Comparing the Susceptibilities to *Microphallus* sp. Infection between a Native Crayfish Species (*Orconectes virilis*) and an Invasive Crayfish Species (*Orconectes rusticus*)

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ABSTRACT

Invasive species, such as rusty crayfish (*Orconectes rusticus*), can threaten the fitness and survival of native species in aquatic ecosystems. In northern Wisconsin, *O. rusticus* out-competes the native virile crayfish (*Orconectes virilis*) for shelter and food resources. The trematode parasite *Microphallus* sp. infects both *O. rusticus* and *O. virilis*. While *Microphallus* sp. is suspected to reduce *O. rusticus* populations and negatively affects behavior in crayfish closely related to *O. virilis*, the relative infection levels between the two species has not been studied. This study examines the difference in *Microphallus* sp. infection level between *O. rusticus* and *O. virilis*. I conducted experimental infections by exposing paired crayfish in one-species and two-species treatments to *Microphallus* sp. cercariae, the larval form of trematodes, collected from *Amnicola limosa* snails. *O. virilis* that were exposed to cercariae were more likely to be infected than those that were not exposed, but there was no difference between experimental and control *O. rusticus*. In addition, experimental infection resulted in significantly higher infection levels in *O. virilis* compared to *O. rusticus*, suggesting *Microphallus* sp. is more effective at infecting the native species than the invasive species. These findings are likely the result of parasite-host coevolution, in which the parasite has had more time to develop adaptations for infecting the native host species. Because *O. rusticus* out-competes *O. virilis*, the fact that *O. virilis* has the additional burden of higher susceptibility to *Microphallus* sp. may accelerate *O. rusticus* invasion.

INTRODUCTION

Invasive species can threaten native species within a community, particularly when competing for the same resources in insular environments. In northern Wisconsin lakes, the invasive species *Orconectes rusticus* has replaced a previous crayfish invader, *Orconectes propinquus* (northern clearwater crayfish), and the true native species *Orconectes virilis*, as the most dominant member of crayfish fauna (Olden *et al.* 2006). The aggressive behavior, rapid growth, and high population densities of *O. rusticus* help secure preferential access to limited crayfish food items such as gastropods, algae, and macrophytes (Klocker and Strayer 2004). At the ecosystem level, the destruction of macrophytes by *O. rusticus* may have the most significant repercussions, altering food webs, trophic interactions, and habitat structures in the relatively unproductive northern lakes (Lodge *et al.* 2000). The widespread consequences of the *O. rusticus*

invasion have prompted scientists to consider the use of a trematode parasite belonging to the genus *Microphallus* as a method of biocontrol.

Microphallus sp. is known to infect both native crayfish and *O. rusticus*. Though the species of this parasite remains unidentified, it is closely related to *Microphallus opacus* (Overstreet *Personal Communications*). The life cycle consists of three stages, beginning in the primary snail host *Amnicola limosa* (Overstreet *Personal Communications*). Snails shed cercariae, the larval stage, into the water, which enter the crayfish and travel to the hepatopancreas where an infection marked by metacercariae, the asexual stage, develops (Caveny and Etges 1971). The final host, though still unidentified for this species of *Microphallus*, then ingests the infected crayfish and the adult trematode attaches to its intestinal wall. Eggs released in the feces of the final host hatch into larvae that infect snails to complete the cycle.

Few prior studies have examined the effects of *Microphallus* sp. on the fitness and survival of *O. rusticus*, *O. virilis*, or *O. propinquus* individually. Roesler (2009) found that the parasite was abundant in lakes with small *O. rusticus* populations and rare in lakes with large *O. rusticus* populations, suggesting that the parasite may impact *O. rusticus* abundance. However, high densities of O. rusticus are known to reduce densities of *A. limosa*, which would lead to lower infection levels of rusty crayfish in lakes with high densities of crayfish even if the parasite has no effect on crayfish fitness. Therefore, because of the complex dynamics between *O. rusticus* and *A. limosa*, the impact of *Microphallus* sp. on *O. rusticus* populations cannot be assessed using snapshot abundances of the crayfish and parasite. Data suggests that *Microphallus* sp. also affects crayfish behavioral interactions since uninfected *O. propinquus* out-compete infected *O. propinquus* for shelter (Towle *Unpublished data*). The relative susceptibilities of

native versus invasive crayfish to *Microphallus* sp., however, have yet to be investigated. Such a study would be particularly informative given the disadvantages *Microphallus* sp. confers to its crayfish hosts and the intimate competitive interaction between *O. rusticus* and *O. virilis*.

Under the Red Queen Hypothesis, the phenomenon of coevolution is a driving force in parasite-host interactions (King 2011). Because parasites and their hosts exert selective pressures on each other, rapid reciprocal adaptations are common. Hosts are constantly developing behavioral defense mechanisms and building up immune system responses to fend off infection. Simultaneously, parasites develop adaptations to increase transmission rate and infection level. This often results in highly-specific host selection among parasites. For example, the coccidian parasite *Isospora felis* is highly evolved to the infection of cats exclusively (Dubey 2009).

Consistent with coevolution, prior studies have reported differences in parasitic infection levels between native and invasive hosts. One study found that an introduced cichlid fish species had both lower parasite species richness and abundance compared with its native competitor (Roche *et al.* 2010). Similarly, a study of amphipod crustaceans revealed a higher prevalence of parasites in the native species than in the invading species (Dunn *et al.* 1998). If *Microphallus* sp. is host-specific and therefore more effective at infecting native host species, higher infection levels in *O. virilis* over *O. rusticus* would be expected. Alternatively, it is also possible that the invasive hosts exhibit higher infection rates because they lack the defenses against the parasite the native hosts may have adapted. One multigenerational study, for example, found that *Microphallus* sp. is more infective to snails exposed to the parasite during the second generation compared to those exposed during the first generation; this indicates the snails are able develop parasite-mediated adaptations to evade infection over time (Koskella 2006). If crayfish hosts are also able to adapt defenses to *Microphallus* sp., higher infection

levels in *O. rusticus* over *O. virilis* would result. The opposing but equally plausible possibilities of higher *O. virilis* infection levels versus higher *O. rusticus* levels warrant further investigation to determine which case actually prevails.

In this study, I experimentally infected *O. rusticus* and *O. virilis* from lakes on the University of Notre Dame Environmental Research Center (UNDERC) property with *Microphallus* sp. cercariae. I postulated that there would be a difference in parasite susceptibility between the two species. Findings may provide insights regarding the role of *Microphallus* sp. role in the competitive relationship between *O. rusticus* and *O. virilis*, which could in turn help predict future population trends to make informed management decisions.

METHODS

Crayfish Collection

I hand-collected 60 *O. rusticus* crayfish from Big Lake and 60 *O. virilis* crayfish from Plum Lake. Crayfish carapace length ranged from 14 to 28 mm, with a mean length of 20 mm. I initially dissected ten crayfish of each species to confirm that the majority of crayfish under 28mm in carapace length were uninfected. After experimentation, an additional 26 *O. rusticus* from Big Lake and 58 *O. virilis* crayfish from Plum and Ottowa Lakes were dissected to obtain a more accurate representation of control infection levels related to carapace length. To dissect, I removed the chelae, legs, and carapace to expose the hepatopancreas within the body cavity. Infection was characterized under a 35X dissection microscope by the presence of 0.5-0.6mm-long, white and translucent metacercariae in the hepatopancreas (Figure 1). I stored crayfish in 10-gallon aquaria with PVC pipe shelter and about four inches of aerated lake water.

Treatment Groups

I divided 40 crayfish of each species into three treatment groups: 1 *O. rusticus* with 1 *O. virilis*; 2 *O. virilis*; and 2 *O. rusticus*. Each pair of crayfish was the same sex and within 2mm of the same carapace length. The first treatment had 20 replicates and the second and third treatments had ten replicates each. The purpose of having two rather than one crayfish of the same species per container for the second and third treatments was to ensure that crayfish surface area was consistent with that of the first treatment. For each treatment, I placed two crayfish in a 1.89L Tupperware container with about 2 inches of well water, gravel substrate, and PVC pipe shelter. Well water was aerated beforehand for roughly 30 minutes and ranged in temperature from 11 to 16 degrees Celsius. I fed crayfish two shrimp pellets twice a week.

Experimental Infection

I hand-collected about 1000 *A. limosa* snails from Big Lake and High Lake. I incubated these snails individually in shallow glass vials filled with about 1mL of well water. After placing the vials under 100-watt fluorescent light for one hour to prompt snails to shed cercariae, I examined them under a 35X dissecting microscope (Fredensborg 2004). Because the *Microphallus* species of interest is known to be closely related to *Microphallus opacus*, we identified clear-bodied cercariae according to the diagram of *M. opacus* found in Caveny and Etges (1971) (Figure 2).

Infected snails were isolated and kept in an aerated aquarium with aquatic plants until they were used for the infection experiment. For experimental infection, these snails were placed in individual Petri dishes with roughly 10mL of well water and exposed to a 100-watt fluorescent light for one hour between 8 and 9 am. Well water varied in temperature from 13 to 16 degrees Celsius and had been aerated beforehand. I then removed the snails, counted and recorded the number of cercariae per Petri dish, and dropped the dish into a glass jar filled with 100mL of

aerated cold well water. Between two and eight cercariae were added to each jar. An additional 60mL of water was added to jars with glass Petri dishes to account for additional displacement of water. Immediately after, I dropped a crayfish pair into the jar at the same time. After one hour, I removed the Petri dish, agitating it to remove any residual cercariae. After four hours, I returned the two crayfish and the water in the jar to their Tupperware container. In 20 days, I dissected crayfish to check for infection. For each crayfish, species, sex, carapace length, treatment group, and number of encysted metacercariae were recorded.

Second Experiment

I placed ten replicates of one *O. rusticus* paired with one *O. virilis*, both of the same sex and within 2mm of the same carapace length, in a 10-gallon aquarium. The aquarium contained eight to ten inches of aerated lake water, cobble for shelter, and a screened pouch of about 1000 *A. limosa* snails. A screened pouch of 26 confirmed infected snails was also added to the tank for the first two days of the experiment. A 100-watt fluorescent light was placed above the tank and came on for one hour in the morning and one hour in the afternoon daily. After 15 days, I dissected crayfish to check for infection and recorded the number of encysted metacercariae.

Statistical Tests

I performed an ANCOVA, linear regressions, and two one-way ANOVAs in SAS (Statistical Analysis System) and two chi-squared tests in SYSTAT to analyze the results. The ANCOVA determined whether the infection levels in crayfish differed between control and experimental treatments, taking carapace length into account. One regression demonstrated the relationship between carapace length and infection level in control crayfish. The second regression demonstrated the relationship between number of introduced cercariae and the number of encysted metacercariae for experimental *O. virilis* and *O. rusticus*. The first one-way

ANOVA test analyzed the results with infection level as the dependent variable and treatment/species as the independent variable. The second one-way ANOVA tested for a difference in infection levels between experimental *O. virilis* and *O. rusticus* across all experimental treatments. To determine whether the introduced cercariae infected crayfish, we used chi-square tests to analyze whether the proportion of infected to uninfected crayfish differed between control and experimental treatments.

RESULTS

A. limosa *Examination*

The clear-bodied cercariae identified during *A. limosa* examination were observed to have either jerk-like or inching motion. Out of the estimated 1000 *A. limosa* examined, 26 were infected, producing a 2.6 percent infection rate for collected *A. limosa*. Additionally, the 26 infected *A. limosa* were observed to shed consistent numbers of cercariae daily.

Infection Levels in Control O. virilis

A linear regression showed a statistically significant positive relationship ($F_{1,66}$= 5.750, R^2=0.0801, p = 0.0193) between carapace length and number of metacercariae in control *O. virilis* (N = 68) (Figure 3).

O. virilis *versus* O. rusticus *Infection Levels*

Many *O. virilis* collected from the field but not used in an infection experiment were found to be infected, but this was not true for *O. rusticus*. To take into account how infected *O. virilis* initially were, we calculated the mean number of metacercariae for each size class of control *O. virilis* and subtracted that value from the number of metacercariae for each experimental *O. virilis* in a corresponding size class. This allowed for an accurate comparison of

susceptibilities to *Microphallus* sp. between *O. rusticus* and *O. virilis* even though some *O. virilis* were likely already infected at low levels. We chose size classes with the criterion of having eight to 12 data points from individuals in the control group to obtain an accurate estimate. The size classes and corresponding mean number of metacercariae in control *O. virilis* were as follows: 15-20 mm (0.375), 20.5-22 mm (0.6), 22.5-23.5 mm (1.917), 24-25 mm (5.91), 25.5-26.5 mm (3.91), 27-29 mm (3.6). These corrected values were used for the *O. virilis* metacercariae in both ANOVAs. Only two out of 26 control *O. rusticus* were infected, so we did not correct for mean number of metacercariae in experimental *O. rusticus* (Callahan *Unpublished data*).

O. virilis in the two-species treatment (N = 14) had the highest mean number of metacercariae at 3.322 (±1.764), followed by *O. virilis* in the one-species treatment (N = 15) with a mean of 0.974 (±0.866) metacercariae (Figure 4). *O. rusticus* in the two-species treatment (N = 20) had a mean of 0.500 (±0.450) metacercariae, and *O. rusticus* in the one-species treatment (N = 19) had a mean of 0.0526 (±0.053) metacercariae (Figure 4). All values are reported as the mean ± standard error. My sample sizes for *O. virilis* were smaller than the numbers I started with because there were some mortalities. A one-way ANOVA also using corrected values for *O. virilis* metacercariae revealed a marginally significant difference ($F_{3,64}$ = 2.59; p = 0.0605) in infection level between *O. virilis* from the two-species treatment and *O. rusticus* in the one-species treatment. A second one-way ANOVA revealed a significant difference ($F_{1,66}$ = 4.337, p = 0.04117) in infection levels between experimental *O. virilis* (N = 29) and *O. rusticus* (N = 39) across all treatments(Figure 5).

Experimental Infection

An ANCOVA showed a marginally significant difference ($F_{2,94} = 2.62$, $p = 0.0784$) in *O. virilis* infection levels between control (N = 68) and experimental (N = 29) treatments, taking carapace length into account (Figure 6). No ANCOVA was performed for *O. rusticus* because the data did not have a linear relationship, and very few *O. rusticus* from the control group were infected.

A chi-square test revealed a statistically significant difference ($\chi^2(1, N=77)=5.680$, $p = 0.017$) in the proportion of infected to uninfected *O. virilis* between control (N = 49) and experimental (N = 30) treatments (Table 1). *O. virilis* from the experimental treatment had a greater proportion of infected to uninfected crayfish compared to that of the control treatment. Conversely, a chi-square test found no statistically significant difference ($\chi^2(1, N=65)=0.0008323$, $p = 0.977$) in proportion of infected to uninfected *O. rusticus* between control and experimental treatments. Only control *O. virilis* within the carapace length range of 18-26 mm were used to ensure that they matched the carapace lengths of the experimental *O. virilis*.

A linear regression demonstrated a non-significant relationship ($F_{1, 27} = 2.260$, $R^2 = 0.0296$, $p = 0.3722$) between the number of introduced cercariae and the number of metacercariae in *O. virilis* (N = 29) (Figure 7). The number of metacercariae for *O. virilis* group was corrected to account for infection prior to experimentation. Another linear regression revealed a non-significant relationship ($F_{1, 37} = 0.796$, $R^2 = 0.01$, $p = 0.5455$) between the number of cercariae introduced and the number of metacercariae in *O. rusticus* (N = 39).

Second Experiment

While all *O. virilis* and no *O. rusticus* were infected in this experiment (Figure 8), my data from the control crayfish treatment suggest that *O. virilis* may have been infected prior to

experimentation. The corrected values for *O. virilis* metacercariae were used. However, I did not have a sufficient sample size due to a high number of *O. virilis* mortalities to run meaningful statistics on these results.

DISCUSSION

The purpose of this study was to determine whether the native *O. virilis* and the invasive *O. rusticus* have different susceptibilities to the trematode parasite *Microphallus* sp. Experimental infection resulted in significantly higher infection levels in *O. virilis* compared to *O. rusticus*. In fact, experimental *O. virilis* were more infected than control *O. virilis* while the same was not true for *O.* rusticus; this suggests *O. virilis* became infected during the experiment but *O. rusticus* did not. These results support the hypothesis and are consistent with prior studies reporting higher parasitic infection levels in native over invasive host species (Roche *et al.* 2010; Dunn *et al.* 1998).

My findings are only meaningful, however, if I was able to successfully infect crayfish through experimental exposure to cercariae. Otherwise, differences in infection levels between species could be attributed to the possibility that *O. virilis* was infected prior to experimental infection. *O. virilis* from experiment exhibited higher levels of infection at lower carapace lengths than what would be expected in control *O. virilis* of the same carapace length, suggesting that I was able to alter infection levels. Additionally, experimental *O. virilis* had a lower ratio of uninfected to infected crayfish compared to control *O. virilis*. This indicates the method of experimental infection used successfully increases the proportion of infected crayfish from the proportion expected in nature. However, comparing the number of cercariae introduced during infection to the number of encysted metacercariae revealed that many cercariae are not

successful at infecting either species even when in close proximity to the crayfish. So while we were able to increase infection levels in general, all cercariae introduced did not necessarily become metacercariae.

My findings indicate that *O. virilis* is more susceptible to *Microphallus* sp. infection than *O. rusticus*. The most likely explanations for this are that the parasite can identify *O. virilis* as a suitable host more easily, or that *Microphallus* sp. is adapted to infect *O. virilis* over *O. rusticus* preferentially. In either scenario, coevolution is a driving force. The longer a parasite coexists with a host species, the more adaptations the parasite is able to develop to facilitate infection (King 2011). *Microphallus* sp. therefore may be more effective at infecting the native *O. virilis* rather than the invasive *O. rusticus* because the two are expected to share a longer evolutionary history.

An alternative outcome of coevolution is that the native host species develops defense mechanisms to better avoid infection, resulting in higher infection levels in the invasive host species that lack these adaptations. My findings, however, do not support this potential outcome of coevolution, indicating that *Microphallus* sp. is better adapted to infecting *O. virilis* than *O. virilis* is adapted to defend against *Microphallus* sp. Furthermore, comparison of infection levels across crayfish treatments reveals that *Microphallus* sp. will not necessarily infect *O. rusticus* in the absence of *O. virilis*; this suggests that the parasite not only prefers to infect *O. virilis*, but it may also be unable to infect *O. rusticus* effectively. A future study could investigate the latter possibility by examining infection levels in experimentally infected *O. rusticus* alone.

A less likely possible reason for the observed differences in *Microphallus* sp. susceptibilities is that the cercariae introduced during experimental infection belonged to a trematode species of *Microphallus* that does not infect *O. rusticus*. However, this is unlikely

considering that *A.limosa* were collected from lakes that have confirmed high infection levels of *O. rusticus* with *Microphallus* sp. Alternatively, perhaps *O. rusticus* have robust immune systems that are well-adapted to fighting off *Microphallus* sp. infection, preventing metacercariae from encysting even if cercariae are able to penetrate the body. Again, this is unlikely because *O. rusticus* and *Microphallus* sp. are not known to share any evolutionary history.

My results, coupled with prior studies' findings that *Microphallus* sp. confers disadvantages to *Orconectes* crayfish, have important implications for the competitive interaction between *O. virilis* and *O. rusticus* (Towle *Unpublished data*). Parasitism has been linked to the mediation of competition dynamics, which by definition entail reciprocal negative effects (Hatcher *et al.* 2008). Thus, if one competitor is more easily affected by a parasite, it is burdened with an additional disadvantage and could more easily be out-competed. In fact, prior studies on the enemy release hypothesis (ERH) have attributed the success of invasive species partly to their evasion of coevolved parasites when they moved to a new location (Coulautti 2004). Considering this, and the fact that *O. virilis* appears to be more susceptible to infection by *Microphallus* sp., the presence of *Microphallus* sp. may expedite *O. rusticus* replacement of native crayfish in northern Wisconsin.

The differences in infection level found between *O. rusticus* and *O. virilis* in this study are likely exacerbated in more natural settings. For example, *O. rusticus* are known to exhibit more aggressive behaviors than *O. virilis*, often excluding *O. virilis* from shelters (Roth and Kitchell 2005). Consequently, *O. virilis* are often pushed into macrophyte habitats that have greater abundances of snails. This could in turn increase *O. virilis*' exposure time to cercariae, increasing infection levels. To explore this, future studies could alter this study's methodology to

better match natural conditions. Crayfish could be taken from the same lakes and placed in containers with more natural substrate and shelters. Ultimately, findings would provide a more accurate representation of the effects of competition on *Microphallus* sp. infection levels.

The most striking finding of this study was that *O. virilis* is more susceptible to infection by *Microphallus* sp. than *O. rusticus*. In addition to providing insight into the evolutionary capacities and host preference of *Microphallus* sp., this has important implications for the competitive relationship between the two crayfish species.

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TABLES

Table 1. Number of Uninfected and Infected *O. virilis* in Control versus Experiment Treatments. A chi-square test revealed a statistically significant difference ($\chi^2(1, N=77)=5.680$, $p = 0.017$) in the proportion of infected to uninfected *O. virilis* between control and experimental treatments.

Treatment	Number Uninfected *O. virilis*	Number Infected *O. virilis*
Control	22	27
Experiment	5	25

FIGURES

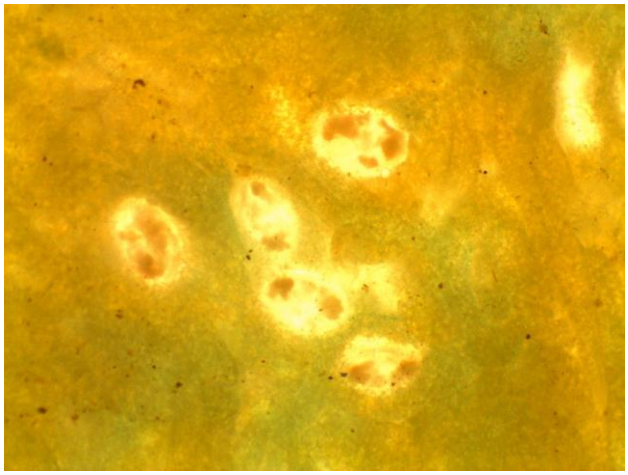

Figure 1. Metacercariae in *O. rusticus* hepatopancreas. This image displays five metacercariae encysted in the hepatopancreas of an *O. rusticus* viewed under a dissecting microscope at X35.

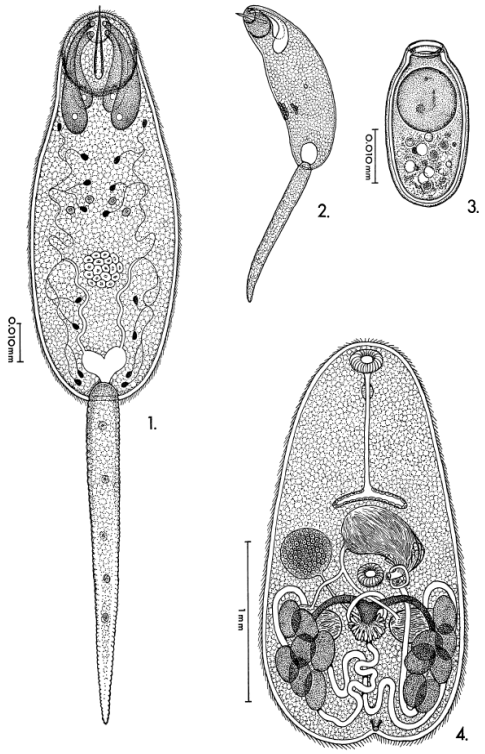

Figure 2. *Microphallus opacus* cercariae. This diagram shows the cercariae of *Microphallus opacus*, a parasite known to be closely related to the *Microphallus* species of interest in this study (1.1, 1.2) (Caveny and Etges 1971).

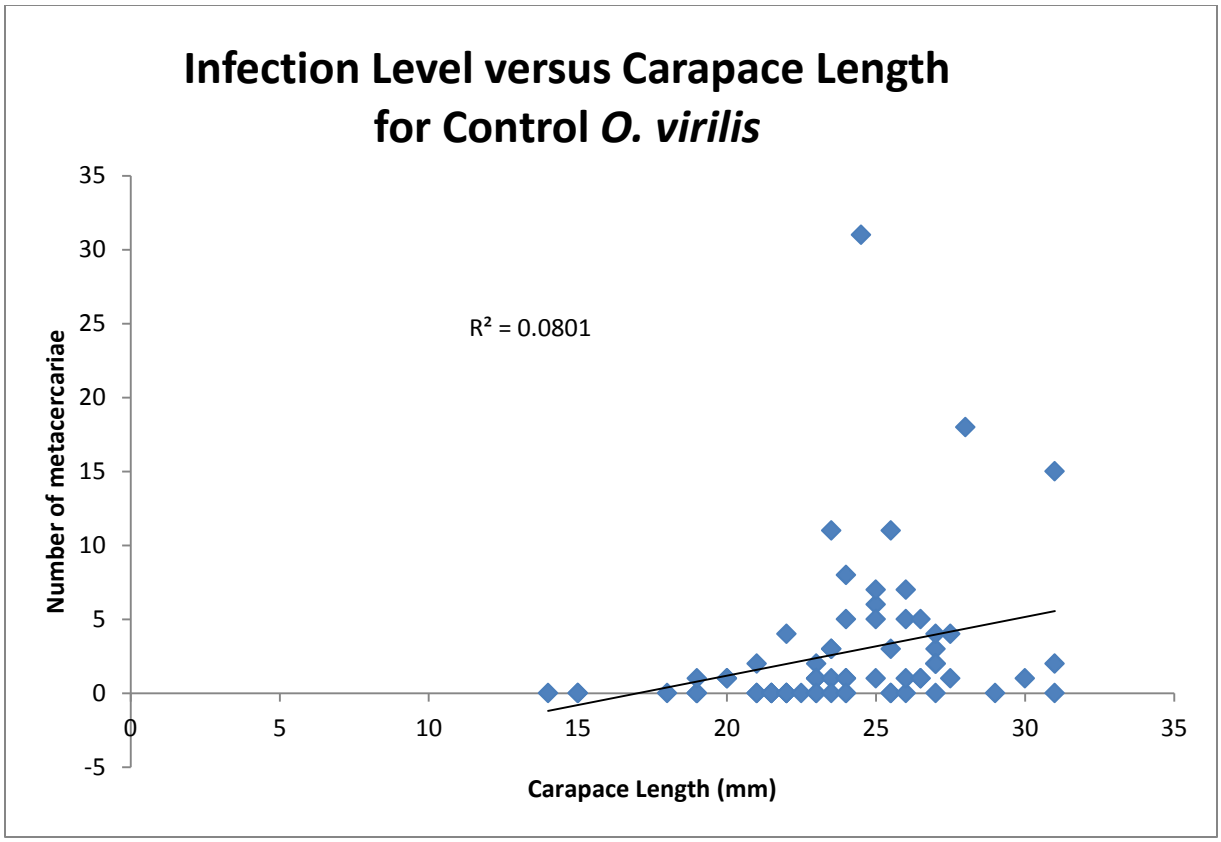

Figure 3. Infection Level versus Carapace Length for *O. virilis* from Control Tank. This graph shows the relationship between carapace length and number of metacercariae in control *O. virilis* (N = 68). Regression analysis revealed a statistically significant positive relationship ($F_{1,66}$=5.750, R^2=0.0801, p = 0.0193) between the two variables. Because carapace length is an indicator of a crayfish's age, this trend is likely attributed to the increased chance of encountering cercariae the longer a crayfish is alive.

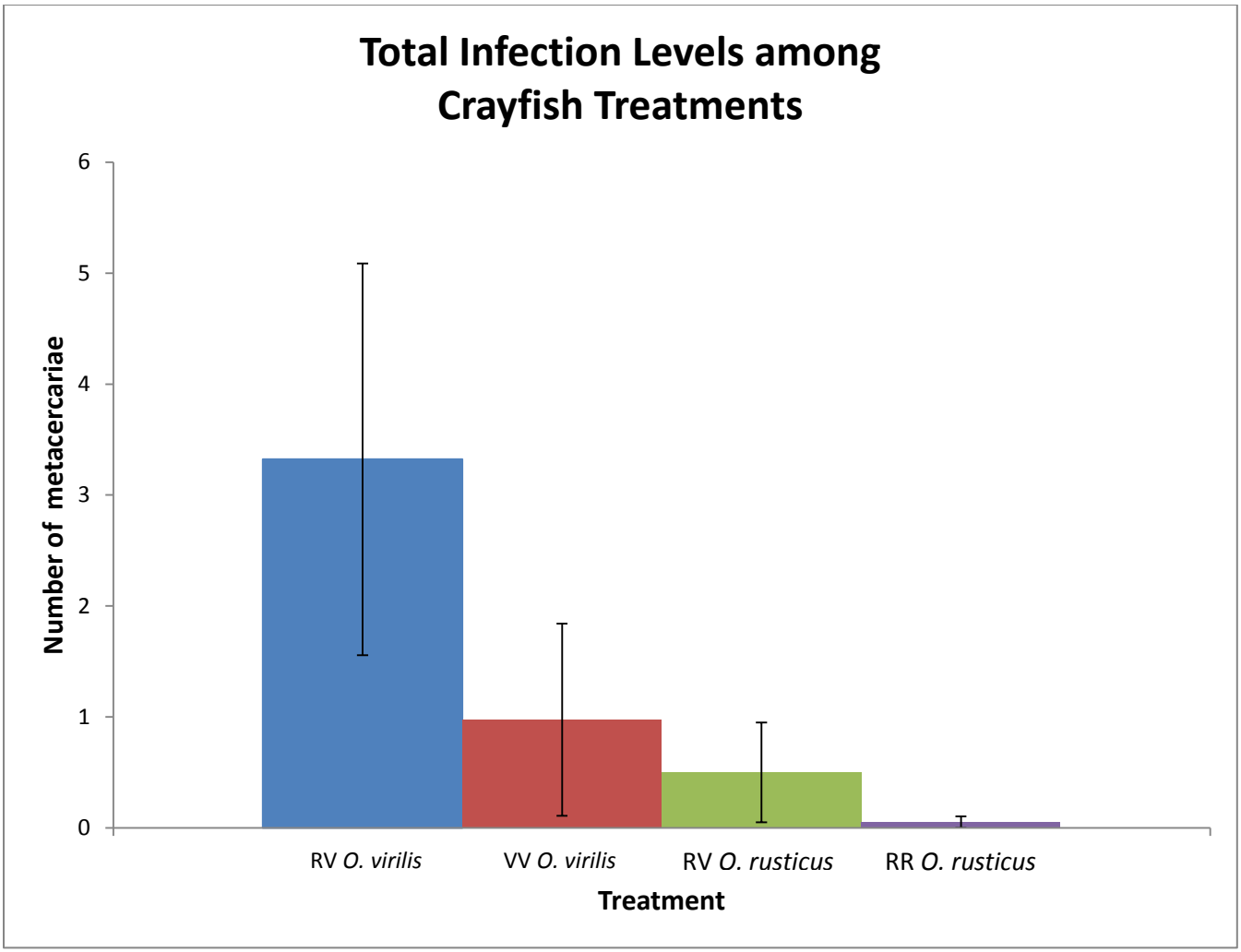

Figure 4. Infection Levels of *O. virilis* and *O. rusticus* among Different Treatments. This graph shows the mean number of metacercariae found in *O. virilis* (N = 14) and *O. rusticus* (N = 20) in the two-species treatment (RV), *O. virilis* (N = 15) in the one-species treatment (VV), and *O. rusticus* (N = 19) in the one-species treatment (RR). RV *O. virilis* had a mean of 3.322 (±1.764) metacercariae; VV *O. virilis* had a mean of 0.974 (±0.866); RV *O. rusticus* had a mean of 0.500 (±0.450); and RR *O. rusticus* had a mean of 0.0526 (±0.053). There is a marginally significant difference ($F_{3,64} = 2.59$; $p = 0.0605$) in infection level between *O. virilis* from the two-species treatment and *O. rusticus* in the one-species treatment.

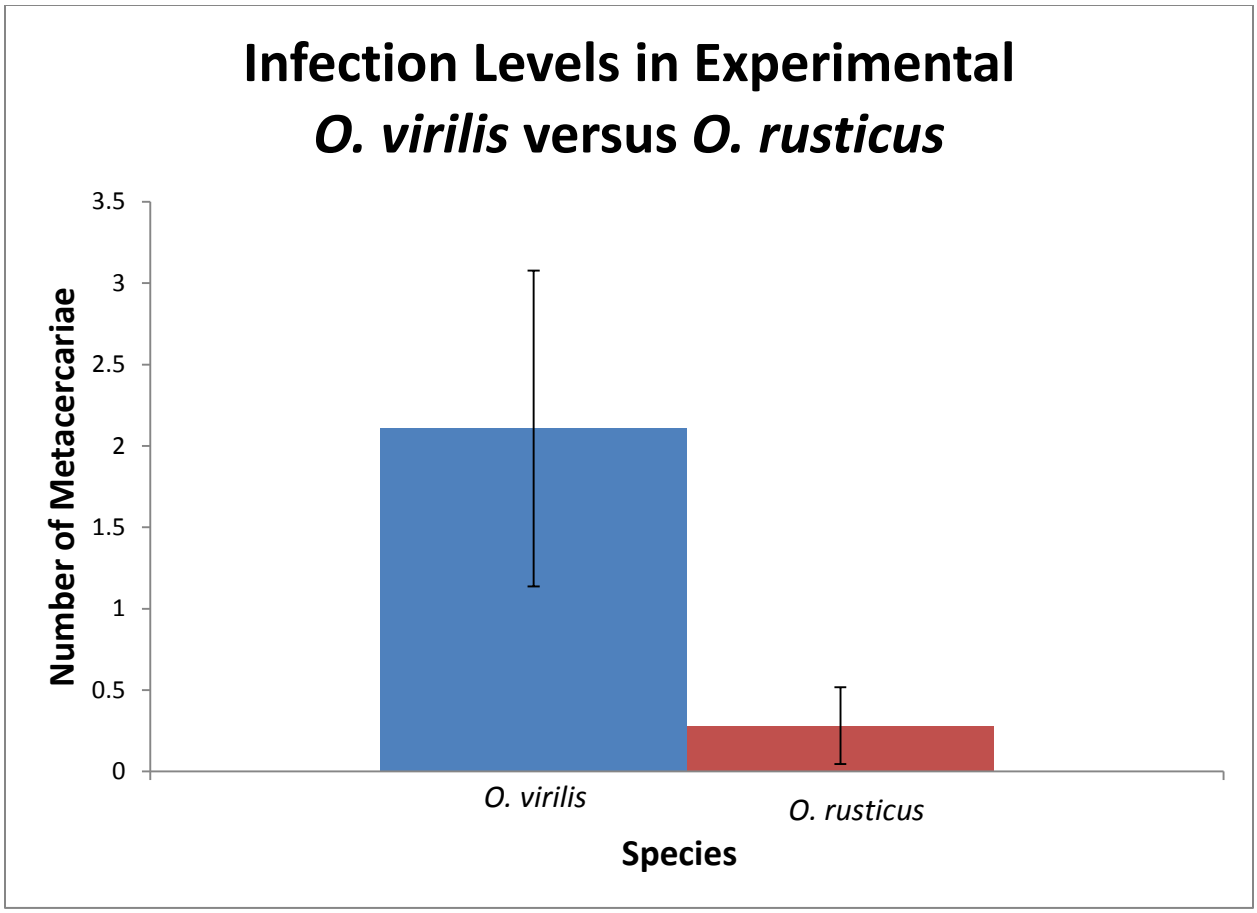

Figure 5. Infection Levels between *O. virilis* and *O. rusticus* in Experimental Treatments. This graph shows the mean number of metacercariae found in experimental *O. virilis* (N = 29) and *O. rusticus* (N = 39). *O. virilis* in the one-species and two-species treatments had a mean of 2.108 (±0.970), and *O. rusticus* in the one-species and two-species treatments had a mean of 0.282 (±0.235). There is a significant difference ($F_{1,66} = 4.337$, $p = 0.04117$) between infection levels in the two species.

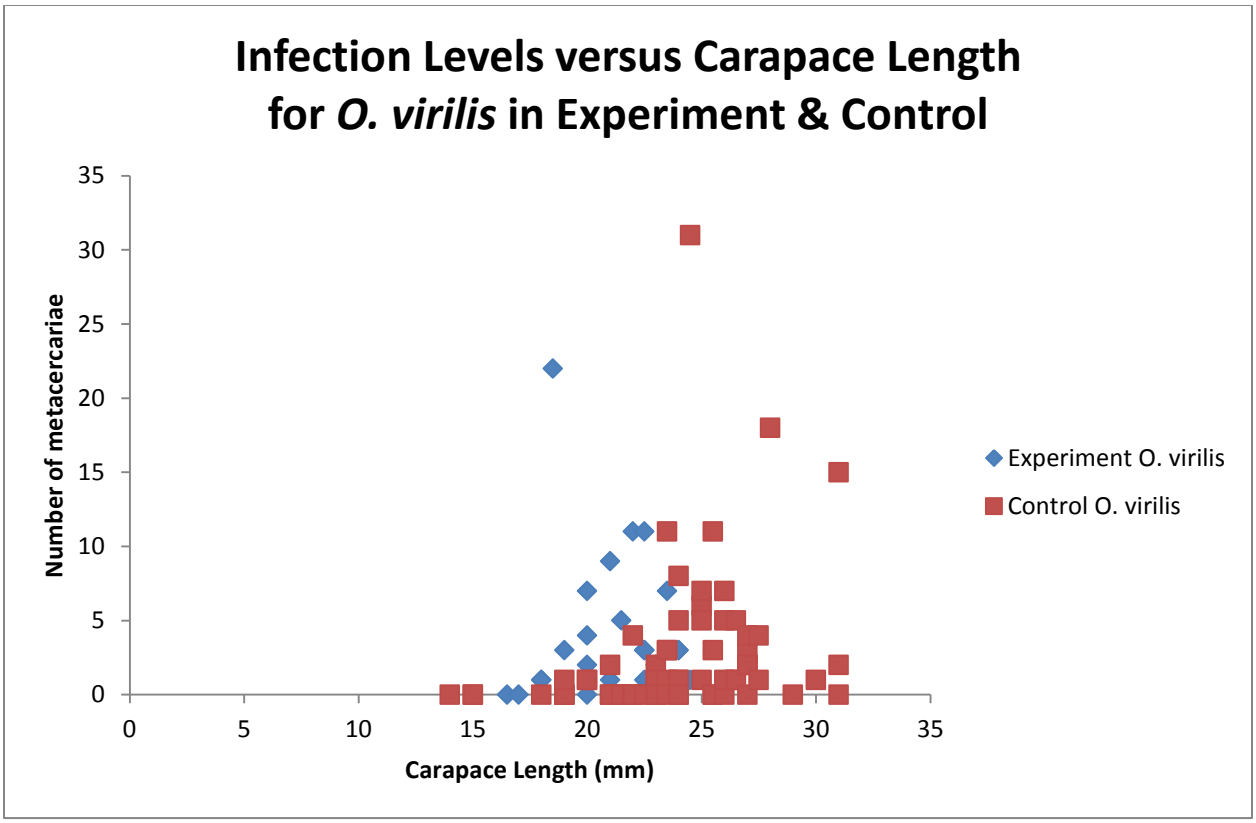

Figure 6. Infection Levels versus Carapace Length in *O. virilis* from Control and *O. virilis* from Experiment. This graph shows the numbers of metacercariae found in each experimental *O. virilis* versus carapace length (N = 29), and the number of metacercariae found in each control *O. virilis* versus carapace length (N = 68). There is a marginally significant difference ($F_{2,94}$ = 2.62, p = 0.0784) in infection level between control and experimental *O. virilis*.

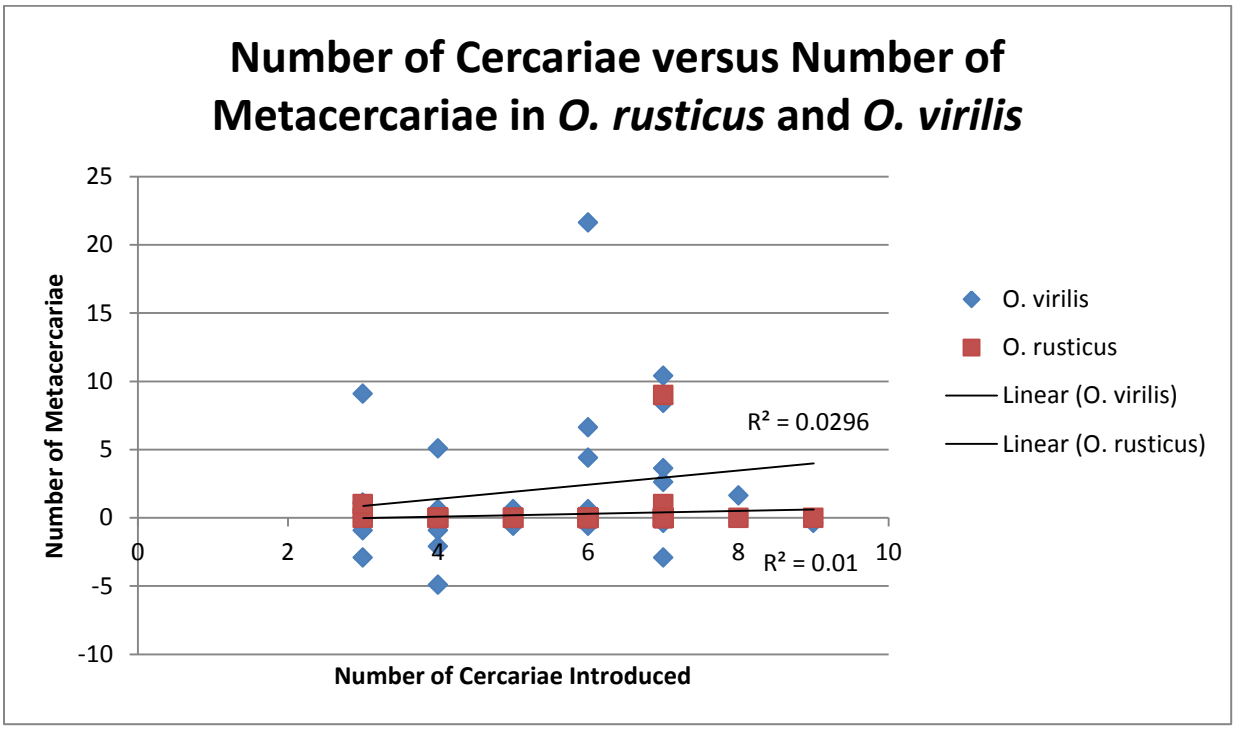

Figure 7. Number of Cercariae Introduced versus Number of Metacercariae in Experimental *O. virilis* and *O. rusticus*. This graph demonstrates the number of introduced cercariae and number of encysted metacercariae in *O. virilis* (N = 29) and *O. rusticus* (N = 39) from all treatment groups. The number of metacercariae for *O. virilis* groups were corrected to account for infection prior to experimentation. There is a non-significant relationship ($F_{1, 27}$ = 2.260, R^2 = 0.0296, p = 0.3722) between the number of introduced cercariae and the number of encysted metacercariae for *O. virilis*, and a non-significant relationship ($F_{1, 37}$ = 0.796, R^2 = 0.01, p = 0.5455) between the two variables for *O. rusticus*.

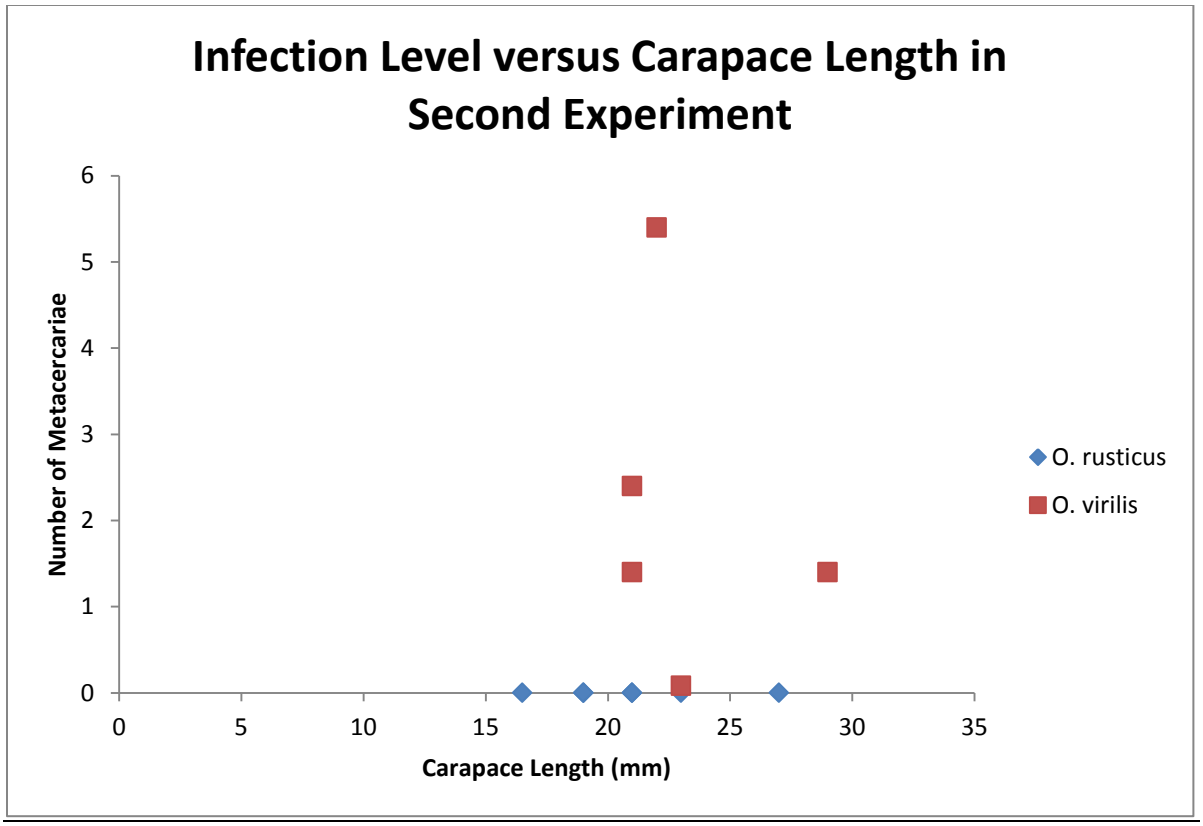

Figure 8. Infection Levels versus Carapace Length for crayfish in the Second Experiment. This graph shows carapace length versus the number of metacercariae found in *O. rusticus* and *O. virilis* from the second experiment. No *O. rusticus* were infected.