Cipangopaludina chinensis: Effects of temperatures and parasite prevalence

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Abstract

Comparison of parasite prevalence between native snail species, *Lymnaea stagnalis*, and invasive snail species, *Cipangopaludina chinensis*, was conducted by euthanizing snails one day after capture from Brown Lake. Looking at if parasitism is a main factor in intraspecific competition of *C. chinensis* at different temperatures, snails were captured, measured for height and width, housed under different temperature settings (20˚C, 25˚C, and 34˚C), and competed against other for a week-long trial. After the week-long trial or when the last snail died, snails were re-measured for growth and checked for parasites. Result from parasite prevalence between two snails species was significant, *C. chinensis* rarely had infections compared to *L. stagnalis*. From the result, native parasites were predicted to be highly specialized for *L. stagnalis* and lack mechanisms to penetrate *C. chinensis*. Result from the intraspecific competition suggested that parasite prevalence was not important as survival (in weeks) in the change in growth. Since parasite prevalence was low, and sample size was small, conclusion on parasite prevalence not being a factor in growth was not robust. Further studies on impact of *C. chinensis* on freshwater systems and native snail species are needed.

Introduction

Climate change is a concerning factor for freshwater ecosystems. Climate change increases the average temperature of water in some aquatic systems. With warmer water temperatures, more exotic species can establish as native species disappear (Rahel and Olden 2008). As native species disappear from the ecosystem, other species dependent on a specific native species may not be able to sustain the loss, leading to a decrease in biodiversity. Biodiversity is important to aquatic systems due to specialized functions of organisms provide to their environment (Covich et al. 2004). For example, better water quality is obtained by breaking down of organic matter from aquatic insects, in return, are food for other aquatic invertebrates and fish. If one species is gone, the system may be affected with negative effects if the species is heavily dependent as food source or ecosystem processes, or little effects.

With climate change, there is evidence that non-native species may shift their ranges and move farther north as more places become more habitable with climate change (Perry et al. 2005). Non-native is defined as an organism not native to a specific via introduction by humans. A non-native species is considered invasive when it causes negative effects to an ecosystem it is not originally from (Colautti and MacIsaac 2004). This may bring exotic species that may eventually become invasive since these species have broader tolerance levels for higher temperature and stress than native species (Zerebecki and Sorte 2011). In addition, increased temperature can hinder the growth rate of native species and be beneficial to the exotic species, making it difficult for natives to compete (Früh et al. 2016). With more exotic species, biodiversity composition of the systems, including vegetation and number of prey/predator species can change (Strayer et al. 1999). This may lead to disruption of trophic cascades in native systems (Wahl et al. 2011).

Invasive species is one of many concerns for freshwater ecosystems. Exotic species such as sea lamprey and zebra mussels are accidentally introduced as stowaways. Other species are introduced for human consumption and recreational purposes, i.e. fishing and hunting (Mills et al. 1994). *Cipangopaludina chinensis* (Chinese mystery snail) was introduced for consumption in the United States of America in the early 1890s from Asia (Wood as cited in Solomon et al. 2009). After its introduction, the species spread to most of the United States via boats and pet trades which includes the Great Lakes region (Solomon et al. 2009). The species occur in high densities in the Great Lakes region Studies by Johnson et al. (2007) and Solomon et al. (2009)

had evidence that *C. chinensis* have some negative effects on native snails species like reduced survival. However, there are not many studies on how the species impact the Great Lake regions' ecosystems.

As temperatures increase, host-parasite interaction can change with the introduction of a new related species. Hosts' development time can increase with temperature, allowing for parasites to increase their rates of infection (Paull et al. 2012). In return, the free-living stage (cercariae) of the parasites are less likely to survive while trying to find the hosts, and hosts developed deformities to avoid infection. In this situation, climate change may have a big impact on community structure especially if the parasite has many stages and hosts. *C. chinensis* most likely has lost many of their native trematode parasites according to the evolution of increased competitive ability hypothesis (EICA), in which species leave behind predators, parasites, and diseases as it becomes established in non-native environments (Blossey and Notzold 1995). However, as *C. chinensis* becomes established in new environments, they are more likely to get infected with many of the native trematode species in the environments due to lack of resistance compared to the native snails (Karatayev et al. 2012). If more invasive hosts are infected with parasites, then disease prevalence will also increase which can spread to native hosts.

The focus of this project is to better understand how climate change affects competition and parasitism within invasive populations. By looking intraspecific competition at different temperatures, one can investigate if parasitism is a factor on survival since rates of infection should increase when hosts' development time increase with temperature. (Paull et al. 2012). The prediction is that *C. chinensis* in a higher temperature setting should have high parasite prevalence and increased rate in development. Parasite prevalence will be observed in both the invasive, *C. chinensis*, and the native species, *Lymnaea stagnalis*. The prediction is that *C.*

chinensis will have a higher parasite prevalence due no previous exposure to the local parasites. Intraspecific competition at different temperatures will be also observed focusing on the species, *C. chinensis*.

Materials and Methods

Sampling location and aquarium setup

Sampling took place at the University of Notre Dame Environmental Research Center (UNDERC), Land O'Lakes, WI. Both native snail species, *L. stagnalis,* and invasive snail species, *C. chinensis,* were obtained at Brown Lake, a eutrophic lake located on UNDERC property. The area is dominated by algae and aquatic plants that supports these snail species along with other various aquatic organisms. The snails were captured using wire mesh strainers near the shore of the lake and housed at the aquarium room on UNDERC property.

Twelve 10L containers were utilized to house the snails. Each container had 3.5-4.0 L of lake water and 3.5 cm layer of rocky substrate. Aquatic grasses were harvested from Brown Lake and added to the tanks as food material. Fluorescent lights were setup and on a timer to mimic natural sunrise and sunset, 6AM – 9PM. Aquarium heaters placed in each tank were set at three different temperatures (20˚C, 25˚C, and 34˚C) with four replicates of each temperature treatment. The pH of each tank was monitored throughout the experiment to ensure stable conditions (pH 7.0-7.9). 3.5-4.0 L of water from each tank were replaced once every week to provide a clean environment.

Parasite prevalence difference between native and invasive species

Parasite prevalence was determined in 60 captured snails of both species by placing each snail in individual compartments at 25˚C overnight. The following day, 20 snails of each species, selected by a random number generator application from Aeiou (True Random Generator) were euthanized by freezing in a freezer at -20°C for 5 hours and left under a fluorescent light table for 12 hours to see if cercariae stage (free swimming stage) of trematrode come out of the snails ‘bodies (Minchella et al. 1985). Afterwards, if no cercariae emerged from the snails after 12 hours, the shells of the snails were cracked open with a hammer to observe if cercariea were embedded within the bodies with a dissecting scope. Snails unable to be inspected the same day were frozen at were frozen at -20°C in the freezer to analyze on a later date. Counts of parasites were not conducted in this part of the project, instead the snails were categorized into if they contained parasites or not.

Intraspecific competition between temperature treatments

Competition was examined using exotic snail species *C. chinensis*. The height and width of each captured snail were recorded. Height was defined as the apex to the bottom of the lip; width was defined from the start of the widest whorl to the edge of the lip (*Fig. 1*). After taking measurements, snails were allocated into tanks at different temperatures: 20°C, 25°C, and 34°C, with a total of 15 snails per temperature setting. Snails were fed with fish flakes for one week. After one week, the food resource was restricted for another week to allow the snails to initiate competition. Competition trial lasted for one week or until the last snail died. Upon the completion of the trial, snails were checked for parasites, measured for their height and width, and placed under a fluorescent light table for 12 hours to allow parasites to emerge. If no cercariae emerged, then the shells were cracked carefully with a hammer to check for embedded parasites and observed with dissecting scope, or they were frozen at -20°C to analyze on a later date. Only 10, randomly selected by a random number generator application (True Random Generator) from each temperature setting, were observed for parasites.

Data analysis

Statistical analysis was completed using SYSTAT (version 13.0, 2016). Differences in parasite prevalence between native and invasive snail species were determined using the Pearson's chi-square test (*Table 1*). A non-parametric one way ANOVA equivalent, Kruskal-Wallis test (*Table 2*), was used test the change in height of the snail based on temperature because the original data was not normal, and a post-hoc test, Conover-Inman test (*Table 3),* was performed. A Kruskal-Wallis test (*Table 4*) was performed on the change in width of the snail based on temperature. Growth, height and width, depending on parasite prevalence was analyzed separately with Mann-Whitney U test since the data was not normally distributed (*Table 5* and *Table 6*). A Pearson's chi-square test was used to analyze if there was a relationship between temperature and parasite prevalence (*Table 7*). A Pearson's Chi-square test was ran on temperature in relation to survival (*Table 8*). Parasite prevalence in relation to survival was tested with Pearson's Chi-square test (*Table 9*).

Results

Parasite prevalence difference between native and invasive species

This result suggested there is a difference in parasite prevalence between the native snail species, *L. stagnalis*, and the invasive snail species, *C. chinensis* (χ^2 = 32.400, df = 1, p = <0.001) (*Table 1*). In the observations, 96.67% of the native snails had a parasite infection mainly at the sporocyst stage (*Fig. 2*), while only 3.33% of the invasive were infected.

Intraspecific competition between temperature treatments

There was a not significant difference in height between temperature treatments of 20˚C and 25˚C (p = 0.441) (*Table 3*); however, differences in height were significant between treatments 20˚C and 34˚C (p = 0.007)) (*Table 3*) and between treatments 25˚C and 34˚C (p = 0.004) (*Table 3*). No significant relationship was found between change in width and temperature (H = 4.002, df = 2, p = 0.135) (*Table 4*). The results illustrated that parasite prevalence did not affect changes in height (U = 19.500, df = 1, p = 0.489) (*Table 5*). Parasite prevalence did not affect the change in width (U = 16.500, df = 1, p = 0.695) (*Table 6*). In the observations, few snails were infected (3.33%). The snails that showed the most change in growth were those with no signs of parasitism and greater survival. Temperature was not a factor in parasite prevalence (χ^2 = 2.069, df = 2, p = 0.355) (*Table 7*).

When comparing treatments to survival, temperature had no significant effect (χ^2 = 33.333, df = 4, p = <0.001) (*Table 8*). From observations, snails exposed to 34°C environments survived fewer days than those within other treatments. Parasite prevalence did not affect survival as seen by equal lifespans of both infected and uninfected snails (χ^2 = 0.689, df = 2, p = 0.708) (*Table 9*).

Discussion

This study shows that the invasive snail species, *C. chinensis*, has a lower parasite prevalence compared to the native, *L. stagnalis*, refuting the original hypothesis. This suggests that the parasite may be host specific, meaning the native species and its relationship with the parasite have been at an evolutionary arms race. Supporting the EICA hypothesis, many of *C. chinensis* did not have parasites indicating that they have lost their native parasites. However, since *C. chinensis* had established a population in a new environment, it is expected for them to be alternative hosts for parasites and increase transmission to definitive hosts. Native parasites may not have or have significant mechanisms to invade C. chinensis. The anatomical structures of the two species are different: *L. stagnalis* have their foot exposed while *C. chinensis* have a trapdoor mechanism that allows retraction of the foot into their shells. A study of exotic mollusks in the Great Lakes concluded no trematode prevalence in *C. chinensis* in their sample size of 30

(Karatayev et al. 2012). A previous study of UNDERC property also identified higher parasite prevalence in *L. stagnalis* and other native freshwater snail species compared to invasive (Hirsch 2007). However, the sample sizes of previous literature as well as this study were not large enough to reach a concrete conclusion of parasite in the snail populations.

The greater survival of snails at 20˚C suggests this is the ideal living condition; sudden increases in temperature could be detrimental to populations as seen by greater mortality in 25˚C and 34˚C. At higher temperatures, snails are thought to have weakened immune defense and be more susceptible to infections (Seppälä and Jokela 2010). In a study with tropical snails in Asia, Teo (2004) reported tropical freshwater snail mortality is high at 32˚C and higher. This implies in introduced areas, *C. chinensis* may be able to persist if bodies of water in the Great Lakes Region are warming up due to climate change. If the waters at UNDERC were to warm up, native snail species and their parasites may not be able to survive as they are adapted to temperate conditions. Further research on native temperate climate snails and their parasites at higher temperatures are needed.

Despite sampling in a pristine environment, there were several limitations to this study. One was time to analyze a larger sample size than 30 snails for parasite prevalence. Cited in Curtis (2002), most of studies snails and trematode infections had analyzed at least 1000 snails in a location with sub-sites. In addition, some of the studies took years, and time at UNDERC was only 10 weeks. With a low sample size, a concrete conclusion cannot be made if temperature was a factor for parasite prevalence, and if parasite prevalence had influence on survival. Another caveat was keeping *L. stagnalis* alive in a controlled setting. Originally, the competition portion of the study was between the two snail species at various temperatures. However, *L. stagnalis* died in one day after placement in the controlled tanks, thus the project focused on the intraspecific competition of *C. chinensis*. There was a suspicion of the pipes that pump Tenderfoot Lake water into the aquarium room were contaminated with copper.

More studies focusing on impacts of *C. chinensis* on freshwater systems are needed. Besides being a potential alternative host for trematodes and increasing the risk of transmission to definitive hosts, other impacts on the freshwater ecosystem are not known. In a study on invasive crayfish species, the native crayfish species used *C. chinensis* as an alternative food source to compete with the invasive species which were consuming native snails (Olden et al. 2009). This was a positive outcome to the native crayfish population to be able to compete with invasive crayfish. However, impacts of *C. chinensis* on the native snail species are relatively unknown. Overall, studies focusing on competition between *C. chinensis* and native species as average temperature increases due to climate change are required.

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Tables

Table 1: Summary of Pearson's Chi-square test on parasite prevalence between native snail species and invasive snail species ($\chi^2 = 32.400$, df = 1, p = <0.001)

	Invasive	**Native**	**Total**
Not Present	19	1	20
Present	1	19	20
Total	20	20	40

Table 2: Summary of Kruskal-Wallis test of change in height dependent on temperature. (H = 7.427, df = 2, p = 0.024)

Group (Temperature ˚C)	**Count**	**Rank Sum**
20	10	191.50000000
25	10	168.50000000
34	10	105.00000000

Table 3: Summary of Conover-Inman test of change in height dependent on temperature.

Group A (Temperature ˚C)	**Group B (Temperature ˚C)**	**Statistic**	**p-Value**
20	25	0.78259331	0.44067618
20	34	2.94323138	0.00660053
25	34	2.16063806	0.03976087

Table 4: Summary of Kruskal-Wallis test of change in width dependent on temperature. (H = 4.002, df = 2, p = 0.135)

Group (Temperature ˚C)	**Count**	**Rank Sum**
20	10	180.50000000
25	10	149.50000000
34	10	135.00000000

Table 5: Summary of Mann-Whitney U test of change in height dependent on parasite prevalence. (U = 19.500, df = 1, p= 0.489)

Group Parasite Present	**Count**	**Rank Sum**
No	29	454.50000000
Yes	1	10.50000000

Table 6: Summary of Mann-Whitney U test of change in width dependent on parasite prevalence. (U = 16.500, df = 1, p = 0.695)

Group Parasite Present	**Count**	**Rank Sum**
No	29	451.50000000
Yes	1	13.50000000

Table 7: Summary of Pearson's Chi-square test on parasite prevalence at different temperature setting. (χ^2 = 2.069, df = 2, p = 0.355)

	Not Present	**Present**	**Total**
20	10	0	10
25	9	1	10
34	10	0	10
Total	29	1	30

Table 8: Summary of Pearson's Chi-square test on the relationship of temperature on survival in weeks. (χ^2 = 33.333, df = 4, p = <0.001)

	0.5 weeks	**1 week**	**2 weeks**	**Total**
20	0	0	10	10
25	0	2	8	10
34	10	0	0	10
Total	10	2	18	30

Table 9: Summary of Pearson's Chi-square test on the relationship of parasite prevalence on survival in weeks. (χ^2 = 0.689, df = 2, p = 0.708)

	0.5 weeks	**1 week**	**2 weeks**	**Total**
Not Present	10	2	17	29
Present	0	0	1	1
Total	10	2	18	30

Figures

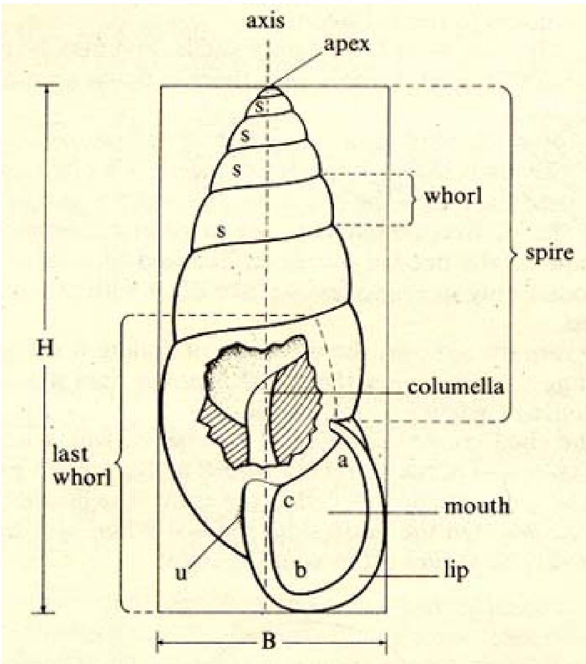

Figure 1: H is the height of shell, B is the width of the shell. (Kerney and Cameron 1976)

Figure 2: Sporocyst stage in *Lymnaea stagnalis*, circled in blue.