Bellamya chinensis and *Lymnaea stagnalis* mortality in the presence of *Macrobdella decora*

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Abstract

Food webs are complex and each trophic level guides the dynamic stability of the whole food web. As humans cause the loss of piscivores and zooplanktivores from lake food webs, parasitic leeches, such as *Macrobdella decora*, lose potential host organisms. In the absence of a host, it has been suggested *M. decora* will parasitize freshwater snails, but there is a lack of research supporting this. At the University of Notre Dame Environmental Research Center – East, the snail species *Bellamya chinensis* and *Lymnaea stagnalis* have similar niches and are prevalent in the lakes, so they were used as research species. Trials were run to observe the mortality of the two snail species in the presence of leeches. The snail species were tested while in the same tank and different tanks. Leeches did parasitize *B. chinensis*. However, *L. stagnalis* did have a significantly higher average mortality than *B. chinensis* in the same tank (treatment: p-value: 0.022; control: p-value: 0.044) and in different tanks (p-value: 0.001), but leech presence did not significantly (p-value: 0.25) change snail mortality. An exact reason for snail species being the factor that controlled snail mortality could not be determined. However, a possible explanation was due to unfiltered fecal material and copper contamination in the trial tanks. Copper is a lethal metal to most gastropods.

Key words: aquatic ecology; food webs; trophic levels; leeches (Macrobdella decora); snails (Bellamya chinensis and *Lymnaea stagnalis)*

Introduction

Food web interactions are complex and are formed by multiple trophic levels regulating the success of one another (Paine 1966; Crowder *et al*.1988; Dunne *et al.* 2002; Boit *et al.* 2012). Therefore, food webs are integral to the success of an ecosystem. Lakes are ecosystems that have been studied extensively and their food webs are still not completely understood (Crowder *et al*.1988; Boit *et al.* 2012). It is understood, however, that the general trophic levels of a lake include piscivores, zooplanktivores, herbivorous zooplankton, and phytoplankton (Carpenter *et al.* 1985). Food webs can be interrupted by human interference through removing important species or whole tropic levels (Pace *et al.* 1999; Rooney *et al.* 2006). When a food web is disturbed, the resulting niche rearrangements are not always clear. Climate change is a key human influence that lake ecosystems are facing. As CO_2 in the atmosphere continues to rise, models, based on a doubling of CO_2 concentration, have predicted that the summer epilimnetic (warmest upper layer of a striated lake) temperature of lakes in Wisconsin will increase by l-7 ºC in the next fifty years (Watson *et al.*1992; De Stasio *et al.* 1996; Wisconsin Initiative 2009). The increased temperatures have been predicted to be lethal to many epilimnetic communities. As these communities disappear, there will be variable trophic interactions that occur (Terborgh and Estes 2013).

Leeches are invertebrates that predate or parasitize a wide range of vertebrates and invertebrates. Leeches have been found on mammals, amphibians, reptiles, and can consume a range of benthic invertebrates including gastropods. Both predator and parasitizing leeches have been shown to maintain the populations of their prey (Schalk *et al.*2002; Sura and Mahon 2012). *Macrobdella decora* are leeches that parasitize animals such as mammals, turtles, and frogs. These leeches generally swim and hunt in the epilimnion of lakes. However, as lakes' epilimnion temperature increases, the leeches' hosts may not survive the new conditions (De Stasio et al. 1996). There has been minimal research done to determine how *M. decora* would react to the absence of a host. It has been suggested that these leeches will shift to parasitizing snails like predatory leeches (Freshwater leech n.d.). The Chinese Mystery Snail (*Bellamya chinensis*) and Great Pond Snail (*Lymnaea stagnalis*) are abundant and have similar niches in many lakes at the University of Notre Dame Environmental Research Center (UNDERC). *B. chinensis* is an invasive species that has an operculum while *L. stagnalis* does not have an operculum (Vermeij and Covich 1978; Brönmark 1992; Nickels 2011). The operculum has the potential to provide protection from *M. decora* (Vermeij and Covich 1978).The shift of *M. decora* to parasitizing either of these species could allow the other to compete for resources more efficiently. This study answered the questions of: Do *M. decora* parasitize *B. chinensis* and *L. stagnalis* in the absence of a host and which snail species has a higher average mortality in the presence of leeches? The hypothesis was that *B. chinensis* will be parasitized less due to their adaptive advantages over *L. stagnalis*, and therefore *L. stagnalis* will have a higher average mortality.

Methods

The experiment was done at the University of Notre Dame Environmental Research Center in the Upper Peninsula of Michigan. *Macrobdella decora* were collected from the edge of Morris Lake (Fig. 1). Minnow traps baited with beef liver and hand trapping were techniques utilized to capture the leeches. Once captured, the leeches were fed beef liver every one to two days. The snails, *Lymnaea stagnalis* and *Bellamya chinensis,* were collected by hand along the edge of Brown Lake (Fig. 1). These two species of snails have similar shell structures and comparable niches (Vermeij and Covich 1978; Brown and Strouse 1988).The specimens were kept in tanks ranging between 19^{o}C and 21^{o}C. The tanks had aerators for a continual oxygen supply, but lacked water filters, so the water was changed at least once a day using water piped from Tenderfoot Lake. The snails were fed Tetramin fish food and lettuce while held in tanks.

Trials were run to observe the mortality of the two snail species in the presence of leeches. Before the trials, the leeches were starved for at least twelve hours to ensure predation would occur (Brown and Strouse 1988). The water temperature of the twelve tanks ranged between 19^{o}C and 21^{o}C. Each tank contained twelve liters of water from Tenderfoot Lake. There were six treatment tanks which held four leeches in each of them (Fig. 2). Two of those tanks contained ten *L. stagnalis* and two other tanks contained ten *B. chinensis*. The remaining two treatment tanks contained five *L. stagnalis* and five *B. chinensis,* respectively. The other six tanks acted as controls and had the same amounts of snails as the treatment tanks, but excluded leeches, so the mortality of the snails could be determined in the absence of predation. The tanks were then checked after twenty-four hours for snail mortality (Brown and Strouse 1988). If a leech left the tank or died, it was replaced and the trial was continued. Snail mortality was determined by checking for the number of dead snails in each tank. Additionally, if a leech parasitized a snail while the observer was present, it was recorded. In the interest of time, leeches and *B. chinensis* that survived previous experiments were reused in subsequent experiments.

The data was not normally distributed after transformation, so a Friedman test was run in place of a two-way ANOVA, with snail species and leech presence as the two factors. This test determined if there was a difference in mortality between snail species while in the presence or absence of leeches. A Wilcoxon Signed Rank test was used to determine whether predation rates differed between the two types of snails in tanks where both were present. The test determined if a snail species had a higher mortality rate when leeches had a choice between preys. These statistics were done using RStudio (RStudio 2012).

Results

A Friedman test was used to analyze how leech presence affected mortality of the two snail species in separate tanks. The species of snail significantly affected mortality (Fig: 3; x^2:10.7877; df: 1; p-value: 0.001), but the presence of leeches did not significantly (x^2: 1.3149; df: 1; p-value: 0.25) affect snail mortality.

A Wilcoxon Signed-Rank test analyzed the prey selection of leeches when presented both snail species at the same time. One Wilcoxon test analyzed snail mortality with leeches present and another one analyzed snail mortality with leeches absent. The average mortality of *L. stagnalis* was significantly higher (Fig: 4; w: 78; p-value: 0.022) than *B. chinensis* with leeches present, but the same experiment was significant (w: 74, p-value: 0.044) in the absence of leeches.

Discussion

Biodiversity of food webs are essential to ecosystem health. As species are depleted, whole trophic layers can be extirpated (Myers *et al.* 2007; Cardinale *et al.* 2012). Humans continue to remove trophic levels through pollution, habitat degradation, and overexploitation. Lakes are especially affected because of their recreational possibilities and abundant natural resources. These exploitations cause declines in the piscivorous and zooplanktivorous species resulting in a top-down shift in the communities (Dobson *et al.* 2006). There are parasites, such as leeches, that rely on these predator species to survive. It has been suggested that, in the absence of a host, leeches such as *M. decora* will shift to parasitizing non-typical prey. Snails were suggested as a possible prey for *M. decora* to parasitize (Freshwater leech n.d.). Snails are a prey source for other species of leeches such as *Nephelopsis obscura* and have similar hematocytes to fish and mammals (Brown and Strouse 1988). In the Upper Peninsula of Michigan, *L. stagnalis* is a native species while *B. chinensis* is invasive, and both are abundant. The objective of the experiment was to determine snail mortality of these two species in the presence of *M. decora.*

The data showed that *L. stagnalis* had a significantly higher average mortality than *B. chinensis*. However, leech presence or absence did not significantly increase or decrease the mortality rate of either species of snails. This suggests that the snails died naturally based upon their adaptations and the environment around them. Similarly, when both snail species were in the presence or absence of the leech at the same time, L. *stagnalis* had a significantly higher mortality rate than *B. chinensis.*

The two experiments had congruent results which suggest that *M. decora* does not parasitize these snail species. It seemed that either L. *stagnalis* naturally died quicker than *B. chinensis,* and/or there was an underlying condition or conditions that were not controlled across the experiments. Also, it was observed that *M. decora* parasitized *B. chinensis* on three separate occasions, but there was no observation of the parasitizing of *L. stagnalis*. However, the tanks were not observed during the whole experiment.

The water quality may have been the variable that promoted mortality. There were no water filters in any tank, so snail fecal material and leech mucous collected in the tanks. Additionally, the water was taken from a Tenderfoot Lake through copper pipes. Copper has been shown to have a high lethality to many gastropods at low concentrations (0.075 mg/L) including fresh water and tropical snails (Ravera 1976; Cunha *et al.* 2007). The water was not tested to determine the copper concentration, so it may have contributed significantly to snail mortality. Many of the *B. chinensis* snails were reused in more than one experiment. Their prolonged exposure to the water could have played a role in skewing the average mortality of later trials.

Leeches did parasitize snails, but there were complicating variables that were not controlled for. The experiments need to be optimized and ran again to collect concrete data for *M. decora* parasitizing of snail species. The trials should be video recorded throughout the experiment to quantify leech predation of snails. Water filters in each tank would minimize organic contamination from the snails and leeches. Also, water excluding inorganic contaminants such as copper would be necessary.

Otherwise, the experiment could be expanded to include leech prey selection between more species of snails. *Helisoma trivolvis* is a freshwater snail species that shares a similar habitat as *L. stagnalis* and *B. chinensis*, but just as *L. stagnalis*, *H. trivolvis* does not have an operculum for protection. Comparing these three snail species would represent more extensive trophic interactions throughout the lake. If *M. decora* would parasitize additional snail species, then algae could become more prevalent due to decreasing snail abundance (Cardinale et al. 2012). Finally, introducing ranging water temperatures to the tanks would simulate climate change in the lakes. Increasing the water temperature from 20 ^{o}C to 30 ^{o}C could allow chemical cues to diffuse more rapidly allowing the leeches to parasitize snails more efficiently (Sura and Mahon 2012) . The results could be used to predict future food web shifts.

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Figures

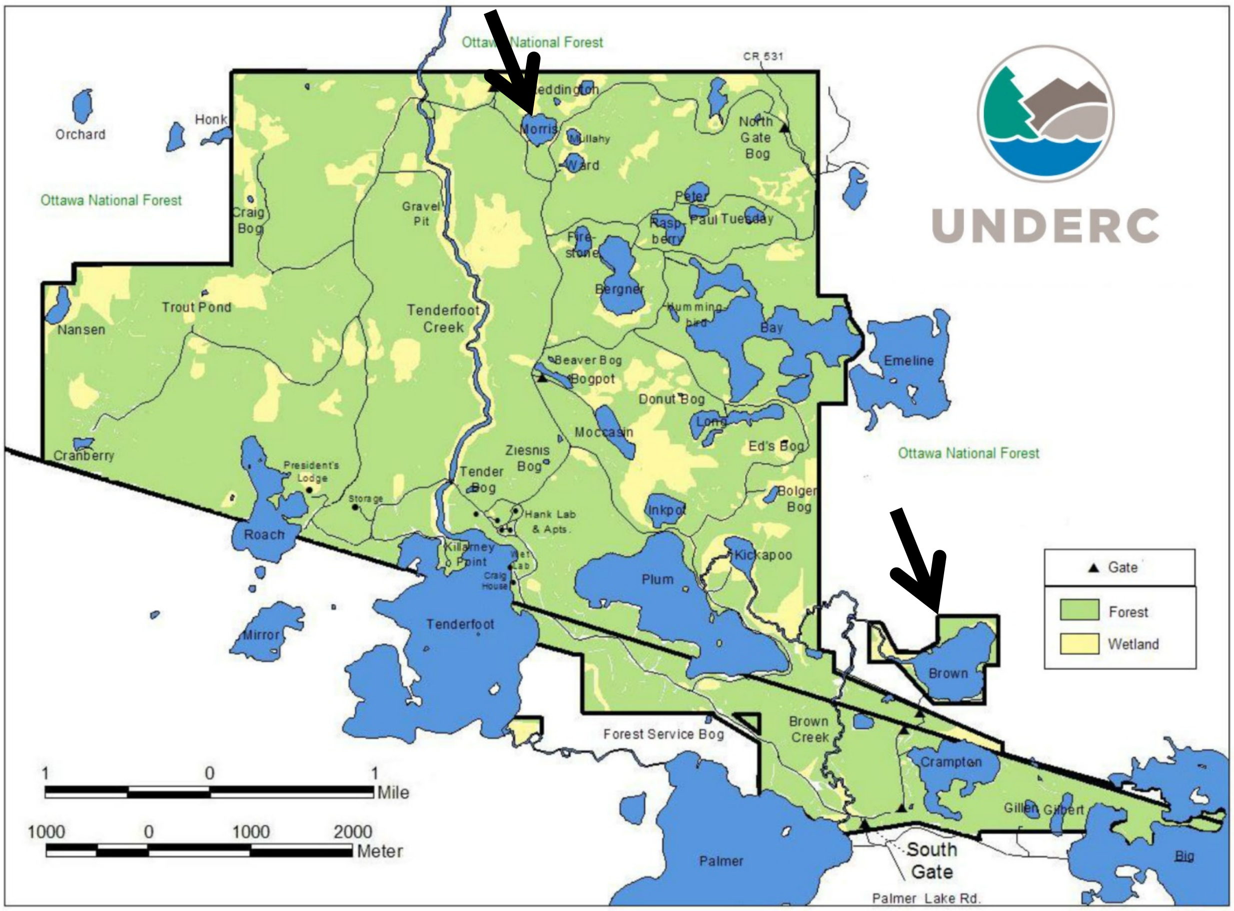

Figure 1: A map of the UNDERC property. The black arrows indicate the lakes that leeches (Morris) and both species of snails (Brown) were collected from.

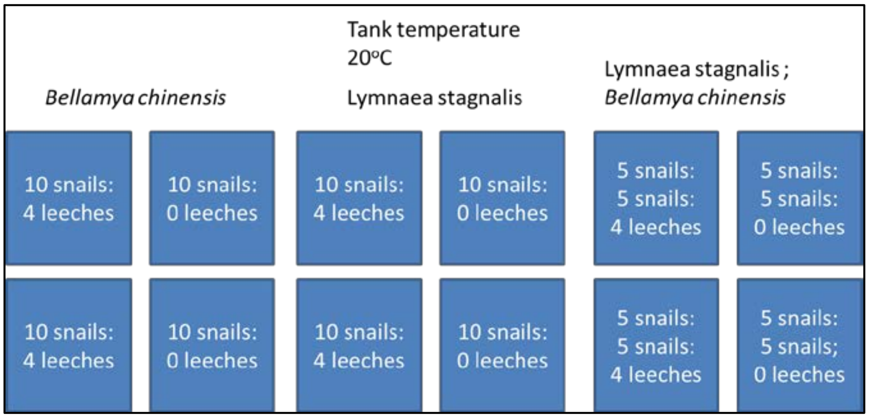

Figure 2: The experiment was set up with twelve tanks. Six of the tanks had four leeches in them and the other six were controls for snail mortality.

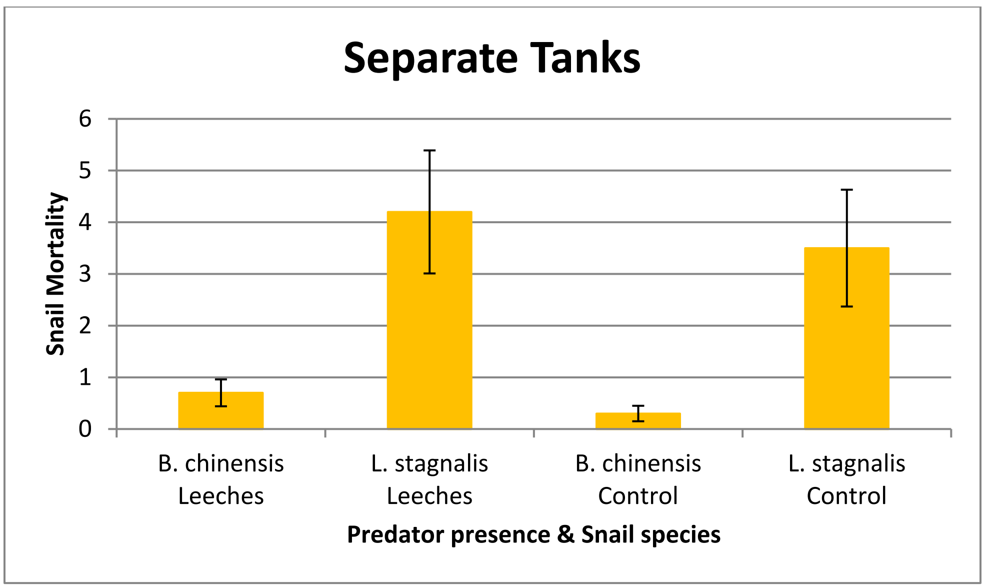

Figure 3: The average mortality of snail species in separate tanks with or without leeches present. The species of snail significantly affected mortality (x^2:10.7877; df: 1; p-value: 0.001), but leech presence did not significantly (x^2: 1.3149; df: 1; p-value: 0.25) affect snail mortality.

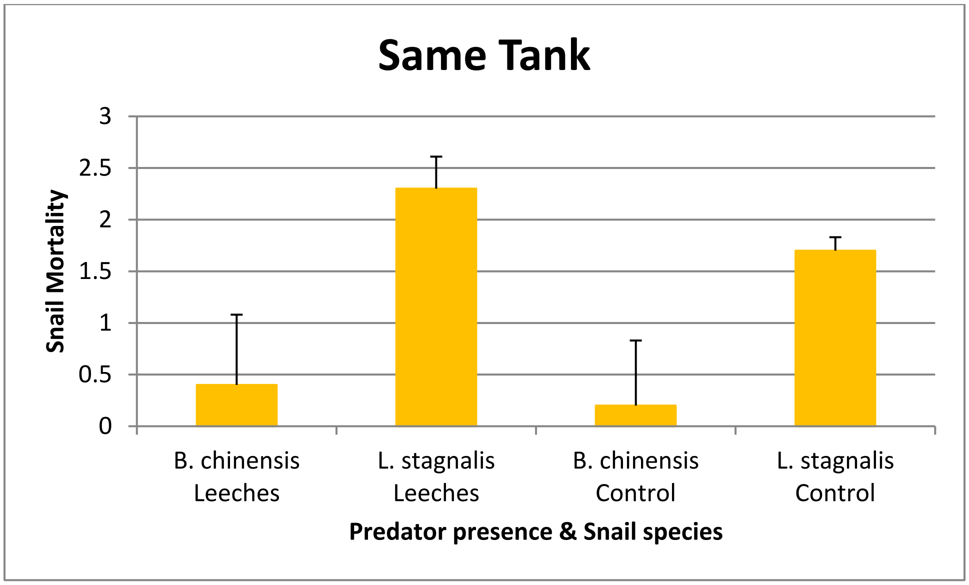

Figure 4: *L. stagnalis* average mortality was significantly (w: 78; p-value: 0.022) higher than *B. chinensis* with them both in the same tank as leeches. *L. stagnalis* also had a significantly (w: 74, p-value: 0.044) higher average mortality in same conditions, but without leeches present.