Crayfish predation and predator induced responses in growth and shell strength of the exotic Chinese mystery snail *Cipangopaludina chinensis*

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Abstract

The effects of Chinese mystery snails (*Cipangopaludina chinensis*) on their surroundings have gone largely unexplored even amidst growing concerns. High population densities and large size are reasons to believe that *C. chinesis* may cause food web disruptions. I ran a laboratory experiment to see if crayfish, which are commonly important predators of snails, will prey upon *C. chinesis.* Additionally, my experiment examined whether crayfish if they prefer native snails over *C. chinesis.* Crayfish preyed upon few *C. chinesis ,* although overall results were inconclusive. Another experiment tested the effect of predation cues on snail growth. While the snails grew over the course of 14 days, but there was no difference between predator treatments (crayfish, pumpkinseed sunfish, and crushed snail) and the control. The shell strength per mm^3 of *C. chinesis* was compared with that of native snails. *C. chinesis* had a weak shell relative to two native snail in the genii *Campeloma* and *Helisoma*. These results may indicate that *C. chinesis* is more susceptible to predation as a youth than similarly sized native snails. It is unclear, though, if native snail predators prefer *C. chinesis* over native snails. A better understanding of the how *C. chinesis* changes food webs in the areas it invades will aid in reducing population sizes and preventing any negative effects of *C. chinesis* invasion.

Introduction

The effects of Chinese mystery snails (*Cipangopaludina chinensis*) on the populations of native organisms have gone largely unexplored even amidst growing concerns from homeowners, anglers, and environmental managers. While it is certain *C. chinensis* is exotic, not enough is known to label it as an invasive species, defined by

Mack et al. (2000) as plants and animals which overcome natural barriers to dispersal, establish self-sustaining populations in exotic habitats, and generate negative ecological or economic impacts. Personal observations have noted that *C. chinensis* may reach population densities of 100 per meter squared and have the capacity to become quite large, roughly the size of a golf balls. At such large densities and sizes alterations in the native foodweb seem inevitable. Any change in food web interactions could lower prey populations for game fish. In northern Wisconsin this could be a serious matter, as much of the population depends on tourism money driven by the recreational fishing industry. They may also be vectors of human disease (USGS 2005). Until such concerns are proven, *C. chinesis* cannot truly be considered an invader.

From the limited literature which exists, we gather that *C. chinensis* first entered the United States as a food source in Asian markets (Woods 1982) and has also made entrances in the aquarium trade (Schmeck 1942). *C. chinensis* now occurs in at least 27 states, from coast to coast (Fig. 1). Such a widespread distribution makes learning about the snail and its impacts even more imperative.

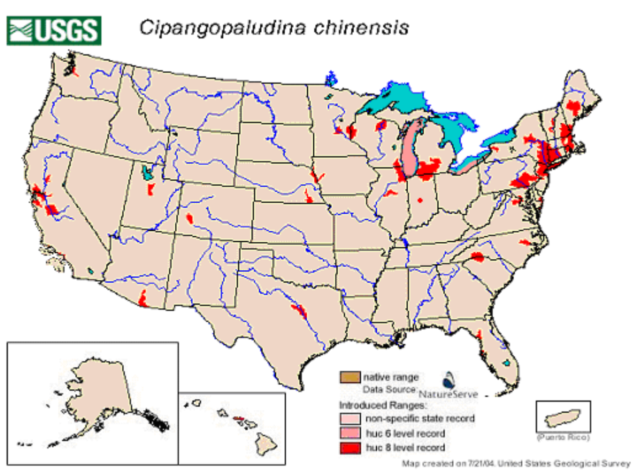

Figure 1: Shading indicates known populations of

Mystery Snails (USGS 2005)

The enemy release hypothesis proposes that some exotic species thrive due to their escaping their natural competitors, pathogens, and predators (Wolfe 2002). Enemey release may help explain how Chinese mystery snails haev effectively established exotic populations in the United States. The large size of the mystery snail could be an example of a defense mechanism in which they avoid natural predators and consequently survive better than native snails. I tested this hypothesis by collecting native crayfish species, which are important predators of snails (Lodge et al. (1994), from University of Notre Dame Environmental Research Center (UNDERC) lakes and streams, native snail species, and varying size of mystery snails.

Even if a species escapes its native predators it must still adapt to the challenges of its new range. Any organism will have to interpret and respond to many different predator cues, in the form of kairomones, and cues from injured organisms in order to survive (Schoeppner et al. 2005). Such cues may include chemical cues from crayfish and sunfish predators and alarm cues from injured conspecifics. By responding either behaviorly or physiologically to such cues, the mystery snail may increase its chances for establishment in new ranges. With the predation experiments outlined earlier I will be able to see how adult mystery snails interact with predators. This experiment will investigate how hatchling *C. chinesis* interact with predators. Many organisms develop differently in the presence of different predators. Streamside salamanders, *Ambystoma barbouri*, delay the hatching of their eggs in the presence of green sunfish which can be voracious predators of salamander hatchlings. The delay in development allows the hatchlings to be larger and perhaps better able to evade predation (Moore et al. 1996).

An exotic freshwater applesnail (*Pomacea insularum)* was found to have different survival rates when born in waters with and without predator cues (Barnes.2006). Like the applesnail and the streamside salamander, I predict that *C. chinesis* develops differently in the presence of predators. I expected the snails grown in the presence of cue to have a slower development and consequently a thinner shell due to the results of Lewis's (2001) study. To test this, I raised *C. chinesis* hatchlings for two weeks in the presence of different predator cues. I will also test to see if mystery snails respond to their own dead by using a crushed snail cue.

The results of these studies will contribute to the knowledge of the overall ecology of this relatively little studied species and could point to how the mystery snail is successful in the areas it has invaded. These studies may also lead to understandings in food web interactions and how an invasive species affects the foot web low on the tropic scale.

Materials and Methods

Predator Cue Growth Experiment

I used twelve aquaria filled with 4 L of Tenderfoot Lake water. I measured the operculum width and shell length of 48 mystery snail hatchlings and then put four in each tank. I had tanks designated for four different treatments - control, crayfish (*Orconectes propinquus*) cue, pumpkinseed (*Lepomis gibbosus***)** cue, and crushed mystery snail cue. The tanks were alternated on the counter space in the lab so that unforeseen factors in placement would not affect all of a single treatment. I tended to the snails every day, with the measurements started on Day 0 and were taken every other day. Cues were added on Day 1 and were added every other day. Crayfish cue water was taken from a tank with one crayfish (1 crayfish/2.5 liters of water) that was fed mystery snail hatchlings throughout the experiment. Pumpkinseed cue was taken from a tank with one fish (1 fish per/ 40 liters of water) that was fed mystery snails. I made the crushed snail cue with a motar and pestal was a mixture of three hatchlings and 30 ml of water. Each dose of cue was 10 ml. When cue was taken from the cue tanks, it was replaced with water from Tenderfoot Lake. Measurements were taken at approximately 8 pm each evening while cues were added the following morning at approximately 8 am. This provided regularity in the sampling regime. The sampling ended after 14 days.

To analyze the data I compared the mean operculum widths for each tank. The purpose of using mean measurements was to eliminate any irregularities or errors from the measurements. I used an analysis of variance (ANOVA) with a Tukey's post hoc test to determine if the snails were significantly different at the beginning and the end of the treatments. I also used a repeated measures ANOVA to determine if there were any differences between treatments over time.

<u>Shell Strength Experiment</u>

Building off the growth experiment, I took the mystery snails from all of the treatments and tested their shell strength. I believed shell strength would provide an indication of predator cue response and a comparative advantage between snails. I had four different treatments - control, crayfish, pumpkinseed, and crushed snail. After testing between mystery snail treatments, I tested six individuals of *Helisoma anceps, Limnaea sp.,* and *Campeloma decisum* against the mean of all the mystery snails. I placed each snail with its operculum down beneath a small plastic container and filled the container with water. I approximated that 1.0 ml of water weighed 1.0 g. Water was added to the container until the snail's shell was crushed. The amount of water plus the weight of the container was used as a measure for the strength of their shell.

An ANOVA test was used to determine if there was any difference between treatments in the mystery snails. In order to compare the data from the different snail species, I standardized the data by computing a ratio of weight needed to crush the shell relative to overall volume of the snail. Volume was estimated by assuming that the snail was spherical and is given by the equation $(4/3)(\Pi)(r^3)$ with the radius being the operculum width. I used the means of the mystery snail trials and the six measurements for each of the native snails to do these calculations. The data was determined not to be normal by a KS test, therefore I attempted to normalize the data using arcsine square root, logarithmic, and inverse transformations, but none were able to normalize the data. I therefore used a Kruskal-Wallis test to explore differences between snail species. Because differences between treatments were very robust and corroborated by nonparametric tests, I felt justified using ANOVA with Tukey post hoc analysis to determine differences between species.

<u>Crayfish Predation Experiment</u>

To determine if crayfish would prey upon mystery snails and to see if they prefer natives over mystery snails, I used ten 40 L aquaria each filled with 20 L of water from Tenderfoot Lake. In each trial I used crayfish (*Orconectes propinquus*) caught from Tenderfoot Lake and Tenderfoot Creek. Each tank contained one crayfish for all of the trials and the crayfish did not eat for 24 hours before each trial. The crayfish were then given 48 hours to prey upon the snails, which again was the same in all trials. In the first trial, I tested large mystery snails against small mystery snails. I used operculum widths as a judge for snail size in all trials. The small snail class was ~15 mm and the large class was ~30 mm. All of the tanks had three snails in them with five of the tanks having only large snails and the other five having only small snails. In the second trial I tested small mystery snails (~15 mm) against local *Campeloma descisum*(~12 mm). Each of the ten tanks had two mystery snails along with two *C. descisum*. The last trial included small mystery snails (~15 mm) and local *Helisoma anceps* (~8 mm). Again, all snail sizes were operculum widths. After 48 hours I removed the crayfish from the tanks and then measured the operculum width of any eaten snails.

Results

Predator Cue Growth Experiment

No tanks (n=4 snails per tank) demonstrated statistically different average snail size at the beginning of the growth experiment, as indicated by ANOVA on the first day of the experiment ($p=0.1$, $df=3$). At day 14 another ANOVA determined that there was no difference between treatments ($p=0.5$, $df=3$). The repeated measures ANOVA found that the snails grew significantly over time($p=0.006$., $df=7$) but no interaction occurred over time between treatments and snail growth ($p=0.49$, $df=21$).

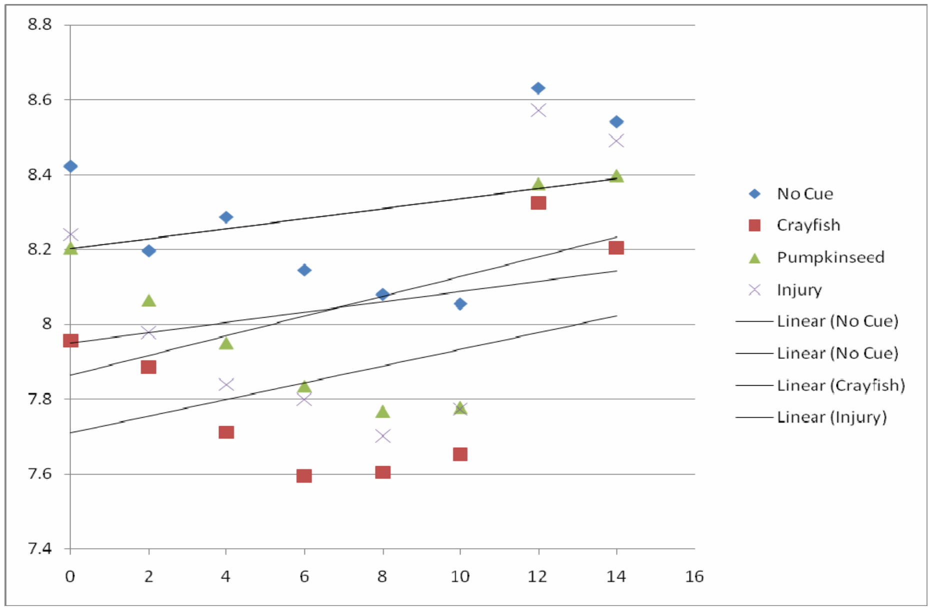

Figure 2. A scatterplot of snail shell length means over the days of the treatment. The regression lines show the upward trend, snail growth.

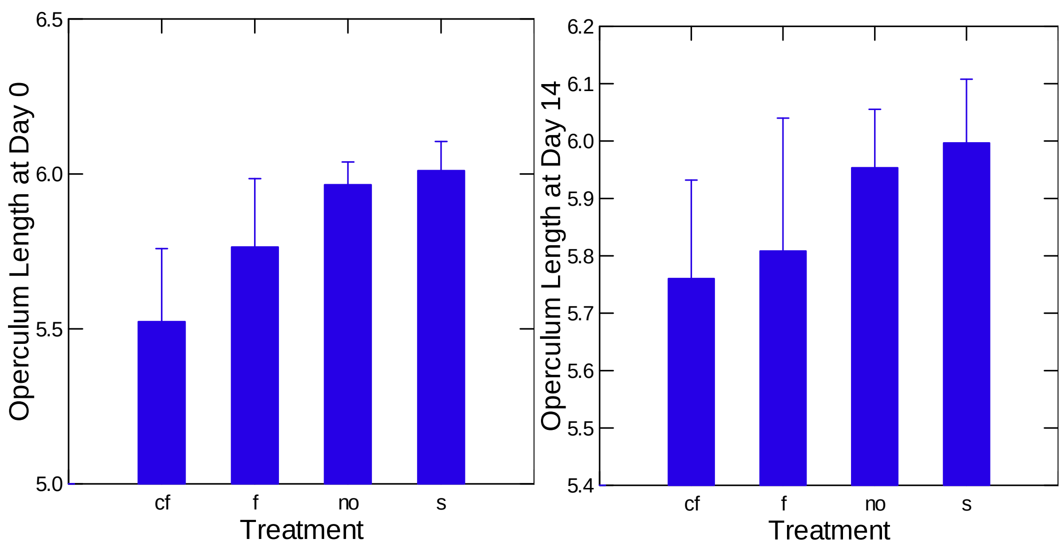

Figure 3. Bar graphs of operculum length against treatment at Day 0 and Day 14. Both show that there was no difference between treatments. (cf = crayfish, f = pumpkinseed, no = control, s = crushed snail)

Shell Strength Experiment

C. chinesis was found to have a significantly weaker shell than *Campeloma* ($p<0.001$) and *Helisoma* ($p=0.037$). There was no difference found between *C. chinesis* and *Lymnaea* ($p=0.893$). *Campeloma* was also found to have a stronger shell than *Helisoma* ($p<0.001$) and *Lymnaea* ($p<0.001$). *Helisoma* was found to have a stronger shell than *Lymnaea* ($p=0.022$).

The differences in the comparison between the ratio crush weight (amount it took to crush the snail) and volume of snail across the snail types were still significant. *Campeloma* still was found to have a stronger shell than *C. chinesis*, *Lymnaea*, and *Helisoma* (all $p<0.001$). *Helisoma* was found to have a stronger shell than *Lymnaea* ($p=0.037$). *C. chinesis* was found to be similar to *Helisoma,* ($p=0.377$) and *Lymnaea* ($p=0.346$). All degrees of freedom are 3 for these tests.

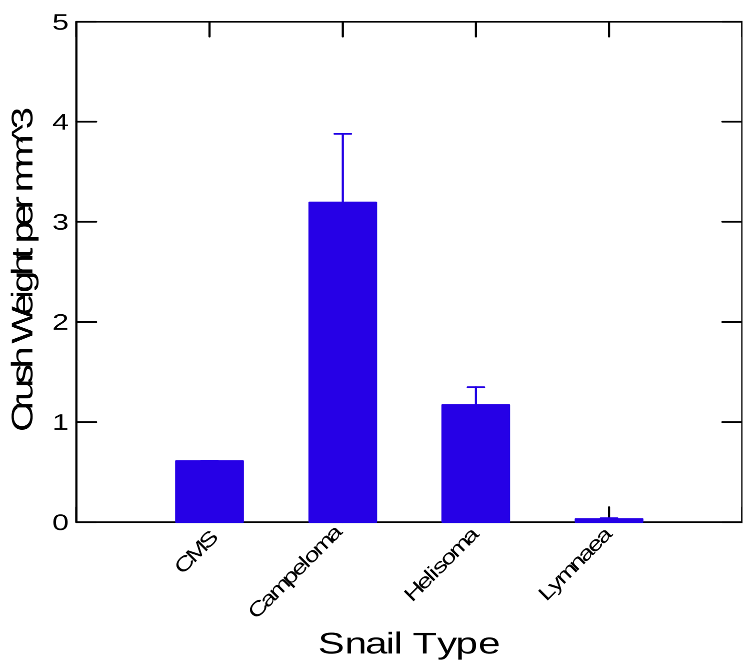

Figure 4. Crush weight per mm^3 over snail type. This shows that *Campeloma* has a significantly higher crush weight per mm^3 than the rest of the snails.

<u>Crayfish Predation Experiment</u>

The only trial resulting in predation was the small versus large mystery snail trial. Overall, the predation experiment did not produce enough data for statistical analysis. It appears that crayfish preferred the small snails over the large snails with seven dead small snails and one dead large snail. The large snail that was dead was 29.0 mm (mean=29.1 mm), while the small snails had a mean of 15.7mm (mean=17.3 mm). The other two trials did not produce any results as none of the snails died in either trial. Both 48 hour trials produced no dead snails, although there was evidence of crayfish attacks on the snails in the form of chipped shells in the *Campeloma* and *C. chinesis* trial. Both snail types had chipped shells which I considered evidence of attempted predation.

Discussion

The large versus small mystery snail trial is what I expected. What interests me the most is the one dead large mystery snail. I did not expect any large mystery snail to be preyed upon. In the experiment I assumed that all dead snails were the result of predation, but it is completely plausible that the snail may have died from other causes. Another study may be able to see if that snail was actually preyed upon, or whether it died due to other causes. It is unfortunate, though, that a bulk of the predation experiments ended with limited predation, but it is good to note that snail behavior was consistent with the literature. Alexander and Covich (1991) found that two freshwater snails, *Planorbella trivolis* and *Physella virgata*, crawled above the waterline of their aquaria in the presence of crayfish cue. In natural environments, snails would crawl up macrophytes to avoid crayfish. Mystery snails did not exhibit this behavior, but *Campeloma* and *Helisoma* snails did. This could explain why none of these snails were preyed upon. I would suspect they do not exhibit crawl out behavior as their main defense is their large size as an adult. More testing could also be done in the area of predation to see if native snails are preferred over mystery snails. A simple solution would be to add less water to the tank so that the native snails will have less area to crawl up. The large amount of water we added to the aquaria was likely a strong source of experimental error. With less area to crawl out of, the crayfish may be able to reach the snails on the sides of the tank making it possible for them to consume snails.

The predator cue growth experiment also did not exactly confirm my hypothesis. The data analysis supports the idea that the mystery snail does not grow differently in response to predator cues. David Lewis (2001) determined that *Amnicola limnosa,* a freshwater snail found in Wisconsin, grew smaller in the presence of crayfish cue. This was due to increased time spent climbing macrophytes which the snails do not eat. *A. limnosa* prefers algal rich benthos, which is also where crayfish are able to prey upon them. His study supported my original hypothesis that there would be a difference in size among treatments. Human error in sampling or equipment error possibly corrupted our data collection. Inconsistency in measuring technique would be the most likely culprit for inaccurate data. Days 0, 12, and 14 were measured by someone other than me. They may have had a different technique than mine which could have caused the data to be off. I would attribute human error to the reasons why results did not come back as expected. As the results stand, though, mystery snails do not grow differently in the presence of predator cues.

No difference was found between the treatments ($p=0.25$, $df=3$). One possible explanation for a lack of significant results could be that the two week time period was not long enough for a change in development speed to occur in the mystery snail. Of course, the explanation that the mystery snail does not respond to these cues is also valid. I was somewhat surprised at the results of the tests between the different snail types. I expected the mystery snail to have a stronger shell relative to the other snail types, but it was not the case. Personal observations have led me to believe that an adult mystery snail has a very hard shell compared with other snail species. A difference between the snails to be noted is that the mystery snail was a hatchling, which was approximately the same size as more mature snails of the other species. Perhaps the mystery snail does not use as many resources to produce a hard shell in its youth while the native snails have developed a strong shell as an adult. The implications of this would be that as hatchling mystery snails, which in earlier trials were shown to be eaten by crayfish, are more susceptible to predation than native snails until they get too large for effective predation. Another study looking at predation with an entire spectrum of mystery snail sizes from hatchling to adult could find where effective predation ends due to various reasons, including size and shell strength.

I believe more needs done in order to understand the overall ecology of *C. chinesis*. The results of this study merely brought confusion to whether or not *C. chinesis* is in fact an invasive species defínted by Mack et al. (2000). Knowing that the Chinese mystery snail will be preyed upon by crayfish and that they have a relatively weak shell as a hatchling shows that the mystery snail is vulnerable. The lack of results in the predation experiment, though, failed to provide more concrete understanding how *C. chinesis* interacts and responds to predators and how they compare to other snails. More research on the topic can shed light into the interactions of the mystery snail and their new potential predators. Ultimately, this could uncover why the mystery snail can reach such high densitiesand possibly even if *C. chinesis* could and should be labeled a true invasive species. The sooner this is done the less potential negative impacts from *C. chinesis* invasion will be realized. Included among them are the lowering of native snail populations, food web disruptions, decreased biodiversity, and ultimately a weakened recreational fishing industry in areas where it is depended on.

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