Microphallus sp. trematode parasites alter use of and competition for shelter in the crayfish *Orconectes virilis* and *Orconectes propinquus*

BIOS 569: Practicum in Field Biology

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2013

ABSTRACT –

Invasive species, such as the crayfish *Orconectes rusticus*, can severely damage freshwater ecosystems and alter the food webs present in these habitats. As *O. rusticus* has increased in density in northern Wisconsin, two other crayfish species, *O. virilis* and *O. propinquus*, have experienced population declines. Because crayfish play an important role in freshwater food webs as consumers that feed on both producers and primary consumers, a decrease in their populations can lead to major food web alterations and reductions in the abundance of native species. A suggested biological control agent for *O. rusticus* is a trematode parasite (*Microphallus* sp.), however, the effects of this parasite on *O. virilis* and *O. propinquus* have not been well studied. This study examines the effects of the *Microphallus* sp. parasite on *O. virilis* and *O. propinquus*. Two different experiments were completed using *Microphallus* infected and uninfected *O. virilis* and *O. propinquus* to test whether infection alters an individual's affinity for shelter and ability to compete for shelter. *O. propinquus* shelter use behavior was strongly affected by *Microphallus* infection while *O. virilis* was not as affected. *O. propinquus* infected individuals spent significantly less time in shelter than uninfected individuals. Uninfected *O. virilis* were better able to compete for shelter than infected individuals. The opposite trend was observed in *O. propinquus*; infected individuals outcompeted uninfected individuals for shelter. These data suggests that both *O. virilis* and *O. propinquus* are affected by *Microphallus* infection, causing *O. propinquus* to be more vulnerable to predation and a reduction in fitness of *O. virilis*. Therefore, *Microphallus* is not a good option as a control of *O. rusticus* populations if maintaining populations of other crayfish is a concern.

INTRODUCTION –

Invasive species are a growing threat to freshwater ecosystems worldwide (Simon and Townsend 2003). Recent research in northern temperate lakes has examined the effects of the invasive crayfish *O. rusticus* on the ecological community and on two other crayfish species, *O. propinquus* (another nonnative crayfish) and *O. virilis* (a native crayfish) (Lodge et al. 1994, Olden et al. 2011, and Szela and Perry 2013).

The *O. rusticus* population has increased significantly in the northern Wisconsin area from about 7% to 36% in the past 20 years (Olden et al. 2006), causing *O. propinquus* and *O. virilis* populations to decline by 8% and 78% respectively (Olden et al. 2011). In some of the tested lakes in the Wisconsin area, resident crayfish species nearly disappeared, although total crayfish abundance continued to rise due to the population increase of *O. rusticus* (Wilson et. al. 2005). Based on these historical data, *O. virilis* is more vulnerable to *O. rusticus* invasion than *O. propinquus* is (Olden et al. 2011).

In freshwater ecosystems with high abundances of *O. rusticus*, ecological impacts range from behavioral shifts in native species to the complete restructuring of food webs (Lodge et al. 1994 and Eby et al. 2006) due to crayfish comprising an important role as mid-level consumers in freshwater food webs (Usio and Townsend, 2004) that feed on both producers and primary consumers and create multilevel interactions in the trophic cascade (Lodge et al. 1994). Furthermore, the introduced *O. rusticus* can ultimately lead to the extirpation of resident crayfish species and reduce macrophyte and invertebrate biodiversity found in freshwater ecosystems (Charlebois and Lamberti 1996). Due to these effects, researchers are interested in parasites that could be used to control *O. rusticus* populations, and a *Microphallus* sp. parasite has been suggested as a potential biological control agent because *Microphallus* parasite abundance is negatively correlated with *O. rusticus* abundance (Roesler 2009). However, the effects of

Microphallus on *O. virilis* and *O. propinquus* are not well understood. If *Microphallus* has a negative impact on *O. virilis* and *O. propinquus* populations, than its use as a biological control agent of crayfish populations may harm resident crayfish populations as well as invasive crayfish populations.

Parasites closely related to *Microphallus* often manipulate their intermediate hosts to complete their life cycle (Levri and Lively 1996), making *Microphallus* a possible manipulative parasite. Manipulative parasites are categorized by the type of phenotypic alterations displayed in the host species (Thomas et al. 2011). Parasitic organisms, such as *Microphallus*, have developed the ability to manipulate the phenotype of their hosts to increase their probability of transmission to higher trophic levels (Lefevre 2008 and Thomas et al. 2011). *Microphallus* is a trematode parasite that infects the hepatopancreas of the crayfish host. The crayfish must be consumed by a vertebrate predator for the parasite to complete its life cycle. Parasites can be full participants in the functioning of ecosystems especially by altering the phenotype of keystone hosts. In this study, the effects of *Microphallus* on the behavior of *O. virilis* (indigenous) and *O. propinquus* (non-indigenous) is examined. Specifically, I will test how infection alters shelter usage and shelter competition behavior.

Although *O. propinquus* and *O. virilis* are similar phyletically and ecologically (Bouwma and Hazlett 2001), it is important to distinguish that *O. virilis* is indigenous to the northern Wisconsin area while *O. propinquus* is non-indigenous. Because it is not known if the *Microphallus* sp. parasite is indigenous to the northern Wisconsin area or not, it is possible that *Microphallus* has different evolutionary histories with *O. virilis* and *O. propinquus*. This difference in the total amount of time that both species have been exposed to *Microphallus* could lead to differences in the way that the parasite affects their behaviors. If *Microphallus* is a manipulative parasite, I expect it would cause a crayfish with which it shares a long evolutionary history to reduce its shelter use.

With these experiments, I test the hypothesis that infection by the *Microphallus* sp. trematode parasite reduces shelter use and the ability of the crayfish to compete for shelter in both *O. virilis* and *O. propinquus*. This is likely to occur because the parasite may manipulate the crayfish hosts to be transmitted to other species and complete its life cycle. I hypothesize that both *O. virilis* and *O. propinquus* will be affected in similar ways by the parasite in regards to shelter use and competition; all infected individuals, regardless of species, will spend less time covered by shelter and will be exposed to predators for longer periods of time in order to maximize the probability of transmission of the *Microphallus* parasite to subsequent hosts. Because shelter use is important for crayfish protection from predation, if *Microphallus* alters crayfish shelter use it may also affect crayfish populations and community interactions. This information can help predict crayfish population trends and also aid in focusing crayfish population management in lakes where crayfish are likely to reach high densities.

MATERIALS AND METHODS –

Crayfish Collection and Husbandry

Crayfish were collected from lakes in the northern Wisconsin and Michigan centered around the University of Notre Dame Environmental Research Center (UNDERC) in Land O' Lakes, WI. These locations included: Forest Lake, Big Muskellunge Lake, Evergreen Lake, Tenderfoot Lake and Plum Lake. Once crayfish were collected, five crayfish of different sizes from each location were dissected and analyzed under a dissection microscope in order to determine general infection levels from these sites. All crayfish used were maintained on UNDERC property in 345 liter cattle tanks. Crayfish were fed shrimp pellets twice a week. The following experimental procedures for shelter affinity and shelter competition were adapted from the procedure used by Capelli and Munjal (1982).

Experiment 1: Affinity for shelter (infected and uninfected)

Crayfish sex and carapace length (measured in millimeters) was recorded and crayfish were then placed by themselves in a 38 liter bucket. Each bucket was filled with roughly 2 cm of sand to mimic the natural habitat, and was filled with aerated well water to a level of 10 cm. An airstone was also placed in each bucket in order to maintain high dissolved oxygen levels. A PVC pipe shelter was also placed in the bucket with the crayfish (crayfish under 23 mm were given a small shelter [6cm x 6cm x 3cm], and those over 23 mm were given a large shelter[7cm x 9cm x 5cm]). In each trial, eleven infected and eleven uninfected crayfish were used in order to accommodate for the competition experiment that would be run using the same crayfish. Five total trials were ran from June to July 2013. All infected and uninfected crayfish were marked on the carapace with a spot of nail polish in order to easily identify individuals. Once placed in their respective buckets, crayfish were left undisturbed overnight as an acclimation period. The following day, crayfish were observed once every hour between 9:00 AM and 3:00 PM (total of seven observations for each crayfish) and their use of shelter was recorded during each observation time. If all periopods (walking legs) of the crayfish were outside of the shelter (even if the tail was still partially in the shelter), it was counted as being “out of shelter” during the observation period. If some periopods were in the shelter, it was considered as “in shelter” for that period.

Experiment 2: Competition/preference for shelter (uninfected vs. infected)

Using the same crayfish from the first experiment, the crayfish were paired based on carapace length (within 1 mm) and sex and placed together in a bucket with one PVC pipe shelter. In these trials, one of the crayfish was infected and the other was uninfected (identified by the different colors of nail polish used to mark each crayfish), which was determined by the preliminary dissections of individuals from each location in order to determine general infection status of those specific crayfish populations. The same recording procedure was followed as described in Experiment 1. After data collection, data was analyzed and a "winner" was determined for each bucket. The winner was the crayfish that was in the shelter for the majority of the seven observations.

Determination of Infection

After the two days of trials, all crayfish used were dissected and the hepatopancreas of each was analyzed for parasite presence under a dissection microscope. The hepatopancreas was placed between to glass slides and gently expanded and flattened. Each individual parasite was counted and the intensity of infection was also documented.

Data Analysis

All statistics were run using SYSTAT. Shelter affinity data was not normally distributed, therefore a nonparametric Kruskal-Wallis Test was run for *O. virilis* and *O. propinquus* separately to determine whether *Microphallus* infection affected the percent of time crayfish spent in shelter. Data was also analyzed by running a linear regression to compare if the number of parasites found in an individual affected the amount of time spent in shelter. Data and results from the competition experiment were tested for statistical significance using a chi-squared test to determine if the presence of the *Microphallus* parasite influenced the ability to compete for shelter.

RESULTS –

There was little difference in shelter use between infected and uninfected *O. virilis* (Fig. 1) ($\chi^2 = 7.13$, $p = 0.119$). However, infection significantly reduced the amount of time that *O. propinquus* spent in shelter (Fig. 2) ($\chi^2 = 28.89$, $p < 0.0001$). Furthermore, a linear regression analyzing if the number of parasites found in the hepatopancreas of the crayfish (independent variable) affects the amount of time the crayfish spends in the shelter (dependent variable) suggests that infection intensity does not have a strong relationship with amount of time spent in shelter was not significant for both *O. virilis* ($R^2 = 0.0078$, $p = 0.485$) and *O. propinquus* ($R^2 = 0.067$, $p = 0.089$).

Competition experiment data (Table 1) revealed that uninfected *O. virilis* are better able to compete for and are more likely to win a shelter competition against *Microphallus* infected individuals ($\chi^2 = 10.889$, $p < 0.001$). Competition data (Table 1) also revealed that infected *O. propinquus* are more likely to win shelter competitions against an uninfected *O. propinquus* ($\chi^2 = 7.118$, $p = 0.0076$).

DISCUSSION –

The idea that biological invasions are preventable is a dictum, there are very few resources available that can successfully aid in controlling invasive species (Simberloff et al. 2005), and even fewer that are capable of controlling these species without overly damaging the native ecosystem and its inhabitants. This experiment aimed at using this belief and testing to determine if the use of a *Microphallus* sp. parasite to control the invasive and dominant crayfish species *O. rusticus* would only minimally affect other species in these freshwater ecosystems, specifically the other two common crayfish species *O. virilis* and *O. propinquus.*

Analyzing the results of the shelter affinity experiment, it can be concluded that infection by *Microphallus* does alter behavior and shelter preferences in *O. propinquus*, but not necessarily in *O. virilis*. In regards to *O. propinquus*, the demonstrated trend followed the original hypothesis that infection would lead to individuals that spent less time in shelter and more time exposed to predators (Garvey et al. 1994) due to *Microphallus* manipulating crayfish behavior to make it more vulnerable to predation. However, in *O. virilis*, although a trend was present that suggests that *Microphallus* infection might alter behavior in a similar way to what was expected, the analysis was not significant. This analysis suggests that *O. virilis* may not be as affected by *Microphallus* infection as *O. propinquus*, especially when taking into consideration the fact that more trials/replicates were run with *O. virilis* than *O. propinquus* due to time constraints.

Differences in evolutionary history between *Microphallus* and the two crayfish hosts may explain the different behavioral effects of the parasite on these two species. As explained in the introduction, while *O. virilis* is indigenous to the northern Wisconsin area, *O. propinquus* is non-indigenous. Because it is not known if *Microphallus* is also indigenous to this area or if it was introduced at some point in history, *O. virilis* and *O. propinquus* may have been exposed to *Microphallus* for different lengths of time, giving one species more time to evolve and possibly neutralize the effects of *Microphallus* in regards to the variables tested in this study, crayfish behavior and shelter competition. If this explanation is true, than it suggests that *O. virilis* has been exposed to the *Microphallus* parasite for a longer time due to its lesser effects on *O. virilis* shelter affinity. However, an alternative explanation is also possible; the *Microphallus* parasite may also have coevolved with one crayfish species in order to maximize its transmission to higher trophic levels. Based on the observed data, this would most likely mean that the *Microphallus* parasite coevolved with *O. propinquus*, suggesting that it shares a longer evolutionary history with *O. propinquus*, and is therefore better able to manipulate it when compared to *O. virilis*, which displayed only slight manipulated behavior and also displayed negative effects in response to *Microphallus* infection (infected individuals were not as able to compete for shelter as uninfected individuals).

Furthermore, another difference between these two species is that *O. virilis* is nocturnal, while *O. propinquus* is diurnal (Hazlett, 1994). This could also alter the ways that *Microphallus* affects these two species. Because *O. virilis* is nocturnal, infection would cause them to be more exposed during darkness, which means that it is protected from predation simply due to predators not being able to see them without light. Therefore, *Microphallus* would have a much less chance of being transmitted to another organism when infecting *O. virilis*. This can also be used to aid in explaining why *O. virilis* was not affected to the same severity as *O. propinquus*.

While infection status (infected or uninfected) altered crayfish behavior, the intensity of infection had no effect on shelter affinity of the crayfish. This is interesting to note because this means that a highly infected crayfish will act similarly to a crayfish that is infected with only a few parasites.

Within the competition experiment, effects of infection on *O. virilis* and *O. propinquus* were complete opposite from one another. Uninfected *O. virilis* won the majority of the competitions for shelter, while infected *O. propinquus* won the majority of competitions. Therefore, the *O. virilis* results support my original hypothesis in that *Microphallus* infection negatively affects crayfish and makes them less able to compete for shelter in the presence of another crayfish. This data also supports previous research that has observed a decrease in defensive behavior and physical fitness in species infected with a closely related species in the *Microphallus* genus (Kunz and Pung 2004). However, the trend seen in *O. propinquus* does not support the original hypothesis. In fact, the infected individuals won the majority of the competitions. A possibility for this is that although *Microphallus* infection in *O. propinquus* does cause individuals to spend less time in shelter, it may also increase aggression, which makes infected *O. propinquus* better able to compete for and occupy available shelter.

Therefore, based on this data, and the observed effects that *Microphallus* sp. has on *O. virilis* and *O. propinquus*, both in terms of their preference for shelter and their ability to compete for shelter, it is not recommended that *Microphallus* sp. be used as a control of *O. rusticus* populations if maintaining other crayfish populations is of value. Specifically, using the similarities in habitat preferences between *O. virilis* and *O. propinquus*, previous research suggests that competition for limited resources, such as shelter, does result in modified predation by fish (Butler and Stein 1985). Furthermore, crayfish interactions and behavior also influence susceptibility to fish predators (Garvey et al. 1994). With this said, changes in shelter affinity and physical fitness induced by *Microphallus* infection, as observed in this experiment, can lead to an increase in predation of crayfish. Because of this increase in possible predation of all crayfish species due to infection by *Microphallus* sp., the use of *Microphallus* sp. as a control agent could lead to population decreases of all of these crayfish species.

Expanding on these results, specifically using the different alterations in behavior caused by *Microphallus* in *O. virilis* and *O. propinquus*, future research should include a survey of multiple lakes that focuses on collecting *O. virilis* and *O. propinquus* in order to determine if *Microphallus* sp. is more abundant in a particular species. This data would aid in determining if one species of crayfish is more vulnerable to *Microphallus* infection.

ACKNOWLEDGMENTS –

I would like to thank Lindsey Sargent, my mentor, for all of her help, advice, and expertise throughout the design, set up, and running of this project. I also would like to thank Sheng Hing for helping in catching crayfish and helping set up for all trials, I would not have finished everything as efficiently as I did without your help. Thank you to Dr. Gary Belovsky and Dr. Michael Cramer for giving me the opportunity to participate in this program and for all of their help in designing this project. Lastly, I would like to thank the Bernard J. Hank Fellowship for funding this research project.

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TABLES –

Species	# of Times Infected Won	# of Times Uninfected Won
Orconectes virilis	2	16
Orconectes propinquus	14	3

Table 1. Competition experiments demonstrate that *Microphallus sp.* infection alters competition for shelter in both species. Infected O. virilis were significantly less likely to win the shelter competition against an uninfected crayfish. Infected O. propinquus were significantly more likely to win the shelter competition against an uninfected crayfish.

FIGURES –

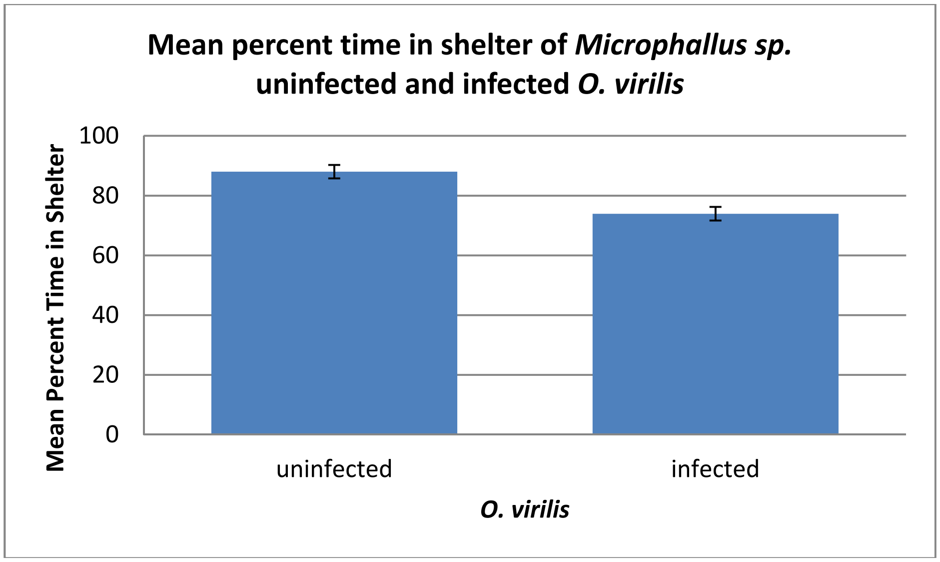

Figure 1. Mean percent time spent in shelter of uninfected and infected *O. virilis* individuals. There was no significant difference in shelter use between uninfected and infected individuals. Uninfected individuals spent a total of 88% (± 1.88) of time in shelter while infected individuals spent a total of 73.94% (± 2.27) of time in shelter. Standard error bars shown.

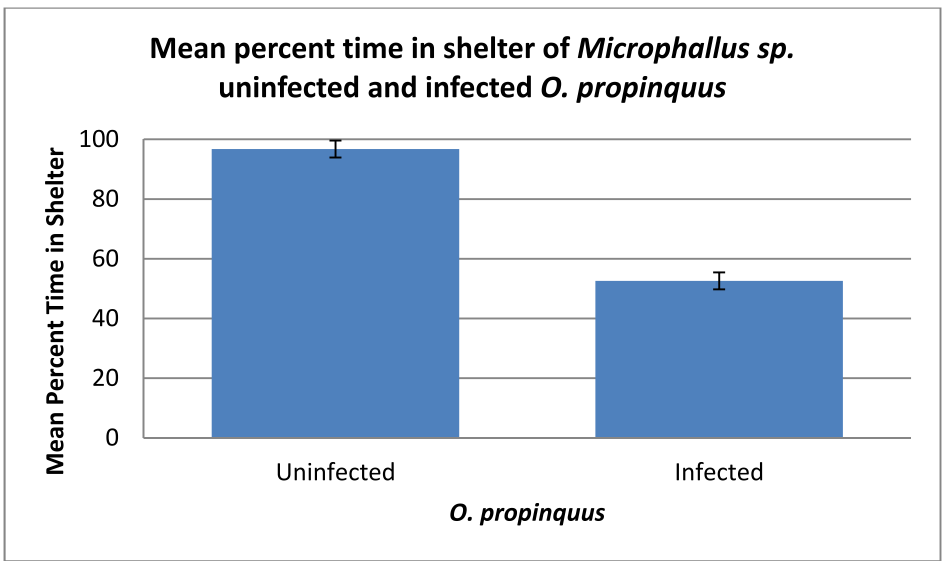

Figure 2. Mean percent time spent in shelter of uninfected and infected *O. propinquus* suggests that *Microphallus sp.* infection causes individuals to spend less time in shelter. Infected individuals spent a total of 52.59% (± 2.83) of time in shelter while uninfected individuals spent a total of 96.75% (± 1.32) of time in shelter. Standard error bars shown.