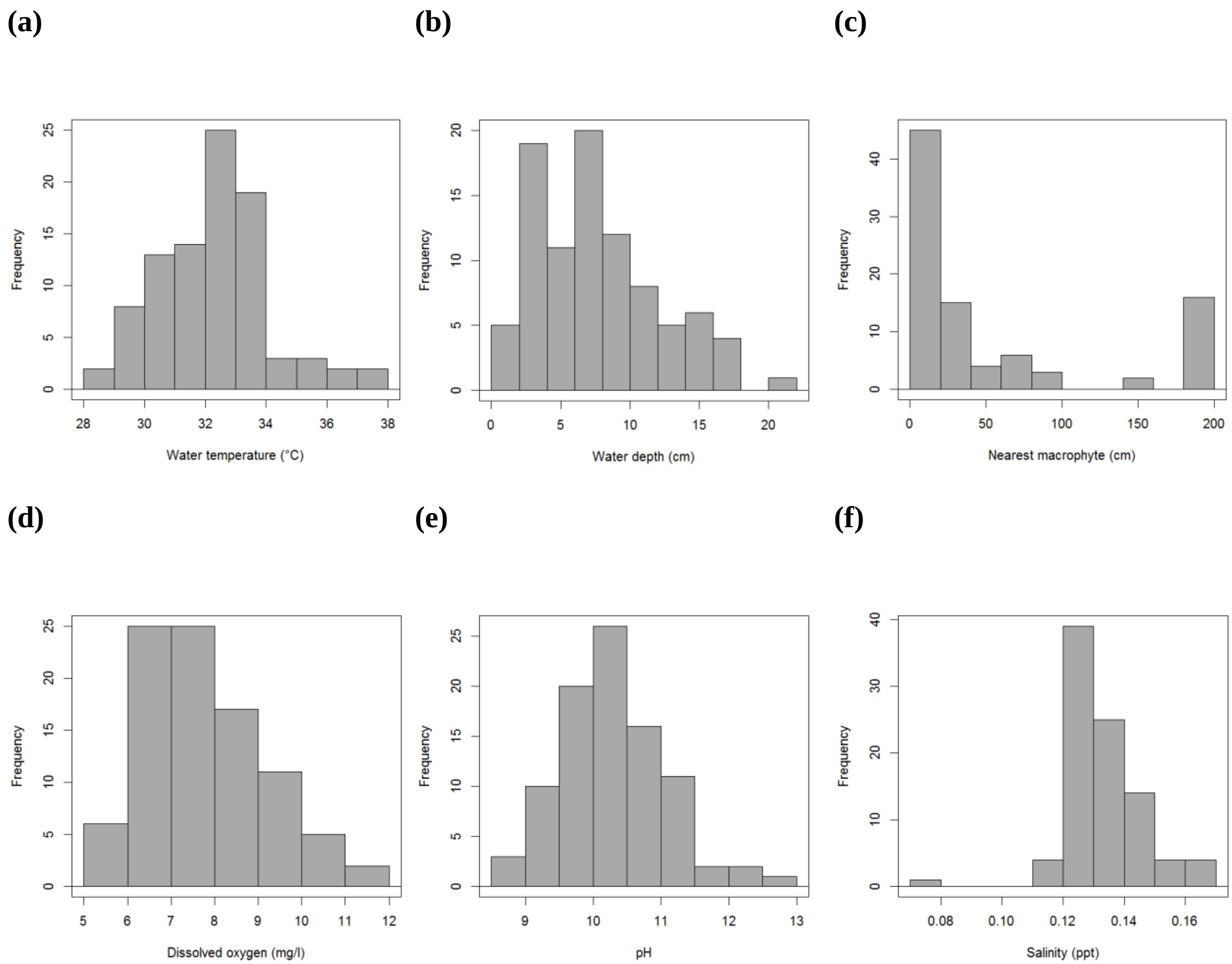


Figure 7. Histograms showing distribution of occupied quadrats (snail density > 0, n=46) according to the habitat variables I measured. Median, mean ± standard deviation for: (a) temperature (°C) = 32.35, 32.31 ± 1.78, (b) water depth (cm) = 7.00, 8.20 ± 4.53, (c) nearest macrophyte (cm) = 21.00, 54.17 ± 73.29, (d) dissolved oxygen (mg/l) = 7.65, 7.78 ± 1.44, (e) pH = 10.31, 10.31 ± 0.756, (f) salinity (ppt) = 0.14, 0.137 ± 0.0136.

Recursive partitioning identified water temperature as the only variable that was significant in predicting snail density ($p < 0.001$). Quadrats which had a water temperature of more than 33.34°C had significantly more snails than those which had a water temperature of 33.34°C or below (Figure 8). As compared to temperature, none of the other variables was significant in predicting snail density.

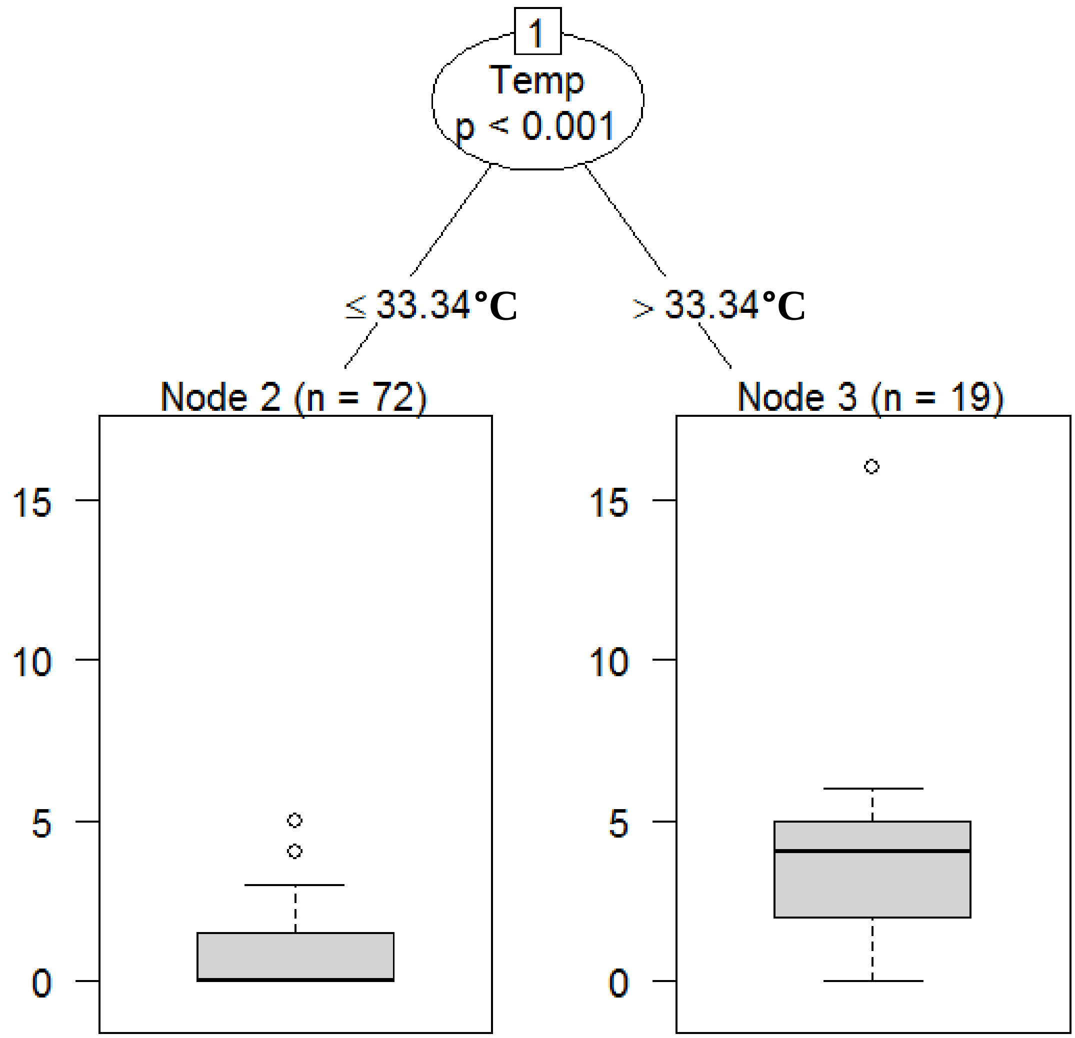


Figure 8. Conditional inference tree from recursive partitioning analysis of golden apple snail habitat distribution data.

Although only water temperature was important in predicting golden apple snail density, there were significant correlations between the habitat variables. Water temperature was significantly correlated with water depth ($r = -0.5026$, $p < 0.0001$) and salinity ($r = -0.3288$, $p = 0.0336$). Depth was also significantly correlated with distance to the nearest macrophyte (r =

0.4308, p = 0.0005) while salinity was significantly correlated with the level of dissolved oxygen (r = -0.3907, p = 0.0031). Dissolved oxygen was significantly correlated with pH (r = 0.5145, p < 0.0001) (Table 2. Correlation coefficients from pairwise correlation analysis of the habitat variables. Significant correlations (adjusted p-value (Holm's method) < 0.05) are highlighted in red.2).

Table 2. Correlation coefficients from pairwise correlation analysis of the habitat variables. Significant correlations (adjusted p-value (Holm's method) < 0.05) are highlighted in red.

	Depth	Dissolved oxygen	Nearest macrophyte	pH	Salinity	Shade	Temperature
Depth		-0.2767	0.4308	-0.1868	0.2066	0.1735	-0.5026
Dissolved oxygen	-0.2767		-0.2319	0.5145	-0.3907	-0.0308	0.2339
Nearest macrophyte	0.4308	-0.2319		-0.0194	0.0751	-0.1213	-0.0476
pH	-0.1868	0.5145	-0.0194		-0.1107	-0.1379	0.31
Salinity	0.2066	-0.3907	0.0751	-0.1107		-0.0933	-0.3288
Shade	0.1735	-0.0308	-0.1213	-0.1379	-0.0933		-0.2877
Temperature	-0.5026	0.2339	-0.0476	0.31	-0.3288	-0.2877	

Attractants data and ANOVA analysis

Overall, boiled papaya leaves attracted the most snails (1421% of the control), followed by fresh papaya leaves (633%) and newsprint (500%). Over the course of the experiment, boiled papaya leaves attracted an average of 5.97 ± 7.13 snails. Fresh papaya leaves attracted 2.66 ± 4.93 snails, newsprint attracted 2.10 ± 4.72 snails, and the control attracted 0.42 ± 0.757 snails.

The difference in the average number of snails attracted by each treatment varied over time (Figure 939). Boiled papaya leaves consistently attracted the most snails. This difference was the largest at the 120 minute mark, where the boiled papaya leaves attracted an average of 9.38 more snails than the second best attractant, the fresh papaya leaves. All three organic attractants attracted around 7 snails after 24 hours

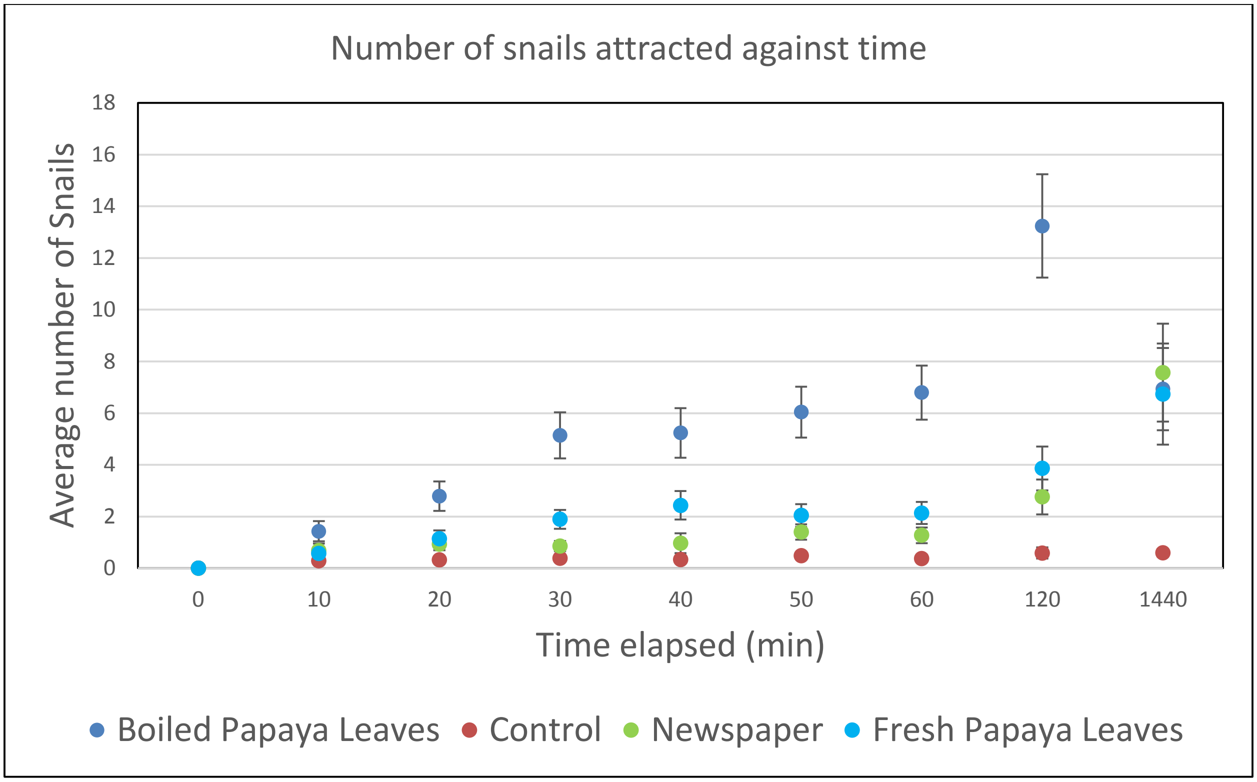


Figure 93. Mean number of golden apple snails attracted by each attractant at each time interval. Error bars indicate the standard error. Each data point represents the mean of 30 observations.

The quadratic model confirmed that all three attractants attracted significantly more snails than the control ($p < 0.05$). The estimated magnitudes of coefficients for B, F, and N also verified that boiled papaya leaves showed the largest attractant effect, followed by fresh papaya leaves (F) and newsprint (N) respectively. The 95% confidence intervals for the estimates of the coefficients for F, B, and N do not overlap, confirming that they all differed significantly from each other in terms of number of snails attracted. Lastly, the number of snails attracted over time also changed significantly, as indicated by the fact that the estimates of the coefficients for both time terms were significant ($p < 0.05$) (Table 3).

Table 3. Estimated coefficients for each term in the quadratic model and their corresponding p-values. All the p-values are significant ($p < 0.05$). The quadratic model used was $Y \sim aTime + bTime^2 + cF + dB + eN$.

	Estimate of coefficient	**Standard error**	**p-value**
Intercept	-1.85	4.00E-01	4.32E-06
Time	4.12E-02	5.40E-03	5.87E-14
Time^2	-2.62E-05	3.64E-06	1.21E-12
F	2.24	4.40E-01	4.51E-07
B	5.55	4.40E-01	< 2e-16
N	1.68	4.40E-01	0.000147

DISCUSSION

My results largely supported my hypotheses, providing justification for urgent control of the golden apple snail in the Singapore Quarry and suggesting that placing organic attractants in the warmer, higher snail density areas of the Quarry can enhance current control methods. These results corroborate prior studies on the golden apple snail diet and the effectiveness of organic attractants (Joshi and de la Cruz 2001, Kwong et al. 2009), but not prior studies on the snails' microhabitat distribution (e.g. Ndifon and Ukoli 1989, Seuffert and Martín 2013). Thus, my study only provides support for the general consensus in the literature that golden apple snails threaten native macrophytes (e.g. Carlsson et al. 2004, Morrison and Hay 2011) and for the notion that using attractants can improve manual control of the golden apple snail (Joshi 2007). Golden apple snail distributions seem to be more site-specific and further research in this area is critical.

Diet of the golden apple snails

The presence of macrophytes in the guts of the golden apple snails I collected confirmed my hypothesis that the golden apple snails in the Singapore Quarry are feeding on the macrophytes there. I found that macrophytes accounted for an average of 22.42% of the diet of the snails I examined (Figure 5), which is similar to Kwong et al.'s (2009) finding that golden apple snails across Hong Kong maintained between 28 to 39% macrophytes in their diet. A diet analysis performed in wetlands in Florida (Morrison and Hay 2011) also found that golden apple snails feed preferentially on macrophytes. These results suggest that the snails' diet preference remains consistent across different habitats. They also supports conclusions from previous observational field studies that herbivory by golden apple snails threaten tropical wetland ecosystems in Southeast Asia (Carlsson et al. 2004, Carlsson, and Lacoursiere 2005). Thus, my results establish that the golden apple snails in the Singapore Quarry behave similarly to populations found elsewhere and are indeed a threat to the macrophyte populations in the Quarry. This implies that control of the snails by the National Parks Board (NParks) is justified. In addition, it becomes important to investigate how NParks can improve its golden apple snail control methods, which is what the second half of my study focuses on.

Microhabitat distribution of the golden apple snails

Recursive partitioning analysis supported the conclusion that the distribution of the golden apple snails in the Quarry depends primarily on water temperature (Figure 7a, 8). There are no prior studies that explicitly examined how water temperature affects golden apple snail distribution. But my findings could be explained by previous studies which found that golden apple snails tend to spend more time active and feeding at higher temperatures (Heiler et al. 2008, Seuffert et al. 2010). I may have observed fewer snails in colder areas because they preferred to bury themselves in the substrate rather than emerge to feed. However, Heiler et al. (2008) also suggested that the snails' increased activity at higher temperatures could be because they are seeking deeper waters to avoid a potential drying out of the habitat caused by the heat. This suggests that the snails' distribution pattern may actually be dynamic, something I did not capture because I performed all my habitat surveys in the morning. More research on the golden apple snail's response to temperature is needed to determine the underlying reason for my findings.

Another issue is that the results of my habitat analysis differ from that of previous habitat studies which found that aquatic pulmonate snail distribution was dependent on water depth (Seuffert and Martin 2010), shade (Ndifon and Ukoli 1989), pH and dissolved oxygen (Ofulla et al. 2013), and distance to nearest emergent macrophyte (Seuffert and Martín 2013). One possible reason for this discrepancy is that water temperature was significantly correlated with several other habitat variables in the Singapore Quarry like water depth and pH (Table 2). This leaves open the possibility that these variables also affect the golden apple snail distribution, as predicted by Seuffert and Martin (2010) and Ofulla et al. (2013).

Another reason for the discrepancy could be because the studies were conducted in different habitats. For example, Seuffert and Martín (2013) examined the distribution of golden apple snails in rivers and suggested that distance to the nearest emergent macrophyte affects golden apple snail distribution because the presence of macrophytes reduce water velocity. This mechanism would not operate in a quarry lake like the Singapore Quarry. Similarly, Ndifon and Ukoli (1989) found that shade affected the snail distribution because it was correlated with the presence of aquatic macrophytes that attracted snails. However, I observed that, in the Singapore Quarry, shade patterns are predominantly shaped by the riparian vegetation. These discrepancies suggest that golden apple snail distribution patterns may be more site-specific than their diet patterns.

Efficacy of organic attractants

The results of my attractants experiment confirmed my hypothesis that while boiled papaya leaves would be the most effective organic attractant, fresh papaya leaves and newspaper would also display an attractant effect (Table 3). This supports reports in the literature that both fresh papaya leaves and newspapers can be used to attract golden apple snails (Joshi 2007, Joshi and de la Cruz 2001). But I also found that boiled papaya leaves were superior to fresh papaya leaves and newspaper in attracting snails (**Error! Reference source not found.**9). Sterry et al. (1982) suggested that herbivorous aquatic snails are able to detect compounds from decaying leaves in water and Thomas (1986) confirmed that freshwater pulmonate snails are attracted to sugars released by decaying leaves. Boiling can mimic the effects of decay because it bursts leaf cells and liberates the nutrients and compounds within them without denaturing these compounds (Suren and Lake 1989). Thus, the boiled papaya leaves may be superior attractants because the boiling process could be liberating compounds that the golden apple snails are attracted to, like short chain carboxylic acids (Sterry et al. 1982). The plausibility of this mechanism is also supported by the fact that the comparative superiority of boiled papaya leaves declined over time.

The experimental results suggested that while boiled papaya leaves are more effective in the short run (< 2 hours), after 24 hours, all three attractants are equally effective. While the effectiveness of the fresh papaya leaves and newspaper continued increasing up till the 24 hour mark, the effectiveness of the boiled papaya leaves peaked earlier and then dropped to the level of the other attractants after 24 hours (Figure 939). One reason for this could be because the compounds released by the boiling dissipated over time. This is supported by Tiwari and Singh (2004) who tested various chemical attractants for freshwater pulmonate snails and found that their effectiveness declined as time passed and the attractants diffused. Another reason for my findings could be because the boiling destroyed the leaves' anti-herbivore defenses (Suren and Lake 1989). Indeed, I observed that the fresh papaya leaves had a water-repellant coating on their surface that the boiled leaves did not have. Given this uncertainty, one possibility I discuss in my section on future research is investigating how exactly the boiled papaya leaves are attracting the golden apple snails.

Limitations and Future Directions

Inference problems

The external validity of my study was limited most significantly by the fact that I conducted all my fieldwork at a single site – the Singapore Quarry. This is problematic because I cannot definitely conclude whether the discrepancies between the results of my study and that of prior studies is due to differences in local conditions or other factors like mistakes in my study design. This is complicated by the fact that the Singapore Quarry represents a relatively unique habitat because it is a disused granite quarry. The impermeable bedrock and steep sides that are characteristic of such quarries often result in unique chemical and physical characteristics of the water columns of granite quarry lakes (Hrdinka 2005).

This inability to exclude the effects of mistakes in my study design is especially acute for my distribution analysis. My novel finding that temperature affects distribution could simply be due to a flaw in my study. However, based on the wide range of habitat variables cited in previous distribution studies (e.g., Ndifon and Ukoli 1989, Ofulla et al. 2013, Seuffert and Martin 2013), golden apple snail distribution does seem to be highly dependent on the effect of local conditions. I am more confident about my gut content analysis results because they agree with the broad consensus in the literature that golden apple snails threaten aquatic macrophyte populations (e.g. Carlsson, and Lacoursiere 2005, Kwong et al. 2009, Morrison and Hay 2011). Finally, the lack of extensive prior studies on attractant effectiveness (Joshi 2007, Joshi and de la Cruz's 2001) means that although my study corroborates their results, I am still wary of extrapolating my conclusions to a wide variety of other habitats. Future studies will be useful for determining whether the results of my study hold in other types of habitats, in addition to helping to elucidate the mechanisms leading to some of my conclusions.

Future directions

To confirm that my results are not specific to the local conditions at the Singapore Quarry and to better understand the mechanisms driving my distribution and attractant findings, my study should be repeated at different sites. These could be other local sites in Singapore that house different ecosystems (e.g. freshwater wetlands) or sites in other climates (e.g. temperate locations like Japan). As I mentioned above, it is important to confirm that my conclusions about the golden apple snail diet and attractants apply under various conditions, but especially critical to repeat my distribution survey in other habitats. In addition, while I expect that future diet and attractant studies will find similar results to mine, I expect that there will be variation in the results of studies that focus on distribution.

Future studies will also be useful to confirm the mechanisms that I suggest to explain my findings. For example, to make it possible to predict the golden apple snail distribution in a particular water body without surveying it, it would be useful to investigate why the golden apple snails in the Singapore Quarry seemed attracted to warmer areas. Was it because they tend to be more active in such areas and are thus more likely to burrow out from under the substrate or did my observations have more to do with my finding that water temperature was correlated with water salinity and depth? It would also be useful to investigate how the boiled papaya leaves were actually attracting the golden apple snails in my experiment. If they were attracting snails because they were releasing attractive compounds into the water, these compounds could be isolated and used to manufacture more potent attractants. Still, despite these uncertainties about the external validity of my study and the mechanisms driving my results, the conclusions of my study can still guide management decisions at the Singapore Quarry itself.

Broader Implications

My study provides justification for urgent control of the golden apple snail in the Singapore Quarry and suggests that NParks' current control method can be enhanced by using boiled papaya leaves placed in warmer areas of the Quarry to concentrate the snails before they are removed manually. It also filled the gap in knowledge in Singapore on the local golden apple snail diet and distribution and established that organic attractants remain effective under local conditions. Specifically, my results confirmed that, in Singapore, golden apple snails still feed preferentially on macrophytes and thus that NParks managers are justified in implementing an intensive control regime. In addition, since I found that golden apple snails naturally concentrate in the warmer parts of the Quarry, managers can place boiled papaya leaves there to further concentrate them and to make their manual removal easier. They may also choose to use newspaper or fresh papaya leaves since these are easier to obtain and are equally effective after 24 hours. Apart from supporting these specific management recommendations, my conclusions about the golden apple snail diet, distribution and susceptibility to attractants at the Singapore Quarry also add to the current body of literature as a case study.

My study enriches the current body of literature as a test case of whether general conclusions about golden apple snail diet, distribution and susceptibility to attractants apply in the unique context of a tropical quarry lake. First, it adds to the large body of literature on how golden apple snails threaten aquatic macrophytes (e.g. Carlsson et al. 2004, Carlsson and Lacoursiere 2005, Kwong et al. 2009, Morrison and Hay 2011). Second, it confirms the pattern that the determinants of golden apple snail distribution seem to be site-specific. Third, it supports prior studies that found papaya leaves and newspaper to be effective golden apple snail attractants (Joshi 2007, Joshi and de la Cruz 2001). Overall, my study adds to the pattern that while golden apple snail diet and susceptibility to attractants seem to remain constant in different environments, it is more difficult to generalize about how habitat affects their distribution. This implies that distribution is especially important to investigate locally, as my study does for the Singapore Quarry.

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