

**Prevalence of Parasitism and Predation in Three Freshwater
Gastropods at UNDERC**

BIOS 569: Practicum in Field Biology

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Abstract. In order to determine the dynamics between snails and three common snail predators, snails of three families (Lymnaeidae, Planorbidae, and Viviparidae) were introduced to two predators (leeches and crayfish) and a survey was utilized to determine trematode prevalence among the three families. Virtually no predation was observed by leeches, marked predation by crayfish was observed only in Lymnaeidae and Planorbidae, and trematode infections were found in all three families, although at different frequencies. Furthermore, crayfish follow foraging theory when preying upon Lymnaeidae and Planorbidae. Future studies should concentrate on the dynamics between exotic mystery snails and native snails to prepare for the inadvertent cohabitation of the species in UNDERC aquatic system.

INTRODUCTION

The recent discovery of the Chinese Mystery Snail (*Cipangopaludina chinensis*), of the Architaenioglossan family Viviparidae, has dramatically increased awareness to the susceptibility of closely guarded habitats such as the University of Notre Dame Environmental Research Center (UNDERC) to invasion by exotic species. Snails can have a profound effect on the benthic communities in which they are found, serving not only as herbivores or detritivores, but as a food source to fish, birds, mammals, and other invertebrates and furthermore as an intermediate host of parasitic trematodes (O'Brien 2006). With the presence of the mystery snails on property, it is imperative to discern the impact they will have on the local benthic communities in which they invade.

Pulmonates are an order of gastropods (Class Gastropoda) in which the gills have diminished and the mantle cavity has evolved into a functional lung. This has allowed pulmonates to become terrestrial; however, many pulmonate families have returned to freshwater habitats (Barnes 1968a, O'Brien 2006).

These species include two of the three families examined in this survey: Lymnaeidae, Planorbidae. Viviparidae are a unique species of snail in that they are operculates and are able to completely close their shell via a calcium carbonate disk attached to their foot (Barnes 1968a).

The focus of this paper is to investigate the effect of the three described families of gastropods on benthic communities as a source prey to three organisms - invertebrate leeches, invertebrate crayfish, and parasitic trematodes – in an effort to assess their roles as a food source, vectors of parasitic disease, and finally to propose ways to control the spread of the mystery snail.

Some species of leeches have been documented to feed off of the body fluids of snails, inducing mortality. For this reason, in recent years, leeches have been approbated as an effective means of controlling snail populations in habitats which they have overpopulated (Gautam and Srimanta 2005). Knowledge of the susceptibility, and resulting predation rate, of UNDERC's snails to leech predation could offer a solution to the problem of invading mystery snails if the local specie of leech prefers these pulmonates to others.

Crayfish are omnivores whose diet consists of macrophytes as well as eggs and invertebrates such as snails. Studies which have examined cohabitation between crayfish and snails have indicated that crayfish can have a considerably negative effect on local populations of snails (Dorn and Wojdak 2004). Once again I will look at crayfish as a potential means of mystery snail management by

assessing the predation of the benthic friendly, and native, crayfish *Orconectes propinquus* on the one exotic and two native families of pulmonates.

Trematodes are parasites which utilize at least two, sometimes three, hosts in a complete lifecycle. Snails, especially freshwater pulmonates, are primarily the first intermediate hosts (and often the second); vertebrates (e.g. fish, birds) are frequently the definitive hosts of adult trematodes. For simplicity, only the life cycle of digenetic trematodes (Order Digenea) will be discussed, since it is the most relevant to this study. Upon hatching, a free swimming miracidium penetrates the epidermis of the first intermediate host and asexually reproduces cercariae. The cercariae leave the first intermediate host and infect a secondary host, in which it develops into a metacercaria and lies dormant until the host is eaten by a definitive host, which it promptly infects (Barnes 1968b).

A trematode can have a substantial effect on its second intermediate host. A study by Levri and Lively (1996) indicated a behavior change which caused infected snails to forage on top of rocks in early morning hours during the prime foraging time for waterfowl, a potential definitive host of trematodes, whereas uninfected snails would not. Another study found that infected pulmonate individuals are more likely to avoid shaded habitats, despite the higher risk of predation by fish which is typically avoided by uninfected individuals who spend a significantly larger amount of time in shaded habitats (Bernot 2003).

Furthermore, it is well documented that trematodes can cause chronic infections that last the life of the snail hosts as they reproduce cercariae. In these lifelong parasitic infections, it has been shown that the trematodes are responsible for, among other things, the castration and retarded growth of their hosts. These two consequences of infection have led many ecologists to propose that snails are actually preyed upon, rather than merely parasitized, by trematodes since their fitness is hindered greatly by the parasite (Brown et al. 1988, Curtis and Hurd 1983, Curtis 2002).

In this study I attempted to determine how these three predators interact with three families of snails commonly found on the UNDERC property by several different factors. First I looked at the overall predation of the predators as a function of snail species. I hypothesized that leeches, crayfish, and trematodes will all show a preference to Lymnaeidae and Planorbidae families and less towards Viviparidae. For leeches and crayfish, this would correlate with typical foraging theory, which suggests that a forager will optimize its nutrient intake while minimizing energy expended in obtaining nutrients (because of the operculum, it can be assumed that it would take more energy for a forager to prey upon Viviparidae) (Quijano et al. 2006). For parasites, the ability for Viviparidae to close its shell completely by way of the operculum would make it a more difficult host to infect.

I also looked at how snail size affects the predation rates of leeches and parasites. I hypothesized that the size of snails would have positive correlation to the risk of predation by both leeches and parasites. Previous studies have shown that, in the case of trematodes, as the axial length (from apex to lower margin of aperture, see Fig.1) increases, the probability of parasite infection increases. This is believed to be due to the fact that axial length is an indication of age and older snails have had more time to become infected (Curtis and Hurd 1983). In the case of leeches, only Viviparidae were used and I hypothesized that medium sized Viviparidae would be preyed upon more because both large and small Viviparidae would have been too hard for the leeches attach to and handle.

METHODS

Study site. Snail specimens were collected from three sites (two lake and one creek) at the UNDERC field station. The property on which the field station is located lies in the Northern Highland Province, on the Michigan-Wisconsin border in Gogebic and Vilas Counties respectively. It encompasses approximately 7500 acres (6150 acres of terrestrial habitat and 1350 of aquatic). The property is surrounded on three sides by the Ottawa National Forest and entry to the property is prohibited to the public. For more information about the field station at UNDERC, including geology, climate or ecological systems, see Belovsky (2004).

Tenderfoot Lake covers approximately 442 acres (max. depth > 10 m) and is a mesotrophic drainage lake, having both inflow and outflow sources. It has several bays perfect for catching Planorbidae, and the weeds just off shore are home to many Lymnaeidae. Brown Lake is a turbid, eutrophic lake with a Secchi depth of less than 1 m and encompasses 63 acres (max. depth = 4.5 m). It is home to an abundant Lymnaeidae population as well as a few sparse Planorbidae populations. Brown Creek exits Brown Lake, leading off property, and contains a plethora of Viviparidae (Belovsky 2004).

Collection. Pulmonates for predation experiments were collected from populations established in three locations based on preferred habitat. Viviparidae were collected from Brown Creek, Lymnaeidae from Brown Lake, and Planorbidae from Tenderfoot Lake. For parasitism experiments, fifty pulmonates were collected from the same locations as those utilized in predation experiments on a separate date. Of these fifty, thirty were selected using the double blind method.

O. propinquus were collected from a single population at Kakabeka Falls in Vilas County, WI; leeches of an undetermined species were purchased from Hilger's Bait and Tackle, Antigo, WI.

Viviparidae prey selection of leeches. Thirty six 22 x 15 cm plastic containers, with a depth of 14cm, were filled with 1200 ml of water from Tenderfoot Lake, and two six-inch segments of coontail, *Ceratophyllum*

demersum, were placed in each. Viviparidae were separated into three size classes by axial height: large (~48.6 mm), medium (~36.4 mm), and small (~7.72 mm). Ten Viviparidae of each class were selected and placed individually into 3.8 L containers. Eighteen leeches were added to random containers (1 per container, 6 leeched containers per size class) and allowed to prey upon the pulmonates for 48 hours. Each size class had 4 control containers. At the end of the trial time, mortality was tabulated for each container.

Prey selection of leeches by family. Twelve snails were selected from each family (~36.4 mm, medium sized, Viviparidae were used) and placed individually into identically set up containers from the previous experiment. Eighteen leeches were added to the containers (1 per container, 6 leeched containers per family) and allowed to prey for 48 hours. Each family had 6 control containers. At the end of the predation period, each snail was once again gauged for mortality.

Prey selection of O. propinquus. Twelve snails were selected from each family (medium sized Viviparidae were used) and placed individually into a total of thirty six 3.8 L containers identical to the ones previously used. For this experiment, only 600 ml of water from Tenderfoot Lake was placed in each container (two six inch segments of *C. demersum* were still used) in order to prevent snails from crawling out of range of the *O. propinquus*. Twenty-four crayfish were added randomly to the containers (1 per container, 8 crayfish

containers per family); 4 containers per family were used as controls. After a 24 hour predation period, each snail was gauged for mortality.

Parasitism of pulmonates. The methods of Minchella et al. (1985) were used to survey parasite prevalence in collected specimens. Each specimen was placed into a separate Petrie dish under a 700,000 lux fluorescent light for 12 hours. The euthanized snails were observed for emerged cercariae with a dissecting microscope. The shells of those without emerged cercariae were carefully cracked, as to not damage or lose embedded cercariae, and examined under a dissecting scope for cercariae.

Statistical Analysis. To determine the relationship between leech predation and snail species and size, a Likelihood Ratio χ^2 test was utilized. A Pearson χ^2 was used to compare crayfish predation among species, as well as axial length of Viviparidae to trematode infection. For correlation of axial length to infection of Planorbidae and Lymnaeidae, an estimate model of logarithmic regression was employed. All statistical work was completed using Systat12 by Systat Software Inc (SSI).

RESULTS

When Viviparidae of three size classes were placed in containers with leeches, there was almost zero mortality. Of the thirty snails, only one (of the 'small' class) was preyed upon by a leech; none of the controls showed mortality. A Likelihood Ratio χ^2 test yielded no significant difference among the size classes

in leech predation ($\chi^2 = 0.062$, $p = .969$). Similar results were found when snails of all three families were introduced to leeches. Of thirty-six snails, only a single Planorbidae was preyed upon. Incidentally, Planorbidae are the smallest of the three families used, which could indicate that leeches only feed off of small snails; however, there were not enough replicates of this phenomena to determine if this was a one time abnormality or active predation. A Likelihood Ratio χ^2 revealed that there was no statistically significant difference in prey selection of leeches across the three families used ($\chi^2 = 0.050$, $p = 0.975$).

When snails were introduced to habitats with crayfish, greater mortality was observed in two families. Mortality was observed in 50% of eight containers of Planorbidae, 37.5% in Lymnaeidae, and 0% in Viviparidae. Upon observing zero mortality of Viviparidae, a second replicate was utilized, replacing Viviparidae containers with a combination of Planorbidae and Lymnaeidae. In this replicate, mortality was significantly greater in Lymnaeidae than in Planorbidae (62.5% and 12.5% respectively). A Pearson χ^2 test indicated a statistically significant difference of prey choice between Lymnaeidae and Planorbidae by *O. propinquus* ($\chi^2 = 8.53$, $p < 0.01$).

Percentage of samples infected with Trematodes showed a prevalence of 30% in Lymnaeidae, 23% in Planorbidae, and 16% in Viviparidae. An estimate model of logarithmic regression showed that for Lymnaeidae there was a positive correlation between increasing axial length and parasitism (Fig. 2a), however the

model created was not significant (Estimates = 0.20, Standard Error = 0.20, $p = 0.31$). A positive correlation was also found in Planorbidae (Fig. 2b), although again, it was not significant (Estimates = 1.3, Standard Error = 0.71, $p = 0.06$). Because of the significantly lower incidence of parasitism in Viviparidae, as well as grouping of infections in only the largest and smallest individuals, with no infections in intermediate snails, a Pearson χ^2 was used, rather than an estimate model, to compare size to infection. For this test, the Viviparidae samples were arbitrarily grouped into two groups, large and small. The Pearson χ^2 revealed a difference between large and small samples that approached significance and associated a positive trend between large axial lengths and increased infections ($\chi^2 = 3.41$, $p = 0.06$).

DISCUSSION

Because there was virtually no predation on the snails by the leeches, I was unable to demonstrate any preference in leeches among families of snails. However, from the data collected, it can be inferred that local leeches do not actively prey upon snails as a food source, although this should be more thoroughly tested with more numerous replicates and more realistic substrates in order to recreate a natural environment in which the two organisms may encounter each other. Similarly, no definite conclusion could be inferred as to whether size plays a role in leech predation of snails since there was virtually no predation of mystery snails.

Predation from crayfish was much more dynamic. From the data, it can be reasoned that mystery snails are not actively preyed upon and, following my hypothesis, Planorbidae and Lymnaeidae follow typical foraging theory. Assuming that both families require approximately the same amount of energy investment during foraging, and that Lymnaeidae has more nutrients due to its larger size, the significant result that Lymnaeidae are preferred correlates to the theory that predators will forage for the most nutrient rich food source.

Trematode prevalence was found to be lowest in mystery snails, and highest in Lymnaeidae, with Planorbidae falling medially in-between. When incidence of parasitism is compared to axial length, a significant trend can only be found in Viviparidae, although it is probable that a larger sample size would strengthen the positive correlation in Lymnaeidae and most certainly in Planorbidae. Because of this, I believe it is safe to conclude that as axial length increases, so does the chance of becoming infected, probably due to time increasing the chances of being infected. These findings mirror those of Curtis and Hurd (1983).

In the future, trematode studies should utilize a much larger sample size over a longer time period. One example, cited in Curtis (2002), included 250,000 dissections in a survey of snail trematodes, although I don't foresee that many dissections as being necessary for a survey of this nature. These dissections

should, however, carry on over several years to look at how parasitism fluctuates over different seasons.

The study of trematodes in snails may be the most important of studies into controlling mystery snails, since trematodes can reduce snail replacement in a community 10-20% (Brown et al. 1988). This is due to the fact that trematodes castrate their host, change its feeding and predator avoidance behavior, and cause physiological changes such as a decreased host heat tolerance (Curtis 2002). From this study, it can be inferred that trematodes are the most intensive predator of Viviparidae of the three predators examined; therefore it is imperative to understand the dynamics between these two.

In the upcoming years, however, much attention should be redirected towards the effect Chinese Mystery Snails have on their communities. They are able to habitate virtually any substrate (personal correspondence with Nick Ward) and without an abundance of natural predators, could soon increase in population in many of the aquatic systems at UNDERC. Therefore, comprehensive knowledge of the effects mystery snails have on their habitat is of dire need. This would naturally include examining the dynamics between Viviparidae and native snails to determine potential competition.

SUMMARY

Chinese Mystery Snails are not actively preyed upon by local leeches or crayfish, and not as susceptible to trematode infections as native snails. This

could aid in their dispersal. Lymnaeidae and Planorbidae, on the other hand, can be shown to follow typical foraging theory with crayfish predators, but not leeches, if, in fact, Lymnaeidae is more nutritious than Planorbidae.

Future studies which replicate my own should include larger sample sizes over longer periods of time (possibly years). Further studies into the subject of invasive mystery snails should concentrate on the role of Viviparidae in aquatic communities and the effect of cohabitation and competition with native snails.

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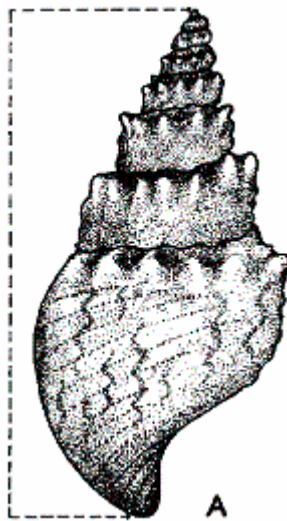


Fig.1. Snail shell illustrating the axial length (dotted line). (Picture taken from http://ecology.hku.hk/vsb/fieldcourse/fc-rock-1_clip_image002.gif)

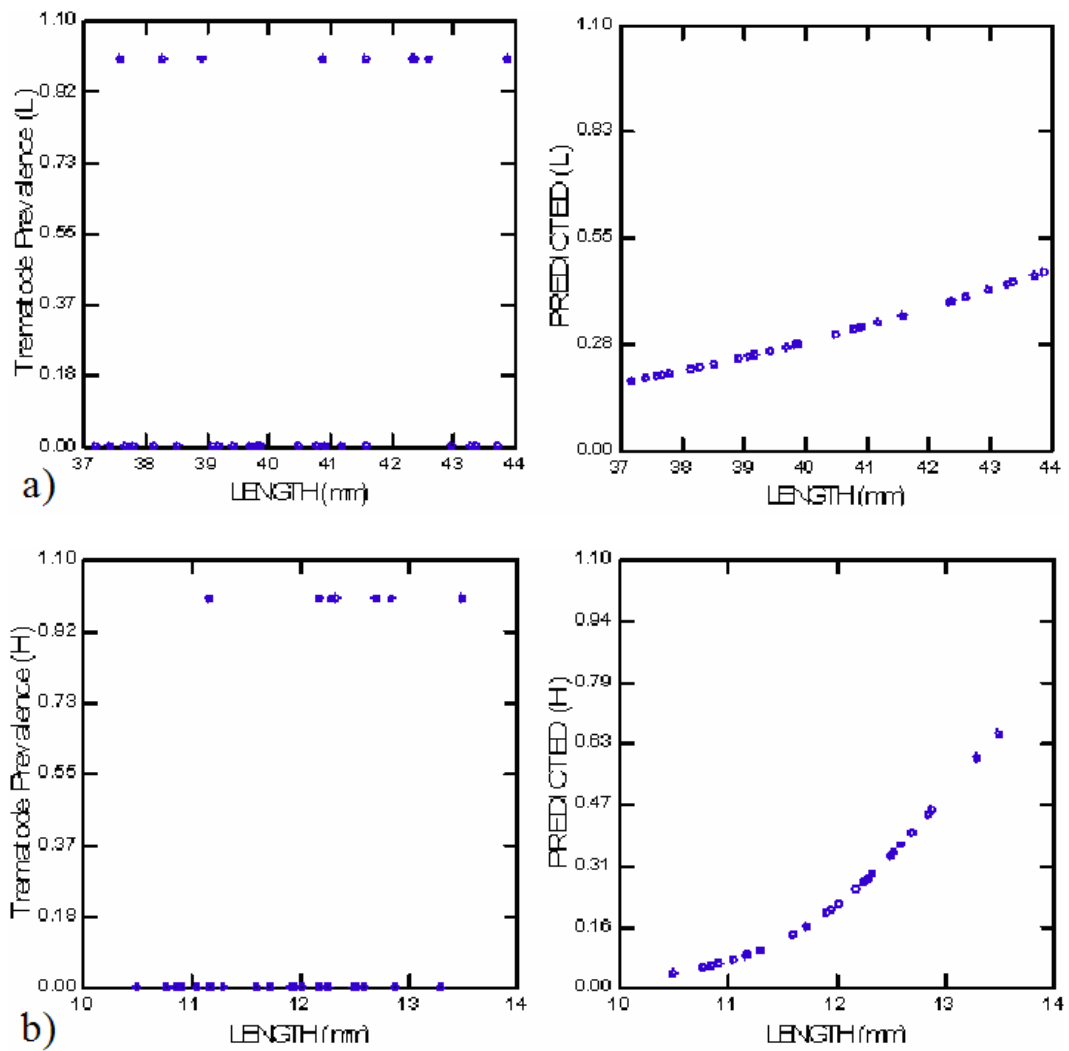


Fig.2. Scatter plot of trematode infection across axial length before and after an estimate model of a logarithmic regression was applied in a) Lymnaeidae and b) Planorbidae. Both log regressions indicate a positive correlation between trematode infection and axial length.