Comparing the predator avoidance and boldness responses of *O. rusticus*, *O. propinquus* and *O. virilis* infected with *Microphallus* spp.

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Abstract

Trematode parasites (*Microphallus* spp.) have been found in lakes in Northern Wisconsin and the Upper Peninsula of Michigan, encysting in the hepatopancreas of crayfish such as *O. rusticus, O. propinquus* and *O. virilis*. *O. rusticus*, an invasive species have been replacing *O. propinquus* (nonindigenous species) while both species have been displacing *O. virilis* (native species). Mechanisms of infection and the effects on crayfish behavior have not been discovered.

Uninfected and infected *Orconectes virilis, O. propinquus* and *O. rusticus* boldness responses were studied in the presence rock bass, *Ambloplites rupestris* in simulated environments where crayfish were initially confined in shelters. After an acclimatization period, crayfish were allowed to exit the shelter and time spent in shelter was observed.

Uninfected *O. rusticus* and *O. propinquus* were bolder (emerged from the shelter more quickly) than *O. virilis.* Additionally, infected *O. rusticus* and *O. propinquus* were significantly bolder than uninfected individuals. This suggests *Microphallus* may have a greater effect on *O. rusticus* and *O. propinquus*, increasing their susceptibility to predation and allowing life cycle completion for *Microphallus*. Parasitism and increased boldness may affect competition interactions and outcomes by stimulating aggressive interactions in *O. rusticus* and *O. propinquus*. This could aid the current displacements in Northern Wisconsin.

With the dominance of *O. rusticus* in several watersheds, *Microphallus* may play an important role by indirectly controlling *O. rusticus* populations. Further study for *O. virilis* is needed as little effect on boldness could suggest a developed resistance or coevolution which may be beneficial considering the ongoing displacements.

Introduction

Although multiple crayfish species are found in lakes throughout Wisconsin, the one species that is of pressing concern is the invasive *Orconectes rusticus*. *O. rusticus* was first discovered in Wisconsin Lakes in 1965 (Capelli 1983). Since then *O. rusticus* has been found to have severe effects on the ecological community including the displacement of resident crayfishes such as *O. propinquus,* an indigenous species supposedly introduced before *O. rusticus*, and *O. virilis,* a native species present before *O. propinquus* and *O. rusticus* (Capelli 1983). Generally *O. rusticus* is more aggressive in comparison to *O. virilis* and *O. propinquus,* which explains their successful invasion of Wisconsin Lakes (Capelli 1983). Another study indicated that *O. rusticus and O. propinquus* outcompete native species *O.virilis* for shelter, which makes them more successful at avoiding fish predation (Garvey et al. 1994).

O. rusticus is currently the most prevalent species in the state of Wisconsin (Olden et al. 2006). The mechanism by which *O. rusticus* replaces other crayfish is primarily through direct aggressive interactions and out competing native species for shelter (Capelli 1983). A variety of control measures have been implemented to control *O. rusticus* populations such as education of those in the fishing industry and use of distribution maps to identify locations where introductions have been most prevalent and where education should be reinforced (Olden et al. 2006). Other management techniques include maintaining healthy sunfish, *Centrarchidae* spp. and bass, *Micropterus* spp. populations to help control *O. rusticus* populations have also been suggested (Olden et al. 2006). Using crayfish for bait is now illegal in Wisconsin because *O. rusticus* were likely introduced and spread by anglers (Wilson et al. 2004).

Recent studies have shown that a trematode parasite (*Microphallus* spp.) may aid in repressing *O. rusticus* populations *(*Roesler 2009). Although the specifics of the effects and mechanisms of *Microphallus* are still being studied, preliminary studies show that parasites alter *O. rusticus* behaviors in a manner that may promote their predation by predatory fish (Sargent unpublished data). *Microphallus* occur as metacercariae in crayfish infecting the hepatopancreas of crayfish and use crayfish as intermediate hosts before completing their lifecycle once the crayfish is consumed by a definitive host (Caveny and Etges 1971, Overstreet et al. 1992). Some closely related species in the *Microphallus* genus use birds, mammals and other vertebrates as definitive hosts (Overstreet et al. 1992). Recent investigations have shown that parasite susceptibility and virulence may differ among host species within the same genus and may not affect behavior equally (Lefevre et al. 2008).

Research on other host-parasite systems has shown that parasite infections can confer reduced predator avoidance behaviors, hyperactivity, sluggishness, fatigue, disorientation, and altered habitat selection (Kunz and Pung 2004). In a study of trematode infections in pulmonate snails, heavier parasite loads led to reduced predator avoidance via reduced use of shelter habitats (Bernot 2003). Additionally, in a study of grass shrimp infected with *Microphallus turgidus,* infected shrimp exhibited behaviors that would reveal their location to predators (Kunz and Pung 2004). Such behaviors primarily facilitate parasite transmission and therefore life cycle perpetuation (Lefevre et al. 2008). Such detrimental behavior has been noted in *O. rusticus, O. propinquus* and *O. virilis,* though the behavioral effects of the parasite are different in each species and have yet to be confirmed. *O. propinquus* likely has the most detrimental behavioral change, reduced time spent hiding; this is likely to increase its susceptibility to predation. Infected *O. rusticus* increase their aggressiveness towards conspecifics and congeners, *O. virilis* and *O. propinquus* (Sargent, unpublished). Such aggressive behavior may increase susceptibility to predation as anti-predator behavior would decrease and boldness increase despite predator presence or threat.

To see how *Microphallus* affects *O. rusticus*, *O. propinquus* and *O. virilis,* avoidance and boldness behaviors in the presence of a predator were studied and compared between infected and uninfected individuals. The primary purpose of this study was to see whether infection has a greater effect in any of these species. Specifically the hypothesis tested was that crayfish will be bolder (spend less time in shelter and emerge from shelter more quickly) when infected with *Microphallus*. It was expected that *O. rusticus* will be bolder when infected because it is more aggressive towards other crayfish and may also be more aggressive (and less timid) in the presence of a fish. Also, a previous study showed that infected *O. propinquus* will spend less time in the shelter compared to uninfected individuals (Sargent, unpublished data). Less time spent in the shelter would suggest increased boldness and altered anti-predator behavior. *O. virilis* will be used as a reference to compare how a native species responds to parasite infection in comparison to *O. rusticus* and *O. propinquus.* Further investigation of how *Microphallus* affect crayfish population densities and distributions may provide insight to managers, such as in which lakes control measures should be most concentrated.

Methods

In order to quantify predator avoidance or boldness behavior, I measured the total time that crayfish spent in shelter and the amount of time before a crayfish emerged from shelter in the presence of a predatory fish in *O. rusticus*, *O. virilis*, and *O. propinquus*. Crayfish leaving the shelter was an indicator of bold behavior and residing in the shelter was an indicator of shy behavior. A total of 10 lakes, Forest, Big Musky, Tenderfoot, Plum (WI), Ottawa, Star, Spider, Papoose, and Plum (MI) Lake were surveyed to determine infection status and specimens were collected for experimental trials. Specimens from Forest Lake were uninfected *O. virilis,* Big Musky Lake individuals were uninfected *O. propinquus.* Star and Papoose Lakes individuals were uninfected *O. rusticus.* Conversely Plum Lake (MI) individuals were infected *O. virilis* while Tenderfoot Lake specimens were infected *O. propinquus.* Spider, Ottawa, and Plum (WI) Lake were infected *O. rusticus.* After each trial I dissected each individual and counted the number of *Microphallus* metacercariae to confirm infection and determine parasite load. All research involving *O. rusticus* was conducted at Trout Lake Research Station.

In each replica tank, a rock bass (*Ambloplites rupestris*), was separated from the crayfish via a screen separating the top and bottom half of the tank. This allowed the crayfish to be aware of the predatory fish and be protected from consumption. A total of two replicate tanks were observed at a time. One tank contained an infected crayfish and the other an uninfected crayfish. Both infected and uninfected crayfish were initially placed in shelters that had a removable plexiglass door that opened by lifting the plexiglass [Figure 2]. Shelters were constructed out of PVC pipe, with a diameter of 6cm and a length of 16.9cm [Figure 2]. One end of the shelter was capped with a PVC lid and a slit made on the other end to fit the plexiglass door, allowing it to be removed easily [Figure 2].

Crayfish were initially placed in the shelter so that they were facing the door. The door was perforated to allow the crayfish to receive chemosensory cues from the fish. After the crayfish was added to the shelter, a rock bass (*Ambloplites rupestris*) was placed in the upper portion of the tank. After 15 minutes of acclimatization, the door was lifted and time spent in the shelter was recorded for 30 minutes. I measured carapace length using calipers and recorded the sex of each crayfish used in the experiment. Each rock bass was also measured prior to running each trial using a fish measuring board. Rock bass, *Ambloplites rupestris* were collected from Tenderfoot Lake using fyke and seine nets and were no smaller than 17 cm.

I ran one-way ANOVA's for *O. propinquus* and *O. rusticus* with total time in the shelter as the dependent variable and infection status as the independent variable. Total time in shelter was transformed using a Log function for *O. propinquus* and a Cosine function for *O. rusticus* to establish normal distribution. I ran a Mann-Whitney's test in lieu of a one-way ANOVA for *O. virilis* because these data did not meet the assumptions of normality. Additionally a Kruskal-Wallis Test was used in lieu of a one-way ANOVA for uninfected individuals of all three species because these data did not meet the assumptions of normality. Significant results were followed by a Dwass-Steel-Chritchlow-Fligner Test to determine which combinations of uninfected species were significant. To analyze whether infected and uninfected crayfish leaving the shelter at any point during the trial was significantly different, a chi-square test was utilized for *O. virilis*. Fisher's Exact test was used for *O. propinquus* and *O. rusticus* for more accuracy due to fewer individuals leaving the shelter.

All the aforementioned analyses were carried out using MYSTAT 12 and SYSTAT 13 with the exception of Fisher's Exact Test which was done using SISA. I also ran a Kaplan-Meier's test which includes whether or not an event happened – the event being whether the crayfish left the shelter – and the time that the event happened in the same analysis. Analysis for this model was computed using R.

Results

O. propinquus and *O. rusticus* on average spent less time in the shelter, regardless of whether they were infected or uninfected in comparison to *O. virilis* [Figure 1]. Means of total time spent in shelter for uninfected (19.12 ± 2.34 minutes) and infected (18.04 ± 2.58 minutes) *O.*

virilis did not vary much [Figure 1]. Means of total time spent in shelter for uninfected and infected *O. propinquus* were 12.32 ± 2.62 minutes and 3.96 ± 1. 37 minutes respectively [Figure 1]. Means of total time spent in shelter for uninfected and infected *O. rusticus* were 9.38 ± 2.12 minutes and 3.81 ± 1.50 minutes respectively.

A Mann-Whitney's test for *O. virilis* showed no significant difference in total time spent in shelter between infected and uninfected individuals ($P = 0.360$) [Figure 1]. A one-way ANOVA for *O. propinquus* showed significant difference in total time spent in shelter between infected and uninfected individuals ($F = 16.291$, $df = 49$, $P < 0.01$) [Figure 1]. A one-way ANOVA for *O. rusticus* showed significant difference in total time spent in shelter between infected and uninfected individuals ($F = 4.325$, $df = 41$, $P = 0.044$) [Figure 1].

A Kruskal-Wallis test for uninfected *O. virilis, O. propinquus* and *O. rusticus* showed significant difference in total time spent in shelter among the three species ($P = 0.006$). Dwass-Steel-Chritchlow-Fligner Test showed significance between the following uninfected pairwise comparisons - *O. virlis* and *O. propinquus* ($P = 0.043$), *O. virilis* and *O. rusticus* ($P < 0.01$), and *O. propinquus* and *O. rusticus* ($P = 0.018$).

Chi-square analyses indicated there was no significant difference between uninfected and infected *O. virilis* leaving the shelter during the entire 30 minute observation (Pearson Chi Square = 0.325, $df = 49$, $P = 0.569$) [Table 1]. Fisher's exact test showed no significant difference between the number of uninfected and infected *O. propinquus* that left the shelter (Pearson Chi Square = 0.758, $df = 49$, $P = 0.428$) [Table 1]. Likewise there was no significant difference in *O. rusticus* infected and uninfected individuals leaving the shelter (Pearson Chi Square = 4.731, $df = 49$, $P = 0.115$) [Table 1].

Kaplan Meier's estimate for *O. virilis* indicated that there was no significant difference in the timing and likelihood that an uninfected or infected individual left the shelter ($\chi^2_{1, 49}$) = 0.5, P = 0.495). The Kaplan Meier's estimate for *O. propinquus* showed a significant difference between infected and uninfected individuals that left the shelter ($\chi^2_{1, 49}$) = 10.5, P = 0.001), indicating that infected individuals were more likely to leave the shelter. In addition, the Kaplan Meier's estimate for *O. rusticus* showed a significant difference for infected and uninfected crayfish that left the shelter ($\chi^2_{1, 41}$) = 8.9, P = 0.002), again indicating that infected individuals were more likely to leave the shelter.

Discussion

Since there was a significant difference between uninfected and infected *O. propinquus* and *O. rusticus* individuals for total time spent in shelters, the data supports the hypotheses that infected *O. propinquus* and *O. rusticus* would spend less time in the shelters, an indicator of an altered anti-predator response or increased boldness in the presence of fish predators. Infected *O. propinquus* and *O. rusticus* spent roughly half the time that their uninfected counterparts spent in shelter suggesting *Microphallus* has a substantial effect on boldness which is likely to increase predation on crayfish by predatory fish. Uninfected and infected *O. virilis* showed no significant differences for total time spent in shelter and therefore are not likely to be affected by infection in terms of predation rate.

Significant differences in time spent in shelters were observed individual ANOVA's and Kruskal Wallis Test comparing all 3 uninfected species and between uninfected and uninfected *O. propinquus* and *O. rusticus*. Differences in average total time spent in shelter appear to be minimal between uninfected *O. propinquus* and *O. rusticus* (P = 0.0182) despite their infected counterparts having fairly similar averages in total time spent in shelter [Figure 1]. This could indicate that *O. rusticus* are naturally bolder than *O. propinquus* when uninfected.

Although no significant differences were noted in any of the species for infected and uninfected individuals that left the shelters for Chi-square analyses and Fisher's Exact Test, time spent in shelter varied drastically among infected and uninfected for *O. propinquus* and *O. rusticus.* If the time frame for each trial was increased, further analyses may yield different results. However, taking the variable of time spent in shelter into account, Kaplan-Meier's estimate results concurred with the significant differences between uninfected and infected *O. propinquus* and *O. rusticus* that left the shelter observed in the one-way ANOVA's.

Other species of *Microphallus* have been found to have varying effects between congeners which could explain the contrasting times spent in shelters. A study on *Microphallus papillorobustus* infections in sand shrimp, *Gammarus insensibilis* and *G. aequicauda* showed equal virulence, causing them to swim to the surface, thus increasing their chance of predation. However, *G. aequicauda* is only susceptible in its juvenile stage whereas *G. insensibilis* remains susceptible to infection in all of its life stages (Lefevre et al. 2008). *Microphallus* may alter boldness responses differently specific to species in a manner that also increases their chance of predation when provided with shelter.

One possible mechanism for the increase in boldness observed in infected *O. propinquus* and *O. rusticus* could be behavioral manipulation of crayfish by the *Microphallus* parasite. Once metacercariae have encysted in hepatopancreas of crayfish, crayfish must be consumed by a definitive host (an unknown vertebrate predator such as a bird or fish) for the completion of the *Microphallus* life cycle to sexual maturity and copulation (Caveny and Etges 1971) (Stunkard 1951). Because there is strong selection for parasites to live in an intermediate host that is eaten by the definitive host, some parasites have developed the ability to alter intermediate host behavior. Increased boldness is likely to increase predation on crayfish. Therefore, the altered behavior observed could be caused by *Microphallus* behaviorally manipulating the crayfish host. A previous study on a species of *Microphallus* that infects an intermediate host, the New Zealand Mud Snail, (*Potamopyrgus antipodarum*) resulted in behavioral changes where snails would expose themselves to high risk locations at specific times associated with predators feeding times (Levri et al. 2007). While the mechanisms and complexity of this system have yet to be explained, the observed increased boldness i.e. less time spent in shelter, could indicate that *Microphallus* is behaviorally manipulating crayfish to leave the shelter, increasing the chances of predation.

Infected and uninfected *O. propinquus* and *O.rusticus* on average spent less time in shelters in comparison to *O.virilis* which indicates that both species are naturally bolder in the presence of predators. Moreover, infected individuals for both species significantly spent less time in shelters. Competition studies have shown that *O. propinquus* have the ability to out compete *O. virilis* due to greater aggressive interactions though *O. rusticus* dominates both species (Capelli 1982). Additionally, parasitism can have indirect effects on competition and predation interactions between native and invasive species, often times indirectly aiding the invasive species invasion (Prenter et al. 2004).

In this case, the presence of *A. rupestris* could induce an aggressive behavior in *O. propinquus* and *O. rusticus* as a defense or threat response which ultimately could result in the crayfish leaving the shelter to exhibit this behavior. This display of aggressive behavior could be a possible explanation of why *O. propinquus* and *O. rusticus* leave the shelters faster than *O.*

virilis, regardless of infection status. *Microphallus* may be better able to behaviorally manipulate species that are already somewhat aggressive such as *O. propinquus* and *O. rusticus.*

One suggestion for why there were no significant differences in time spent in shelter for uninfected and infected *O. virilis* could be also due to a long coevolutionary history between native species *O. virilis* and *Microphallus*, further confounding the possible parasitism effects on *O. virilis* boldness. *O. virilis* is suspected to have colonized much of Northern Wisconsin and the Upper Peninsula of Michigan 10,000 years ago following the glacial retreat (Capelli 1982). While no records date the first observance of *Microphallus* in crayfish and the species of this study was not identified, it is quite likely that a long term relationship may have developed, culminating in parasite-host coevolution. In this case, while parasite virulence may have increased over time, *O. virilis* resistance may have simultaneously evolved to a threshold level where both organisms are operating on the Red Queen Principle. Certain models have stipulated that parasitism; particularly long-term relationships may eventually lead to commensalism or mutualism if virulence levels change (Toft and Karter 1990) in which case infected *O. virilis* would seemingly have no obvious detriment with regards to increased boldness. Regardless, this absent effect on increased boldness may prove beneficial for native *O. virilis* considering the ongoing displacements via *O. rusticus.* Interspecific prey competition studies have shown that *O. virilis* incurred in risky behaviors via increased swimming and activity in the presence of predators (Garvey et al. 1994) which may suggest infection of parasites may or may not have a large effect on boldness behavior due to the unpredictable behavior and former observations of bold behavior.

Conversely, *Microphallus* may have been introduced along with the invasion of *O. propinquus* and *O. rusticus,* and therefore are only successful at behaviorally manipulating these introduced species. The hormones or other regulatory compounds released by *Microphallus,* a suspected mechanism for behavioral manipulation, may not work on *O. virilis* or affect *O. virilis* in the same manner it does in its congeners. Parasites introduced from invasive species have been known to have both positive and negative effects on native species (Dunn et al. 2012), where in this case the effects on *O. virilis* have yet to be discovered, behavior manipulation is not an obvious occurrence with regards to this study. This would suggest a positive effect on *O. virilis* considering their ongoing displacements by their congeners and unclear effects of parasitism.

Uninfected and infected *O. rusticus* were overall bolder than *O. virilis.* Infected *O. rusticus* were also bolder than their uninfected counterparts that may be rooted in the idea of their domineering aggressive behavior and how this interplays with competition and predation as aforementioned. This data would therefore suggest *O. rusticus* and *O. propinquus* to be the species most highly likely to be preyed on when the effects of parasitism influences this natural bold behavior. While parasitism may aid their aggressive behavior and ability to outcompete their conspecifics aiding their invasion and ability to compete for shelter, (Capelli 1983) (Sargent, Unpublished) *Microphallus* may have caused population declines in several lakes in Northern Wisconsin (Roesler 2009), further supporting the findings. Surveys showed that parasite loads were greater where *O. rusticus* population densities were lower and vice versa, suggesting that *Microphallus* may be controlling or lowering *O. rusticus* populations (Roesler 2009). This decline may have been caused by increased predation on *O. rusticus* due to increased boldness in infected individuals. Reduced *O. rusticus* populations could lead to recovery of some native lake species of crayfish and the recovery of ecosystems. This tradeoff between parasitism, boldness and aggressiveness may therefore be beneficial in slowing down a species composition shift and continued invasions. The ecology and complex interactions with *Microphallus* and their hosts in lake communities may be naturally countering the invasion of *O. rusticus* though such interactions may take time to have observable effects.

Further study is needed to determine the mechanism by which *Microphallus* alters crayfish boldness behavior in order understand why there are different effects on *O. virilis, O. propinquus* and *O. rusticus.* A long term study on predator boldness or aggressive behavioral responses for *O. virilis* may shed further light on different anti-predation alterations *Microphallus* may have on *O. virilis*. This would be more accurate considering the fact that native species may have coevolved with *Microphallus* being more resistant or less susceptible to effects of parasitism. Studies have suggested that invasive species are successful in that their native parasites are not brought along with them and they are less susceptible to native parasites in the invaded area. While infections may still occur, invasive species typically have fewer parasite species (Torchin and Mitchell 2004). Identification of *Microphallus* parasites to species is warranted to see if there may be a species causing the most influence in behavior alterations, and it is possible that the infected *O. virilis* used in this study could have had a different species of *Microphallus*. This study could also benefit with the actual assessment and quantification of crayfish aggressiveness in the presence of a predator using an ethogram for a more comprehensive study of behaviors in infected crayfish versus uninfected. It is also important to identify how parasites may contribute to fresh water community level effects, especially for parasites like *Microphallus* that have multiple hosts, this should be considered before the utilization of *Microphallus* as bio-control for *O. rusticus.*

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References cited

Bernot, R.J. 2003. Trematode Infection Alters the Antipredator Behavior of a Pulmonate Snail. *Journal of the North American Benthological Society* 22(2): 241-248.

Capelli, M.G. 1983. Displacement of Northern Wisconsin crayfish by *Orconectes rusticus*. *Limnology Oceanography* 27(4): 741:745.

Capelli, M.G. and B. L. Munjal. 1982. Aggressive interactions and resource competition In relation to species displacement among crayfish of the genus *Orconectes. Journal of Crustacena Biology* 2(4): 486-492.

Caveny, B.A. and F.J. Etges. 1971. Life History Studies of *Microphallus opacus* (Trematoda: Microphallidae). *The Journal of Parasitology* 57(6): 1215-1221.

Dunn, A.M., M.E. Torchin, M.J. Hatcher, P.M. Kotanen, D.M. Blumenthal, J.E. Byers, C.A.C. Coon, V.M. Frankel, R.D. Holt, R.A. Hufbauer, A.R. Kanarek, K.A. Schierenbeck, L.M. Wolfe and S.E. Perkins. 2012. *Functional Ecology* 26: 1262-1274

Garvey, J.E., R.A. Stein, and H.M. Thomas. 1994 Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. *Ecology* 75(2): 532-547.

Kunz, A.K. and O.J. Pung. 2004. Effects of *Microphallus turgidus* (Trematoda: Microphallidae) on the predation, behavior, and swimming stamina of the grass shrimp *Palaemonetes pugio. Journal of Parasitology* 90(3): 441-445.

Lefevre, T, C. Lebarbenchon, M. Gathier-Clerc, D. Misse, R. Poulin, and F. Thomas. 2008. The ecological significance of manipulative parasites. *Trends in Ecology and Evolution* 24(1): 42-48.

Levri, E.P., S.J. Lunnen, C.T. Itle, L. Mosquea, B.V. Kinkade, T.G. Martin, and M.A. DeLisser. 2007. Parasite-induced alteration of diurnal rhythms in a freshwater snail. *Journal of Parasitology* 93(2): 231-237.

MYSTAT 12 ®. 2008. Cranes Software International Ltd. Illinois, Chicago.

Olden, J.D., J.M. McCarthy, J.T. Maxted, W.W. Fetzer, M.J., and V Zanden. 2006. The rapid spread of rusty crayfish (Orconectes rusticus) with observations on native crayfish declines in Wisconsin (U.S.A.) over the past 130 years. *Biological Invasions* 8:1621-1628.

Overstreet, R.M., R.W. Heard, and J.M. Lotz. 1992. Microphallus fonti sp. n. (Digenea: Microphallidae) from the Red Swamp Crawfish in Southern United States. *Memorias Instituto Oswaldo Cruz, Rio de Janeiro* 87(1) 175-178.

Prenter, J., C. MacNeil, J.T.A. Dick, and A.M. Dunn. 2004. Role of parasites in animal invasions. *Trends in Ecology and Evolution* 19(7): 385-390

R. 1997. GNU project, Inc. Auckland, New Zealand.

Roesler, Craig. 2009. Distribution of a Crayfish Parasite, Microphallus sp. In Northern Wisconsin Lakes and Apparent Impacts on Rusty Crayfish Populations. Wisconsin Department of Natural Resources, Hayward.

Sargent,L., K. Towle, A. Baldridge and D. Lodge. 2012. Endoparasite impacts on rusty crayfish (Orconectes rusticus). Report for the Wisconsin Department of Natural Resources.

Stunkard, H.W. 1968. The asexual generation, life cycle and systematic relations of *Microphallus limuli*. *The Biological Bulletin* 134(2) 332-343.

SYSTAT 13 ®. 2008. Cranes Software International Ltd. Illinois, Chicago.

Toft, C.A. and A.J. Karter. 1990. Parasite-host coevolution. *Trends in Ecology and Evolution* 5(10): 326-369

Torchin, M.E. and C.E. Mitchell. 2004. Parasites, pathogens, and invasions by plants and animals. *Frontiers in Ecology and the Environment* 2(4): 183-190

Uitenbroek, D. G. (1997) "SISA-Binomial", Simple Interactive Statistical Analyses (SISA), Inc. Available: http://www.quantitativeskills.com/sisa/distributions/binomial.htm.

Wilson, K.A., J.J. Magnuson, D.M. Lodge, A.M. Hill, T.K. Kratz, W.L. Perry, and T.V. Willis. 2004. A long-term rusty crayfish (*Orconectes rusticus*) invasion: dispersal patterns and community change in a north temperate lake. *Canadian Journal of fisheries and aquatic sciences* 61:2255-2266.

Species	**Infection Status**	**Left Shelter**	**Stayed in Shelter**	**Chi-Square/ Fisher's exact Test P-Value /**
O. virilis	Uninfected	13	12	0.569
	Infected	15	10	
O. propinquus	Uninfected	21	4	0.428
	Infected	23	2	
O. rusticus	Uninfected	18	3	0.115
	Infected	21	0	

Table 1. Chi-square and Fisher's exact Test on infected and uninfected *O. virilis, O. propinquus,* and *O. rusticus* leaving the shelter.

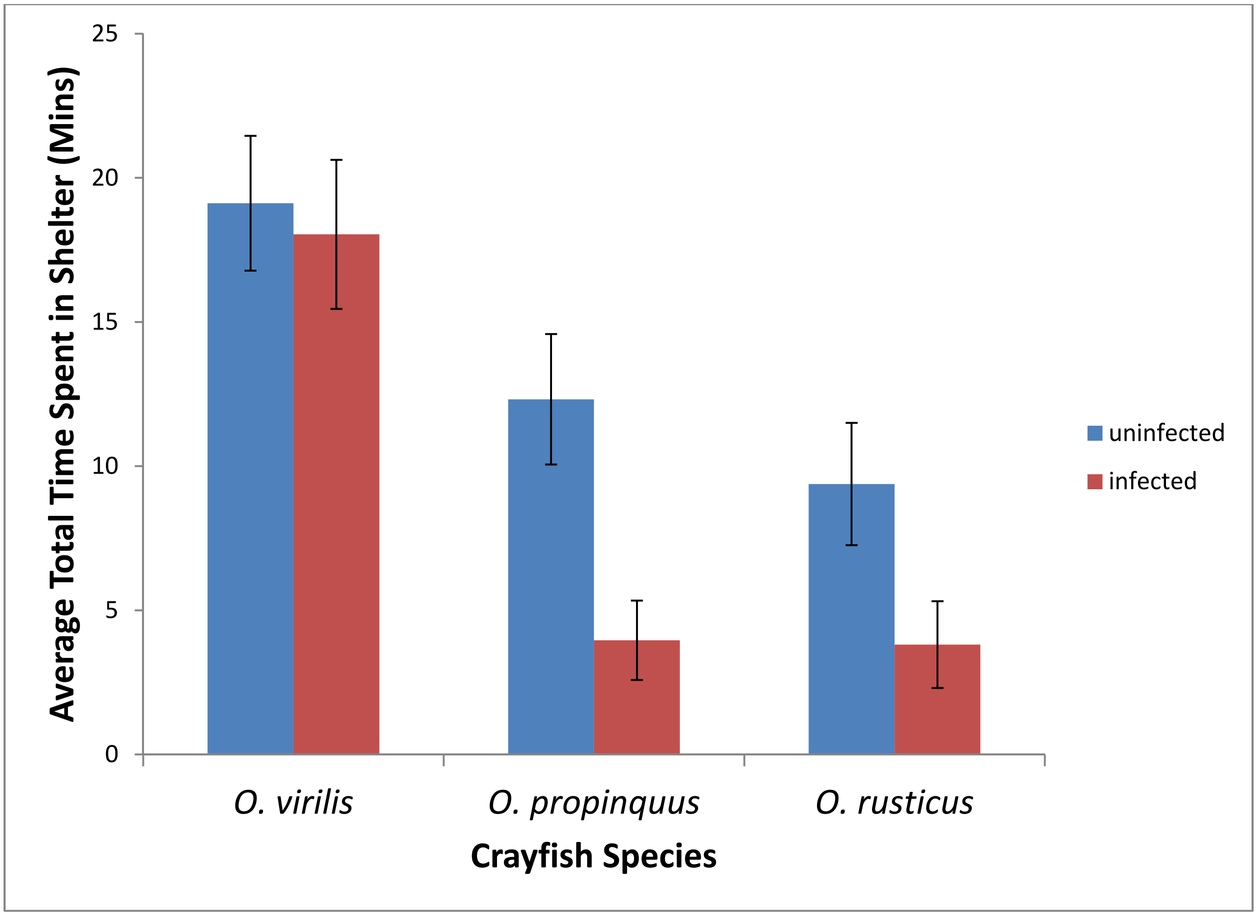

Figure 1. Means of total time spent in shelter (minutes) for infected and uninfected *O. virilis*, *O. propinquus*, and *O. rusticus*. Errors bars are representative of standard error for each mean.

Figure 2. Experimental design displaying PVC shelters, plexiglass doors, and screen separating crayfish from rock bass