The Effect of *Microphallus sp.* Infection on the Shelter Competition Behavior of Northern Clearwater Crayfish (*Orconectes propinquus*)

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BIOS 35502: Practicum in Field Biology
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Abstract

Invasive species, such as rusty crayfish (*Orconectes rusticus*), are a threat to native aquatic ecosystems and biodiversity, stimulating the need to research methods to control their invasion. One suggested form of a biocontrol is the crayfish parasite *Microphallus sp.* which has been shown to possibly reduce rusty crayfish numbers in northern Wisconsin lakes. However, the effect of *Microphallus sp.* on other crayfish species is not well researched. This study will examine the effect of *Microphallus sp.* infection on northern clearwater crayfish (*Orconectes propinquus*) host behavior through a shelter competition experiment. A survey was conducted on the University of Notre Dame Environmental Research Center (UNDERC) property to determine the distribution and prevalence of the *Microphallus sp.* parasite. Shelter competitions between *Orconectes propinquus* were designed to determine whether the presence of the *Microphallus sp*. parasite influences the ability of crayfish to compete for shelters. It was found that there is a significant association between the presence/absence of *Microphallus sp*. and the location of *Orconectes propinquus* inside or outside the shelter, where uninfected crayfish out compete the *Microphallus sp*. infected crayfish for the shelter. Therefore, the use of *Microphallus sp*. as a biocontrol to reduce invasive rusty crayfish populations is not recommended due to the negative effects it can have on other crayfish species.

Introduction

Invasive species critically affect native ecological integrity. Methods to control invasive species must be researched to reduce damage to natural ecosystems. A well-studied invasive species is rusty crayfish (*Orconectes rusticus*), which has spread from its native range in the Ohio River basin to northern Wisconsin (Klocker and Strayer 2004). Rusty crayfish threaten native ecosystems and biodiversity by disrupting both lentic and lotic benthic food webs (Lodge et al. 2000).

Studies have shown that rusty crayfish have become the dominant crayfish species in northern Wisconsin, replacing the native crayfish fauna (Olden et al. 2006). Rusty crayfish are able to displace the native crayfish due to their aggressive behavior, rapid development and growth, reproductive interference with native species (Klocker and Strayer 2004), destruction of aquatic plant habitat (Olsen et al. 1991), competition for limited resources (Garvey et al. 1994), and dislodgment of native crayfish from their shelters which makes them more susceptible to fish predation (Hill and Lodge 1993, Hayes et al. 2009). Rusty crayfish have been producing negative effects on communities and ecosystems across a wide geographic range, stimulating the need to research methods of controlling their invasion.

One suggested form of a biocontrol is the crayfish parasite *Microphallus sp.,* which has been shown to possibly reduce rusty crayfish numbers in northern Wisconsin lakes (Roesler 2009). *Microphallus sp.* is a trematode parasite that infects the hepatopancreas and gills of the crayfish host (Stunkard 1951). The initial hosts of *Microphallus sp*. are snails, which release cercariae that develop into metacercariae inside the hepatopancreas of the infected crayfish (Caveny and Etges 1971). The metacercariae are 0.5-0.6 mm long and white, translucent in color. Once the infected crayfish is ingested, the adult trematode attaches to the final host's intestinal wall, releasing eggs to infect the primary hosts, snails (Stunkard 1951).

A study in northern Wisconsin lakes suggested that lakes with *Microphallus sp.* present had low densities of rusty crayfish, while lakes lacking *Microphallus sp.* had high densities of rusty crayfish (Roesler 2009). However, the effect of *Microphallus sp.* on other crayfish species is not well researched, a necessary step in determining the costs of use of *Microphallus sp*. as a biocontrol to reduce the numbers of invasive rusty crayfish. This study will examine the effect of *Microphallus sp.* infection on northern clearwater crayfish (*Orconectes propinquus*) host behavior through a shelter competition experiment. The use of *Orconectes propinquus* will also help determine the general effects the *Microphallus sp.* parasite has on crayfish behavior, providing insight into how *Microphallus sp*. may be affecting rusty crayfish populations.

Microphallus sp. has been shown to reduce the physical fitness in other closely related crustaceans, such as grass shrimp. Kunz and Pung (2004) concluded that *Microphallus turgidis* infected grass shrimp had a weakened swimming stamina and were less defensive and motionless in the presence of a predator. Therefore, infected grass shrimp were more likely to be consumed than the uninfected grass shrimp (Kunz and Pung 2004).

Another study examining food foraging of snails discovered that *Microphallus sp.* infected snails altered their behavior by remaining on top of rocks where the chance of predation is much greater instead of retreating to the bottom of the rocks which provides more cover (Levri 1999). Levri and Lively (1996) demonstrated that *Microphallus sp.* might be capable of parasitic manipulation, where the parasite alters the behavior of the host to increase the chance of predation, improving the chance of transmission to the next host. *Microphallus sp.* infected snails tended to forage in the early morning when the next host, waterfowl, is most active, while uninfected snails foraged more during the day when the waterfowl was less active (Levri and Lively 1996). This time-specific behavioral manipulation may enhance the transmission of *Microphallus sp.* to the waterfowl host. Helluy and Thomas (2003) also found that *Microphallus papillorobustus* infected gammarids diverged from their normal predator evasion behavior, making them twice as likely to be preyed upon by birds than uninfected gammarids.

Therefore, my hypothesis is that *Microphallus sp.* has the ability to alter the behavior of *Orconectes propinquus*, so crayfish infected with *Microphallus sp.* will be less aggressive in the shelter competition whereas an uninfected crayfish will dominate the shelter. This lack of shelter would make the infected crayfish more susceptible to predation, potentially reducing their overall abundance and inclusive fitness (Garvey et. al 1994).

Materials and Methods

A survey was conducted on the University of Notre Dame Environmental Research Center (UNDERC) property to determine the distribution and prevalence of the *Microphallus sp.* parasite. *Orconectes propinquus*, *Orconectes virilis*, and *Orconectes rusticus* were hand captured in the littoral zone of Tenderfoot, Palmer, Plum, Brown, and Big Lakes, with a goal of 10 crayfish per lake site.

Captured crayfish were dissected to determine the presence or absence of the *Microphallus sp.* parasite. Crayfish were dissected by severing the thoracic ganglia to reduce any pain sensation. Then the legs, chelae, and carapace were removed to expose the hepatopancreas within the body cavity. The hepatopancreas was examined under a dissecting microscope, using a dissecting needle to separate the finger-like projections of the hepatopancreas to expose the metacercariae. For each crayfish, the number of parasites, species, sex, carapace length (mm), and lake site were recorded. The parasite prevalence was calculated for each lake sample site to determine how the abundance of the parasite varied between lakes.

The *Microphallus sp.* survey revealed that *Orconectes propinquus* with a carapace length between 20-30 mm had the largest variance in infection level. Therefore, crayfish with a carapace length greater than 20 mm were hand collected at Tenderfoot Lake, where *Orconectes propinquus* were abundant. Shelter competition tanks were designed using 48 cm in diameter round aquaria filled with eight cm of water and lake sediment to cover the bottom. Large gravel rocks were removed to prevent the engineering of temporary shelters, and screen was attached on top to prevent the escape of specimens. A 10x5 cm PVC pipe cut lengthwise was weighted and placed in the middle of each aquarium. Two *Orconectes propinquus* of the same sex and with carapace lengths within one mm of each other were simultaneously placed in opposite sides of the aquaria. The carapace of each crayfish was marked with colored paint to differentiate between the specimens. The trials began between 17:00 and 18:00, and ran overnight for 14 hours to allow the crayfish to acclimate to the new environment. The individual inside of the shelter after the period of acclimation was recorded, and the crayfish behavioral interactions were observed for one hour, noting any confrontations and shelter dominance shifts.

At the end of the trial, both specimens were dissected and the hepatopancreas was examined under a dissecting microscope to determine the presence or absence of the *Microphallus sp.* parasite. This procedure was repeated for 30 trials. Between trials, test specimens were held in 48 cm diameter retention aquaria with eight cm of water and PVC pipe shelters.

All statistics were run using SYSTAT. The survey data was analyzed using a two-sample t-test, one-way ANOVA, and linear regression to determine the indicators of parasite susceptibility. A chi-square test was used to determine whether the presence of the *Microphallus sp*. parasite influences the outcome of the shelter competition.

Results

Microphallus sp. was found to be distributed throughout all lakes sampled on UNDERC property. The prevalence and mean number of parasites differed among the surveyed lakes (Table 1), with Tenderfoot Lake having the highest mean number of parasites (42.8) and the highest prevalence (90%). *Microphallus sp*. was present in *Orconectes propinquus, Orconectes rusticus, and Orconectes virilis*. A linear regression showed a statistically significant ($F_{1,48}$=39.501, R^2=0.451, p<0.0001) positive relationship between carapace length and the number of parasites within the crayfish (Figure 1). A two-sample t-test showed no statistically significant difference (t(48)=0.006, p=0.995) of infection levels between males and females. A one-way ANOVA revealed no statistically significant difference ($F_{2,47}$=0.101, p=0.904) of infection levels between *Orconectes propinquus, Orconectes rusticus,* and *Orconectes virilis*.

A chi-square test revealed a statistically significant association (χ^2(1,N=60)=5.173, p=0.023) between the presence/absence of *Microphallus sp*. and the location of *Orconectes propinquus* inside or outside the shelter. The uninfected individuals tended to be present in the shelter while the *Microphallus sp*. infected individuals tended to be outside of the shelter (Table 2). A chi-square test limited to trials where one crayfish was infected while the other crayfish was uninfected revealed a statistically significant (χ^2(1, N=30)=5.533, p=0.011) association between the presence/absence of *Microphallus sp*. and the location of *Orconectes propinquus* inside or outside the shelter. The *Microphallus sp*. infected individuals lost the shelter competition more often, while the uninfected crayfish tended to be present in the shelter (Table 3). There was also a statistically significant difference (t(56)=2.267, p=0.027) of the mean number of parasites in the crayfish between the location of inside or outside of the shelter (Figure 2). The mean parasite count of crayfish within the shelter was 1.46 (±0.605) while the mean parasite count of crayfish outside of the shelter was 16.16 (±6.16).

Discussion

The goal of this study was to determine the effect *Microphallus sp*. infection has on the shelter competition behavior of *Orconectes propinquus*. A survey of the prevalence and distribution of *Microphallus sp*. on UNDERC property provided insight into the dynamics of the parasite. *Microphallus sp*. was found in all crayfish species surveyed, and one sex is not more susceptible to infection than the other. The positive relationship between carapace length and parasite count suggests that crayfish size is the best indicator of susceptibility to parasitism.

The presence of the *Microphallus sp*. parasite was found to influence the outcome of the shelter competition. These results suggest that *Microphallus sp*. is capable of altering the behavior of *Orconectes propinquus*, where infected crayfish are unable to compete with uninfected individuals for the shelter. This result supports the hypothesis of this study and is consistent with previous studies that have demonstrated a reduction of defensive behavior and physical fitness in related crustaceans due to *Microphallus sp*. infection (Levri and Lively 1996, Helluy and Thomas 2003, Kunz and Pung 2004).

These results may provide insight into the factors affecting rusty crayfish in northern Wisconsin, where it was suggested that the presence of *Microphallus sp.* limits rusty crayfish populations (Roesler 2009). The lack of shelter exposes crayfish to a higher risk of predation, as shown by Garvey et. al (1994). Therefore, in lakes where *Microphallus sp*. is present, infection may be exposing more crayfish to the risk of predation due to the loss of a shelter, causing a potential population crash.

Future experimentation is needed to determine the driving factors behind *Microphallus sp*. behavioral alteration in crayfish. A potential mechanism is parasitic manipulation, as suggested by Levri and Lively (1996). *Microphallus sp*. infection might manipulate the behavior of *Orconectes propinquus* to stay out of the shelter. As a result, the susceptibility of *Orconectes propinquus* to predation would increase, improving the rate of *Microphallus sp*. transmission to the final host. An interesting result is that in both trials where neither specimen entered the shelter, both crayfish were infected. Another potential mechanism is the physiological stress induced by the necrosis of the hepatopancreas due to *Microphallus sp*. infection, as shown in estuarine crabs (Robaldo et. al 1999). This physiological stress may require crayfish to expend more energy to cope with the hepatopancreas tissue atrophy, stimulating infected crayfish to allocate less energy to confrontation and shelter competition. Another possible factor is that *Microphallus sp*. does not directly alter the behavior of crayfish, but rather, tends to infect crayfish that routinely forage outside of shelters.

The high prevalence of *Microphallus sp*. on UNDERC property may have many different implications. The presence of *Microphallus sp*. infection was shown to have a negative effect on the ability of *Orconectes propinquus* to compete for a shelter. A potential increase in parasite transmission rate due to the lack of shelter may set in motion a population spike of *Microphallus sp*. at the cost of a population crash of crayfish. Therefore, the use of *Microphallus sp*. as a biocontrol to reduce invasive rusty crayfish populations as suggested by Roesler (2009) is not recommended due to the negative effects it can have on other crayfish species populations.

For future experimentation, different experimental errors would need to be corrected. The surveillance of UNDERC property only examined a limited number of specimens from only a few lakes on property. Future experimentation would include a more thorough survey to better understand the distribution, prevalence, and dynamics of *Microphallus sp*. A more representative gradient of carapace size from each lake would need to be collected to standardize the prevalence among sites due to the relationship between parasite count and carapace length. An interesting result is that despite the small survey sample size, *Microphallus sp*. was discovered in all lakes and all crayfish species when it had previously not been observed on UNDERC property.

The nature of the shelter competition was challenging to obtain a clear disparity between specimen infection levels due to only being able to quantify the presence or absence of *Microphallus sp*. through an invasive technique. A more thorough surveillance might allow for one test specimen from a lake with a high prevalence of *Microphallus sp*. and one test specimen from a lake with a low prevalence of *Microphallus sp*. An alternative method is selective infection by exposing certain crayfish to cercariae from infected snails. These experiments would allow for a greater difference of infection levels to better understand the effects of *Microphallus sp*. on the shelter competition behavior of *Orconectes propinquus*. Future expanded experiments could also examine the shelter competition behavior of rusty crayfish to better understand the driver of potential rusty crayfish population crashes in northern Wisconsin, and if *Microphallus sp*. infection has similar results in different species.

Acknowledgements

I would like to thank my mentor, Lindsey Sargent, for all of her advice and feedback throughout the designing and implementation of this experiment. I would also like to thank Katie Weaver for her collaboration on the surveillance of UNDERC lakes and crayfish collection. Thank you to Dr. Michael J. Cramer, Heidi Mahon, Maggie

Mangan, and Collin McCabe for all of their advice and help along the project. I would also like to thank the generous funding through the Hank Family endowment which allowed me to perform this research this summer.

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Figures and Tables

Table 1. Results of *Microphallus sp.* survey on UNDERC property.

Lake	Number of Captured Crayfish	Dominant Crayfish Species	Mean Parasite Count	Prevalence
Big Lake	13	*O. rusticus*	11	61.53%
Plum Lake	10	*O. virilis*	2.1	40%
Brown Lake	7	*O. virilis*	7.57	85.71%
Tenderfoot Lake	10	*O. propinquus*	42.8	90%
Palmer Lake	10	*O. virilis*	2.6	60%

Table 2. Location of *Orconectes propinquus* inside or outside of the shelter (rows) by the presence or absence of *Microphallus sp.* infection (columns). There is a statistically significant association ($\chi^2(1,N=60)=5.173$, $p=0.023$) between the presence/absence of *Microphallus sp.* and the location of *Orconectes propinquus* inside or outside the shelter. The uninfected individuals tended to be present in the shelter while the *Microphallus sp*. infected individuals tended to be outside of the shelter.

	Infected	Not Infected
Inside Shelter	12	16
Outside Shelter	23	9

Table 3. Location of Orconectes propinquus inside or outside of the shelter (rows) by the presence or absence of Microphallus sp. infection (columns), limited to trials where one specimen was infected while the other specimen was uninfected. There is a statistically significant ($\chi^2(1, N=30)=5.533$, $p=0.011$) association between the presence/absence of *Microphallus sp*. and the location of *Orconectes propinquus* inside or outside the shelter. The *Microphallus sp*. infected individuals lost the shelter competition more often, while the uninfected crayfish tended to be present in the shelter.

	Infected	Not Infected
Inside Shelter	4	11
Outside Shelter	11	4

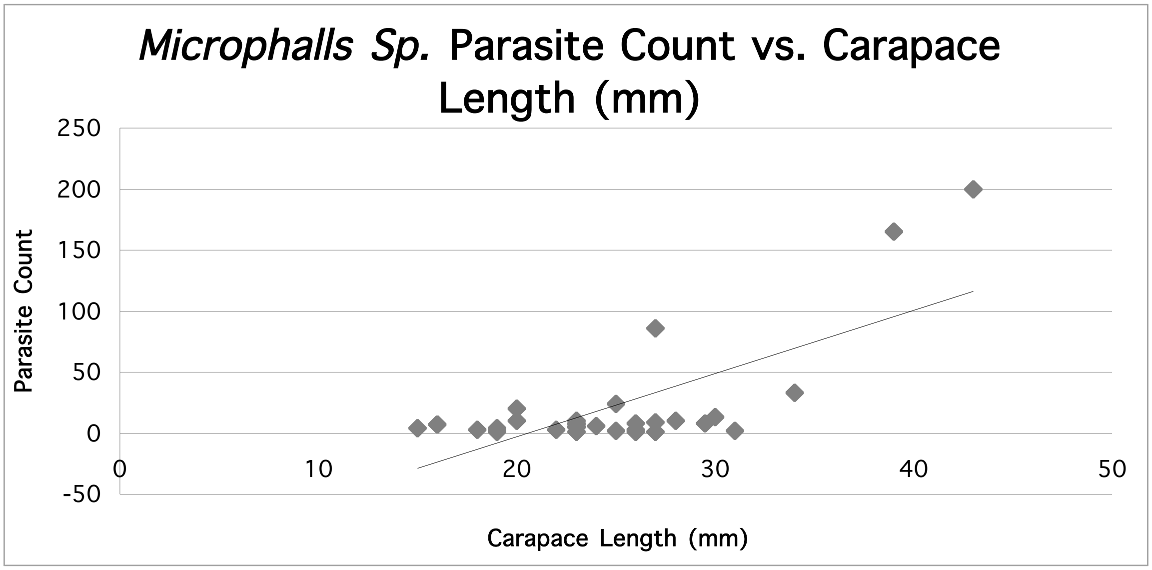

Figure 1. Relationship between *Orconectes propinquus* carapace length and *Microphallus sp.* parasite count. There is a statistically significant ($F_{1,48}$=39.501, R^2=0.451, p<0.0001) positive relationship between carapace length and the number of parasites.

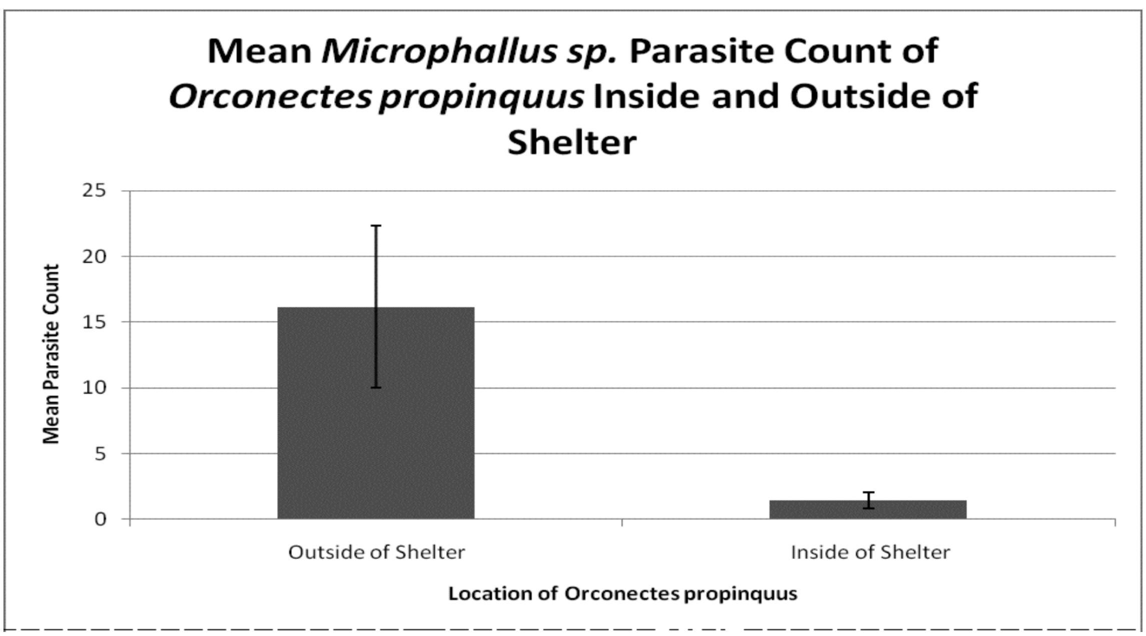

Figure 1. Mean parasite count difference between *Orconectes propinquus* inside and outside of the shelter. There is a statistically significant difference (t(56)=2.267, p=0.027) of the mean number of parasites in the crayfish between the location of inside or outside of the shelter. The mean parasite count of crayfish within the shelter is 1.46 (±0.605) while the mean parasite count of crayfish outside of the shelter is 16.16 (±6.16). Standard Error bars shown.