Predation-induced responses of freshwater snails through morphological defenses and avoidance behavior

BIOS 35502: Practicum in Field Environmental Biology

Nora Nickels

Advisor: Heidi Mahon

2011

Abstract

Aquatic organisms have been shown to respond to predation with rapid evolution due to the adaptive morphological and behavioral responses they acquire in order to better survive predator-prey interactions. These adaptive responses have variable costs and benefits as the organism works to avoid predation. Freshwater snails have adapted to crayfish predation in various ways. We observed morphological traits and behavioral responses of three groups of freshwater snails on the UNDERC property – *Bellamya chinensis*, *Helisoma anceps* and *Helisoma trivolvis,* and *Lymnaea stagnalis*. All were exposed to a crayfish predator, *Orconectes propinquus*. We took morphological measurements of shell length, thickness, and strength. We predicted that snail species with the thickest shells would show the highest amounts of avoidance behavior. We found that shell thickness did not necessarily increase with shell strength, and that *Bellamya chinensis* had the strongest shells along with the highest amounts of avoidance behavior. We also found that *Bellamya chinensis* had the highest palatability to crayfish predators. We believe that shell strength, snail palatability, and the presence of an operculum all help to determine the amount of avoidance behavior that a snail species will exhibit in response to predation. Observing the costs and benefits of morphological and behavioral adaptations of aquatic species are an important part of studying energy flow throughout aquatic communities.

Introduction

Predator-prey interaction is an important ecological phenomenon that is studied in order to observe energy movement throughout a community. Predators can affect prey through direct consumption or through inducing defensive strategies that can be energetically costly (Preisser, et al. 2005). Adaptation to predation often occurs in populations experiencing a specific and continuous predation. These conditions may cause prey species to respond by altering their behavioral and morphological defenses (Dalesman, et al. 2008). The theory of this type of local adaptation is supported by studies that have demonstrated that predation can select for rapid divergent evolution in aquatic organisms (Dalesman, et al. 2008). For example, in aquatic gastropods, evidence has been found that suggests flexibility in response to prior or prolonged exposure to predation cues. Each adaptive response of avoiding predation seems to have variable costs and benefits depending on the context (Covich 2010).

These adaptive traits of freshwater gastropods can be morphological or behavioral. Variability in shell thickness can be adaptive in reducing vulnerability to shell-breaking predators. In areas of abundant gastropod predators, heavy-shelled or spinose species are able to provide a competitive advantage over more vulnerable, thin-shelled species, at least for some period of time (Covich 2010). However, the offset is that these morphological adaptations require species to increase energy expenditures for locomotion to carry heavier, stronger shells along the surface, out of the water, or into the sediment (Covich 2010). Thin-shelled species are typically smaller, faster moving and more agile. Thick shells also require sufficient calcium and take longer to construct than thin shells, therefore requiring more resources to maintain (Covich 2010). The presence of an operculum is another morphological adaptation that has been shown to be advantageous for protection from predators by preventing the predator access to the foot of the gastropod (Kelly and Cory 1987).

While predators are actively feeding, gastropods have been observed to have specific behavioral responses. These responses include burrowing into the sediments, shaking to escape from a predator's grasp, movement to crevices, rocks, or vegetation, and crawling out of the water or towards the water line (Alexander and Covich 1991). Studies have demonstrated that chemical cues trigger different types of behavioral responses depending on the type of predator present, making them a good example of predation-induced responses (Covich 2010). In fact, crawl out behavior is chemically mediated due to the mucous trail gastropods utilize during movement (Alexander and Covich 1991). Freshwater snails have been shown to reduce feeding in the presence of predators (Sura and Mahon 2011), which would reduce the amount of energy obtained by the snail.

Many invertebrate (aquatic insects, leeches, crayfishes, crabs) and vertebrate (such as fish and turtles) predators include gastropods in their diet (Covich 2011). In this experiment, crayfish were used as model predators to observe predation-induced responses of freshwater snails through examination of their behavioral responses and morphological defenses.

The purpose of our study was to use three parts to assess the predator-prey interactions: 1) measure force necessary to penetrate the shell of different snail species, specifically snails with differing shell length and thickness; 2) test the presence of chemical cues in snails by exposing crayfish predators to crushed pellets of snail meat; and 3) observe behavioral responses of different species of snails using the model predator to induce responses.

The general hypothesis that is expected from these experiments is that snails with thick shell morphologies will require the highest forces for shell penetration and will display significantly less avoidance behavior.

METHODS

Study Organisms – We used freshwater snails from three different families that can be found throughout North America. *Helisoma anceps* (Planorbidae) are dextral, non-operculate snails that are abundant on inorganic substrate in shallow, eutrophic waters (Harman and Berg 1971). We collected *Helisoma anceps* from Brown Creek (Figure 1) in Wisconsin on the University of Notre Dame Environmental Research Center (UNDERC) property. *Helisoma trivolvis*

(Planorbidae) are smaller, sinistral, and non-operculate snails that can be found on either inorganic substrate or living vegetation in shallow, eutrophic waters (Harman and Berg 1971). We collected *Helisoma trivolvis* from Tenderfoot Lake on UNDERC property (Figure 1). The two *Helisoma* species (length 9.7 ± 0.4 mm; thickness 0.3 ± 0.01 mm) were grouped together for the purpose of our experiment. *Lymnaea stagnalis* (Lymnaeidae) are dextral, non-operculate snails with thin, fragile shells. They can be found in warm, shallow lakes, usually within one meter from the surface on aquatic plants or inorganic substrate (Harman and Berg 1971). *Lymnaea stagnalis* snails (length 34.3 ± 0.8 mm; thickness 0.2 ± 0.02 mm) were collected from Plum Lake on UNDERC property (Figure 1). *Bellamya chinensis* (formerly *Cipangopaludina chinensis*) (Viviparidae) is a large, dextral, and operculate snail that is considered an invasive species (Johnson 2009). Since being introduced to the United States multiple times through aquarium trade or culinary purposes, *Bellamya chinensis* can be found throughout the United States, including the Great Lakes area, in slow-moving, lentic waters (Jokinen 1982). *Bellamya chinensis* snails (length 33.7 ± 0.1 mm; thickness 0.3 ± 0.02 mm) were collected from Brown Creek on UNDERC property (Figure 1). *Orconectes propinquus* were our model crayfish predator used in this study. The crayfish are now considered native in all parts of Wisconsin in freshwater lakes and streams (Olden et al., 2006). We collected *Orconectes propinquus* crayfish (50.3 ± 2.7 mm) from Tenderfoot Lake, but the species can be found in Plum Lake and Brown Creek as well, suggesting that the crayfish might have fed upon the snails in the wild.

Shell strength experimental design – We determined the amount of force necessary to penetrate the shells of snails in order to mimic the energy necessary for a crayfish to crush a shell in order to consume it (Alexander and Covich, 1991). The amount of pressure in kg/mm was measured using a penetrometer for twenty snails from each species (*Bellamya chinensis, Helisoma* spp., and *Lymnaea stagnalis*). We measured the shell length and shell thickness for each snail. Snails were randomly selected from the total group of snails collected. We predicted that increased shell thickness would correlate with increased amount of pressure necessary to penetrate the shell.

Palatability experimental design – In order to determine if snails produced chemical cues to deter crayfish predators, we tested the preferentiality of crayfish when exposed to alginate pellets made with snail meat and control pellets, which had no snail meat. We randomly selected a crayfish to be exposed overnight to twelve control pellets and twelve treatment pellets made from one species of snail. We repeated this procedure with six crayfish for each species of snail. All crayfish were kept without food for three days prior to testing. We recorded how many pellets and which kind of pellets the crayfish either nibbled on or consumed overnight.

Behavioral testing – Twenty-four snails from each species group were used in observing snail behavior when exposed to a crayfish predator. We measured the length and thickness of each snail. Each species was acclimated in aerated holding tanks with its conspecifics prior to experimentation. We randomly chose three individuals of the same species and placed them in the experimental tank for one hour at a time; we observed behavior at the end of the hour. The experimental tank contained a rocky substrate, wooden dowels to mimic plants that extend above the water line and a container holding a live crayfish. The crayfish used was one that was exposed to the pellets of the same species of snail being tested, so that if the crayfish had consumed any part of the snail meat pellets it had the potential of excreting chemicals that could be detected by the live snails. We also randomly selected a conspecific to crush and keep at the bottom of the container with the crayfish, mimicking the circumstance of a snail being injured by a crayfish. The container had mesh siding in order for chemical cues from either the crayfish or the crushed conspecific to be detected in the water by the experimental snails (Aizaki and Yusa 2009). After the observation period, we recorded whether the snails exhibited one of three distinct behaviors: 1) crawling across the substrate away from the contained crayfish, 2) climbing up the wall or the mimicked vegetation, and 3) doing nothing. We predicted that species of snails that were observed to have the thinnest shell thicknesses would display significantly more drastic behavioral responses in the form of more movement away from predators (i.e. more movement towards the water line, more crawling away, etc.).

Statistical Analyses – We used a regression to measure the relationship between shell length and shell thickness of all the snails. We also used a regression to measure the relationship between shell thickness and the force necessary to penetrate the shell. A one-way ANOVA was used to compare the force necessary to penetrate the shell and the three species groupings of snails used. We used a Pearson's Chi-squared analysis to compare the number of snails that performed an avoidance behavior between the three species groupings of snails. A one-way ANOVA was used to compare shell thickness between the snails that performed specific avoidance behaviors, such as climbing, crawling, or no movement. We used a two-way ANOVA to compare the number of alginate pellets consumed with pellet type (control or treatment) and snail species group. All statistical data was normalized using a Lilliefors test and a Shapiro-Wilk test. We set our alpha value at $p = 0.05$.

RESULTS

Shell thickness did increase significantly with shell length for all species of snails ($F_{1,58} = 4.6272$, $p = 0.0356$, $R^2 = 0.0739$; Figure 2). The force necessary to penetrate the shell did not significantly increase with shell thickness ($F_{1,58} = 2.3812$, $p = 0.1283$, $R^2 = 0.0394$; Figure 3). However, the force required to penetrate the shells of *Bellamya chinensis* was significantly higher than the force required to penetrate the shells of both *Helisoma* spp. and of *Lymnaea stagnalis* ($F_{2, 57} = 6.9778$, $p = 0.00195$; Figure 4).

Bellamya chinensis showed significantly less behavior avoidance than either *Helisoma* spp. and *Lymnaea stagnalis* (Pearson's value = 13.4478, $p = 0.00118$; Table 1). When observing the behavior distributions for all three species (Figure 5), we saw that *Bellamya chinensis* showed the highest percentage of individuals (42%) that showed no movement when exposed to a predator. Only 17% of *Helisoma* spp. showed no avoidance behavior, and all of the *Lymnaea stagnalis* snails showed some sort of avoidance behavior (Figure 5). Also, shell thickness was not significantly related to any sort of specific avoidance behavior (i.e., climbing, crawling, no movement) ($F_{2,69} = 0.0965$, $p = 0.9081$).

Finally, crayfish consumed a significantly higher amount of alginate pellets made out of *Bellamya chinensis* meat than of pellets made from both *Helisoma* spp. and *Lymnaea stagnalis.* Crayfish also showed a preference for treatment pellets over control pellets, showing that pellet type and snail species had an interacting effect on the amount of pellets the crayfish consumed ($F_{1,30} = 11.6868$, $p = 0.0002$; Figure 6).

DISCUSSION

Although shell length was positively related to shell thickness, the force necessary to penetrate the shell was not significantly related to shell thickness. We had predicted that snails with thicker shells would require higher forces in order to puncture the shells. Our results showed that force, not thickness, was significantly related to both species and the tendency to participate in avoidance behavior. *Bellaymya chinensis* required significantly higher amounts of force in order to puncture their shells than did either *Helisoma* spp. or *Lymnaea stagnalis.* Further, *Bellamya chinensis* performed significantly lower amounts of avoidance behavior than either of the other two species groups. Therefore, it is force rather than thickness that can be significantly related to avoidance behavior, verifying that a species that requires a larger average force for a predator to break shell and consume the snail will show less avoidance behavior.

We believe this result shows that a thicker shell does not necessarily provide more protection from the risk of crayfish predators. Rather, it is the chemistry that gives a shell a stronger density requiring a larger force to penetrate that provides more protection; more protection morphologically allows for a lower necessity to escape using avoidance behaviors. Studies have shown that although shell thickness is important to snail predation, different species of snails that have varying mean thicknesses are at risk to different groups predators (Brown 1998), supporting the idea that risk of consumption does not have a linear relationship with thickness of snail. Further, snail shells are composed of a biocomposite material, and 90-95% of this material is made up of calcium carbonate that gives the shell the majority of its weight and thickness (Marin and Luquet 2004). The other small percentage is an organic matrix made up of proteins that stabilize the calcium carbonate, giving the shell its strength (Marin and Luquet 2004). It is therefore this matrix of protein that provides the strength of the snail, and the calcium carbonate that provides the thickness.

Snail shells also grow with a focus in length, not thickness (Kemp and Bertness 1984). This type of growth could be due to the fact that a higher thickness does not necessarily lead to evolutionary success for snail species. Since crayfish use their chelae to chip away at the aperture margin of the shell near the foot of the snail (Olden, et al. 2009), the predator needs to overcome the strength, not thickness, of the snail shell. Nystrom and Perez (1998) found that in certain areas, snails are consumed by crayfish as they are encountered, and all size classes of snails are reduced in number by crayfish predation. Because we found that shell length positively correlates with shell thickness, we believe that this supports the idea that thickness does not affect a snail's susceptibility to its predators. Our results also showed that snails of specific classes of shell thickness did not display specific avoidances behaviors. That is, climbing, crawling, and the absence of movement did not vary significantly with shell thickness. This result also supports the theory that shell thickness does not play a role in affecting the avoidance behavior of snails.

Another morphological trait of snails that has been shown to provide defense against predators is the presence of an operculum (Kelly and Cory 1987). *Bellamya chinensis*, the only species of the three species tested to have an operculum, showed significantly lower amounts of avoidance behavior. We believe these results show that the presence of an operculum could also lead to less avoidance behavior due to its benefits of protection morphologically.

The results from the palatability experiment showed that crayfish consumed the highest amounts of pellets made from *Bellamya chinensis*. *Bellamya chinensis* had the highest levels of palatability for crayfish predators. We believe that this signifies that *Helisoma* spp. and *Lymnaea stagnalis* are more likely to have chemical cues used to deter away predators. *Helisoma spp.* and *Lymnaea stagnalish* had significantly weaker shells and also lacked an operculum. Since some snails use chemical cues in the presence of certain predators (Covich 2010), we believe that use of chemical cues by *Helisoma* spp. and *Lymnaea stagnalis* could be another predator-prey response acquired to further detract predators due to their lack of morphological protection.

During the collection of study organisms, we acquired a fourth species of snail, *Campeloma decisa,* from Brown Creek on the UNDERC property (Figure 1). Because we only collected six organisms of this species, we could not use them in our experimental design. However, we measured the strength of their shells and observed their behavior when exposed to a crayfish predator. All six snails performed avoidance behavior. The mean force necessary to penetrate their shell fell between the mean force from the shells of *Bellamya chinensis* and the mean force from the shells of the other two species groups. Since *Campeloma decisa* lacked an operculum and did not have a higher mean force required to puncture their shells, they fell in a category more similar to *Helisoma* spp. and *Lymnaea stagnalis*. Therefore the fact that they performed avoidance behavior fit into our model, demonstrating that the presence of operculum and shell strength affect the amounts of avoidance behavior performed by a species of snails (Table 2). Due to the small amount of *Campeloma decisa* snails collected, we were unable to run palatability experiments with the species.

Testing these experiments with *Campeloma decisa* and other species of snails would prove to be interesting areas for future research. Another possible future experiment would be exposing crayfish to all types of species of snails in the form of alginate pellets and measuring preferences of palatability based on snail species. Furthermore, experiments that could analyze the chemical makeup of snail meat could lead to more information regarding what chemical cues the snails are releasing and what parts of the snail the chemicals are embedded within. Another study that we had anticipated performing was testing handling times of crayfish with snails by measuring how long it takes for crayfish to consume a snail and correlating that with shell thickness or strength. Due to the behavior of our model predators, we were unable to test this study. We had also anticipated using a second model predator, such as a freshwater fish, yet time constraints did not allow that either.

Overall, we believe that shell strength, operculum, and palatability are all important morphological defenses that influence the amounts of avoidance behavior that a species of snail will display. Knowing how snails respond with behavior and morphology to predation is important to understand when studying community dynamics. Different types of predator-prey interactions can increase the diversity of gastropod assemblages by reducing the dominance of any one shell type or behavioral response. Therefore, observing the costs and benefits of morphological and behavioral adaptations are an important part of acquiring knowledge on greater ecological concepts such as predator-prey interactions and the flow of energy throughout aquatic communities.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Heidi Mahon, to whom I am indebted for the rest of my being. I would like to thank Dr. Gary Belovsky, Dr. Michael Cramer, Shayna Sura, and Matt Igleski for their insight and also for the materials and use of the UNDERC property. A special thanks to Kevin Park, Marielle Saums, Ashley Stiffarm, Victoria Blackhorse, and Casey Smith for their help. I would like to thank the Bernard J. Hank Family Endowment for their generosity and funding that made this study possible. Finally, I would like to thank the UNDERC-East class of 2011 for providing me with entertainment, laughter, and plenty of chaos.

LITERATURE CITED

Aizaki, K. and Y. Yusa. 2009. Field observations of the alarm response to crushed conspecifics in the freshwater snail *Pomacea canaliculata*: effects of habitat, vegetation, and body size. *J. Ethol.*, 27(1):175-180.

Alexander, J. E. and A. P. Covich. 1991. Predation risk and avoidance behavior in two freshwater snails. *Biol. Bull.* 180: 387-393.

Brown, K. M. 1998. The role of shell strength in selective foraging by crayfish for gastropod prey. *Freshwater Biology* 40: 255 – 260.

Covich, A. P. 2010. Winning the biodiversity arms race among freshwater gastropods: competition and coexistence through shell variability and predator avoidance. *Hydrobiologia* 653: 191-215.

Dalesman, S., S. D. Rundle, and P. A. Cotton. 2008. Crawl-out behavior in response to predation cues in an aquatic gastropod: insights from artificial selection. *Evolutionary Ecology* 23: 907 – 918.

Harman, W. N. and C. O. Berg. 1971. The freshwater snails of central new york with illustrated keys to the genera and species. *Search: Agriculture*, *Entomology* 4: 1 – 65.

Johnson, P.T.J., J.D. Olden, C.T. Solomon, and M.J. Vander Zanden. 2009. Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia*, 159:161-170.

Jokinen, E.H. 1982. Cipangopaludina chinensis (Gastropoda, Viviparidae) in North America; review and update. *Nautilus*, 96:89-95.

Kelly, P. and J. Cory. 1987. Operculum closing as a defense against predatory leeches in 4 british fresh-water prosobranch snails. *Hydrobologia* 144: 121 – 124.

Kemp, P. and M. D. Bertness. Snail shape and growth rates: evidence for plastic shell allometry in *Littorina littorea. Proc. Natl. Acad. Sci.* 81: 811 – 813.

Marin, F. and G. Luquet. 2004. Molluscan shell proteins. *Comptes Rendus Palevol*, 3: 469 – 492.

Nystrom, P. and J. R. Perez. 1998. Crayfish predation on the common pond snail (*Lymnaea stagnalis*): the effect of habitat complexity and snail size on foraging efficiency. *Hydrobologia* 368: 201 – 208.

Olden, J. D., E. R. Larson, and M. C. Mims. 2009. Home field advantage: native signal crayfish

(*Pacifastacus leniusculus*) out consume newly introduced crayfishes for invasive Chinese mystery snail (*Bellamya chinensis*). *Aquatic Ecology* 43: 1073 – 1084.

Olden, J.D., J.M. McCarthy, J.T. Maxted, W.W. Fetzer, and M.J. Vander Zanden. 2006. The rapid spread of rusty crayfish (*Orconectes rusticus*) with observations on native crayfish declines in Wisconsin (U.S.A.) over the past 130 years. *Biol. Invas.*, 8:1621-1628.

Preisser, E. L. ,D. I. Bolnick, and M. F. Benard. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology* 86:501-509.

Sura, S. A. and H. K. Mahon. 2011. Effects of competition and predation on the feeding rate of the freshwater snail, *Helisoma trivolvis.* In press.

TABLES

Table 1. Snails of different species showing avoidance behaviors. *Helisoma* spp. and *Lymnaea stagnalis* showed significantly higher amounts of avoidance behavior than *Bellymya chinensis* (Pearson's value = 13.4478, p = 0.00118).

Species	No Avoidance Behavior	Avoidance Behavior
Bellamya chinensis	10	14
Helisoma spp.	4	20
Lymnaea stagnalis	0	24

Table 2. Summary of morphological and behavioral defenses. Based on our results, we believe that the presence of an operculum, palatability to crayfish, and a higher average mean force required to puncture the shell leads to lower rates of avoidance behavior in a snail species.

Snail Species	Mean Force (kg/mm)	Operculum Present	Palatability	% Performing Avoidance Behavior
Bellamya chinensis	2.0965	Y	P	58%
Helisoma spp.	1.4145	N	NP	83%
Lymnaea stagnalis	1.4875	N	NP	100%
Campeloma decisa	1.8500	N	Not Determined	100%

FIGURES

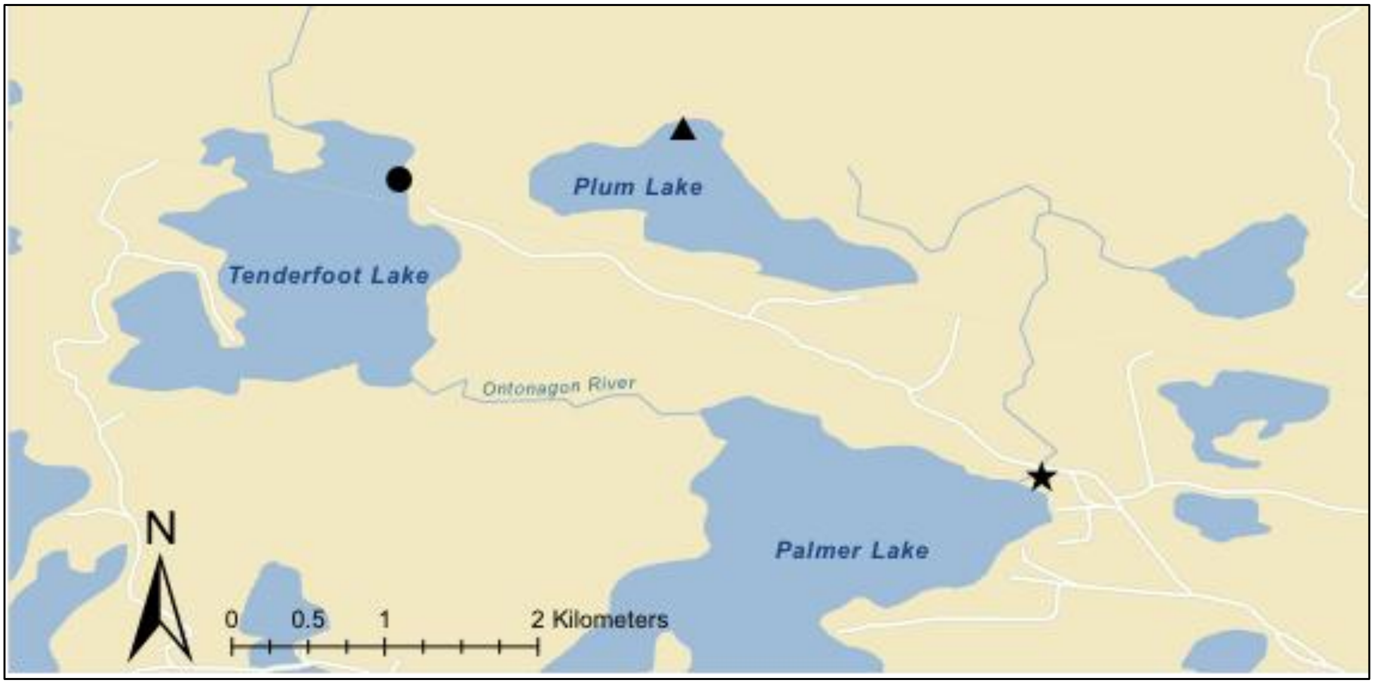


Figure 1. Map of sampling sites on UNDERC property in Wisconsin. The circle shows the area of Tenderfoot Lake where *Helisoma trivolvis* and *Orconectes propinquus* were collected. The triangle shows the area on Plum Lake where *Lymnaea stagnalis* were collected. The star shows the area on Brown Creek where *Helisoma anceps* and *Bellymya chinensis* were collected.

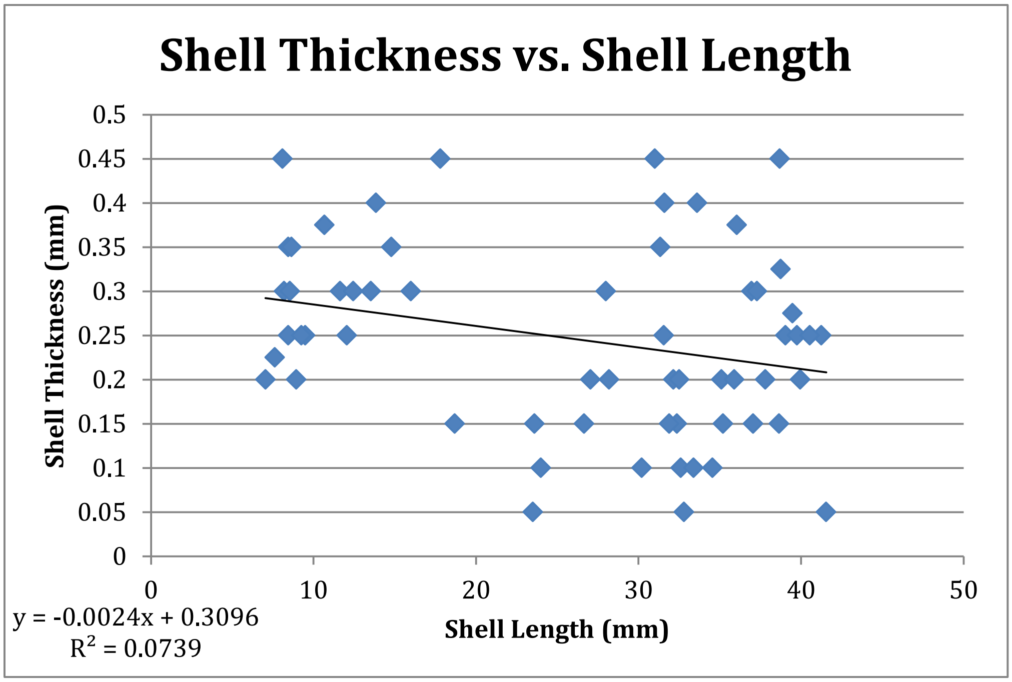


Figure 2. Relationship between shell thickness and shell length for all snail species. Shell thickness was seen to have a positive relationship with shell length for all species ($F_{1,58}$ = 4.6272, p = 0.0356, R^2 = 0.0739).

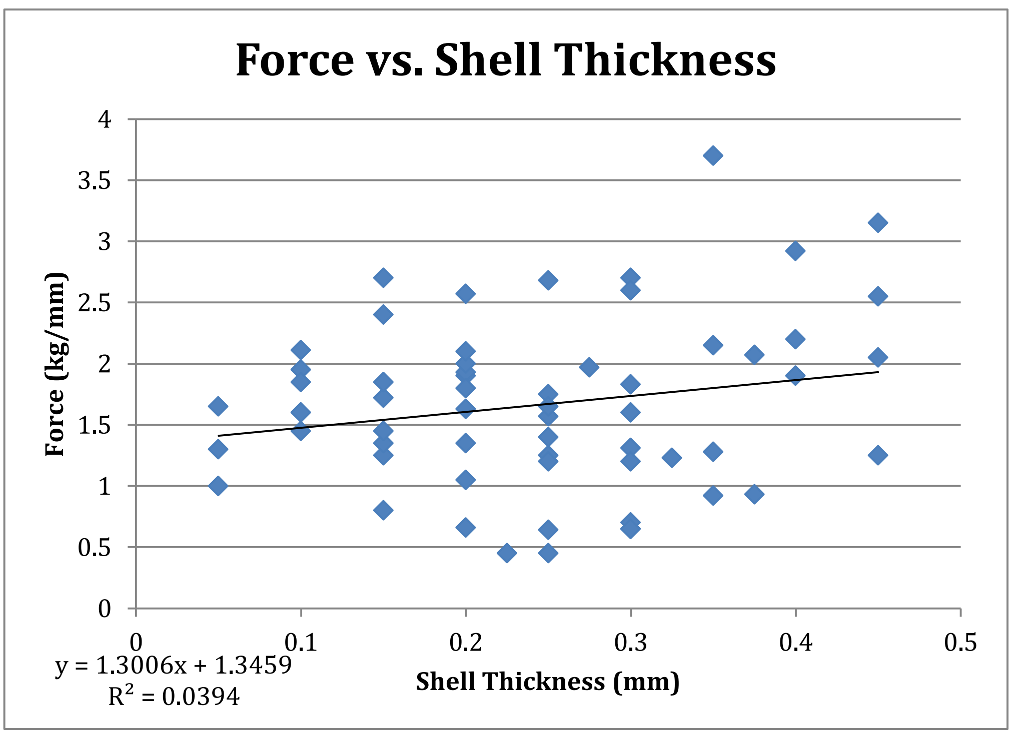


Figure 3. Relationship between shell strength and shell thickness. The force required to penetrate a snail shells did not significantly increase with shell thickness for all species ($F_{1,58}$ = 2.3812, p = 0.1283, R^2 = 0.0394).

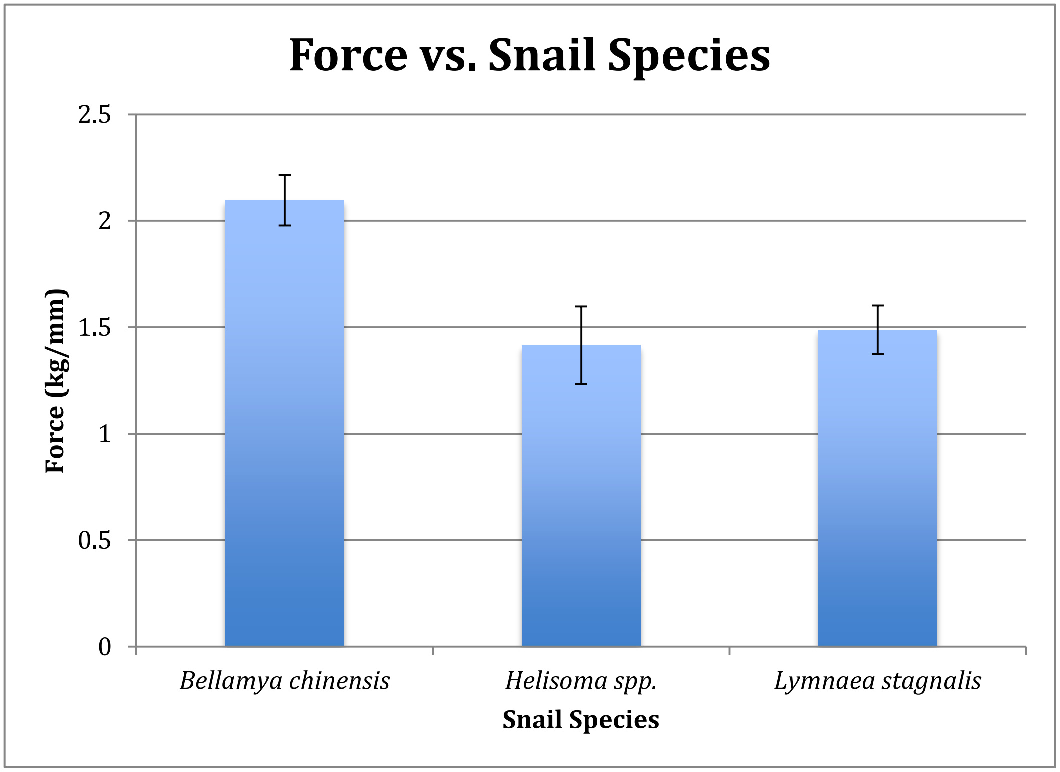
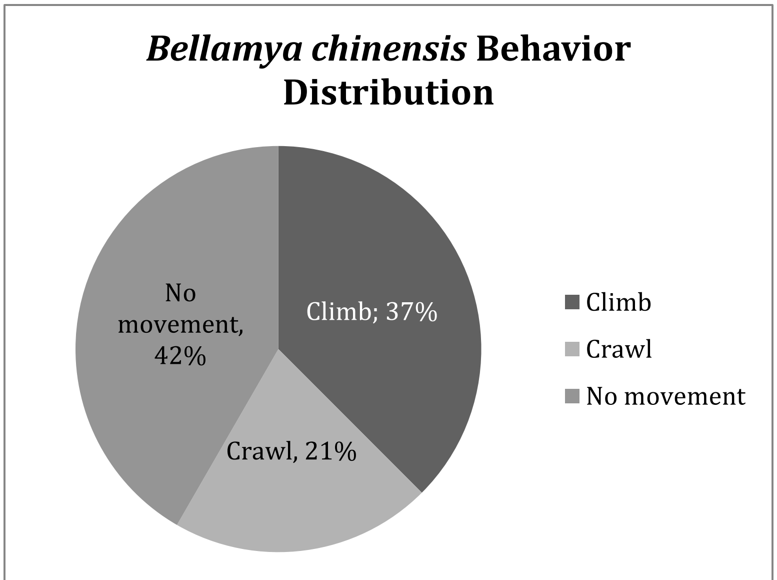
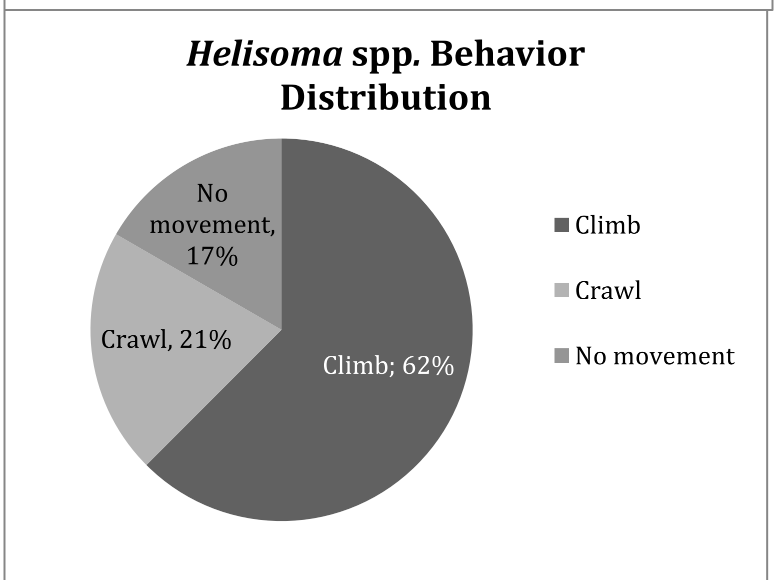


Figure 4. Mean force required for shell puncture of three different species groups. *Bellamya chinensis* required a significantly stronger amount of force in order to penetrate the species' shells ($F_{2, 57} = 6.9778$, $p = 0.00195$). The average force required for *Bellamya chinensis* was 2.10 ± 0.02 kg/mm. The average force required for *Helisoma* spp. was 1.41 ± 0.32 kg/mm. The average force required for *Lymnaea stagnalis* was 1.49 ± 0.33 kg/mm.


Bellamya chinensis Behavior Distribution
No movement, 42%
Climb; 37%
Crawl, 21%
Climb
Crawl
No movement


Helisoma spp. Behavior Distribution
No movement, 17%
Crawl, 21%
Climb; 62%
Climb
Crawl
No movement

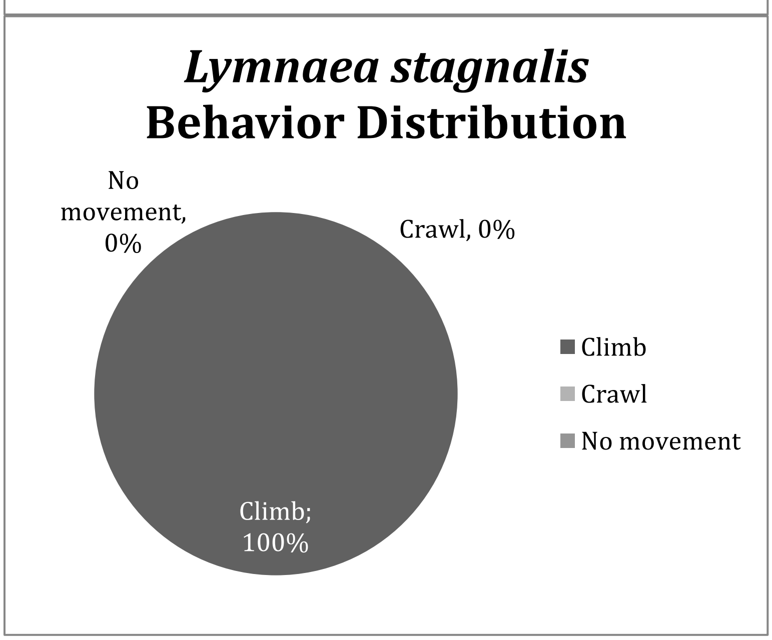

Lymnaea stagnalis Behavior Distribution
No movement, 0%
Crawl, 0%
Climb; 100%
Climb
Crawl
No movement

Figure 5. Behavior Distributions of all three species groups. Each graph shows the percentage of individuals that reacted by climbing, crawling, or not moving in response to the presence of a predator. *Bellamya chinensis* had the greatest percentage of individuals that did not respond with avoidance behavior.

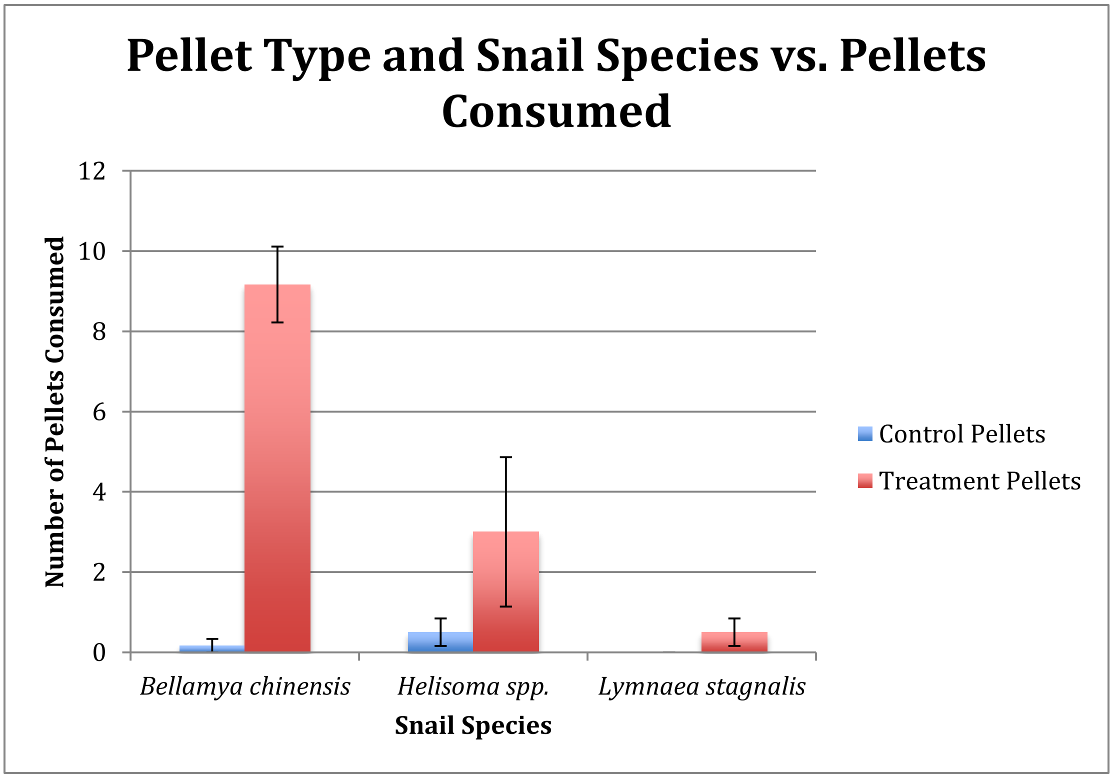


Figure 6. Palatability tests of all three species groups. Crayfish consumed a significantly higher amount of treatment pellets and *Bellamya chinensis* pellets ($F_{1,30}$ = 11.6868, p = 0.0002). *Helisoma* spp. and *Lymnaea stagnalis* appeared to be less palatable to crayfish predators.