Microphallus spp. Effects on Orconectes Rusticus Feeding Behavior

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Abstract
The rusty crayfish (Orconectes rusticus) is an invasive species that has dominated the lakes of Northern Wisconsin and other waterways. The rusty crayfish is native to the Ohio River basin and its dispersal is attributed to poor fishing practices. This crayfish species has negative effects on the submerged macrophyte species of lakes, in turn reducing the abundance and richness of macroinvertebrates and some species of fish. A trematode parasite, Microphallus spp., has been shown to affect the natural behavior patterns of rusty crayfish. This study investigates whether infection with Microphallus spp. affects the feeding behavior of the rusty crayfish. Rusty crayfish were collected from infected and non-infected sites and dissected to confirm the infection statuses reported in previous years. More specimens were collected from the confirmed infected and uninfected sites and were used in behavioral experiments. Crayfish were placed in a controlled environment with six prey items (live mayflies) for thirty minutes to determine their feeding behavior. Analysis showed significant results: infected crayfish on average ate three times less often than uninfected crayfish, indicating that the parasite likely affects the feeding behavior of the rusty crayfish in a negative manner, reducing its consumption. This behavioral change could be attributed to chemical signals released by the trematode parasites that alter the behavior of the rusty crayfish.

1.0 Introduction
Parasites have traditionally been understudied by ecologists and their effects are often challenging to study and understand. For example, the Toxoplasma parasite has been studied in over 15,000 research articles, 500 reviews and several books; yet according to Astrid Tenter [1] “there are still many aspects of its biology, natural life cycle, and the epidemiology of T. gondii infections of which we know relatively little.” It took scientists over sixty years to comprehend T. gondii’s full life cycle after it was first described in 1908, which demonstrates how much time and effort it takes to understand the effects and life history of a parasite [1]. Parasitic interactions with hosts often lead to behavioral changes in the infected hosts [2]. T. gondii is currently under study for its affect on human reaction time, tendency for accident, behavior changes, and even mental illness [3]. These effects demonstrate the importance of parasitology and understanding parasitic effects on host species.

The rusty crayfish (Orconectes rusticus) is an invasive species of crayfish that is displacing the native virile crayfish (Orconectes virilis) in the northern temperate lakes of Wisconsin [4]. In the past 50 years, the rusty crayfish has spread from its native habitat in the Ohio River drainage to many water ways in Illinois, Michigan, Wisconsin, Minnesota, and parts of 11 other states [4]. Rusty crayfish can reduce macrophytes by as much as 80% through direct predation as they invade northern temperate lakes [5]. In addition, native virile crayfish are often extirpated following the introduction of rusty crayfish, and the impact of rusty crayfish on macrophytes also leads to reductions in the populations of some fish [5].

The crayfish of northern Wisconsin and the UP of Michigan are infected by trematode parasites (Microphallus spp.). Infection causes the rusty crayfish to have higher aggression levels than virile crayfish, which may increase ability of the rusty crayfish to displace virile crayfish [6]. The infected crayfish must be consumed by a predator for the trematode parasite to complete its lifecycle [7]; therefore, the parasite should af-
fect the crayfish in a way that promotes its consumption. For example, Sargent et al. [6] found that infection with Microphallus increased the boldness of rusty crayfish in the presence of fish predators, likely increasing the predation rate of infected individuals in the field.

Sargent, et al [6] determined that infected rusty crayfish were less inclined than uninfected individuals to hide when in the presence of predatory fish. By allocating less time to hiding, infected rusty crayfish can attribute more time to foraging. This study tests the hypothesis that when a predator cue is present, infected crayfish will consume more prey than uninfected crayfish. If this hypothesis is supported, it would suggest that parasitized individuals would be more vulnerable to predation because they spend more time consuming prey, but they may also have a fitness benefit in terms of growth and reproduction because of increased food intake. This study will provide information about how infection alters rusty crayfish impacts, and may allow managers to better predict rusty crayfish impacts in lakes with and without Microphallus parasites.

2.0 Methods

For this experiment, it was necessary to collect both infected and uninfected rusty crayfish. To confirm the infection status of previously studied populations, lake surveys were performed by hand in which varying sizes of males were caught from lakes that had infection levels previously described by Sargent [7]. These specimens were preserved in ethanol and brought back to the lab for dissection. To dissect the crayfish, the cephalothorax was gently pulled back from the chelipeds and walking legs to expose the hepatopancreas. This organ was then viewed under a dissecting microscope between two glass slides and the trematode cysts present were counted or a lack of cysts was noted.

Lake Ottawa is in the Upper Peninsula of Michigan near Iron River, MI. This lake was chosen due to its partial infection status, where one side of the lake contains Microphallus parasites and the other side does not. Due to the duel nature of this lake, we were able to collect both infected and uninfected individuals on the same day (6/17/14), with site one (46.0882, -88.7636) containing uninfected and site two (46.0768, -88.7728) containing infected individuals. The other two lakes utilized for collection are located in Northern Wisconsin and are named Star and High Lake. Star Lake (46.0222, -89.4720) is located near Sayner, WI and contains uninfected individuals. High Lake (46.1497, -89.5472) is south of Boulder Junction, WI and contains moderate infection levels. These lakes were sampled on the same day (7/7/14).

These four sites were chosen to collect the rusty crayfish for the behavior study, and a minimum carapace length of 26 mm was set to certify that the infection status would be consistent within each site. Thirty rusty crayfish from each site were collected with a goal of twenty behavioral trials per site - more crayfish were collected than necessary in case individuals were lost in transit or damaged by other crayfish. Collections took place at an infected and uninfected site, one to two days prior to the behavioral experiments so that the crayfish would have fasted for 24 to 48 hours before the trials.

The experiment was set up on the shore of Big Lake, a lake in Northern Wisconsin adjacent to the UNDERC research property, because rusty crayfish cannot be brought onto UNDERC property due to their invasive nature. For the behavioral experiments, two tanks (60 cm long x 50 cm wide x 35 cm deep) were set beside each other and filled with approximately 10 cm of lake water. Lake water was collected from Big Lake, which contains abundant fish that prey on crayfish, such as smallmouth bass, largemouth bass, and rock bass; therefore, some fish cues were expected to be present during the trials. Each tub received sand sprinkled on the bottom to replicate crayfish habitat. Six mayflies were placed into each tub followed by an infected crayfish in one tub and an uninfected crayfish in the other. The experiment proceeded for thirty minutes, and then the crayfish were removed from the tubs and thoroughly checked for mayfly hitchhikers. Following each experiment, the specimen’s carapace length was measured and its sexual form recorded. The crayfish were then placed in their own ethanol bags and labeled with their site and trial number. The number of mayflies the crayfish consumed was recorded and the bins were replenished with new mayflies for the next experiment.

A total of 68 behavioral experiments were completed, 20 from both Lake Ottawa sites, eleven from Star Lake, and seventeen from High Lake. Star Lake experiments were limited due to high crayfish mortality in transit. After all trials were completed, the crayfish were preserved in...
ethanol and dissected to confirm their infection status and the number of parasites present in each crayfish was recorded.

### 2.1 Data Analysis

To determine whether infected or uninfected populations ate more often, a chi-squared test was performed, analyzing the determined infection status versus the number of mayflies consumed. To ascertain if the distributions varied for amount of mayflies eaten between infected and uninfected populations, a non-parametric Kruskal-Wallis test was executed.

### 3.0 Results

Following the dissections, analysis showed that seven crayfish of the 37 from infected sites were uninfected specimens. Two of these specimens were from Lake Ottawa and the other five were from High Lake. These specimens were counted with the uninfected data resulting in a total of 38 uninfected and 30 infected specimens. Twenty-three of the 38 uninfected specimens consumed at least one or more mayflies during the trials, equating to 61% of the uninfected specimens eating. Just 20% of the infected specimens consumed one or more mayflies with only six of the 30 eating (Figure 1).

The average carapace length of the uninfected population was 31.8 mm, with a standard error of 0.5 mm. The infected populations measured slightly larger with an average of 33.2 mm and a standard error of 0.7 mm. Uninfected crayfish ate significantly more often than infected crayfish (chi-squared test: chi-squared = 9.66, P = 0.0019). In addition, uninfected crayfish ate significantly more mayflies than infected crayfish (Kruskal-Wallis test: chi-squared = 11.03, p = 0.0009; see Figure 2).

### 4.0 Discussion

The data did not support the original hypothesis that the infected populations would eat more mayflies than uninfected populations because they spent less time fearing fish predator cues. The results actually pointed to the opposite relationship between infection with Microphallus and crayfish feeding. In fact, the uninfected individuals ate over three times as often when compared to infected individuals. These results were unanticipated, but with such little knowledge of the Microphallus parasites, it is easy to misconceive their effects. It is possible that the lake water did not contain enough fish chemical cue to cause differences in boldness behavior. Future research could test the feeding rate of infected and uninfected crayfish with a predator actually present in the tank, perhaps behind a screen. The infected crayfish were, on average, larger than the uninfected individuals used in this study, but that did not seem to affect the results. If anything, the larger individuals would be expected to eat more prey, so more equal populations would have provided even more significant results.

A similar study conducted by Sargent [7] found a significant reduction in feeding behavior in crayfish that were experimentally infected. The results of Sargent’s study [7] mirror this study, and it concluded that the reduction in feeding could be attributed to two varying causes. The crayfish host is either responding to the infection as a sickness and therefore its normal behavior patterns are being disrupted, or the parasite is manipulating the crayfish’s behavior in a way that will allow the parasite to move into its definitive host [7].

These results could be interpreted in many ways, but most likely, the parasite is directly interfering with the rusty crayfishs’ survival functions, similar to how the Toxoplasma parasite is implicated in compromising human tendency for accidents [3]. One possible reason for this would be the prey item chosen in this study. The Microphallus parasite needs the crayfish to be consumed so that it can move further up the food chain and complete its life cycle [7]. With this thought process, it would not be advantageous to have the crayfish die from starvation, but it would be beneficial to drive the crayfish away from certain prey items or habitats. The mayfly larvae used in this experiment were all found clinging to the underside of stones submerged in lakes. It would be beneficial for the Microphallus parasite to drive crayfish predation away from rocky habitats and toward open vegetation where they are more likely to be preyed upon. A similar interaction is documented in pill bugs infected with Acanthocephalan parasites, where uninfected individuals show a negative phototaxis and infected individuals display a positive phototaxis [8]. This leads to an un-proportional consumption by predators towards infected individuals.

Another possible explanation is that the Microphallus parasite causes the rusty crayfish to
mindlessly wander, leaving it open to fish predation. This type of parasitic behavioral change can be related to the fungal parasite, *Ophiocordyceps unilateralis*, also known as the “zombie fungus”, which causes ants to wander aimlessly through the forest until they encounter a suitable habitat for the fungus to grow. The fungus then causes the ant to latch its mandibles onto a branch or leaf stem just before the fungus kills its host and sprouts from its corpse [9]. While the Microphallus does not seem to affect crayfish in this extreme of a manner, it is not a far leap to presume that the Microphallus parasite could have developed mechanisms that alter the host’s natural thought process and cause the crayfish host to behave in abnormal ways.

**5.0 Conclusions**

Evidence shows that the Microphallus parasite is detrimental to rusty crayfish populations, meaning that it could be a vital tool for lake management against the rusty crayfish invasion. This parasite not only seems to reduce rusty crayfish populations [7], but may also reduce rusty crayfish impacts leading to increased macrophytes, macroinvertebrates, and fish populations in infected lakes. The infection may also cause a positive feedback loop where higher infection leads to less predation on snail hosts for the parasite, which in turn leads to increasing parasite prevalence.

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References


