Microphallus spp. Effects on *Orconectes rusticus* Feeding Behavior

BIOS 569: Practicum in Field Biology

Kraig Esswein

Advisor: Lindsey Sargent

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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 since its release from its native habitat in the Ohio River basin. 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 rusty crayfish's natural behavior patterns. I investigated whether or not 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 that 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 their consumption. This

behavioral change could be attributed to chemical signals released by the trematode parasites that alter the behavior of the rusty crayfish.

Introduction

Parasites have traditionally been understudied by ecologists, and their effects are often challenging to study and understand. Take the Toxoplasma parasite for example; over 15,000 research articles, 500 reviews and several books are devoted to it yet according to Astrid Tenter "there are still many aspects of its biology, natural life cycle, and the epidemiology of T. *gondii* infections of which we know relatively little" (Tenter 2000). 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 (Tenter 2000). Parasitic interactions with hosts often lead to behavioral changes in the infected hosts (Dobson 1988). T. *gondii* is currently under study for its affect on human reaction time, tendency for accident, behavior changes and even mental illness (Dubey, 2008). These effects demonstrate the importance of parasitology and of understanding of 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 (Olsen et al. 1991). 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 (Olden et al. 2005). Rusty crayfish can reduce macrophytes by as much as 80% as they invade north temperate lakes (Wilson et al. 2004). 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 fishes (Wilson et al. 2004).

The crayfish of the north woods region are infected by trematode parasites (*Microphallus* spp.). Infection causes the rusty crayfish to have higher aggression levels than virile crayfish, which may increase the rusty crayfishes ability to displace virile crayfish (Sargent et al. in prep 2013). The infected crayfish must be consumed by a predator for the trematode parasite to complete its lifecycle (Sargent et al. 2014), therefore the parasite should affect the crayfish in a way that promotes its consumption. For example, Sargent et al. (in prep) 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 (in prep) determined that infected rusty crayfish were less inclined to hide when in the presence of predatory fish than uninfected individuals (Sargent et al. 2013). By allocating less time to hiding, infected rusty crayfish can attribute more time to foraging, implicating the trematodes in a relationship between infection status and rusty crayfish impacts through consuming macrophytes and macroinvertebrates. In this study, I test the hypothesis that when 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.

Collection Sites

Crayfish collection took place at two sites on Lake Ottawa in the Upper Peninsula of Michigan. Collection also took place at one site on High Lake and one site on Star Lake, which are both located in Northern Wisconsin. Coordinates for Lake Ottawa site one are (46.0882, -88.7636) and site two are (46.0768, -88.7728). Coordinates for High Lake are (46.1497, -89.5472). Coordinates for Star Lake are (46.0222, -89.4720).

Materials and Methods

For this experiment, I needed to collect both infected and uninfected rusty crayfish. To confirm the infection status of previously studied populations I performed lake surveys, hand catching varying sizes of males from lakes with infection levels previously described in Lindsey Sargent's papers. I preserved these specimens in ethanol and brought them back to the lab for dissection. To dissect the crayfish, I gently pulled the cephalothorax back from the chelipeds and walking legs to expose the hepatopanceas. I viewed this organ under a dissecting microscope between two glass slides and counted the trematode cysts present or noted a lack of cysts.

Based on these dissections, I determined that all specimens from Lake Ottawa site one and Star Lake were not infected with the trematode parasite and that all specimens with a carapace length of 25 mm or higher from Lake Ottawa site two and High Lake were infected. I chose these four sites to collect the rusty crayfish for my behavior study, and set a minimum carapace length of 26 mm to certify that the infection status would be consistent within each site. I collected thirty rusty crayfish from each site with a goal of twenty behavioral trials per site, the higher collection number providing a cushion for any individuals lost in transit or damaged by other crayfish. Collections took place at two sites, 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 behavioral experiment set up consisted of two tanks (60 cm long x 50 cm wide x 35 cm deep) 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, I expected some fish cue to be present during the trials. Each tub received sand sprinkled on the bottom to replicate crayfish habitat. I placed six mayflies 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 I took the crayfish out of the tubs and thoroughly checked them for mayfly hitchhikers. Following each experiment I measured the specimens carapace length and recorded their sexual form. I then placed the crayfish in their own ethanol bags and labeled them with their site and trial number. After this I recorded how many mayflies the crayfish consumed and replenished the bins with new mayflies to perform the next experiment.

I completed a total of sixty-eight behavioral experiments, twenty 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 causing a need to recollect. After I completed all trials, I preserved the crayfish in ethanol and dissected each crayfish to confirm their infection. I also recorded the number of parasites present in each crayfish.

To determine whether infected or uninfected populations ate more often I performed a chi-squared test. To ascertain if the distributions varied for amount of mayflies eaten between infected and uninfected populations I executed a non-parametric Kruskal-Wallis test.

Results

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

Analysis revealed that the average carapace length of the uninfected population measured at 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: χ^2 = 9.66, P = 0.0019). In addition, uninfected crayfish ate significantly more mayflies than infected crayfish (Kruskal-Wallis test: of χ^2 = 11.03, p = 0.0009; Figure 2).

Discussion

My data did not support my original hypothesis that the infected populations would eat more mayflies than uninfected populations because they spend less time fearing fish predator cues. Very significant 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 effects of the *Microphallus* parasite 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 Lindsey Sargent found a significant reduction in feeding behavior in crayfish that were experimentally infected (Sargent et al. 2014). The results of this study mirror that of my own 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 crayfishes behavior in a way that will allow the parasite to move into its definitive host (Sargent et al. 2014).

These results could be interpreted in many ways but the most likely is that the parasite is directly interfering with the rusty crayfishes survival functions similar to how the Toxoplasma parasite is implicated in compromising human tendency for accidents (Dubey 2008). One possible reason for this would be the prey item chosen in this study. The *Microphallus* parasite's end goal is to get the crayfish consumed so that it can move further up the food chain (Sargent et al. 2014). 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. 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 towards open vegetation where they are more likely to be preyed upon.

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 behavior 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 (Anderson et al. 2009). While the *Microphallus* does not seem to affect crayfish in this extreme of a manner, it is not a far leap to presume that it could have developed mechanisms that alter the hosts natural thought process and cause the crayfish host to behave in abnormal ways.

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 (Sargent et al. 2014), 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 feed back 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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Figures

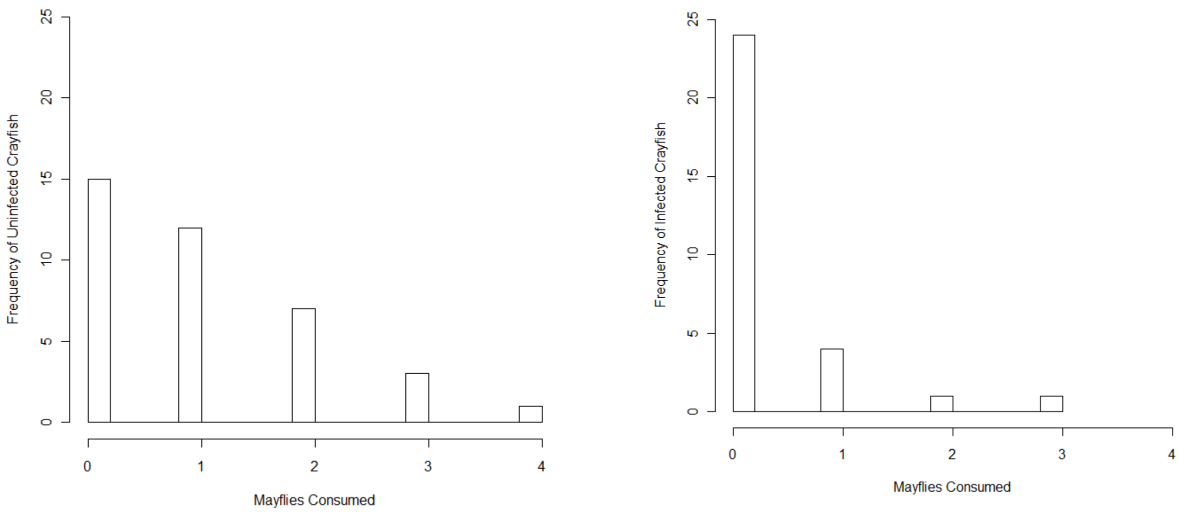

Figure 1. Histograms showing the frequency of crayfish consuming 0, 1, 2, 3, or 4 mayflies. On the left is the uninfected crayfish and the right is infected crayfish. Analysis showed that uninfected crayfish ate significantly more frequently than infected crayfish.

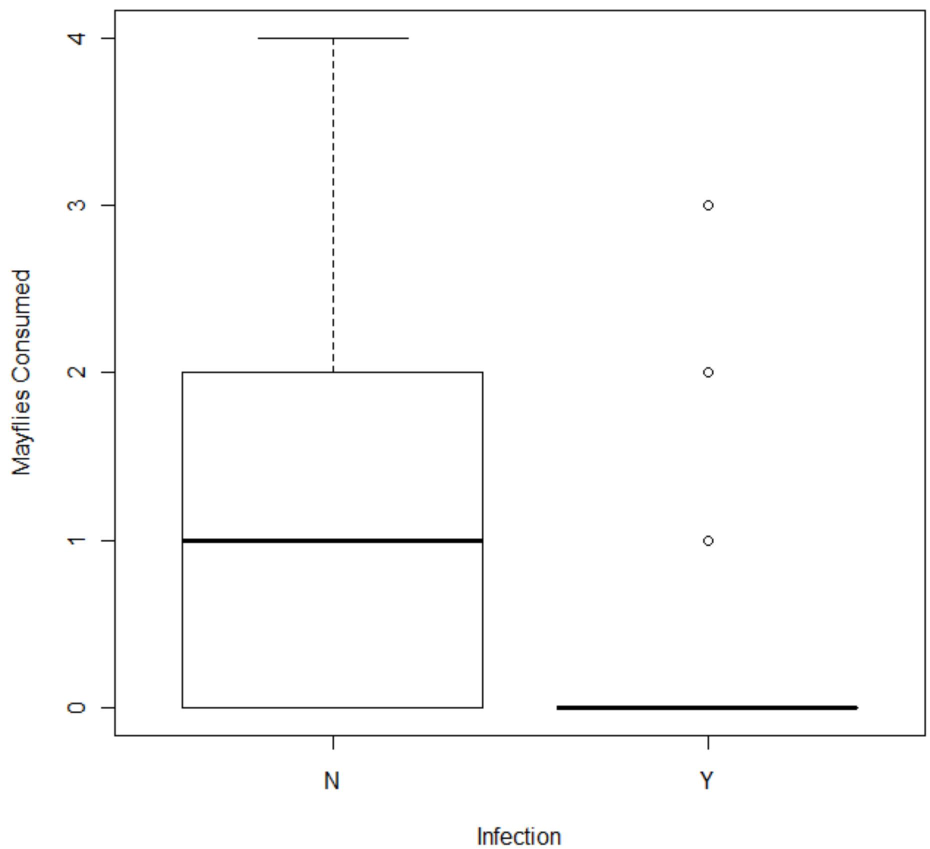

Figure 2. Boxplots of the infected (Y) and uninfected (N) results, clearly distinguishing that the uninfected consumed mayflies more often on average and that the infected specimens that did consume mayflies were outliers.