Shelter Use and Aggression Changes in Rusty Crayfish (*Orconectes rusticus*) Infected with *Microphallus Sp.*

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Abstract

Rusty crayfish (*Orconectes rusticus*) is an invasive species in northern Wisconsin and the upper peninsula of Michigan, disrupting ecosystems and displacing native crayfish. A trematode parasite, *Microphallus sp*., has been known to infect rusty crayfish and is thought to possibly be decreasing invasive crayfish populations. Recent investigations have shown that this parasite reduces a closely related species, *O. propinquus,* ability to compete for shelter. This study examines behavioral changes in rusty crayfish pertaining to shelter use and aggression. Results showed no difference in shelter use, but increases in aggressive actions, suggesting that infected crayfish are not negatively affected by infection. This suggests that *Microphallus sp*. is not a parasite that inhibits rusty crayfish population growth.

Introduction

Invasive species are a detriment to the environment in which they occupy. They often disrupt food webs within the ecosystem, displacing native species that occupy the same niche as well as harming other species in different trophic levels. The rusty crayfish (*Orconectes rusticus*) has taken root in northern Wisconsin lakes over the past 40-50 years, displacing native species *Orconectes propinquus* and a previous invader, *Orconectes virilis*,in addition to disturbing trophic interactions (Capelli and Magnuson, 1989).

Rusty crayfish are thought to have travelled from their native area of the Ohio River basin to northern Wisconsin lakes through human activity such as baiting for recreational fishing (Capelli and Magnuson 1983). Rusty crayfish were first identified in Trout Lake in Wisconsin in 1979, but may have been introduced as early as the 1960s (Olden et al., 2006). This invasive species population has exploded while depleting native species densities. Over the past 50 years, *O. rusticus* has increased from 7% to 36% in the number of occurrences in records concerning northern Wisconsin and Michigan lakes while *O. virilis* has decreased from 54% to 34% (Olden et al. 2006). *O. rusticus* cannot thrive in water with pH levels lower than 5.5 (Berrill et al. 1985) and calcium concentrations less than 2.5 mg/L (Capelli and Magnuson 1983). Most lakes in northern Wisconsin, however, have pH and calcium levels that are higher than these thresholds, making these lakes hospitable for *O. rusticus* (Olden et al. 2006). In addition, *O. rusticus* has been shown to be more fit than *O. virilis* and *O. propinquus* in four levels of interspecific competition (Hill and Lodge 1999) as well as showing more aggressive behavior than native species (Pintor and Sih 2008). The combination of environmental compatibility and ecological fitness makes the rusty crayfish a strong invasive species. In addition, rusty crayfish are known to damage the ecosystem in which it invades. Previous studies have shown that macrophyte species richness declined as much as 80 percent in the presence of *O. rusticus*, which suggests that this invasive species may be dramatically changing littoral zone biota (Wilson et al. 2004). These changes have dramatically altered ecosystems of northern Wisconsin and Michigan lakes.

A parasite trematode, *Microphallus sp*., has been suggested as a potential biocontrol for *O. rusticus* (Roesler 2009). While the exact species of the parasite is unknown, members of the family Mircrophallidae have a three-stage life-cycle. A snail thought to be *Amnicola limosa* (personal communication, Robin Overstreet, University of Southern Mississippi) is the first host and releases cercariae to the crayfish, which houses the metacercariae in its hepatopancreas. The crayfish is then consumed by the final host, which is unknown, but hypothesized to be either a species of bird or fish (Roesler 2009). Earlier studies have shown that *Microphallus sp*. can affect the behavior of its initial host. Levri (2009) describes the snail *Potamopyrgus antipodarum* as not retreating to safe shelter after food has been removed from exposed rocks, which leaves the snail vulnerable to predation. This was inferred to mean that *Microphallus sp*. may alter the behavior of its first host in order to more expediently proceed to its final host.

In addition to its first host, *Microphallus sp*. may be affecting populations of its intermediate host, the rusty crayfish. Other trematodes are known to change the behavior of their hosts such as *Euhaplorchis californiensis*, which affects its intermediate host, *Fundulus parvipinnis*, such that it performs actions that are more conspicuous to its avian predator, expediting the parasitic life cycle (Lafferty and Morris 1996). In addition, a rusty crayfish relative, *Orconectes propinquus*, experiences abnormal behavior when infected with *Microphallus sp* (Towle unpublished).

It has been shown that *Microphallus sp.* is present in lakes with low rusty crayfish population densities while the parasite is either not prevalent or absent in lakes with high *O. rusticus* densities (Roesler, 2009). This suggests that *Microphallus sp*. is affecting population growth of rusty crayfish in addition to its previous host, snails. How *Microphallus sp*. is accomplishing this decrease in rusty crayfish population, however, is unknown. One possibility is that the parasite is using similar manipulations of crayfish behavior as compared to what was observed in Leviri (2009) in snails. This could lead to infected crayfish exhibiting atypical behavior such that they would have decreased fitness, therefore lowering the *O. rusticus* populations

It is with this consideration that I investigated shelter use and aggression behavior changes of *O. rusticus* individuals that are infected with *Microphallus sp*. I hypothesized that rusty crayfish infected with *Microphallus sp.* will exhibit changes in territory use and aggression, therefore not instigating any threat to other crayfish in shelter competition. This project was accomplished through direct observation of specimen behavior in a laboratory setting through two experiments: shelter occupation, and direct confrontation in the presence of limited shelter resources.

Materials and Methods

Crayfish collection and infection

I collected *A. limosa* from High Lake and Big Lake, which contain crayfish with Microphallus metacercariae, in order to determine locations with high Microphallus sp. infection rates of snails. Snails and surrounding water were viewed under a dissection microscope for released cercariae after direct light exposure. After this preliminary research, snails were collected from High Lake, where cercariae were most abundant.

I hand trapped *O. rusticus* from Big Lake and dissected 10 individuals in order to determine size ranges of uninfected crayfish. The remaining crayfish were housed in two aquaria: one containing a mesh sack of aquatic plants and the other containing a mesh sack of aquatic plants and 1000 *A. limosa* that were collected from High Lake. Crayfish remained exposed to potential infection between 15 and 18 days. 100 watt fluorescent bulbs above each aquarium exposed snails to additional extra light to both aquaria for three hours a day in order to stimulate cercariae release from infected snails from the mesh pouch.

I also collected *O. rusticus* from two sites on Plum Lake that had differing levels of *Microphallus sp*. infection. Crayfish were housed between five and eight days in aquaria before the experiment.

Shelter Usage Experiment

Trials were conducted in circular tanks 25.5cm in diameter that contained a thin layer of sand and 10cm of well water. A 5x10 PVC pipe cut lengthwise was placed in the center of each tank. All tanks contained an airstone and screen secured over the opening to prevent crayfish escape. Each crayfish was placed one in one aquaria between 14:00 and 22:00 and was left overnight. I then recorded their position once every hour the next morning beginning at 9:00 for five hours. Categories were as follows: in shelter, peeking, middle of the aquarium, and edge of the aquarium. Peeking was defined as the crayfish being between 25% and 75% exposed.

I then recorded the carapace length and sex of each crayfish before extracting the hepatopancreas in order to determine infection of *Microphallus sp*. Hepatopancreases were flattened between two glass slides and viewed under a dissecting microscope.

Fifteen crayfish from both the laboratory infection aquarium and the control aquarium were used in this experiment. I also performed 9 replicates for both Plum Lake crayfish locations. Crayfish not infected but housed in the laboratory were tested between June 21 and 24 while all other crayfish were tested between July 9 and 14.

Shelter Competition Experiment

Shelter competition tanks were designed using 25.5cm diameter circular aquaria filled to 10cm depth of well water with sand covering the bottom and a 5x10cm PVC pipe cut lengthwise placed in the center, acting as a shelter. Screen placed on top of the aquaria served to prevent crayfish from escaping. I paired crayfish from both the control and experimental aquaria with crayfish from the control aquarium by sex and carapace lengths within one millimeter of each other. Both members of each pair were marked on the carapace with nail polish in order to distinguish between them. Crayfish were placed in individual aquaria between 13:00 and 22:00 and remained overnight in order to become accustomed to their new environment. I then moved crayfish from their solitary aquarium to another identical aquarium that contained its matching crayfish within the shelter. The introduced crayfish behavior was recorded for 15 minutes as tallies regarding the following categories of actions: approaching the shelter with an occupant, approaching the shelter without an occupant, entering the shelter with an occupant, entering the shelter without an occupant, meral spread, chela strike, initiating an attack, and interlocking with an opponent, all of which are described in Bruski and Dunham (1987). In addition, I counted the number of tail flips that the introduced crayfish and the original occupant performed, naming these losses and wins, respectively.

I then dissected the introduced crayfish in order to examine the hepatopancreas for *Microphallus sp*. infection. Hepatopancreases were viewed under a dissecting microscope while flattened between two glass plates.

Fifteen crayfish from the aquarium containing no snails and thirteen crayfish from the aquarium containing *A. limosa* were tested for behavior. Sample size was reduced in the second aquarium due to mortalities. In addition, 20 crayfish from Plum Lake were tested. Crayfish not infected in the laboratory were observed between June 21 and June 24 and crayfish experimentally infected as well as crayfish collected from Plum Lake were observed between July 9 and July 14.

Data analysis and statistics

Data quantification for the shelter usage experiment was accomplished by assigning points to each of the four position categories where in the shelter yielded zero points, peeking gave 0.5 points, and in the middle and at the edge of the aquarium were worth one point. Points from every hour were totaled for each crayfish.

I quantified data collected from the shelter aggression experiment by totaling the number of times that the observed crayfish performed an action. These totals were then grouped into two categories. Persistence included approaching the shelter with and without an occupant as well as entering the shelter with and without an occupant. Aggression was defined as performing a meral spread, chela strike, or initiating an attack.

All statistics were done using MYSTAT. I performed chi-square test in order to confirm crayfish infection success in addition to a chi-square test to examine infection rates of males versus females. Shelter use was examined via a two-sample t-test for both crayfish experimentally infected and infected crayfish from Plum Lake. Two-sample t-tests compared infected and uninfected crayfish in both shelter seeking and aggressive behavior. A chi-square test determined differences in shelter seeking behavior.

Results

Infection

This study showed that the method that I used to infect rusty crayfish with *Microphallus sp*. is effective for small-scale laboratory infections. A chi-square test determined that the proportion of infected crayfish to uninfected crayfish was higher in the experimental aquarium compared to the control aquarium (χ^2(N=61)=24.71, p<0.001). Infection rates of males and females, however was an non-significant trend (χ^2(N=61)=2.379, p=0.123).

Shelter Use

There was no difference in shelter use between infected and uninfected crayfish. Infected and uninfected crayfish occupied shelters at the same frequency between infected and uninfected crayfish (t(22)=0.406, p=0.689). Statistical analysis could not be performed on crayfish from Plum Lake due to lack of data variance. No tested uninfected crayfish yielded any shelter points where infected crayfish had on average 0.2±0.153 points.

Shelter Aggression

Results from the shelter aggression experiments show no statistical significance in behavior between infected and uninfected rusty crayfish, though I did observe non-significant trends. Infected and uninfected crayfish shelter-seeking behavior was compared through a one-way ANOVA comparing laboratory infected crayfish with uninfected crayfish and crayfish that were exposed to cercariae, but were found to be uninfected (Figure 1). Separating both of these categories of uninfected crayfish gave potential to see possible differences in uninfected crayfish behavior between the two weeks of trials. The results of this test showed no statistical significance ($F_{2,27}$=3.16. p=0.058), but there was a trend in differences between infected and uninfected crayfish where uninfected crayfish executed more shelter-seeking actions. Tukey's test showed that crayfish not exposed to cercariae were significantly more shelter-seeking than laboratory infected crayfish (p=0.0478). A two-sample t-test comparing all infected and uninfected laboratory crayfish showed that infected crayfish were less shelter-seeking than infected crayfish (t(28)=-2.273, p=0.031). In addition, two-sample t-test comparing all infected and uninfected crayfish observed in the second week of trials showed no difference in shelter-seeking behavior (t(33)=-1.256, p=0.218) (Figure 3).

There was no difference in aggressive behavior between infected and uninfected *O. rusticus*. A two-sample t-test comparing all laboratory uninfected crayfish to laboratory infected crayfish resulted in no significance (t(28)=0.343, p=0.734) (Figure 2). When the uninfected crayfish were separated by the aquarium they occupied in order to determine if the time tested influenced aggression, a one-way ANOVA showed a trend in aggression ($F_{2,27}$=2.586, p=0.094). All infected and uninfected crayfish examined during week two of experimental trials were compared via two-sample t-test, which showed that infected crayfish displayed more aggression (t(33)=2.06, p=0.047) (Figure 3).

Discussion

This study showed that *O. rusticus* infected with *Microphallus sp*. exhibit differences in shelter-seeking behavior, but not shelter use or aggression. Shelter-seeking behavior was significantly lower in laboratory infected crayfish, but not compared to uninfected crayfish that were observed during the second week of trials. Uninfected crayfish were consistently approaching and entering the shelter with or without an occupant, regardless of whether or not they were the dominant crayfish. This shows that there is a decreased shelter-seeking behavior in laboratory infected crayfish (Figure 1). These results, however, are not consistent with what I observed with infected *O. rusticus* collected from the field. All crayfish that were examined in July showed no difference in shelter-seeking behavior. Instead, these crayfish were significantly more aggressive (Figure 3). In addition, crayfish examined in June were more active than individuals observed in July.

Previous studies have shown that there is a difference in activity in the crayfish *Cambarus latimanus*. As the temperature increased in non-breeding seasons, agonistic behavior decreased (Thorp 1978). This is consistent with our raw counts in that both laboratory infected crayfish and infected and uninfected crayfish from the field were less active than uninfected crayfish that were examined three weeks prior. During the weeks I conducted trials, I observed highs between 14˚C and 18˚C in the first trial week in June, but highs ranging from 21˚C to 32˚C in the second trial week during July (The Weather Channel Monthly Planner for Minocqua, WI). This variation could have possibly affected the crayfish activity levels during these testing weeks as well as between these periods of time. In order to amend this variation, I compared behaviors between infected and uninfected individuals that I observed during the same week, which shows no difference in shelter-seeking behavior, but a difference in aggression such that infected rusty crayfish are more aggressive than uninfected individuals.

These results suggest that Microphallus has different effects on *O. rusticus* than *O. propinquus*. Towle (unpublished) found that uninfected northern clearwater crayfish were found in shelters in the presence of a conspecific more frequently than *O. propinquus* infected with *Microphallus sp*. This may suggest that *Microphallus sp*. has different effects on the two species found in northern Wisconsin, which may explain how rusty crayfish have displaced the other crayfish species. Both *O. propinquus* and *O. rusticus* have been shown to have hierarchal behaviors (Fero and Moore 2007 and Fero et al. 2006). This confirms that there is a difference in *O.propinquus* and *O. rusticus* behavior when infected with *Microphallus sp*. These behavior changes would expose more native individuals to predation, lowering their populations, while rusty crayfish would remain unaffected.

This study also investigated the effects of controlled infections in addition to crayfish infected before collection. While the method used in the laboratory showed to be effected in infecting rusty crayfish, infections were at minimal levels with most crayfish only having one metacercariae in their hepatopancreas. In addition, these crayfish were only infected for at the most 18 days, which may not be sufficient time for Microphallus individuals to take effect on their host’s behavior. At the same time, these infected crayfish have a relatively controlled infection rate, however small. Exposure times are known and the resulting infection levels were almost all the same, which is not the case considering crayfish that are collected from the field having already being exposed. While crayfish from the field are infected at higher and varying levels, there is no way of knowing how infected these individuals are or how long they have been infected. This gives variation that may add complications to research investigating the effects of Microphallus sp. on its host.

Future studies could therefore investigate more effective methods of Microphallus infection. Increased ratios of *A. limosa* to *O. rusticus* within aquaria may help to infect more crayfish in addition to infecting at higher levels. In order to ensure that Microphallus have fully developed into metacercariae within the hepatopancreas, crayfish would require more time after exposure than what was given in this experiment. This might also give higher percentages of infected crayfish. The method of infection used in this study was effective, but would need to be adjusted to accommodate for higher and more developed infections. With these controlled infections,

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Figures

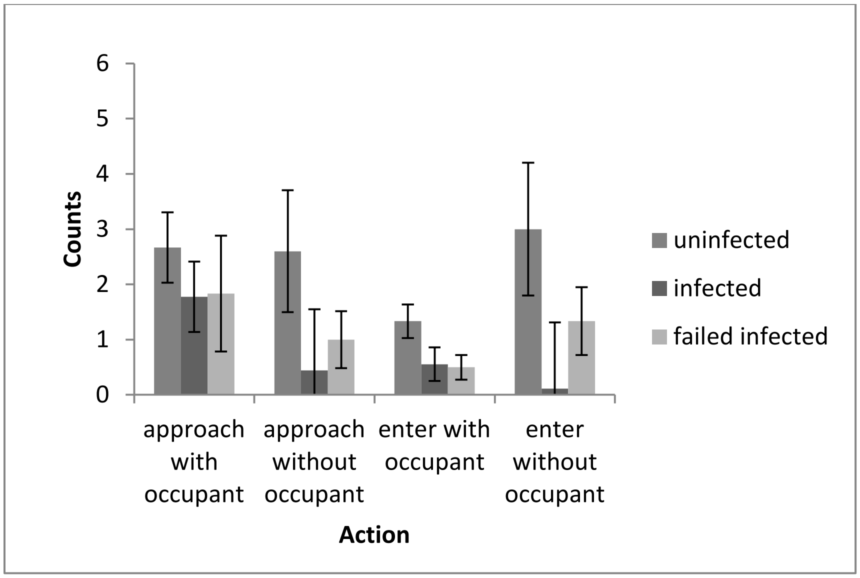

Figure 1. Shelter seeking behavior of laboratory crayfish. ANOVA showed a trend in shelter seeking behavior ($F_{2,27}$=3.16. p=0.058) and post-hoc Tukey test showed significance between uninfected and infected crayfish where uninfected crayfish were more shelter-seeking (p=0.0478). In addition, uninfected crayfish were more shelter-seeking compared to all infected laboratory crayfish and uninfected crayfish (t(28)=-2.273, p=0.031)

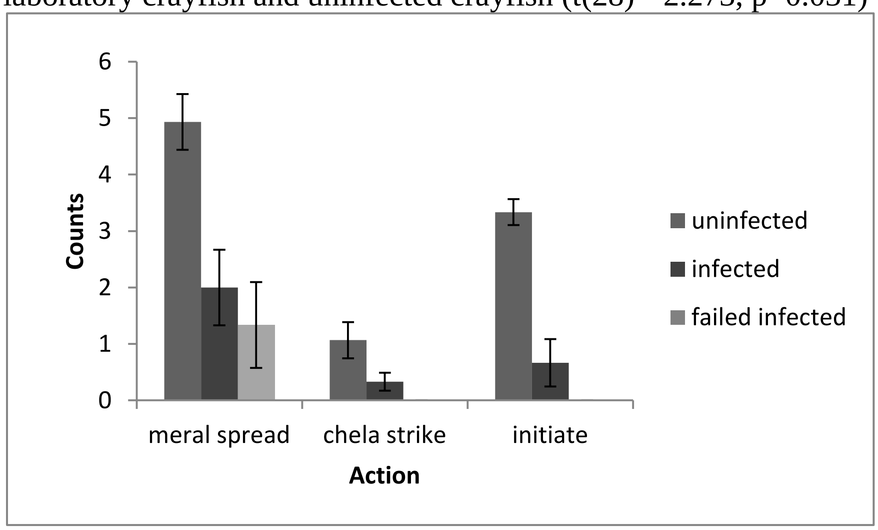

Figure 2. Aggressive behavior of laboratory crayfish. No significance was found through a two-sample t-test combining all uninfected crayfish (t(28)=0.343, p=0.734) nor was there a difference between all three categories of crayfish ($F_{2,27}$=2.586, p=0.094).

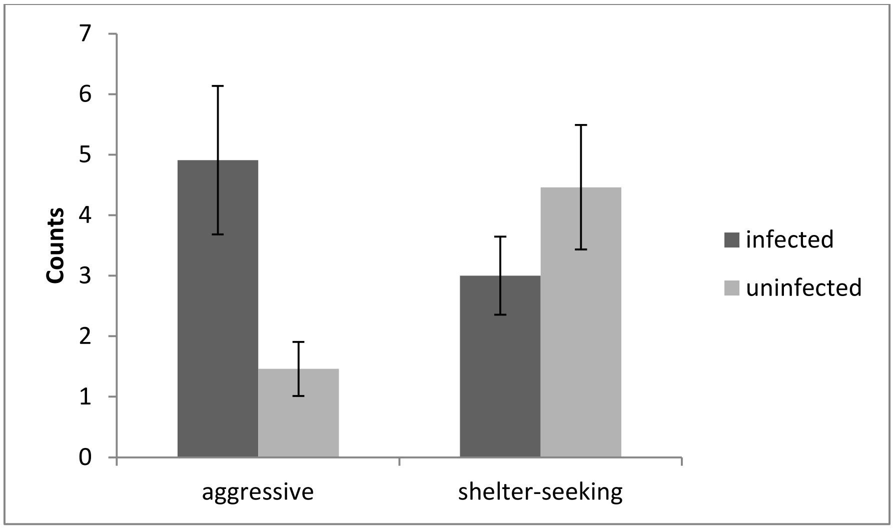

Figure 3. Aggressive and shelter-seeking behavior of crayfish observed in July. Two-sample t-tests showed no difference in shelter-seeking actions between infected and uninfected crayfish (t(33)=-1.256, p=0.218) but infected crayfish were significantly more aggressive than uninfected individuals (t(33)=2.06, p=0.047).