The Reproductivity of Chinese Mystery Snails (*Cipangopaludine chinensis*), an Invasive Snail to North America

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Abstract

The Chinese Mystery snail, *Cipangopaludine* (*Bellamya*) *chinensis*, is invading freshwater ecosystems in North America and Europe. This species of snail, which is native to Asia, was first introduced to the United States of America in the 1980s in the Asian food markets. Due to release of snails from aquaria and boating activity by sticking to boats that move between freshwater systems, *C. chinensis* has spread in freshwater ecosystems across North America at an alarming rate and has recently been found in watersheds across Nova Scotia, namely within the Shubenacaide watershed, the largest in the province. To better understand the spread of this species, researchers have tried to estimate the number of young born per female per year (fecundity). While, previous research has focused solely on fecundity, our research shows that fecundity may not be the best indicator of reproductivity and that reproductive rates vary depending on competition levels due to the number of snails present. Better understanding of the snail's lifecycles will provide us with more tools to predict and mitigate their spread throughout Nova Scotia's aquatic habitats.

Introduction

The Chinese Mystery snail, *Cipangopaludine chinensis*, is an invasive (non-native) mollusc in North America (Stephen *et al.*, 2013). *C. chinensis* is from the mystery snail family, Viviparidae. This species is native to Burma, Thailand, South Vietnam, China, Korea, Japan, the Philippines, and the Island of Java (Indiana Department of Natural Resources, 2005). *C. chinensis* eat zooplankton and phytoplankton (Indiana Department of Natural Resources, 2005). They can feed by grazing or filter-feeding through the use of its radula (for grazing) or by breathing water (filter feeding) (Olden *et al.*, 2013). Females give birth to live young in contrast to native species that reproduce by laying eggs in jelly sacks attached to solid surfaces, the egg sack is called an egg capsule (British Columbia Ministry of Environment, Lands and Parks, 2000). *C. chinensis* have a shell feature called a trap-door which can close to provide extra protection when conditions are unfavourable (Indiana Department of Natural Resources, 2005). This species is very hardy, adult snails have been shown to be able to survive for at least four weeks of air exposure (out of water) and smaller juvenile snails lasted for 3 to 14 days during air exposure (Havel, 2011). Therefore, the characteristic of this invasive species makes *C. chinensis* very resilient and difficult to eradicate.

Invasive species are difficult to manage in terms of geographic spread and population control (Havel, 2011). *C. chinensis* has spread to the United States of America, Canada, and Europe (Olden *et al.*, 2013). Current reports in Canada identify *C. chinensis* to have established populations in southern Ontario, British Columbia, Quebec, New Brunswick, Newfoundland, and Nova Scotia (McAlpine *et al.*, 2016). The main transportation vector for the spread of *C.*

chinensis is human activities including boating, aquarium releases, and garden ponds (Havel, 2011; Indiana Department of Natural Resources, 2005; McAlpine *et al.*, 2016; Harried *et al.*, 2015; Solomon *et al.*, 2010; Haak, 2015). Despite the range and abundance of this species being relatively high, little is known about *C. chinensis.*

Haak (2015) reported that only 10-20% of introduced species become invasive in their new ecosystem. Invasive species conclusively cause ecological and economic harm. Haak (2015) mentions five stages necessary for a species to successfully becoming invasive which are arrival, establishment, growth and reproduction, displacement of native species, and community domination. Since *C. chinensis* has already arrived in North America and established populations, it is important to study and better understand the stage of growth and reproduction to manage the spread of this species. Accordingly, attempts have been made to study the effects of *C. chinensis* on native communities. One study, by Solomon *et al.* (2010), found that the introduction of *C. chinensis* in Wisconsin lakes did not affect the assemblage of native snail species. However, this study did not consider the abundance of native species. Native species may have been on the decline even though the lake had the same variety of snail species as was historically reported. Another study, by Johnson *et al.* (2009), found that native snail species were in decline and some became almost extinct when *C. chinensis* and non native crayfish were introduced into the same test vessel. Olden *et al.* (2013), focused on the microbial community and found that an increase in *C. chinensis* caused a significant shift in bacterial community composition. To summarize, literature regarding *C. chinensis* is reporting contrasting results, with some works identifying a hazard to native species (Olden *et al.*, 2013, Johnson *et al.*, 2009) while others have not (Solomon *et al.*, 2010).

Due to the importance of monitoring and predicting the growth and reproduction stage of the invasive species, research has been completed to calculate the number of births per adult female snail per year (Stephen *et al.*, 2013). Stephen *et al.* (2013) estimate that an adult female produced 27.2-33.3 young per year which attributed to 2.2-3.7 million annual production of young for one reservoir in Nebraska. Although Stephen *et al.* (2013) conducted research that provided valuable information about the estimated number of births per year per adult female snail, they were not able to accurately comment on the reproductive rate.

The research presented in this paper aims to identify the reproductive rate of *C. chinensis* and to determine the impact that competition between snails in the same aquatic ecosystem has on the rate of reproduction while comparing results with fecundity to determine if fecundity can be used as an effective predictor of reproductive rate or population size.

Methods

Adult C. chinensis Culture

A total of 70 snails were collected from Loon Lake, Dartmouth, NS (44º41’52’’N 63º29’41’’W) and placed in a cooler with approximately 5 L of Loon Lake water. The snails were transported in the cooler back to the laboratory at Saint Mary’s University. They were left over night in the cooler. The following day, the snails were individually removed from the cooler, scrubbed clean with a soft bristled tooth brush and transferred to the culture aquarium. The aquarium, labelled as *C. chinensis* adult aquarium, was a 29 gallon aquarium fitted with a filter, lid, and UV hood lights. The light was placed on a timer set for 12 hr light: 12 hr dark. A thermometer was placed in the aquarium to monitor the temperature (20 ± 2°C). The aquarium floor was coated with Loon Lake sediment and, initially, the aquarium was filled with Loon Lake water (pH 7.3, Conductivity 403 $\mu S \cdot cm^{-1}$, dissolved oxygen was 80%, total chlorine (Clt) concentration was 0.0 ppm). Also, potted aquatic plants purchased from the PetSmart in Dartmouth, NS were placed in the aquarium. Three times a week, ¼ of the aquarium's water was changed with dechlorinated water (DeCl). Therefore, over time, the aquarium changed from Loon Lake (LL) water to DeCl water which allowed the snails to slowly adjust to their new environment. Additionally, whenever the water was changed (i.e. three times per week), the snails were fed 7 algae discs (Wardley Algae Discs RM 112811) and if there were offspring produced, they were counted and removed to a separate aquarium for juvenile snails.

Juvenile C. chinensis Culture

Juvenile snails were counted and collected from the adult aquarium and transferred to a smaller aquarium (5.5 gallons). The juvenile aquarium was fitted with a lid, a filter, UV light (set on a timer to 12 hr light: 12 hr dark), and filled with LL water (no sediment). There were a few (approximately 3) pieces of aquatic plants placed in the aquarium for the juvenile snails to climb on. Three times a week, ¼ of the water was changed with LL water. LL water was used for the water changes because it contained more calcium (Ca) (LL water was 23 ppm versus DeCl water was 0 ppm). Pervious research has shown that although adult snails are not adversely affected by a lack of Ca, juvenile snails require Ca for shell development (Haak, 2015). Once a week, the juvenile snails received one algae disc (same type of food as prepared for the adult snails).

Week 7

After 7 weeks of *C. chinensis* remaining and adjusting to the lab culture, 35 of the initial 66 adult snails were randomly removed from the aquarium, placed in individual plastic bags, and frozen. The removal of over half the culture population effectively reduced the competition by half. The remaining 31 snails, still in the adult aquarium, were kept on the same feeding and water change schedule (three times a week) and received the same amount of food. This simulated a high competition environment and then a low competition environment.

Dissecting Adult Snails and Fecundity

The 35 snails removed from the adult aquarium at week 7 were dissected to determine sex and, in the case of females, fecundity. Fecundity was determined by the presence of embryos, whether the embryos were in yolk or complete shell stage, and number of embryos per female. Sex was determined by examination of testis, tentacles, and the presence or absence of developing embryos.

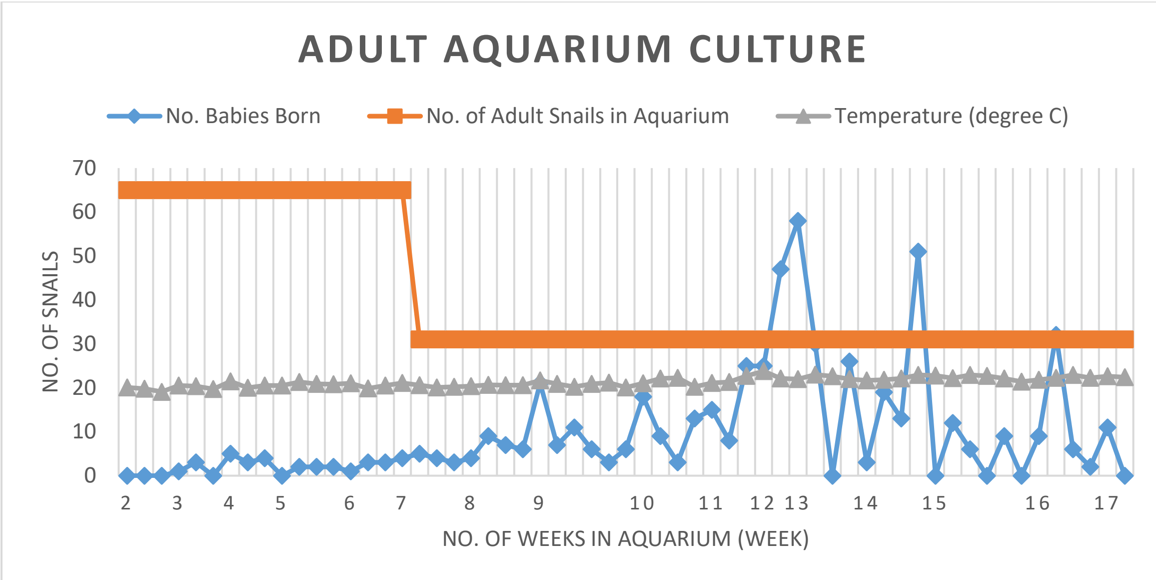

Figure 1: The reproductive rates of an aquarium containing 66 adult Chinese Mystery snails (*C. chinensis*). The initial culture contained 33 females and 33 males.

Results:

When more than half the population was removed from the aquarium at week 7, effectively decreasing the competition, the reproductive rate increased. Weeks 12 and 13 saw a 6-fold increase in reproduction compared to weeks 0 to 7. The ratio of male snails to female snails determined by dissection was approximately 1:1 (18 males: 17 females from a total of 35 dissected). The 35 snails used for the dissection acted as representative of the overall aquarium population (the initial 66 snails). Using the male: female ratio of 1:1 to estimate the number of females of the 31 snail remaining in the aquarium, it was estimated that 15.05 females remained in the snail culture. The number of females and the number of babies born were used to compare the reproductive rate during a high competition scenario, 66 snails in the aquarium, and low competition scenario, 31 snails in the aquarium. The reproductive rates were calculated using the following equation:

$$\left(\frac{No.of\ Babies\ Born}{No.of\ Adult\ Femaeles}\right)\left(\frac{No.of\ Weeks\ in\ Year}{No.of\ weeks\ in\ Culture}\right) = reproduction\ rate\ (high/low\ comp.)$$

From week 0 to 7 there were 33 baby snails born and from week 7 to 17 there were 542 baby snails born. The calculated reproductive rates were 7.42 babies/female/year and 181.83 babies/female/year, respectively. Therefore, the number of offspring produce by each female varied widely depending on availability of resources and space (competition). The reproduction rates calculated here are different than those previously published (Stephen *et al.*, 2013) that estimated reproductivity to be 27.2-33.3 young per female per year, but, as shown by the presented research, the number of young born each year heavily depends on the competition level of the ecosystem.

Furthermore, Stephen *et al.* (2013) found a strong correlation between shell size and fecundity, or the number of embryos found. To create a comparable result the 17 females from the 35 used for dissection were examined for fecundity by determining the number of embryos each female carried and whether the embryos were in yolk or complete shell phase of development. Figure 2 compares the shell size of the adult female *C. chinensis* with the number of embryos present. There was no significant correlation between number of embryos developing and female shell size (r^2=0.0966, p=1.15385 x 10^{-7}). These results contrast the results of Stephen *et al.* (2013) that had found a clear and strong correlation between shell size of adult female *C. chinensis* and 'brooded' embryos.

The number of brooded embryos found from the 17 females dissected varied from 5 to 35 embryos. Most of the embryos were in the fully developed shell phase. However, one snail that contained only 5 embryos had yolk stage and no shell stage embryos. There was a total of 404 embryos found, which resulted in an average of 23.76 embryos per female per year. This estimated value (23.76 young per female per year) is lower than published values by Stephen *et al.* (2013) that estimated average reproduction to be 27.2 to 33.3 young per female per year.

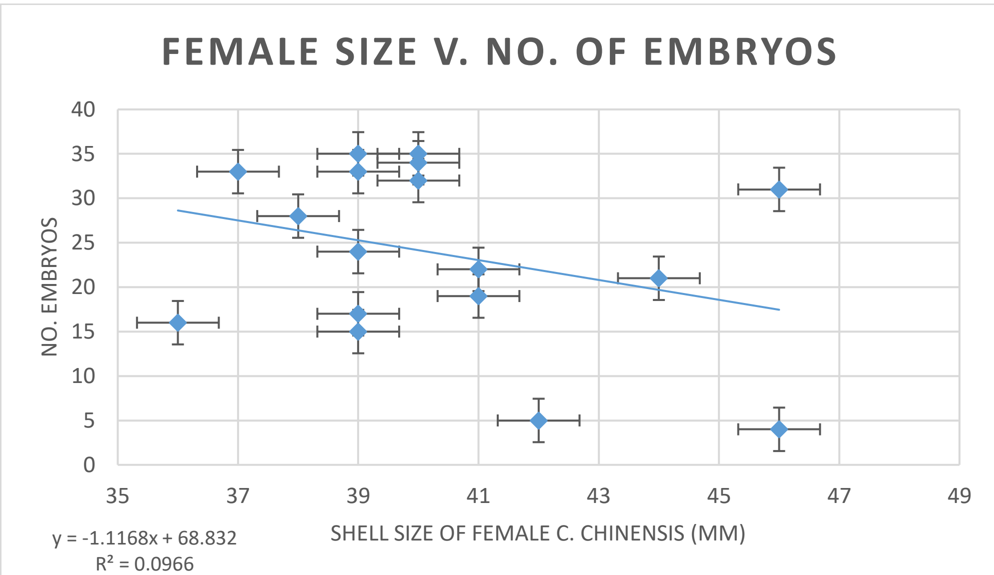

Figure 2: The shell size of each adult female *C. chinensis* was measured from the tip of the spire to the lip of the aperture. Error bars are 1 standard deviation. (p-value calculated using the Student's T-test, p= 1.15385 x 10^{-7})

Discussion:

Reproductive Rate

An established *C. chinensis* population is growing and spreading in Atlantic Canada. It is therefore important to study and understand this species (McApline *et al.*, 2016). Reproductive rate is an indicator of potential population size and spread of an invasive species. The findings presented in this paper conclude that many potential factors can affect reproduction. These potential factors include competition and temperature (Olden *et al.*, 2013, Haak, 2015), but there are other factors involved such as the presence of other invasive species (Johnson *et al.*, 2009). This research shows a connection between competition and reproductive rate. This is significant because as *C. chinensis* moves to invade new freshwater systems the competition level will be relatively low and so, a population boom could occur. A sudden increase in competition would negatively affect native species, particularly cases where another invasive species is already present, because *C. chinensis* can out compete native snails for available resources (Johnson *et al.*, 2009). Additionally, *C. chinensis* can change the water chemistry by excreting large amounts of nitrogen and by encourage microbial growth (Kingsbury *et al.*, unpublished, Olden *et al.*, 2013). Previous research (Stephen *et al.*, 2013) used shell size as a predictor of fecundity, but as these results have shown shell size may not be an adequate predictor of fecundity and fecundity did not effectively determine reproduction rate. The reproductive rate for a low competition scenario was higher than for a high competition scenario, but lower than the estimated reproductive rate calculated from fecundity. Therefore, fecundity did not effectively predict reproductive rate which may be due to females being constantly fertilized or perhaps a short gestation period.

Fecundity and Shell Size

All the females dissected were of breeding and birthing age, estimated to be at least one year old (Stephen *et al.*, 2013), with shell between 36-46 mm. As illustrated by Figure 2, there was no significant correlation between shell size and number of embryos found during the dissection. This result was surprising as Stephen *et al.* (2013) showed a very clear and strong correlation between the two factors: shell size and brooded embryos. Because of the similarities between this study and Stephen *et al.* (2013); the same male to female sex ratio of 1:1 and females of a similar size, 36-46mm which was encompassed by the range studies by Stephen *et al.* (2013) 28.5-57.1 mm, it was expected that the results would have been similar. Although the range of the shell sizes of female *C. chinensis* studied by Stephen *et al.* (2013) was a wide range than studied of this project, most of the shell sizes studied by Stephen *et al.* (2013) were in the 35-45mm range. More females in the 45-60 mm range need to be collected to conclusively determine if there is a correlation between shell size and number of brooded embryos. Furthermore, Stephen *et al.* (2013) based their estimation on snails collected from a single survey. To more accurately assess fecundity of *C. chinensis*, reproductivity over a prolonged period of time should be observed or multiple surveys completed over the span of a few years). The contrasting results presented in this paper with that of Stephen *et al.* (2013) are significant because it is possible that freshwater stakeholders and researchers are relying on data that may be heavily varied based on the other ecological factors. This research has shown that one contributing factor that affects population size is same species competition, but there may be other ecological factors to consider, such as temperature or other species present in the water body.

Reproduction and Temperature

By observing a culture of *C. chinensis* under controlled settings, we were able to determine how temperature and competition affect reproductivity. Previous research by Haak (2015) states that below 18°C there was no observed reproduction. So, in order to continue reproduction over the colder months, the temperature in the aquarium was monitored and regulated to be 21±3°C. This note on temperature from Haak (2015) is important because it may explain the difference in results between this study and Stephen *et al.* (2013) who collected their snails in September. Unfortunately, Stephen *et al.* (2013) did not make note of the water temperature of Wild Plum Lake during their survey. The temperature, which in more northern climates is cooler later in the year, has been shown by Haak (2015) to have significant changes on reproductivity and fecundity. By controlling the temperature in the culture, it is likely that the results are reproducible and that temperature did not adversely affect results.

Potential Risks of C. chinensis on Native Species

There are currently other invasive species in North America, such as zebra muscles and rusty crayfish, that have had great ecological impacts. *C. chinensis* is at risk of becoming another invasive species that will out-compete, out-reproduce and change inhabited environments that would negatively affect native species. The current literature on how and if *C. chinensis* impact native species is inconsistent. Some researchers have found no significant ecological changes caused by *C. chinensis* (Harried *et al.*, 2015; and Solomon *et al.*, 2010). The two big questions to be answered about an invasive species' effects on native communities are 1) how the invasive species is affecting the native population in terms of resource competition (e.g. food and space) and 2) how is the invasive species causing other adverse side effects to the native species such as spreading diseases, changing water chemistry, pushing out native species. Solomon *et al.* (2010) attempted to answer the question of if *C. chinensis* is out competing native snail species in lakes in North Wisconsin. They surveyed 44 lakes to assess the assemblage of native snail species. Solomon *et al.* (2010) compared their results with data from a 1930s survey and concluded that there was the same assemblage of native snail species as was previously reported despite the occurrence of *C. chinensis*. However, that study failed to compare the quantity of each native species. It is possible that although there were the same types of native snail species found, their abundance was in decline.

A second study from Wisconsin looked at the infection patterns of *C. chinensis* and compared *C. chinensis* with two native species, *Physella gyrina* and *Bithynia tentaculate* (Harried *et al.*, 2015). Harried *et al.* (2015) found that *C. chinensis* was able to encapsulate the parasites in their shell that they had been exposed to (*Cyathocotyle bushiensis* and *Sphaeridioterma pseudoglobulus*) and only two of the 147 *C. chinensis* collected from 22 Wisconsin lakes were infected with *Aspidogaster conchicola* or *C. bushiensis*. These parasites commonly infect bivalves and water fowl. Therefore, based on these results, it is unlikely that the negative impacts on native communities by *C. chinensis* are due to disease and/or parasite spreading. Most likely, any decrease of native species, especially native bivalves, caused by *C. chinensis* is due to outcompeting the native species. *C. chinensis* can push out native species by expanding their population due to their high reproductivity. For example, research by Johnson *et al.* (2009) found that in experiments of outdoor mesocoms, native snail species (*Lymnaea stagnalis* and *Physa gyrina*) had reduced mass after exposure to mesocoms containing *C. chinensis*. Furthermore, Johnson *et al.* (2009) found that when *C. chinensis* is combined with another invasive species like the crayfish, native species were almost extinct. Lastly, Johnson *et al.* (2009) suggests that *C. chinensis* was able to change the native algal communities. Olden *et al.* (2013) found that at high population densities *C. chinensis* can change the microbial community to diversify feeding techniques. *C. chinensis* which is considered to be a grazer (Indiana Department of Natural Resources, 2005) can switch it's feeding mechanism to filter feeding. Olden *et al.* (2013) found filter feeding techniques used by snails with shell sizes $\geq$ 44 mm, showing there may be a connection between shell size and feeding technique. However, the strongest indicator of shifts in the microbial community was based on population size. Therefore, determining the population size in a specific water body is important for predicting impacts on native communities. As this research has shown, there is a need for additional research to developing better methods to predict *C. chinensis* population size and growth. Another side effect of large *C. chinensis* populations is a change in water chemistry caused by fecal and pseudo-fecal waste produced by *C. chinensis* in large quantities. Because fecal waste from *C. chinensis* is high in nitrogen, the results from Olden *et al.* (2013) suggest that *C. chinensis* having a large impact on the microbial community may affect the benthic community and pelagic food webs in freshwater ecosystems. Certainly, further research is needed to assess the nitrogen excretion of *C. chinensis* and the impact that *C. chinensis* may have on sensitive native species.

One main issue brought forward by this research is that the reproductive rate of *C. chinensis* is unclear, contested, and potentially far higher than previously predicted. Here we show that there is a variation in reproductive rate depending available resources. The common practice is to use fecundity to estimate reproductivity (Stephen *et al.*, 2013; Haak, 2015), but we have shown fecundity may be impacted based on the time of year the snails are collect surveyed, the temperature of the lake, and the competition level. Therefore, previous fecundity studies may not accurately reflect (and potentially underestimate) the reproduction rates of these snails. Further research into management practices for stopping or slowing the spread of *C. chinensis* may be needed. For species conservation, research should focus on the effects *C. chinensis* may be having on native ecosystems, especially in areas of sensitive species.

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