CONTEMPORARY EVOLUTION AND PARASITISM ALTER THE ECOLOGICAL IMPACTS OF AN INVASIVE CRAYFISH

A Dissertation

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Invasive species can substantially alter ecological communities and ecosystem processes. I examined whether contemporary evolution (evolution that occurs on ecological timescales) and parasitism within the invaded range affect the success and impacts of an invasive species, the rusty crayfish (Orconectes rusticus). Previous research indicates that both contemporary evolution and parasitism can be important; however, community ecologists still lack a broad understanding of how these factors affect communities.

My research on contemporary evolution indicates that growth rate has diverged between the native and invaded ranges since O. rusticus were introduced to north temperate lakes. Rapid individual growth, which leads to increased reproduction in crayfish, may be selected for in invasive populations because crayfish are introduced at low densities. Faster growth in the invaded range contributes to the impacts of O. rusticus by allowing them to reach high densities and replace congeners. These findings suggest that including evolutionary potential in risk assessments may enhance our ability to predict invasion success and impacts.
My research also indicates that parasitism affects *O. rusticus* invasion success and impacts. I found that trematode parasites (*Microphallus* spp.) were associated with declines in *O. rusticus* population growth. I also examined the behavioral effects of *Microphallus* on crayfish feeding, aggression, shelter use, and predator avoidance. Infection substantially altered crayfish behavior, and had different effects on *O. rusticus* and its congener. In a mesocosm experiment to test how these parasite-induced behavioral changes impact the benthic aquatic community, infected *O. rusticus* had a greater per-capita impact on macrophytes and macroinvertebrates than uninfected *O. rusticus* when fish were present, likely due to increased boldness.

Finally, I used a survey of anglers to investigate whether a change in Missouri policy to prevent crayfish introductions would have the intended consequences of protecting game fish populations and the fishing industry. I found that banning crayfish from the bait trade is unlikely to reduce angler spending, but instead could increase the revenue generated by the fishing industry by protecting game fish populations. Overall, my dissertation provides new information about the importance of contemporary evolution, parasitism, and human behavior in controlling invasion success and community composition.
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CHAPTER 1:
INTRODUCTION

1.1 Nonindigenous species and invasion success

Nonindigenous species can substantially alter ecological communities and
ecosystem processes, sometimes causing ecological and economic harm (Keller et al.
dominant nonindigenous species which reach high biomass and outcompete native
species have received much attention from ecologists over recent decades (e.g. Blossey
Gurevitch et al. 2011). One might expect native species to consistently have higher
fitness than nonindigenous species because native species have the benefit of years of
natural selection in response to the local abiotic and biotic environment. However,
nonindigenous species also leave behind organisms such as parasites, predators, or
consumers that may regulate their populations in the native range, and escape from these
organisms may allow nonindigenous species to be especially successful in the introduced
range (Keane and Crawley 2002, Torchin et al. 2002, Inderjit and van der Putten 2010).
Further, there is recent evidence that nonindigenous species can rapidly adapt to
conditions within the introduced range (Sakai et al. 2001, Cox 2004, Hairston et al. 2005,
Huey et al. 2005), and therefore, the relatively short length of coevolutionary history
between a nonindigenous species and the biotic and abiotic environment may be unimportant.

1.2 Nonindigenous species and evolution

Whether or not nonindigenous species are ecologically different from native species has recently received much attention (e.g. Davis et al. 2011, Simberloff and Vitule 2013). However, a recent literature review indicates that dominant nonindigenous species typically have greater impacts on lower trophic levels than dominant native species (Paolucci et al. 2013), likely because they do not share a long coevolutionary history with other organisms in the community. This likely allows nonindigenous predators, consumers, or pathogens to have greater effects on naïve, native organisms in lower trophic levels. It is important that ecologists and managers understand the influence of coevolution on interspecies interactions in order to manage the many ecosystems that now contain a mix of nonindigenous and indigenous species. Further, biological invasions provide natural experiments that allow us to examine the importance of coevolution and interspecies interactions in shaping community structure and composition in native communities.

Nonindigenous species may diverge further from native species because they may adapt to conditions during an introduction. Nonindigenous species are initially introduced at low densities. Low density populations are likely to grow exponentially, and theory suggests that \( r \)-selected traits are more beneficial in exponentially growing populations (Lewontin 1965). Therefore, we would expect \( r \)-selected traits to evolve in introduced populations. These traits may contribute to the strong impacts of invasive
species by allowing them to spread rapidly and reach high densities. Recent empirical data support this theory. For example, when raised in common conditions, cane toads (*Bufo marinus*) from the edge of their invaded range grew more rapidly and reached reproductive maturity earlier than those from longer established populations (Phillips 2009). In addition, comparing native and invaded range populations indicates that faster growth, wide environmental tolerance, shorter generation time, and increased reproductive capability has evolved in the invaded range in a number of populations (Whitney and Gabler 2008). However, few studies have used reciprocal transplant or common garden experiments (as opposed to field observations) to compare differences in invasive traits between populations, and most of these studies focus on introduced plants (Koskinen et al. 2003, Lee et al. 2003, Phillips 2009). Therefore, it remains unclear how often invasive traits evolve in nonindigenous animal populations.

1.3 Nonindigenous species and parasitism

In addition to *r*-selection, parasites (or lack thereof) may be important in determining whether nonindigenous species achieve high demographic success (Prenter et al. 2004, Dunn et al. 2012). The study of the impact of parasites on invasions has largely focused on how release from home-range parasites can lead to invasion success. Few studies, however, have examined how parasites encountered in the introduced range control populations of nonindigenous hosts, but this interaction may be equally important (Colautti et al. 2004, Mitchell et al. 2006). A growing body of literature suggests that parasites can alter interactions between pairs of invasive and native species (Bauer et al. 2000, Tompkins et al. 2003, Georgiev et al. 2007, Dunn et al. 2012). The numerous
introduced parasites or pathogens that have catastrophic effects on naïve, native populations (e.g. chytrid fungus, crayfish plague, and hemlock woolly adelgid) indicate that a lack of coevolutionary history between hosts and pathogens can dramatically reduce naïve host populations (Vogt 1999, Orwig et al. 2002, Fisher et al. 2009). However, in other cases, parasites and pathogens may be more detrimental to the host with which they share the longest coevolutionary history (Dunn et al. 2012). For example, an acanthocephalan parasite can successfully manipulate the behavior of a native gammarid host, increasing predation on this species and transmission of the parasite to higher trophic levels, but this parasite is unable to alter the behavior of a nonindigenous gammarid (Bauer et al. 2000). Even though these host-parasite interactions are ecologically important, we still lack a general framework for predicting the relative impacts of particular types of parasites (such as those that are trophically transmitted) on hosts with which they share a long coevolutionary history and those with which they do not. Additional research examining host-parasite interactions among species that share different lengths of coevolutionary history would help address this problem.

1.4 Crayfish in north temperate lakes as a model system

Long term trends, ecological impacts, and interspecies interactions surrounding the introduction of nonindigenous crayfish in north temperate lakes in Wisconsin and Michigan have been well-studied (e.g. Olden et al. 2006, Kreps et al. 2012, Peters and Lodge 2013). Therefore, crayfish in north temperate lakes provide an ideal model system for examining emerging topics in invasion biology such as the effects of contemporary
evolution (evolution that occurs on ecological timescales) and parasitism on the impacts of nonindigenous populations.

Rusty crayfish, *Orconectes rusticus*, are native to streams in the Ohio River drainage and were introduced by anglers to northern Wisconsin and Michigan lakes in the mid 1960s (Olden et al. 2006). *O. rusticus* are a major driver of community composition in northern Wisconsin lakes where they reduce the abundance and richness of macrophytes and benthic macroinvertebrates (Capelli 1982, Lodge et al. 1994, Wilson et al. 2004). *O. rusticus* also reduce the abundance of some species of fish due to a combination of direct consumption of substrate-nesting fish eggs, reduction of macrophyte habitat, and competition for macroinvertebrate prey (Dorn and Mittelbach 1999, Wilson et al. 2004, Baldridge and Lodge 2013). Reductions in fish abundance have economic consequences in northern Wisconsin and Michigan where recreational fishing is an important economic activity (Keller et al. 2008). Crayfish population records indicate that *O. rusticus* remain at low densities in some lakes where they are introduced, but reach ‘outbreak’ densities in other lakes where they alter the ecological community (Roth et al. 2007, Kreps et al. 2012).

Two other crayfish are also common in northern Wisconsin and Michigan lakes. The virile crayfish, *O. virilis*, is native to northern Wisconsin and Michigan. The northern clearwater crayfish, *O. propinquus*, is nonindigenous to this region and was likely introduced by anglers in the mid 1930s (Capelli and Munjal 1982). These two species have lesser impacts on macrophytes, macroinvertebrates, and fish than *O. rusticus* due to differences in consumption rates of macrophytes and macroinvertebrates, differences in growth rates, and differences in densities between species (Olsen et al.)
As O. rusticus and O. propinquus have spread in northern Wisconsin and Michigan, they have displaced other crayfishes. O. propinquus has replaced O. virilis in many lakes where it has been introduced, and O. rusticus has replaced both congeners and is now the most dominant member of the crayfish fauna in Wisconsin (Capelli 1982, Capelli and Magnuson 1983, Olden et al. 2006). These crayfish species replacements have been largely attributed to greater aggression in O. rusticus and a superior ability of O. rusticus to compete for shelter and avoid fish predation (Hazlett et al. 1992, Didonato and Lodge 1993, Garvey et al. 1994, Hill and Lodge 1999). Crayfish reduce their susceptibility to predation primarily through utilizing shelter (Garvey et al. 1994, Soderback 1994). In laboratory experiments, O. rusticus displaces both congeners from shelter, and O. propinquus displaces O. virilis from shelter (Capelli and Munjal 1982), which is consistent with the observed patterns in lake-wide species replacements. Further, in whole-lake studies, O. virilis shifts its distribution from cobble habitat (where shelter is abundant in interstitial spaces) to macrophyte habitat (where it is more exposed) in lakes with O. rusticus (Peters and Lodge 2013).

1.5 Crayfish parasites

Because crayfish and their interactions have strong impacts in north temperate lakes, parasites that affect crayfish in this region are especially likely to be ecologically important. Recently, a number of cryptic trematode parasites in the same species complex as Microphallus opacus and M. fonti were identified in crayfish in northern Wisconsin lakes (Roesler 2009, Overstreet 2011). These microphallid parasites are common and widespread within northern Wisconsin, and the most common of these
*Microphallus* genotypes has been found to frequently infect all three common orconectid crayfishes (Roesler 2009, Overstreet 2011). It is currently unclear whether these parasites were recently introduced to northern Wisconsin lakes, perhaps along with a nonindigenous crayfish, or whether they are native to the region and coevolved with a native crayfish. Previous to my dissertation research, it was unclear how *Microphallus* affected crayfish density, behavior, and ecological impacts.

*Microphallus* must infect multiple hosts in order to complete its life cycle. *Microphallus* reproduces asexually within its first secondary host, a hydrobiid snail (Overstreet 2011). Free-swimming cercariae (larval trematodes) then exit the snail and infect a crayfish if one is encountered. The parasites enter the gill chamber of the crayfish, encyst in the hepatopancreas (digestive organ), and remain there until the crayfish is consumed by a definitive host. The definitive host for these microphallids is currently unknown, but closely related trematodes use one or more bird, fish, or mammal species (Bray et al. 2008, Overstreet 2011). *Microphallus* then reproduces sexually within the definitive host and sheds eggs which may be consumed by hydrobiid snails.

While *Microphallus* metacercariae (encysted trematodes) likely cause little damage to crayfish tissue, they could affect crayfish by altering their behavior. Other microphallid species behaviorally manipulate snail, gammarid, and shrimp intermediate hosts to increase transmission to higher trophic levels (Levri 1999, Kunz and Pung 2004, Helluy and Thomas 2010), and it is possible that crayfish are similarly manipulated by their microphallids. The mechanisms used by parasites to alter host behavior are poorly understood, but there is evidence that parasites can alter concentrations of hormones or neurotransmitters in their hosts (Poulin 2010). This can be achieved by parasites that
infect the brain of their hosts as well as those that are only found in the host’s body cavity (Maynard et al. 1996, Overli et al. 2001, Poulin et al. 2003). Behavioral responses to infection that are beneficial to the host but not the parasite are also often associated with infection. For example, infected hosts may reduce their activity or bask to increase their body temperature (Moore 2002). If *Microphallus* alters crayfish behavior in north temperate lakes, the effects of these parasites on the community and ecosystem could be substantial because small differences in crayfish behavior among species can cause crayfish species replacements (Garvey et al. 1994, Soderback 1994, Pintor and Sih 2009) and differences in per capita impacts on lower trophic levels (Olsen et al. 1991).

1.6 Dissertation overview

The major goals of my dissertation research were to examine how contemporary evolution and parasitism alter the success and ecological impacts of an invasive species (Figure 1.1). I used nonindigenous crayfish in north temperate lakes to test how these factors affected crayfish traits, density, interspecies interactions, and impacts on lower trophic levels. In addition, I used my background on the impacts of nonindigenous crayfish combined with an angler survey to evaluate a policy approach to managing crayfish introductions.
Figure 1.1 Major interactions between *O. rusticus* and other organisms in north temperate lakes. Solid arrows indicate trophic interactions, and dashed arrow indicates competitive interactions. My dissertation focuses on how contemporary evolution and parasitism alter (A) *O. rusticus* traits and population growth, (B) interactions between *O. rusticus* and predatory fish, (C) interactions between *O. rusticus* and congeners, and (D) impacts of *O. rusticus* on lower trophic levels (macrophytes and macroinvertebrates). In addition, I investigated the consequences of policy surrounding (E) crayfish bait-bucket introductions.
In Chapter 2 (published as Sargent and Lodge 2014), I conducted two common garden experiments to evaluate evolution in crayfish invasions. I tested for differences in growth rate, survival, and response to predators in native and invaded range populations of *O. rusticus*. I hypothesized that low conspecific densities during introductions into lakes would select for increased investment in growth and reproduction in invasive populations. I collected crayfish as eggs from *O. rusticus* populations from both the native and invaded range and reared them in common garden experiments in invaded range lakes and mesocosms, the latter in which I also included treatments of predatory fish presence and food quality. I also tested for differences in egg size between the two ranges to determine whether differences in this aspect of maternal investment could explain differences in growth. Overall, this research examines whether evolution within the introduced range contributes to the strong impacts of *O. rusticus* in north temperate lakes. Studies such as this one demonstrate the importance of evolution in controlling the impacts of invasive species.

In Chapter 3 (published as Sargent et al. 2014), I investigated whether *Microphallus* parasites could contribute to previously documented alternate states in the abundance of *O. rusticus* in north temperate lakes. The first secondary host of *Microphallus* is a hydrobiid snail, and *O. rusticus* reduces the density of snails through direct predation and destruction of macrophyte habitat. Therefore, if *Microphallus* substantially reduces *O. rusticus* fitness, these parasites may reinforce a state of low crayfish abundance, and, at the other extreme, abundant crayfish may repress these parasites, reinforcing a state of high crayfish abundance. To examine these relationships, I used trap catch data to examine *O. rusticus* abundance in 109 sites in 16 lakes. Within
each site, I dissected crayfish to determine parasite prevalence (% of crayfish infected). The following year, I trapped the same sites to assess crayfish population growth between years. I also examined hydrobiid snail abundance at a subset of sites to examine the relationship between snail abundance and parasite abundance within crayfish. Finally, with experiments, I examined whether infection affected *O. rusticus* growth and feeding behavior because these effects are likely to have strong impacts on crayfish demographic success. While the importance of enemy release in facilitating invasions has often been emphasized, few studies have addressed the role of parasites in the invasive range in controlling demographic success of potential invaders. The results from this chapter contribute to our understanding of the effects of parasites on invasive species densities.

In Chapter 4 (published as Reisinger et al. in press), I examined whether infection with *Microphallus* altered the behavior of *O. rusticus*, *O. propinquus*, and *O. virilis*. I collected infected and uninfected crayfish of all three species from the field and examined their shelter affinity in laboratory experiments in the presence and absence of a conspecific. Crayfish avoid predation largely by utilizing shelter, so any significant impacts on shelter use would likely alter crayfish mortality rates. I also observed crayfish agonistic interactions to determine how infection alters competitive interactions. Finally, I measured crayfish behavior (boldness) in the presence of a predator by quantifying how quickly crayfish emerged from shelter with a predatory fish present. I then compared my results to those from previous behavioral studies to determine whether *Microphallus* was likely to alter direct or apparent competition between these crayfish species in lakes via behavioral modifications. This study is one of the first to test whether parasites alter
crayfish behavior, and these results contribute to our understanding of the role of parasites in controlling competitive interactions between native and invasive species.

In Chapter 5 (unpublished), I used a large mesocosm experiment to test whether the behavioral changes associated with infection could alter the per capita impacts of *O. rusticus* on lower trophic levels. Results from Chapter 3 and Chapter 4 indicate that infected *O. rusticus* consume fewer macroinvertebrates and are bolder in the presence of predatory fish than uninfected individuals. Therefore, I predicted that when predators are absent, infected crayfish would have reduced impacts on macrophytes and macroinvertebrates compared to uninfected crayfish. However, when predators are present, I expected infected crayfish to have the greatest impact on lower trophic levels because of increased boldness. Results from this chapter reveal whether parasites in this ecosystem can substantially alter invasive species impacts merely by modifying their behavior.

Finally, in Chapter 6 (unpublished), I investigated whether a change in policy to prevent crayfish introductions and to protect the fishing industry from crayfish impacts would have the intended consequences. I conducted this research in Missouri, where policymakers have recently considered prohibiting the sale of crayfish as bait. I used a mail survey of anglers in Missouri to determine how banning crayfish would affect angler behavior and the fishing industry. I was especially interested in whether banning crayfish from the bait trade would cause anglers to fish less often and whether anglers would be likely to collect crayfish directly to use as bait if crayfish were not available from bait shops. If directly collecting crayfish (and introducing them to new waters) increases in response to banning crayfish from the bait trade, this behavior could be a substantial new
source of crayfish introductions. Results from this chapter inform whether banning crayfish from the bait trade will be an effective way to reduce new crayfish introductions in Missouri and if this policy change will likely impact the revenue generated by the fishing industry.

Overall, my dissertation uses a combination of small scale laboratory experiments, large mesocosm experiments, a field survey, and a survey of human behavior to investigate the factors that control the introduction and impacts of an invasive species. My dissertation focuses on the effects of contemporary evolution and parasitism within the invaded range because the effects of these factors are likely to be important in invasions but are not well understood. These studies will not only inform invasion biology, but also elucidate the importance of contemporary evolution and parasitism in communities in general.

1.7 References


CHAPTER 2:
EVOLUTION OF INVASIVE TRAITS IN NONINDIGENOUS SPECIES:
INCREASED SURVIVAL AND FASTER GROWTH IN INVASIVE POPULATIONS
OF RUSTY CRAYFISH (*ORCONECTES RUSTICUS*)

2.1 Abstract

The importance of evolution in enhancing the invasiveness of species is not well understood, especially in animals. To evaluate evolution in crayfish invasions, we tested for differences in growth rate, survival, and response to predators between native and invaded range populations of rusty crayfish (*Orconectes rusticus*). We hypothesized that low conspecific densities during introductions into lakes would select for increased investment in growth and reproduction in invasive populations. We reared crayfish from both ranges in common garden experiments in lakes and mesocosms, the latter in which we also included treatments of predatory fish presence and food quality. In both lake and mesocosm experiments, *O. rusticus* from invasive populations had significantly faster growth rates and higher survival than individuals from the native range, especially in mesocosms where fish were present. There was no influence of within-range collection location on growth rate. Egg size was similar between ranges and did not affect crayfish growth. Our results, therefore, suggest that growth rate, which previous work has shown

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1 In *Evolutionary Applications* with coauthor D. M. Lodge. 2014. doi:10.1111/eva.12198
contributes to strong community-level impacts of this invasive species, has diverged since *O. rusticus* was introduced to the invaded range. This result highlights the need to consider evolutionary dynamics in invasive species mitigation strategies.

### 2.2 Introduction

Evolution in nonindigenous populations contributes to the success and harmful impacts of some invasive species (Siemann and Rogers 2001, Handley et al. 2011), but how often this phenomenon occurs, especially for animals, is inadequately understood. Nonindigenous species can dramatically change biotic communities and ecosystem processes, sometimes causing extensive ecological or economic harm (Sala et al. 2000, Keller et al. 2009, Butchart et al. 2010). However, only a small percentage of nonindigenous species have strong, community-level impacts (Ricciardi and Kipp 2008). *r*-selected life history traits such as rapid growth and high fecundity are common among many of those nonindigenous species that have strong impacts (Sakai et al. 2001, van Kleunen et al. 2010, Lamarque et al. 2011), and characteristics of the environment and biotic community within the introduced range are also often important for invasion success (Catford et al. 2009). A subset of introduced species already possess *r*-selected life history traits upon arrival in a novel habitat, but other species may evolve toward these traits in response to selection in the invaded range.

Theory suggests that populations with lower conspecific densities should have greater *r*-selection (Lewontin 1965), and recent empirical data support this theory. For example, cane toads (*Bufo marinus* Linnaeus) from populations on an expanding range edge grow more rapidly than those from longer established populations when raised in
common conditions and, therefore, reach reproductive maturity more quickly (Phillips 2009). In addition, a recent review of trait evolution in nonindigenous populations reveals that some invasive populations have evolved faster growth, wide environmental tolerance, shorter generation time, and increased reproductive capability in the invaded range (Whitney and Gabler 2008). However, few studies have tested for differences in invasive traits between populations using reciprocal transplant or common garden experiments (as opposed to comparative field observations), and almost all of these studies focus on introduced plants (but see Koskinen et al. 2002, Lee et al. 2003, Philips 2009). Therefore, it remains unclear how often invasive traits evolve in nonindigenous animal populations. Though evolution within the invasive range may alter the impact of nonindigenous species, evolutionary potential is rarely included in risk assessments and policy decisions involving species introductions (Whitney and Gabler 2008).

To evaluate the likelihood of evolution influencing crayfish invasiveness, we conducted a series of common garden experiments to test whether differences existed in growth rate, survival, and response to predators in young of year (YOY) rusty crayfish (*Orconectes rusticus* Girard) from native and invasive populations. *O. rusticus* is one of many species of crayfish that have been introduced globally (Lodge et al. 2012). *O. rusticus*, in particular, causes major community-level impacts in their invaded range. *O. rusticus* is native to streams in the Ohio River drainage in Ohio, Indiana, and Kentucky and was introduced by anglers to northern Wisconsin and Michigan lakes in the mid 1960s as well as to Illinois, Minnesota, Ontario (Canada), the Laurentian Great Lakes, and portions of 11 other states (Olden et al. 2006, Peters et al. 2014).
For this study, we focused on comparing well-studied invasive *O. rusticus* populations from northern Wisconsin to lesser studied native populations from the Ohio River drainage. Where *O. rusticus* has become abundant in Wisconsin and Michigan lakes, it has displaced resident crayfishes, reduced the abundance and richness of macrophytes and macroinvertebrates, and caused declines in the abundance of panfish (*Lepomis* spp.) (Capelli 1982, Lodge et al. 1994, Wilson et al. 2004, Olden et al. 2006). Faster growth of *O. rusticus* also contributes to the displacement of resident crayfishes (Hill et al. 1993, Garvey et al. 1994). In addition, *O. rusticus* reaches higher densities than other crayfishes in this region, which contributes to its strong impacts (Wilson et al. 2004). To our knowledge, the community-level impacts of *O. rusticus* in the native range have not been investigated. Pintor and Sih (2009) found that *O. rusticus* from an invasive population grew more rapidly than *O. rusticus* from a native population when competing with congeners in mesocosms. However, because adult crayfish collected from the field were used in this study, it is unclear whether this result was due to evolution or to environmental differences between the two collection locations. Here we use experiments to test for divergence in *r*-selected traits, specifically YOY growth rate and survival, in invasive *O. rusticus* populations in Wisconsin.

To determine whether there are widespread growth rate and survival differences between *O. rusticus* from native and invasive populations, we first reared crayfish from both ranges in enclosures in three lakes within the invaded range in summer 2011. We selected lakes with different abundances of predatory fish and macroinvertebrate prey to determine whether differences existed in growth rate or survival among different invasive range environments. Then in summer 2012, to provide evidence for the hypothesis that
there is a genetic basis for the differences we observed, we reared crayfish in mesocosms
where we controlled temperature and varied the presence of predatory fish and food
quality to determine which factors were important in controlling *O. rusticus* growth rate
and survival. Previous research indicates that predatory fish can reduce crayfish feeding
activity (Stein and Magnuson 1976, Hill and Lodge 1995) and growth (Hill and Lodge
1999). We hypothesized that crayfish from the invaded range would respond less (i.e.
smaller reduction in feeding activity) to predatory fish than those from the native range
because there is likely to be a greater fitness benefit to allocating time to feeding (growth)
within the invaded range. In addition, food quality (Hill et al. 1993) and temperature
(Mundahl and Benton 1990) are important for crayfish growth. It is possible that rapid
growth of invaded range crayfish can only be achieved in locations with abundant, high
quality food resources. Our study is the first to test whether food quality and predator
abundance have different effects on *O. rusticus* from native and invasive populations.
Finally, we investigated the potential influence of maternal effects on results by testing
for the effects of clutch and initial egg weight. Our study examines whether there are
growth differences between replicated populations of rusty crayfish from the invaded and
native range, and whether the observed differences have an environmental or genetic
basis.

2.3 Methods

2.3.1 Lake common garden experiment

To test whether differences exist in growth rate and survival between native and
invasive *O. rusticus*, we reared YOY crayfish from native and invasive populations in
enclosures in invaded range lakes in summer 2011. We hand collected berried females (females with eggs attached to their abdomen) from the Great Miami (39°56’N, 83°44’W and 39°56’N, 83°42’W) and Little Miami (38°54’N, 83°34’W) river drainages in the native range in Ohio, USA and from High Lake (46°08’N, 89°32’W), Big Lake (46°11’N, 89°26’W), and Papoose Lake (46°10’N, 89°48’W) in the invaded range in Wisconsin, USA. Because temperatures are warmer in the native range than in the invaded range, *O. rusticus* females extrude eggs earlier in the native range. We collected females from streams in the native range in late April and from lakes in the invaded range in late May. Each female was placed in an individual container (18 x 18 cm) in the laboratory with constantly aerated well water, a shelter constructed from a polyvinyl chloride pipe, and gravel substrate. Eggs hatched, and young became independent from females three to four weeks after collection. Females were removed from containers once young were independent. YOY were fed a combination of spirulina disks and shrimp pellets *ad libitum* while in the laboratory. YOY were placed in lakes one to two weeks after they became independent from females (in late May for native range YOY and early July for invaded range YOY). All YOY were removed from lakes on September 9th after native range crayfish were in lakes for 15 weeks and invaded range crayfish were in lakes for 10 weeks.

Within lakes, crayfish were each housed in an individual clear plastic container (18 x 18 x 12.7 cm) with large rectangular holes (14 x 8 cm) cut into each side and replaced with window screen. Screened sides prevented crayfish escape, but allowed crayfish to be in contact with the physical and chemical lake environment and to receive visual cues from predators. Four to six grams of natural woody debris (sticks) were
added to each container and four stones were glued to the bottom to increase container weight and provide shelter. Containers were placed between 0.25 – 0.5 m depth at one of two sites (sites were 50 - 100 m apart) in each of three lakes, Big Lake, High Lake, and Papoose Lake in Wisconsin, USA. Two sites were used in each lake to hedge against total loss of crayfish in the case of disturbance by humans, other animals, or storms. Each site contained 1 YOY from each brood (13 invasive females and 13 native females), so that there were a total of 26 YOY housed at each site and 52 YOY housed in each lake.

Lakes were chosen based on different invasion histories. Papoose Lake had high densities of *O. rusticus* (35 *O. rusticus* per trap in 2011), and therefore we expected this lake to have reduced macrophyte, macroinvertebrate, and panfish populations resulting from *O. rusticus* impacts (Wilson et al. 2004). High Lake had low densities of *O. rusticus* (4 *O. rusticus* per trap in 2011), and Big Lake had moderate densities of *O. rusticus* (19 *O. rusticus* per trap in 2010).

Total length of YOY was measured every six to eight days. Growth rate was calculated as the difference between initial and final length divided by days in the experiment. Mortalities that occurred within the first three weeks of the experiment were replaced with individuals from the same brood that were housed in the laboratory with the same husbandry and conditions as provided after hatching.

Data from a previous preliminary experiment in Big Lake indicated that Big Lake YOY grown in containers (and fed only though natural colonization of the containers by macroinvertebrates) were equal in length to those YOY growing outside of containers at the end of the summer. Therefore, we did not add food to the experimental containers.
Containers in all three lakes were quickly colonized by macroinvertebrates which provided food for crayfish. To provide an index of any differences in food availability among lakes, six containers without crayfish were placed in each lake in June. Macroinvertebrates were removed from these containers in August and preserved in 70% ethanol. We compared the ash free dry mass of these macroinvertebrates among lakes.

We also assessed temperature and the abundance of predatory fishes in each lake because they might affect crayfish growth rates. Hourly temperature was recorded at each site using temperature loggers (Onset Computer Corporation). Predatory fish abundance was assessed in each lake using three fyke nets set for one night at the end of June and one night at the end of July. Fyke nets were set within 50 m of crayfish sites, and thus were intended to measure fish activity at those specific locations.

2.3.2 Mesocosm common garden experiment

To examine whether differences in growth rate and survival observed between YOY crayfish from native and invasive populations could be genetically based, and to identify important factors influencing growth rate and survival, we raised crayfish in common conditions in mesocosms in summer 2012. We used a 2x2x2 factorial design to examine the effects of range, predators, and food quality on the growth rate, survival, and behavior of rusty crayfish. As in the 2010 lake experiments, berried females were collected earlier from native range locations due to differences in reproductive timing between the two ranges. We hand collected berried females in early April from the Little Miami (38°54’N, 83°34’W and 39°47’N, 83°51’W) and Scioto River (40°00’N, 83°23’W) drainages in Ohio, USA. In early May, we collected berried females from High Lake (46°08’N, 89°32’W), Big Lake (46°11’N, 89°26’W), and Papoose Lake.
(46°10’N, 89°48’W) in Wisconsin, USA. Housing of females in the laboratory and all other husbandry practices were the same as for lake experiment unless specified below. YOY from the native range were placed in experimental mesocosms in late May, and YOY from the invaded range were placed in experimental mesocosms in late June. While native and invasive YOY were placed in mesocosms at different times during the summer, we controlled temperature and food availability so that all crayfish experienced the same environmental conditions throughout the experiment (as described below).

Within each mesocosm, ten invaded range and ten native range YOY crayfish were each housed individually in a clear plastic container with screened sides (identical to those used in lake experiments), so that the growth of each crayfish was independent and was not affected by the other crayfish in the mesocosm. Two stones were glued to one side of the bottom of the container to provide shelter for crayfish. On the opposite side, we attached a small nylon nut and bolt which held disks of prepared food (described below) securely in place. Crayfish, therefore, had to choose between feeding and hiding. Total length of crayfish was measured once every seven days, and crayfish were removed from the experiment after seven weeks. We replaced mortalities that occurred within the first two weeks of the experiment with crayfish from the same range and, if possible, the same brood. Replacement crayfish were housed in the laboratory with the same husbandry and conditions as provided after hatching.

Mesocosms consisted of 416 L plastic tanks with flow-through, aerated well-water, and were located in a wooded area on the shore of Trout Lake (Wisconsin, USA), under a suspended tarp to reduce light, falling debris, and heat load. There were 12 mesocosms in total, with 20 YOY *O. rusticus* in individual containers (10 invasive and
10 native) reared in each mesocosm. Temperature was maintained in each mesocosm by a 300 W heater, and each mesocosm was aerated to maintain high dissolved oxygen (8-10 mg/L) and uniform temperature. Hourly temperature was recorded in each mesocosm using temperature loggers (Onset Computer Corporation; mean temperature = 18.1°C, summertime range = 10 - 25°C). These temperatures are cooler than summer invaded range lake temperatures (mean temperature was 24.3°C in lake epilimnia during the first 7 weeks of crayfish growth in 2011); however, we were only able to heat well water to this extent in early summer, and needed to keep temperatures consistent later in the summer so that invaded range crayfish experienced the same conditions as native range crayfish. We tested whether temperature was different during native and invaded range crayfish growth periods using ANOVA of average weekly temperature in each mesocosm. To examine whether predators had an effect on growth, six of the twelve mesocosms contained predatory fish (three bluegill, *Lepomis macrochirus* Rafinesque, and three smallmouth bass, *Micropterus dolomieu* Lacépède). Bluegill ranged in size from 9.5 to 13 cm total length during the experiment, and smallmouth bass ranged in size from 10.5 to 14.5 cm total length. Fish were fed *O. rusticus* (three per mesocosm) once per week and earthworms (*Lumbricus terrestris* Linnaeus) twice per week for the duration of the experiment. Fish readily consumed both food types. Bluegill and smallmouth bass are common in both the native and invaded range of *O. rusticus* (Boschung et al. 1983).

To examine the effect of food quality of crayfish growth, half of the crayfish in each mesocosm were fed high quality food and half were fed low quality food, which we created by mixing 500 ml of plant and animal matter with 20 g of sodium alginate and
750 mL of water. Using methods similar to those used by Cronin et al. (2002), we solidified food by pouring dissolved calcium chloride (14 g calcium chloride in 500 mL water) over a thin sheet of this food mixture. Food was cut into 2.5 – 5 g squares and secured in each container weekly with the nylon nut and bolt. Except for a few of the largest crayfish at the end of the experiment, some food remained in each container at the end of each week, so the amount of food was ad libitum. High quality food consisted of 40% macrophytes (Potamogeton amplifolius Tuck, Potamogeton richardsonii (Bennett) Rydberg, and Sagittaria graminea Michaux) and 60% animal matter (earthworms and bluegill filets). Low quality food was made from the same organisms, but contained 80% macrophytes and 20% animal matter. All food was frozen after it was made, and thawed within one day of placing it in the experiment. Native and invasive crayfish were fed from the same batch of food during the same week of growth.

2.3.3 Statistical analyses

For the lake common garden experiment, we used ANOVA to examine the effects of range (native or invasive) and lake on growth rate (mm/day). We also included initial length of YOY and maternal identity (clutch) as covariates to account for potential effects of these variables. Only crayfish that survived for the entirety of the experiment were used in the growth analysis. We also used ANOVA to examine the effect of the collection location within each range on growth. For this analysis, we ran one ANOVA for native range crayfish and one ANOVA for invaded range crayfish, and also included the effect of lake (where YOY were housed) in each model. Initial length and clutch were not included in this second analysis because they were found to be unimportant in the first growth model. Because native and invaded range crayfish were placed in lakes
at different times, we also conducted an analysis of weekly growth to better account for the varying effects of temperature and crayfish length throughout the summer. We used a linear mixed effects model to examine the effects of range, lake, length of crayfish (at the start of the week), and average temperature (during the one week growth period) on weekly crayfish growth. We included crayfish identity in this model as a random effect.

For the mesocosm common garden experiment, we used a linear mixed effects model to examine the effects on crayfish growth rate of range, fish, food quality, and all interactions between these variables. In addition, mesocosm nested within fish treatment was included as a random effect. We also included the effects of average temperature and initial length in the model to account for potential effects of these variables. Although initial length was found to be unimportant in the lake common garden analysis, we included it in this analysis because YOY in the mesocosm common garden were housed in the lab slightly longer; therefore, variance in initial size was greater and could have had a more substantial effect on growth. Because each crayfish was reared in a separate, individual container within the 12 mesocosms and was provided with its own food, the growth of each crayfish was independent and was not affected by the growth of other crayfish in the same mesocosm. Therefore, we analyzed the effects of range, food quality, temperature, and initial length at the individual crayfish level, and controlled for the influence of the mesocosm by including it as a random effect. On the other hand, fish treatment was applied at the mesocosm level, and thus we nested the effect of fish within tank in the analysis, so that we conducted this analysis at the mesocosm level. We did not test for the effect of clutch in this analysis because YOY from females were not evenly divided between treatments as in the lake experiment. YOY used in this analysis
came from 53 different clutches, with an average ± standard error (SE) of 3.75 ± 0.26 crayfish per clutch. It is, therefore, unlikely that the genotype of any one parent would drive growth rate trends. We included all crayfish that survived for at least 30 days in this analysis, so that we could increase our sample size while allowing sufficient time for crayfish to grow. There was no significant effect of survival time (30 – 50 days) on growth rate (invasive: \( P = 0.84, R^2 = 0.0005 \); native: \( P = 0.57, R^2 = 0.006 \)). As for the lake experiment, we tested the effect of collection location within each range on growth rate. For this analysis, we used separate linear mixed models for crayfish from the native and invaded range and included the effects of fish treatment, average temperature, and initial length as fixed effects as well as mesocosm nested within fish treatment as a random effect. Food quality was not included because it was found to be unimportant in the first growth model.

For both the lake and mesocosm experiments, we used Cox Proportional Hazards Models to test the effect of range on YOY survival. We included lake as a fixed effect in the lake experiment model and predatory fish treatment and food quality as fixed effects in the mesocosm experiment model. We also included mesocosm nested within fish treatment as a random effect in the mesocosm experiment model.

To examine whether maternal investment was important for growth rate differences between native and invaded range crayfish, we compared egg mass between native and invaded range females in spring 2012. We obtained blotted wet weight for five to nine eggs from each of six females from the native range and nine females from the invaded range. Because female size may also affect egg mass, we analyzed these data using ANCOVA to test the effects of range (native or invasive) and maternal carapace
length on egg weight. We found that maternal carapace length was an important predictor of egg mass, and therefore could use maternal carapace length as an index of egg size for those crayfish grown in the common garden. We could not directly measure egg size for crayfish used in the experiment because removal of eggs from females causes egg mortality. We used ANCOVA to test the effects of range, maternal carapace length (as a index of egg size), and their interaction on the initial and final length of YOY, and a linear model to examine how range, maternal carapace length, and their interaction influenced YOY growth rate. We also included fish treatment and average temperature as fixed effects in the linear model because these were important factors controlling growth rate. To examine how maternal carapace length (as a proxy for egg size) influenced YOY survival, we added maternal carapace length to the Cox Proportional Hazards Model for the mesocosm experiment.

In the mesocosm experiment, we also tested whether crayfish behavior differed between fish treatments by recording the location of each YOY when containers were opened once a week to measure crayfish and replace food. Starting in the fourth week of crayfish growth, we recorded the crayfish as ‘in shelter’ if it was under or motionless next to the rocks or screened sides of the container and ‘out of shelter’ if it was found away from the rocks and screen. We quantified the percentage of observations that were classified as ‘out of shelter’ for all native and invaded range crayfish in each mesocosm. We tested the effects of range and fish treatment on the percent of observations out of shelter in a mixed effects model with mesocosm included as a random effect.
2.4 Results

2.4.1 Lake common garden experiment

Over the course of the summer, *O. rusticus* from invasive populations grew more rapidly than *O. rusticus* from native populations ($F_{1, 83} = 22.13, P = 0.0033$); lake ($F_{2, 82} = 73.87, P < 0.0001$) and the interaction between lake and range also significantly affected growth rate ($F_{2, 82} = 8.56, P = 0.0175$; Figure 2.1A). Crayfish from invasive populations grew about 20% faster than crayfish from native populations in Big Lake and High Lake, but growth rates were similar between native and invaded range crayfish and about 30% slower in Papoose Lake (Figure 2.1A). There was no significant effect of clutch or initial length on growth rate, nor any other significant interactions between range, lake, clutch, or initial length ($P > 0.4$; Table 2.1).

We tested for differences in temperature between lakes to determine if temperature could cause differences in YOY growth rate observed between lakes. Temperature did not differ greatly between lakes. Average temperature in Big Lake (24.5°C) was very similar to that recorded in Papoose Lake (24.4°C) and High Lake (24.1°C; Figure 2.2).
Figure 2.1 (A) Growth rate of *O. rusticus* from native and invasive range populations in lake common gardens. (B) Percent survival of native and invasive range crayfish over the course of the lake common garden experiment.
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<td>0.06</td>
<td>0.8213</td>
</tr>
<tr>
<td>Lake*Initial Length</td>
<td>2</td>
<td>0.20</td>
<td>0.8248</td>
</tr>
<tr>
<td>Lake*Clutch</td>
<td>30</td>
<td>0.66</td>
<td>0.7929</td>
</tr>
<tr>
<td>Initial Length*Clutch</td>
<td>12</td>
<td>0.31</td>
<td>0.9597</td>
</tr>
<tr>
<td>Range<em>Lake</em>Initial Length</td>
<td>1</td>
<td>&lt;0.01</td>
<td>0.9466</td>
</tr>
<tr>
<td>Lake<em>Initial Length</em>Clutch</td>
<td>4</td>
<td>0.38</td>
<td>0.8167</td>
</tr>
</tbody>
</table>
Figure 2.2 The cumulative number of degree days (°C x days) in Big Lake, High Lake, and Papoose Lake in summer 2011.
In contrast to temperature, we found substantial differences in fish and invertebrate abundance between lakes. Predatory fish species collected in fyke nets included bluegill, pumpkinseed (*L. gibbosus* Linnaeus), smallmouth bass, largemouth bass (*M. salmoides* Lacépède), rock bass (*Ambloplites rupestris* Rafinesque), and yellow perch (*Perca flavescens* Mitchell). Predatory fish were most abundant in High Lake (397 fish per trap night) followed by Big Lake (33 fish per trap night) and then Papoose Lake (19 fish per trap night; Table 2.2). Thus, growth rate differences were not consistent with inhibition of feeding in the presence of predatory fishes. Differences in invertebrate colonization among lakes were consistent with differences in growth rate of crayfish among lakes: invertebrates colonizing containers were most abundant in High Lake (0.066 ± 0.37 g ash free dry mass ± SE) followed by Big Lake (0.025 ± 0.006 g ash free dry mass ± SE), the two lakes where growth rates were highest, and then Papoose Lake (0.007 ± 0.001 g ash free dry mass ± SE; Figure 2.3).
## TABLE 2.2

FISH SPECIES AND NUMBERS COLLECTED IN FYKE NETS IN HIGH LAKE, PAPOOSE LAKE, AND BIG LAKE IN SUMMER 2011

<table>
<thead>
<tr>
<th>Lake</th>
<th>Species</th>
<th>June Fyke Nets</th>
<th>July Fyke Nets</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Lake</td>
<td>Bluegill</td>
<td>529</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>Rock Bass</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Pumpkinseed</td>
<td>24</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Yellow Perch</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Largemouth Bass</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Smallmouth Bass</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Papoose Lake</td>
<td>Bluegill</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Rock Bass</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Pumpkinseed</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Yellow Perch</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Largemouth Bass</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Smallmouth Bass</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Big Lake</td>
<td>Bluegill</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Rock Bass</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Pumpkinseed</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Yellow Perch</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Largemouth Bass</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Smallmouth Bass</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Quantities represent the total number of each species collected from three fyke nets.
Figure 2.3 Average quantity of invertebrates from different orders that colonized control containers (containers without crayfish) ± SE in High Lake, Big Lake and Papoose Lake in summer 2011.
We also tested for within-range variation in growth rates to determine whether growth rate differences occur throughout the native and invaded range or were dependent on sampling location. While there was a significant impact of range on growth rate in the lake experiment, there was little within-range variation. For crayfish from the invaded range, lake of origin (population) was not a significant predictor of growth rate ($F_{2, 52} = 2.41, P = 0.1011$), and there was no significant interaction between population and the lake where YOY were grown ($P > 0.5$). Similarly, for crayfish from the native range, river of origin (population) was not a significant predictor of growth rate ($F_{1, 29} = 0.57, P = 0.4589$) and there was no interaction between population and the lake where YOY were grown ($P > 0.5$).

Crayfish from the invaded range also grew more rapidly than those from the native range in the weekly growth analysis ($F_{1, 695} = 7.74, P = 0.0069$), and there was still a significant effect of lake on growth rate ($F_{1, 695} = 17.48, P < 0.0001$). Temperature was also important for weekly growth ($F_{1, 695} = 17.48, P < 0.0001$), and there was an interaction between length and lake indicating that there was no effect of length on growth rate in some lakes, but larger crayfish grew more slowly in other lakes ($F_{1, 695} = 8.53, P = 0.0002$). There was also an interaction between range and length whereby larger native range crayfish grew more slowly, but there was no effect of length on growth rate in invaded range crayfish ($F_{1, 695} = 10.34, P = 0.0014$).

In addition to differences in growth rate, we also tested for differences in survival. Native range crayfish were about 12% less likely to survive than invaded range crayfish within invaded range lakes (Cox Proportional Hazards Model coefficient = 1.1206, $z_{1, 201}$
Neither lake nor the interaction between lake and range were significant predictors of survival ($P > 0.1$).

2.4.2 Mesocosm common garden experiment

Growth results in the mesocosm experiment were similar to those from the lake experiment. Crayfish from invasive populations grew about 50% to 120% faster (depending on fish treatment and food quality) than crayfish from native populations ($F_{1,145} = 21.41, P < 0.0001$; Figure 2.4A). We also found effects of fish presence and temperature on growth. Growth rates were about 40% lower in mesocosms with fish present ($F_{1,11} = 5.48, P = 0.0412$; Figure 2.4A) and increased with temperature ($F_{1,145} = 6.00, P < 0.0001$). Despite our attempts to control temperature, crayfish from native populations experienced slightly warmer temperatures on average than crayfish from invasive populations ($F_{1,145} = 6.01, P = 0.0154$). Average temperature experienced by native range crayfish ($\pm$SE) was $18.4 \pm 0.2 ^\circ C$, while average temperature experienced by invaded range crayfish ($\pm$SE) was $17.8 \pm 0.2 ^\circ C$. The slower growing, native range crayfish experienced warmer temperatures; therefore, the positive effect of temperature on growth rate was weaker than the effect of range. There was no significant effect of food quality ($F_{1,145} = 0.39, P = 0.5328$) or initial length ($F_{1,145} = 0.55, P = 0.4586$) on crayfish growth. Average initial length $\pm$ SE of YOY at the start of the experiment (when placed in the mesocosms) was $13.9 \text{ mm} \pm 0.2 \text{ mm}$ for crayfish from invasive populations and $13.2 \text{ mm} \pm 0.2 \text{ mm}$ for crayfish from native populations. There was a significant interaction between initial length and range ($F_{1,145} = 6.99, P = 0.0093$), indicating that growth rate decreased with initial size in invaded range crayfish ($R^2 = 0.03$), but increased with initial size in native range crayfish ($R^2 = 0.08$). All other interactions
between range, fish treatment, food quality and initial length were non-significant ($P > 0.1$; Table 2.3).

Figure 2.4 (A) Growth rate of *O. rusticus* from native and invasive range populations in mesocosm common gardens. (B) Percent survival of native and invasive range crayfish over the course of the mesocosm common garden experiment. Treatments include predatory fish absent or present x high or low quality food.
### TABLE 2.3
ANOVA TABLE FOR GROWTH RATE WITHIN THE MESOCOSM EXPERIMENT

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>1</td>
<td>21.41</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Fish</td>
<td>1</td>
<td>5.49</td>
<td>0.0412*</td>
</tr>
<tr>
<td>Food Quality</td>
<td>1</td>
<td>0.39</td>
<td>0.5328</td>
</tr>
<tr>
<td>Initial Length</td>
<td>1</td>
<td>0.55</td>
<td>0.4586</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>26.00</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Range*Fish</td>
<td>1</td>
<td>1.16</td>
<td>0.2846</td>
</tr>
<tr>
<td>Range*Food Quality</td>
<td>1</td>
<td>0.023</td>
<td>0.8787</td>
</tr>
<tr>
<td>Fish*Food Quality</td>
<td>1</td>
<td>1.67</td>
<td>0.1986</td>
</tr>
<tr>
<td>Range*Initial Length</td>
<td>1</td>
<td>6.99</td>
<td>0.0093*</td>
</tr>
<tr>
<td>Fish*Initial Length</td>
<td>1</td>
<td>2.28</td>
<td>0.1340</td>
</tr>
<tr>
<td>Food Quality*Initial Length</td>
<td>1</td>
<td>0.03</td>
<td>0.8557</td>
</tr>
<tr>
<td>Range<em>Fish</em>Food Quality</td>
<td>1</td>
<td>0.06</td>
<td>0.8042</td>
</tr>
<tr>
<td>Range<em>Fish</em>Initial Length</td>
<td>1</td>
<td>1.54</td>
<td>0.2172</td>
</tr>
<tr>
<td>Range<em>Food Quality</em>Initial Length</td>
<td>1</td>
<td>0.83</td>
<td>0.3638</td>
</tr>
<tr>
<td>Fish<em>Food Quality</em>Initial Length</td>
<td>1</td>
<td>0.21</td>
<td>0.6513</td>
</tr>
<tr>
<td>Range<em>Fish</em>Food Quality*Initial Length</td>
<td>1</td>
<td>0.68</td>
<td>0.4107</td>
</tr>
</tbody>
</table>
Also similar to the lake experiment, no significant effect existed of within-range lake or river of origin on growth rate for either invaded range or native range crayfish ($F_{1, 92} = 0.24$, $P = 0.7881$, and $F_{1, 52} = 1.31$, $P = 0.2859$, respectively). In addition, there were no significant interactions between within-range population and any other variable in the models ($P > 0.1$).

As in the lake experiment, crayfish from native populations were about 12% less likely to survive during the experiment than crayfish from invasive populations across treatments (Table 2.4; Figure 2.4B). In addition, crayfish that received low quality food were roughly 13% less likely to survive than crayfish that received high quality food (Table 2.4; Figure 2.4B). Significant interactions existed between range, fish treatment, and food quality on crayfish survival (Table 2.4; Figure 2.4B). Overall, within crayfish from the invaded range, individuals had the lowest survival when fish were absent and they received low quality food. Within crayfish from the native range, individuals had the lowest survival when fish were present and they received low quality food.
TABLE 2.4
COX PROPORTIONAL HAZARDS MODEL FOR CRAYFISH SURVIVAL IN THE
MESOCOSM COMMON GARDEN EXPERIMENT

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (native)</td>
<td>1.122</td>
<td>3.16</td>
<td>0.0016*</td>
</tr>
<tr>
<td>Fish (present)</td>
<td>0.106</td>
<td>0.25</td>
<td>0.8000</td>
</tr>
<tr>
<td>Food Quality (low)</td>
<td>1.128</td>
<td>3.18</td>
<td>0.0015*</td>
</tr>
<tr>
<td>Range*Fish</td>
<td>0.042</td>
<td>0.09</td>
<td>0.9300</td>
</tr>
<tr>
<td>Range*Food Quality</td>
<td>-1.178</td>
<td>-2.67</td>
<td>0.0075*</td>
</tr>
<tr>
<td>Fish*Food Quality</td>
<td>-0.988</td>
<td>-1.88</td>
<td>0.0600</td>
</tr>
<tr>
<td>Range<em>Fish</em>Food Quality</td>
<td>1.254</td>
<td>1.99</td>
<td>0.0470*</td>
</tr>
</tbody>
</table>

A total of 333 crayfish were used in this analysis.

2.4.3 Maternal effects

Overall, there was little evidence for significant effects of egg weight on growth rate or survival. There was no significant difference in egg weight between crayfish from native and invasive populations ($F_{1, 11} = 3.16$, $P = 0.1030$; Figure 2.5), and no interaction between range and maternal carapace length on egg weight ($F_{1, 11} = 0.01$, $P = 0.9250$; Figure 2.5), indicating that native and invaded range females of the same size produced eggs of the same size. However, larger females from both ranges produced significantly larger eggs than small females ($F_{1, 11} = 24.82$, $P = 0.0004$; Figure 2.5).
Further, while larger females produced larger young, there was no effect of maternal size on growth rate or survival. At the beginning of the mesocosm experiment (when YOY were placed in mesocosms), there was a significant positive relationship between maternal carapace length (as a proxy for egg size) and carapace length of offspring ($F_{1,142} = 33.21, P < 0.0001$). There was also a significant interaction between maternal carapace length and range on offspring size ($F_{1,142} = 5.26, P = 0.0233$), indicating that maternal carapace length had a greater positive effect on invaded range offspring than native range offspring. At the end of the experiment, maternal carapace length still had a positive influence on offspring size ($F_{1,142} = 6.32, P = 0.0131$), but the
interaction between maternal carapace length and range on offspring size was non-significant ($F_{1, 142} = 0.08, P = 0.7810$). The relationship between maternal carapace length and offspring growth rate during the experiment was also non-significant ($F_{1, 142} < 0.01, P = 0.9823$; Figure 2.6), and there were no significant interactions between maternal carapace length and any other variable in the growth model ($P > 0.1$). Further, there was no significant effect of maternal carapace length on survival (coefficient $= 0.0268, z_{1, 332} = 0.29, P = 0.77$) nor any significant interaction between maternal carapace length and any other variable in the Cox Proportional Hazards Model ($P > 0.4$). Overall, these results indicate that egg weight did not drive the observed differences in growth rate and survival between native and invaded range crayfish.

![Figure 2.6 Relationship between maternal carapace length (as a proxy for egg size) and growth per day in native and invasive range O. rusticus.](image-url)
2.4.4 Crayfish behavior

Crayfish behavior differed between fish treatments. Crayfish in mesocosms without fish were more likely to be found outside of shelter \( (F_{1, 23} = 27.11, P < 0.0001) \). In addition, there was a non-significant trend suggesting that crayfish from native populations spent more time outside of shelter than crayfish from invasive populations \( (F_{1,23} = 3.24 P = 0.0878) \). Invaded range crayfish were found outside of shelter (±SE) 75 ± 5% of the time in mesocosms without fish and 52 ± 2% of the time in mesocosms with fish, and native range crayfish were found outside of shelter 86 ± 1% of the time in mesocosms without fish and 59 ± 7% of the time in mesocosms with fish. There was no interaction between range and fish treatment on behavior \( (P = 0.65) \).

2.5 Discussion

2.5.1 Growth rate differences

In both lake and mesocosm common garden experiments, invasive crayfish had faster growth rates than native crayfish. Data indicate that these growth rate differences were not due to differences in egg weight between the two ranges. Overall, these findings are consistent with evolution of faster growth rates within the invaded range. While larger females initially produced larger young (presumably because of the positive relationship between maternal carapace length and egg weight), there was no significant effect of maternal length on growth. In addition, in both lake and mesocosm experiments we found that within each range young collected as eggs from different lakes or streams had similar growth rates. These results provide further evidence that the observed growth
rate differences are due to differences that characterize the ranges (native vs. invaded) rather than sampling locations within each range.

While our data are consistent with evolution of faster growth in invasive populations of *O. rusticus*, we cannot completely rule out the influence of maternal effects. However, maternal effects are less likely to control the differences we observed in growth rates than genetic differences because eggs from the largest females in our study were roughly 3x larger than the eggs from the smallest females, and we did not detect a significant effect of this difference in egg size on YOY growth rate (Figure 2.4). Other research has found little influence of maternal effects on offspring quality in other decapods (Tropea et al. 2012, Swiney et al. 2013), or that maternal effects scale with female size (Sato and Suzuki 2010). However, there could potentially be other maternal effects such as differences in hormones or specific nutrients within eggs that could affect growth rate. We intentionally collected females from lakes with variable invertebrate prey availability, and there was no effect of within-range lake or river of origin on growth rate in either common garden experiment. We therefore expect that the differences in growth rates we observed were most likely genetically based.

Within the lake common garden experiment, we placed crayfish from the native range in the lakes earlier than crayfish from the invaded range because of differences in timing of reproduction. Growth differences, therefore, could have been due to differences in temperature and/or food availability during the initial weeks of YOY growth. However, because we were able to control the external environment including temperature and food availability in mesocosms, our results are consistent with a genetic basis for the observed differences in growth rates between native and invaded range
crayfish. Lake experiments suggest that this phenomenon occurs not only in the laboratory, but also in natural environments.

Although we were not completely successful controlling temperature in the mesocosm experiment, the differences in temperature experienced by invasive and native range crayfish are not consistent with the differences in growth rate between populations (i.e., if temperature had been the primary driver of growth rates, the differences in growth rate would have been in the opposite direction from those we observed). Still, because crayfish were reared at different times in the mesocosm experiment, it is possible that an unmeasured factor affecting growth rate could have influenced our results. However, this is unlikely because we observed consistent differences in growth rate between native and invaded range crayfish across mesocosms where we varied important factors such temperature, food availability and predator presence. Eggs were also exposed to the environment within their lake or river of origin for a few weeks before collection. We also think this is less likely than genetic differences to be responsible for the observed differences in growth rate because we collected eggs from diverse environments within each range and the majority of egg development occurred in identical conditions in the lab.

Our results suggest that food availability differences among lakes were important for differences in crayfish growth rate. In the lake common garden experiment, the positive relationship between prey abundance and crayfish growth rate suggests that food availability was an important driver of growth rate differences; however, we found no effect of food quality on growth rate in the mesocosm experiment. It is possible that less food was available in the lake with the lowest invertebrate biomass (Papoose Lake) than
we provided in the low quality food treatment of the mesocosm experiment (which still contained 20% animal matter). We expect that providing less food in the mesocosm experiment would have made food quality an important predictor of growth rate in this experiment as well.

Predatory fish presence, in contrast, was a significant predictor of crayfish growth rate in the mesocosm experiment but not in the lake experiment (i.e., the lake with the slowest growth had the lowest abundance of predatory fish). We expect that the effect of fish was more pronounced in the mesocosm experiment because fish were completely absent from some mesocosms but were present at different densities in all lakes.

Behavioral data suggest that reduced growth rates associated with fish presence are likely due to the behavioral response of crayfish to fish. Results indicated that crayfish spent more time hiding (and therefore not consuming food) when fish were present. Together these data suggest that nonconsumptive effects of fish do reduce crayfish growth rates, but in natural systems, low densities of predators can have similar effects to high densities of predators.

The mesocosm experiment also revealed that initial length and temperature were important predictors of YOY growth rate. Invaded range crayfish were slightly larger on average than native range crayfish at the start of the mesocosm experiment (by an average of 0.7 mm carapace length), and there was a significant interaction between initial length and range on growth. Larger invaded range crayfish tended to grow slower over the course of the experiment, which is consistent with the well-documented pattern of declining growth rate with increasing size in many animals (Ricklefs 1967). Native range crayfish did not get as large as invaded range crayfish in the mesocosm study.
which may be why there was no decline in growth rate for these individuals and the
largest individuals grew most rapidly. We also observed a negative relationship between
native range crayfish length and growth rate in the lake study likely for the same reason.
Native range crayfish were largest at the end of the lake study because they had a longer
growing period. As observed in previous studies (e.g. Mundahl and Benton 1990),
crayfish grew faster in warmer water, but this was clearly not sufficient to overshadow
the differences between native and invasive populations.

While crayfish from northern Wisconsin grew more rapidly than those from the
Ohio River drainage, it is unclear whether this would lead to larger young within the
invaded range compared to those within the native range because of temperature
differences between these two locations. Previous studies examining growth of YOY *O.
rusticus* have found YOY carapace lengths ranging from 9 – 16.5 mm in September in
northern Wisconsin (Lorman 1980) and YOY carapace lengths ranging from 8 – 17 mm
in September in northern Kentucky (Prins 1968). In a preliminary study in 2010, we
collected YOY crayfish from Big Lake in northern Wisconsin in August, which ranged
from 9.5 – 15.5 mm carapace length. While these measurements are restricted to specific
locations within each range, and not necessarily representative of growth rates throughout
each range, they suggest that if there are differences in crayfish size between these two
ranges, they are not large.

2.5.2 Survival differences

Not only did native range crayfish have reduced growth, they also had reduced
survival in both the lake and mesocosm common garden experiments. This could be a
result of local adaptation of invasive *O. rusticus* populations to environmental conditions
in the invaded range, especially if some characteristics in the mesocosm experiment more closely resembled lakes in northern Wisconsin than streams in the Ohio River drainage. We expect calcium concentration was lower in lakes and mesocosms than it is in Ohio streams, and flow also differs between these environment types. Differences in growth rates could also be attributed to local adaptation of the invasive population to environmental characteristics such as these. However, if the differences in growth rates observed between the two ranges were due to local adaptation to calcium concentration or flow rate, we would expect to see larger YOY at the end of the summer in the native range where temperatures are warmer.

In the mesocosm experiment, food quality and predatory fish presence had similar effects on native and invaded range crayfish growth, but these factors differentially influenced native and invaded range crayfish survival. Invaded range crayfish were more likely to survive than native range crayfish in all treatments except when food quality was low and no fish were present. Higher mortality within this group was unexpected, but may be due to the combination of rapid growth and low quality food, which could potentially lead to higher mortality due to unavailability of essential nutrients. In addition, crayfish in this group had the fastest growth rates on the low quality diet, and thus likely ate a greater quantity of low quality food than other crayfish. Therefore, secondary metabolites from macrophytes in the low quality diet could also be responsible for the observed increase in crayfish mortality within this group. Crayfish from the native range had the lowest survival when food quality was low and fish were present. This finding suggests a strong behavioral response of native range crayfish to fish that results in reduced feeding or increased energy expenditure. This response was not
observed in invaded range crayfish. Because of high mortality, those crayfish that had
the greatest behavioral response to fish may not have survived long enough to be
included in the growth results, which may be why there is no interaction between range
and fish presence apparent from the growth data. In addition, higher mortality of native
range crayfish may also explain the trend that surviving native range crayfish spent more
time outside of shelter than surviving invaded range crayfish. If predation pressure is
similar between the two ranges, we expect it will be more beneficial for invaded range
crayfish to favor feeding over predator avoidance because there should be a greater
fitness benefit associated with fast growth in this range.

2.5.3 Mechanisms leading to growth rate evolution in invasive populations

The finding that *O. rusticus* from invasive populations have faster growth rates
than those from native populations was consistent with our expectations of how natural
selection within the invaded range would alter this trait. Larger crayfish produce more
eggs than small crayfish (Savolainen et al. 1997, Skurdal et al. 2011), so crayfish with
faster growth have greater reproductive output. Life history theory predicts that optimal
life-history strategies will differ between density-regulated and non-density-regulated
populations, with higher fitness associated with high reproductive rates in non-density-
regulated populations (Roughgarden 1971, Burton et al. 2010). Invasive populations are
non-density-regulated in the early stages of an introduction. Some previous studies have
found evidence for evolution of *r*-selected life history traits in invasive populations or
during range expansion (Burton et al. 2010, Phillips et al. 2010, Flory et al. 2011);
however, other studies have found no evidence for the evolution of these traits (e.g.
Bossdorf et al. 2004, Cripps et al. 2009). We expect that there may be a more lasting
effect of life history evolution in aquatic invasive species compared to most terrestrial species. Range edges, or locations with low conspecific densities, are scattered throughout the invaded range for most aquatic species. Lakes are insular environments and uncolonized lakes are spread throughout the invaded range; therefore, many aquatic invasive populations are serially introduced into locations with low conspecific densities. Thus, we hypothesize that compared to invaders in most terrestrial or marine environments, aquatic invaders will experience exponential growth more often, and there will be a stronger or longer lasting effect of $r$-selection in these populations.

Evolution of increased competitive ability (EICA) is another mechanism which could lead to evolution of rapid growth in invasive populations. EICA postulates that release from natural enemies such as predators and parasites allows nonindigenous species to allocate more resources towards growth (Keane and Crawley 2002, Inderjit and van der Putten 2010). However, because there are native congeners in northern Wisconsin lakes, there are many predators and parasites that readily consume or infect $O.\ rusticus$. Predatory fish are important in controlling $O.\ rusticus$ populations (Roth et al. 2007) and high levels of parasitism by trematodes have been observed in some lakes (Roesler 2009). Therefore, we think this mechanism is less likely to be responsible for the higher growth rates observed in invasive $O.\ rusticus$ than life history trait selection.

Hybridization may enhance the likelihood that nonindigenous populations will evolve invasive traits (Ellstrand and Schierenbeck 2000). Within the invaded range, $O.\ rusticus$ hybridizes with a resident congener, $O.\ propinquus$ Girard (Perry et al. 2001). $O.\ rusticus$ is competitively superior, and hybrids produce offspring that are most likely to backcross with $O.\ rusticus$ (Perry et al. 2001). It is unclear whether $O.\ propinquus$
alleles remain in invasive *O. rusticus* (hybrid) populations over time (Perry et al. 2001). Since the early 1980s, no *O. propinquis* have been detected in any of the lakes where we collected *O. rusticus*, and *O. propinquis* has never been detected in Big Lake (Lodge unpublished data); therefore, we were not examining populations that hybridized recently. *O. propinquis* grow more slowly than *O. rusticus* (Hill et al. 1993), so rapidly growing invasive *O. rusticus* represent novel genotypes that are dissimilar from both parental populations. It is, however, possible that earlier hybridization and introgression provided increased additive genetic variance or created novel epistatic interactions that allowed *O. rusticus* to evolve faster growth rates in the invaded range.

2.5.4 Community impacts of growth rate divergence

Rapid growth rates in invasive *O. rusticus* have had major community-level consequences. *O. rusticus* has a greater impact on the ecological community than congeners, *O. virilis* Hagen and *O. propinquis*, and often causes declines in macrophyte and macroinvertebrate abundance and richness when replacing these species (Wilson et al. 2004). The ability of *O. rusticus* to replace *O. propinquis* has been attributed in part to its faster growth rate and ability to outcompete smaller individuals for shelter (Hill et al. 1993, Garvey et al. 1994, Hill and Lodge 1994). Faster growth also causes *O. rusticus* to escape predation from gape-limited fish more rapidly (Stein 1977). Understanding how often nonindigenous organisms evolve invasive traits is crucial for understanding the costs and consequences associated with introducing species to new locations.
2.5.5 Implications for management of invasions

We recommend that the potential for populations to evolve increasingly invasive traits be considered when moving species to new locations. Even though a species may not be problematic in its native range, or may be unproblematic initially in a new location, traits such as rapid growth and high reproductive output that may increase ecological impacts can evolve within the invaded range. This may be especially likely to occur in populations which are serially introduced to insular environments such as aquatic organisms in lakes.

Risk assessments that do not include evolutionary potential may underestimate the likelihood of a species to cause ecological and economic harm. Species that are likely to hybridize with native species may also be especially likely to evolve in response to selection within the invaded range because of increased additive genetic variance in hybrids. Crayfish in North America are a prime example of organisms that are likely to evolve invasive traits when introduced to new locations because they live in patchy insular environments (lakes or stream drainages), and because they are likely to encounter native crayfishes with which hybridization may be possible. Especially when introduced to new locations within North America, crayfish are often exposed to closely-related, native species with which they are likely to hybridize (Perry et al. 2002). Seventy-five percent of the world’s crayfish species are found within the United States (Lodge et al. 2000). Despite these problems, many states in the United States do not regulate the movement of crayfish or encourage voluntary practices to restrict moving crayfish, and many other states have legislation that only restricts moving certain species that are known to be problematic (Peters and Lodge 2009, Dresser and Swanson 2013).
Our research suggests that invasive traits can evolve in nonindigenous crayfish populations, and this risk could be considered when weighing the costs and benefits of moving crayfish to new locations.

2.6 Acknowledgments

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2.7 Data archiving statement

Data underlying the main results of this study are available from the Dryad Digital Repository.
2.8 References


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CHAPTER 3:
TREMATODE PARASITE ALTERS GROWTH, FEEDING BEHAVIOR, AND
DEMOGRAPHIC SUCCESS OF INVASIVE RUSTY CRAYFISH (ORCONECTES
RUSTICUS)²

3.1 Abstract

Nonindigenous species can cause major changes to community interactions and ecosystem processes. The strong impacts of these species are often attributed to their high demographic success. While the importance of enemy release in facilitating invasions has often been emphasized, few studies have addressed the role of parasites in the invasive range in controlling demographic success of potential invaders. Here we examine whether a trematode parasite (Microphallus spp.) can contribute to previously documented alternate states in the abundance of invasive rusty crayfish (Orconectes rusticus) in north temperate lakes in Wisconsin, USA. Microphallus infect O. rusticus after emerging from their first intermediate host, a hydrobiid snail. As previously documented, O. rusticus reduce densities of hydrobiid snails through direct predation and destruction of macrophyte habitat. Therefore, if Microphallus substantially reduce O. rusticus fitness, these parasites may reinforce a state of low crayfish abundance, and, at

the other extreme, abundant crayfish may repress these parasites, reinforcing a state of high crayfish abundance. From samples collected from 109 sites in 16 lakes, we discovered (1) a positive relationship between crayfish infection intensity and hydrobiid snail abundance, (2) a negative relationship between parasite prevalence and crayfish abundance, and (3) a negative relationship between parasite prevalence and crayfish population growth. With experiments, we found that infection with *Microphallus* reduced foraging behavior and growth in *O. rusticus*, which may be the mechanisms responsible for the population reductions we observed. Overall results are consistent with the hypothesis that *Microphallus* contributes to alternate states in the abundance and impacts of *O. rusticus*.

3.2 Introduction

Nonindigenous species that reach high densities or spread rapidly may cause extensive ecological and economic harm (Sala et al. 2000, Keller et al. 2009, Butchart et al. 2010), and parasites (or lack thereof) may be important in determining whether nonindigenous species achieve this high demographic success (Prenter et al. 2004, Dunn et al. 2012). Understanding the mechanisms that allow some nonindigenous species to become invasive in some habitats but not others may enable us to better predict invasion outcomes and control these species (Lodge et al. 1998). The study of the impact of parasites on invasions has largely focused on how release from home-range parasites can lead to invasion success: escape from natural enemies is a leading hypothesis that nonindigenous species escape population regulation from coevolved predators, parasites, and pathogens in the introduced range and therefore can outcompete native species.
(Keane and Crawley 2002, Torchin et al. 2002, Inderjit and van der Putten 2010). Fewer studies have examined how parasites encountered in the introduced range control invaders, but this interaction may be equally important in controlling invasion success (Colautti et al. 2004, Mitchell et al. 2006). Here we use observational and experimental data to explore whether alternate states in abundance and impacts of a major invader in north temperate lakes, the rusty crayfish (*Orconectes rusticus*), are mediated by infection with trematode metacercariae (encysted larval trematodes).

Parasites have the potential to control community composition, especially if they alter the abundance or life history traits of a host species that has large impacts on other species or ecosystem processes (Thomas et al. 2000, Wood et al. 2007). Crayfish can have unusually large impacts on aquatic ecosystems because of their large size compared to other aquatic invertebrates, their omnivorous feeding habits, and their ability to reach high population densities (Hobbs and Lodge 2010). Invasive *O. rusticus* are native to streams in the Ohio River drainage and were introduced by anglers to northern Wisconsin and Michigan lakes in the 1960s (Olden et al. 2006). The lifespan of these crayfish ranges from 3-4 years (Lorman 1980), and young hatch once per year in the spring (Prins 1968). Some young of year reach reproductive maturity after one growing season, but others do not mature until after their second growing season (Lorman 1980). *O. rusticus* are a major driver of community composition in northern Wisconsin lakes where they reduce the abundance and richness of macrophytes and macroinvertebrates (Capelli 1982, Lodge et al. 1994, Wilson et al. 2004, Olden et al. 2006). *O. rusticus* also reduce populations of fishes, especially bottom-nesting panfish, probably via a combination of nest predation (Dorn and Mittelbach 1999, Baldridge and Lodge 2013), competition for
food resources, and destruction of macrophyte habitat (Wilson et al. 2004). This has major economic consequences in northern Wisconsin and Michigan where recreational fishing is an important economic activity (Keller et al. 2008).

Recently, a group of trematode parasites (*Microphallus* spp.) belonging to the *M. opacus* and *M. fonti* species complex were identified in crayfish in northern Wisconsin lakes (Roesler 2009, Overstreet 2011). Genetic data indicate that this group of parasites is made up of multiple closely related microphallid species which are indistinguishable morphologically (Overstreet 2011). These parasites infect both native (*O. virilis*) and nonindigenous (*O. propinquus* and *O. rusticus*) orconectid crayfish (Overstreet 2011), so it is currently unclear whether these parasites were recently introduced to northern Wisconsin lakes, perhaps along with a nonindigenous crayfish, or whether they are native to the region and coevolved with a native crayfish. Previous research conducted by the Wisconsin Department of Natural Resources indicates that *Microphallus* are currently common, occurring in roughly half of 25 lakes examined (Roesler 2009); however, occurrence of this parasite was likely underestimated because few crayfish (mean *N* = 14) were examined from each lake. Our preliminary research indicates that as in other trematode-host relationships (Fredensborg et al. 2004), parasite abundance within crayfish increases with crayfish size. This is because older hosts, including crayfish, have been exposed to more parasites, which persist within the host for multiple years. Individual crayfish can be infected with many thousands of metacercariae (Roesler 2009).

Orconectid crayfish are infected with *Microphallus* after free-swimming trematode cercariae emerge from a hydrobiid snail host (Overstreet 2011). The parasites enter the gill chamber and then encyst in the hepatopancreas (digestive organ) of the
crayfish and remain there until the crayfish is consumed by an unknown definitive host; closely related trematodes use a number of bird, fish, turtle, and mammal species as definitive hosts (Bray et al. 2008, Overstreet 2011). Parasites can cause substantial damage to organs and connective tissue initially as they enter the host and migrate to the hepatopancreas (Meissner and Bick 1999), but are thought to do little damage to host tissue once they encyst (Jensen et al. 1998). However, some necrosis of tissue surrounding metacercariae has been observed in infected decapods (Robaldo et al. 1999). Some species of microphallid metacercariae also cause major changes to host behavior, which can affect host fitness (Levri 1999, Kunz and Pung 2004, Helluy and Thomas 2010).

Because *O. rusticus* cause declines in hydrobiid snail populations through direct predation and reduction of macrophyte habitat (Weber and Lodge 1990, Lodge et al. 1994), we hypothesize that *Microphallus* may contribute to maintaining the two alternate states in crayfish densities observed in lakes in this region. Crayfish population records indicate that *O. rusticus* remain at low densities in some lakes where they are introduced, but reach ‘outbreak’ densities in other lakes (Roth et al. 2007, Kreps et al. 2012). Feedbacks between abundances of predatory fish, macrophytes, and crayfish may drive lake communities towards either high or low densities of crayfish (Roth et al. 2007). Impacts of *O. rusticus* are not observed in lakes where populations remain at low densities (Lodge et al. 1998, Rosenthal et al. 2006); therefore, *Microphallus* could increase diversity and abundance of native macrophytes and invertebrates if they substantially reduce *O. rusticus* densities or impacts. Observations by Roesler (2009) suggest that a negative relationship may exist between the abundance of *O. rusticus* and
the abundance of Microphallus. Alternate states in crayfish abundance have been previously attributed solely to interactions among fish, macrophytes, and crayfish (Roth et al. 2007) without consideration of the impact of parasites in mediating these interactions. If Microphallus have substantial negative impacts on O. rusticus fitness, these parasites may reinforce a state of low crayfish abundance in a lake, and high crayfish abundance may repress these parasites (Figure 3.1).

To explore whether Microphallus interactions could lead to alternate states in crayfish density, we tested the hypotheses that hydrobiid snail density positively correlates with Microphallus abundance in O. rusticus (Interaction 1 in Figure 3.1) and that Microphallus reduces O. rusticus fitness to the extent that the crayfish population declines (Interaction 2 in Figure 3.1). In addition we investigated the mechanism by which Microphallus may affect O. rusticus populations by testing how infection alters growth and feeding behavior in O. rusticus. Because Microphallus infect the hepatopancreas (digestive organ) of crayfish, it is likely that infection could alter digestion and growth. Growth is important for crayfish fitness because larger crayfish produce more offspring (Savolainen et al. 1997, Skurdal et al. 2011) and can better avoid fish predation (Stein et al. 1977). Finally, to assess the strength of the impact of Microphallus on crayfish relative to a better known interaction, we compared the impact of parasites on crayfish feeding behavior to the impact of the presence of a fish predator. The impacts of predatory fish on O. rusticus populations (Didonato and Lodge 1993, Kershner and Lodge 1995, Tetzlaf et al. 2011) and community interactions (Hill and Lodge 1995, Hill and Lodge 1999) are well known and used to inform management (Hein et al. 2006), but the impact of parasites on O. rusticus may also be substantial. To
our knowledge, ours is the first study to test the potential effects of parasites on *O. rusticus* population growth.

![Diagram of crayfish, Microphallus, snails, and macrophytes interactions]

**Figure 3.1** Hypothesized interactions between *O. rusticus* crayfish, *Microphallus* parasites, snails and macrophytes, and definitive hosts. We have depicted the definitive host as a bird, but the definitive host is currently unknown for these parasites. Black arrows indicate consumer-resource interactions, white arrows indicate the effect of parasites on hosts, and dashed arrows indicate movement of *Microphallus* parasites. Arrow size is indicative of the hypothesized net strength of the interaction or quantity of parasites. (A) In a lake with few crayfish, (1) abundant *Microphallus* cercariae emerge from abundant snails and infect crayfish, (2) these parasites reduce crayfish densities by reducing host fitness, and (3) the smaller crayfish population has little impact on snails. (4) Definitive hosts release abundant parasite eggs, which are consumed by snails. (B) In a lake with many crayfish, (1) few *Microphallus* cercariae emerge from snails (in low abundance), and therefore (2) *Microphallus* have little impact on the crayfish population, and (3) abundant crayfish reduce host snail abundance through trophic interactions. (4) Definitive hosts release few eggs. The interaction between *O. rusticus* crayfish and snails is well established in the literature (Weber and Lodge 1990, Lodge et al. 1994). We test (1) the effect of hydrobiid snail abundance on *Microphallus* abundance in crayfish and (2) the effect of *Microphallus* on *O. rusticus* density and fitness.
3.3 Methods

3.3.1 Snail and *Microphallus* dynamics

In order to determine whether snail density is a good predictor of parasite abundance in *O. rusticus* (Interaction 1 in Figure 3.1), we used SCUBA to survey 75 sites in 8 northern Wisconsin lakes for hydrobiid snail density in July of 2011, using the same sampling devices and methods as used in earlier studies on these organisms in this region (Lodge et al. 1998, Kreps et al. 2012). At each site, snails were sampled at a haphazard location within the littoral zone at a depth randomly selected from three predetermined depths (0.75 m, ½ of Secchi depth or ¾ of Secchi depth). On soft sediments (sand or muck), snail density was sampled using a cylindrical polyvinyl chloride (PVC) sampler (0.018 m²), which was pushed roughly 5 cm into the substrate to remove a sediment core. Macrophytes within the sampler were removed along with the core. In areas with substantial macrophyte cover, we used a sampler of the same diameter that consisted of two hinged PVC halves, which were closed around macrophytes near the sediment surface before the sampler was pushed into the substrate. A net (1 mm mesh) attached to the top of the sampler was then zipped closed to capture macrophytes within the sample. On cobble substrate, a ring (0.5 m²) was placed on the substrate and all cobbles within the ring were gently removed and placed in a sieve bucket (1 mm mesh), and then carefully transferred to the surface. All samples were sieved (1 mm mesh) and preserved in 70% ethanol. Snails were picked from each sample in the laboratory and all hydrobiid snails were identified to species according to Burch (1989). Hydrobiid species were pooled for analysis.
We collected *O. rusticus* from within 20 m of each snail sampling site using modified Gee minnow traps baited with 120 ± 10 g beef liver in July and August of 2010 (as in Lodge et al. 1986). Crayfish were placed on ice in the field and frozen within 6 h of collection for preservation of metacercariae. We later thawed crayfish and examined the dissected hepatopancreas by flattening it between two glass slides (top slide with 8 mm² grid) and counted metacercariae using a dissecting microscope. Metacercariae were distributed uniformly throughout the hepatopancreas, which allowed for accurate estimates to be obtained by sub-sampling. If crayfish were highly infected (> 1,000 metacercariae), metacercariae were only counted in half of the hepatopancreas. In a few very highly infected individuals, the hepatopancreas was sub-sampled by counting metacercariae in three gridded squares and visually estimating hepatopancreas area.

We used a linear mixed effects model to examine the relationship between hydrobiid snail abundance and mean metacercariae count per crayfish. P values were calculated using likelihood ratio tests for all mixed effects models. We included in the analysis all sites where we found at least one crayfish of 30 to 40 mm carapace length (to control for size effects on parasite count), and used parasite abundance (parasites per host, including uninfected individuals) at a site as the dependent variable. The independent variable was snail abundance and lake was included as a random effect. Metacercariae count was log transformed (log₁₀ (metacercariae count + 0.5)) to meet assumptions of normality. Because *O. rusticus* can dramatically reduce snail populations, there were relatively few sites where both snails and crayfish were detected, and crayfish were often rare at sites where snails were detected. Therefore, there were too few
crayfish to accurately assess the effect of hydrobiid snail abundance on parasite prevalence (% of crayfish infected).

3.3.2 *Microphallus* impact on *O. rusticus* dynamics

To test the hypothesis that parasites reduce *O. rusticus* fitness to the extent that the crayfish population declines, we examined whether *Microphallus* abundance or prevalence (% of hosts that are infected) correlated with *O. rusticus* population trends in northern Wisconsin lakes (Interaction 2 in Figure 3.1). In July and August of 2010, we surveyed crayfish populations at 109 sites within 16 lakes and determined parasite abundance and parasite prevalence for 3-10 crayfish from each site using the same methods describe above. Sample size was lower in areas with low trap catch because fewer crayfish were available for dissection. We used data from all lakes to examine the relationship between parasite prevalence and crayfish abundance.

Because *O. rusticus* reduce snail densities (likely leading to lower infection levels in crayfish), it was also important to assess the impact of *Microphallus* on *O. rusticus* population trends between years. In any one year, the correlation between parasite abundance and *O. rusticus* abundance could be attributed to the impact of *O. rusticus* on snails (leading to fewer parasites emerging from snail hosts and infecting crayfish) instead of the impact of parasites on crayfish fitness. Therefore, for 10 lakes we examined how parasite abundance in crayfish was related to changes in crayfish abundance the following year. To accomplish this, a subset of sites (82 sites within 10 lakes) were trapped again in summer 2011 (8 lakes) or 2012 (2 additional lakes).

To assess the effect of *Microphallus* prevalence on *O. rusticus* abundance in 2010, we used a linear mixed effects model with parasite prevalence as the independent
variable and lake included as a random effect. We also used correlation analysis to examine the relationship between parasite prevalence and mean parasite abundance per crayfish. In addition, we assessed the effect of parasite prevalence on crayfish population trends (measured as the percent change in trap catch from 2010 to either 2011 or 2012) using a linear mixed effects model that included parasite prevalence, initial crayfish abundance, and their interaction as independent variables. Lake was included as a random effect. We excluded one lake (Lake Ottawa) from the crayfish population trend analysis to meet assumptions of normality of variance; most sites from this lake were outliers on residuals vs. fitted values plots and quantile-quantile plots. Trap catch was very low in Lake Ottawa in 2010 compared to previous years and to the following year. We expect that the unusual population estimate in 2010 was due to trapping artifacts.

3.3.3 Microphallus impact on *O. rusticus* growth

To examine the impact of infection with *Microphallus* on *O. rusticus* growth (a possible mechanism driving interaction 2 in Figure 3.1), we collected *O. rusticus* from a lake with moderate *Microphallus* prevalence (Big Lake, Vilas County, Wisconsin, USA) to examine differences in growth between infected and uninfected crayfish. We identified sites within Big Lake where crayfish were likely to be infected or uninfected with preliminary dissections of crayfish from a number of locations and metacercariae quantification. After identifying these locations, we collected 20 *O. rusticus* from sites with low infection prevalence and 20 *O. rusticus* from sites with high infection prevalence using baited minnow traps. Crayfish ranged from 26 to 40 mm carapace length. We housed each crayfish in an individual, perforated, plastic container (13 x 35 x 19 cm) placed between 0.5 – 0.25 m depth at one of two locations within Big Lake. We
used two locations to avoid the potential loss of all experimental animals to human tampering or storms. Each crayfish was fed five spinach leaves (approximately 5 g) and five shrimp pellets (Aquatic Ecosystems; approximately 0.6 g) each week, and carapace length for each crayfish was recorded over a four week period. It is likely that the crayfish diets were supplemented by small invertebrates colonizing the containers. Containers were marked so that the location where the crayfish was collected (and therefore its likely infection status) was unknown to the person feeding and measuring the crayfish. We quantified metacercariae in the hepatopancreas at the conclusion of the experiment. We used ANCOVA to compare percent growth of uninfected and infected crayfish with initial carapace length as a covariate.

In addition to examining the effect of infection status (infected or uninfected), we experimentally determined the relationship between growth and infection intensity (number of metacercariae within infected individuals). To accomplish this, we hand-collected 44 crayfish from a lake with high infection prevalence and a greater range of infection intensities (Plum Lake, Vilas County, Wisconsin, USA). Crayfish ranged from 20 to 46 mm carapace length. Crayfish were housed at two sites within Plum Lake and fed using the same methods described above. Carapace length for each crayfish was recorded over a five week period. We analyzed results using multiple regression to assess the effects of initial carapace length and infection intensity on percent growth.

3.3.4 *Microphallus* impact on *O. rusticus* feeding behavior

We tested the effect of infection on feeding behavior by comparing feeding latency (the amount of time before a crayfish retrieved a section of earthworm) between infected and uninfected *O. rusticus*. We used both laboratory-infected and field-infected
O. rusticus in trials. Field-infected O. rusticus were collected in August 2011 from different locations in Plum Lake. Previous surveys allowed us to predict locations where infected and uninfected crayfish were located. These crayfish were housed in 38 L aquaria filled with aerated well water. Aquaria contained sections of PVC pipe for shelter. O. rusticus for the laboratory-infected treatment were collected from Lake Ottawa (Iron County, Michigan, USA) in June 2011. Half of the crayfish collected were infected with Microphallus via exposure to hydrobiid snails collected from Plum Lake. Laboratory-infected crayfish were housed in 38 L aquaria filled with aerated well water and exposed to roughly 4,000 hydrobiid snails contained within a screened pouch for a period of six weeks. Control crayfish were housed in identical aquaria with screened pouches that did not contain snails. All screened pouches also contained macrophytes (Potamogeton amplifolius) coated with periphyton to provide food for snails. In late August, all crayfish (field and laboratory infected) were moved to tanks equipped with PVC shelters and constantly refreshed, aerated, well water. All crayfish were fed shrimp pellets ad libitum.

Experimental trials were carried out in October and November 2011. During the experimental trial, crayfish were contained in a modified bucket (25 cm diameter x 15 cm height) with a screened top and a screened hole (20 cm x 8 cm) in each side. Each modified bucket contained a shelter constructed from a section of PVC pipe scaled to the size of the crayfish (carapace length < 30 mm, 6 cm length x 5 cm diameter PVC pipe; carapace length ≥ 30 m, 8 cm length x 7.5 cm diameter PVC pipe). The modified bucket was placed inside a 68 L tank filled to a depth of 30 cm with well water.
In order to assess the effect of predatory fish presence and infection on feeding behavior, each crayfish was tested with a smallmouth bass present in the outer tank and with no fish present. Smallmouth bass are voracious predators of *O. rusticus* and are common in northern Wisconsin and Michigan lakes (Peters 2010). Crayfish could receive visual and chemical cues from the predatory fish, but could not be contacted or consumed by the fish during the trial. We randomized whether each crayfish received the smallmouth bass treatment first or second. Bass trials were always conducted in the same tanks so that chemical cues from bass were not introduced to non-bass trials. Crayfish were placed alone in a bucket with a shelter and aerated well water for one day before the first trial and for one day between the two trials.

Crayfish and fish were placed in experimental conditions 24 h before the start of each trial, and crayfish were fasted during this 24 h period. At the start of the trial, we dropped a 1 cm section of earthworm into the center of the modified bucket. We checked to see if crayfish had obtained the worm every 2 to 3 min until 10 min had passed and then every 20 min until 70 min had passed. After each crayfish was tested once without bass and once with bass, we dissected the crayfish and quantified *Microphallus* metacercariae.

Using survival analysis, we tested whether infection status (infected or uninfected) or intensity was related to whether or not a crayfish fed. Specifically, we used two different Cox Proportional Hazards Models (one with infection as a categorical variable and one with infection as a continuous variable) (Cox 1972). We also examined the effect of fish presence and the effect of infection treatment (whether the crayfish was
laboratory-infected (collected from Lake Ottawa) or field-infected (Plum Lake)). Crayfish identity was included in each model as a random effect.

3.4 Results

3.4.1 Snail and *Microphallus* dynamics

We found a significant positive relationship between hydrobiid snail density and average *Microphallus* count per crayfish (coefficient 0.003, $\chi^2(1) = 17.69, P < 0.001$; Figure 3.2). While there were likely snails in some locations where they were undetected by our sampling methods (because we were able to detect *Microphallus* metacercariae within crayfish at these locations), infection intensity was greater on average in locations where we did detect snails, indicating that our methods were sufficient for determining relative snail abundance between sites.

We found *Microphallus* metacercariae within crayfish in all of the 16 lakes that we sampled, indicating that these parasites are more prevalent than previously reported (Roesler 2009). Abundance of *Microphallus* metacercariae differed substantially within many of the lakes that we sampled. For example, in Plum Lake, we found average infection levels ranging from 0 – 1999 metacercariae per crayfish at different sites, but infection intensity was similar among crayfish collected from the same site (effect of site on infection intensity: $F_{7, 32} = 8.00, P < 0.001$). The average lakewide abundance (metacercariae per crayfish) of *Microphallus* also differed substantially among lakes (mean ± SE = 80 ± 14.5, range = 1-80). The average lakewide parasite prevalence was 63% (range = 8% – 92%, SE = 6%).
Figure 3.2 Relationship between abundance of snails and mean metacercariae count per crayfish ($P < 0.001$) with lake included as a random effect. 95% confidence intervals are indicated by dashed lines. Data points represent 75 sites in 8 northern Wisconsin lakes. Parasite abundance represents average metacercariae count for all crayfish between 30 and 40 mm (from one to ten individuals) collected from a site.

3.4.2 *Microphallus* impact on *O. rusticus* dynamics

Snapshot abundances of crayfish and parasites in 2010 indicated a significant negative relationship between parasite prevalence and crayfish abundance (coefficient -0.094, $\chi^2(1) = 5.01, P = 0.025$); however, parasite prevalence explained only 5% of the variance in trap catch. We found no sites with high crayfish abundance (> 30 crayfish per trap) and high parasite prevalence (> 80% parasite prevalence). Parasite prevalence and average number of metacercariae per crayfish at a site were significantly positively
correlated \( T = 3.27, P = 0.002, df = 106, R = 0.3 \). Therefore, if there is an effect of parasites, these trends could be attributed to either the impacts of parasite prevalence or infection intensity.

We also found a significant relationship between parasite prevalence and \( O. rusticus \) population dynamics between years. Overall, there was a negative effect of parasite prevalence on percent increase in trap catch (coefficient -2.174, \( \chi^2(2) = 28.24, P < 0.001 \); Figure 3.3). Crayfish abundance declined when prevalence exceeded about 80% (Figure 3.3). A significant negative relationship also existed between initial crayfish abundance and percent increase in trap catch, indicating that sites with high initial abundance were unlikely to increase in trap catch (coefficient -4.957, \( \chi^2(2) = 32.24, P < 0.001 \); Figure 3.3). Finally we observed a significant interaction between initial crayfish abundance and parasite prevalence, whereby parasite prevalence had a weaker effect on population trends at locations with high crayfish abundance (coefficient 0.034, \( \chi^2(1) = 4.01, P = 0.045 \); Figure 3.3). For example, parasite prevalence explained 45% of the variance in population trends in locations with fewer than 30 crayfish per trap, but only 0.5% of the variance in locations with greater than 30 crayfish per trap (Figure 3.3). Thus, sites with low and medium initial crayfish abundance increased in population size when parasites were rare and decreased when prevalence was high, but sites with high initial crayfish abundance were not as strongly affected either way (Figure 3.3).
Figure 3.3 Relationship between the percent of crayfish between 30 and 40 mm carapace length that were infected in 2010 (parasite prevalence) vs. the change in trap catch from year 2010 to year 2011 or 2012. Each data point is a site within a lake. Trap catch was treated as a continuous variable in the statistical analysis, but data are grouped by crayfish abundance in 2010 for ease of visualizing trends (A = less than 20 crayfish per trap, B = 20-30 crayfish per trap, C = greater than 30 crayfish per trap). Dashed lines indicate 95% confidence intervals. Parasite prevalence was a significant predictor of change in crayfish trap catch ($P < 0.001$) as was initial crayfish abundance ($P < 0.001$).

3.4.3 *Microphallus* impact on *O. rusticus* growth

Infection status significantly influenced crayfish growth. In Big Lake, growth was greater in uninfected crayfish ($n = 10$) than in crayfish with low intensity infections ($n = 26$; mean metacercariae per crayfish $\pm$ SE $= 11 \pm 5$; $F_{1,35} = 8.05$, $P = 0.008$; Figure 3.4). There was also a significant interaction between initial length and infection status ($F_{1,35} = 7.12$, $P = 0.012$; Figure 3.4), indicating that growth in small crayfish was especially reduced by infection (by about 33% for 25mm crayfish) and large crayfish grew little regardless of infection status.
Plum Lake had higher infection intensities and greater parasite prevalence than Big Lake, and few of the crayfish used in our experiment were uninfected (5 out of 39). Because there was a greater range in infection intensity in Plum Lake (range =1-3995 metacercariae per crayfish, mean = 638) we were able to assess the effect of infection intensity on growth in this lake. As in Big Lake, growth was greater in small crayfish ($F_{1, 33} = 14.64$, $P < 0.001$, $R^2 = 0.11$), but no significant relationship existed between number of metacercariae within infected crayfish and crayfish growth ($F_{1, 33} = 14.64$, $P = 0.829$). Combined, our results indicate that infected crayfish grew more slowly than uninfected individuals (Big Lake), but that the intensity of infection did not affect individual growth (Plum Lake).
3.4.4 *Microphallus* impact on *O. rusticus* feeding behavior

Infection status altered crayfish feeding behavior, but infection intensity did not (Table 1). When we examined infection status as a categorical variable (infected or uninfected), there was a significant negative effect of infection on feeding, and a significant interaction between infection source (i.e., field-infected or laboratory-infected) and infection status (Table 1; Figure 3.5), indicating that the crayfish we infected experimentally fed significantly less than control crayfish, but there was no difference in feeding behavior between naturally infected and uninfected crayfish (Figure 3.5). Uninfected crayfish from Lake Ottawa and Plum Lake displayed similar feeding behavior ($Z_{1.42} = -1.20, P = 0.23$); thus differences between the two groups were driven by differences in feeding behavior of infected crayfish. The naturally infected crayfish had higher infection intensities (metacercariae per crayfish ± SE = 682±80) than those that were experimentally infected (metacercariae per crayfish ± SE = 30±7). There was also a significant effect of fish treatment on crayfish feeding behavior, indicating that crayfish were less likely to feed when a smallmouth bass was present (Table 1). The effect of fish was similar in magnitude to that of infection; crayfish were roughly 19% less likely to feed when infected and 23% less likely to feed when fish were present (Table 1).

When infection was treated as a continuous variable in the Cox Proportional Hazards model, the only significant predictor of feeding behavior was fish treatment (Table 1); number of metacercariae (once it exceeded zero) did not significantly influence feeding behavior (Table 1).
TABLE 3.1

RESULTS OF COX PROPORTIONAL HAZARDS MODELS EXAMINING THE EFFECT OF INFECTION STATUS (INFECTED VS. UNINFECTED) AND PARASITE ABUNDANCE WITHIN CRAYFISH ON CRAYFISH FEEDING BEHAVIOR

<table>
<thead>
<tr>
<th>Factor</th>
<th>Infection Status</th>
<th>parasite abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>Z</td>
</tr>
<tr>
<td>Infection</td>
<td>-1.185</td>
<td>-2.95</td>
</tr>
<tr>
<td>Source of Infection</td>
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<td>-1.28</td>
</tr>
<tr>
<td>Fish</td>
<td>-1.227</td>
<td>-2.40</td>
</tr>
<tr>
<td>Infection*Source</td>
<td>1.712</td>
<td>2.63</td>
</tr>
<tr>
<td>Infection*Fish</td>
<td>0.731</td>
<td>1.02</td>
</tr>
<tr>
<td>Source*Fish</td>
<td>0.910</td>
<td>1.27</td>
</tr>
<tr>
<td>Infection<em>Source</em>Fish</td>
<td>-0.668</td>
<td>-0.72</td>
</tr>
</tbody>
</table>
Figure 3.5 Results of feeding experiment comparing feeding activity between infected and uninfected crayfish during a 70 minute trial. 95% confidence intervals are indicated by dashed lines. Crayfish that were experimentally infected \((N = 40)\) fed less than control crayfish \((N = 24)\) (Panel A: \(P = 0.004\)), but no significant difference existed in feeding behavior between infected \((N = 44)\) and uninfected \((N = 18)\) crayfish collected from Plum Lake (Panel B: \(P = 0.30\)).
3.5 Discussion

Our combined results are consistent with *Microphallus* reducing *O. rusticus* fitness via reductions in feeding and growth to the extent that crayfish population growth is reduced. We found no sites with both high crayfish abundance and high parasite prevalence, and we observed a negative relationship between snapshot (within the same year) estimates of crayfish abundance and parasite prevalence. Snapshot trends may be due to either parasites reducing crayfish fitness in locations with high parasite prevalence or to crayfish reducing populations of hydrobiid snails to the extent that parasite prevalence declines. The population growth results, however, are unambiguous and provide strong support for our hypothesis that *Microphallus* reduces crayfish population growth. Sites with high parasite prevalence in 2010 were associated with reduced crayfish population growth in the subsequent year, and crayfish populations declined in sites with the highest parasite prevalence (Interaction 2, Figure 3.1). These results cannot be attributed to crayfish reducing host snail densities. Locations with high initial crayfish abundance (> 30 crayfish per trap) did not substantially increase in trap catch even when parasite prevalence was low, likely because these populations were already near carrying capacity. Therefore, our results indicate that parasite prevalence is a strong predictor of crayfish population trends in areas with low to moderate initial crayfish abundance.

While parasite prevalence was a significant predictor of snapshot estimates of crayfish abundance, there was substantial variation in crayfish abundance that was not explained by parasite prevalence. Residual variation may result from past parasite dynamics, but parasite prevalence and infection intensity have remained consistent in locations sampled annually or biannually from 2010-2012 (Sargent, unpublished data).
Residual variation may also result from previously explored factors such as the availability of suitable habitat (Lodge 1993, Kershner and Lodge 1995) or predator abundance (Roth et al. 2007). Overall, our findings suggest that *Microphallus* prevalence is a strong predictor of *O. rusticus* population growth from year to year and a significant but weaker predictor of overall crayfish abundance.

In addition, we found higher average *Microphallus* count per crayfish in locations where snails were abundant. Research in other ecosystems has also found that the distribution and abundance of the first intermediate host can be important for determining infection levels in the second intermediate host (Thieltges 2007, Thieltges and Reise 2007), and invasive crayfish can reduce host snail and trematode abundances (Mkoji et al. 1999). In combination with the observed relationship between crayfish population trends and parasite prevalence and the well-established impact of *O. rusticus* on snail populations (Weber and Lodge 1990, Lodge et al. 1994, Lodge et al. 1998), these results suggest that alternate states in crayfish abundance that have been previously attributed solely to interactions between predatory fish and crayfish (Roth et al. 2007) are likely also partly a result of *Microphallus* parasites.

These dynamics are less likely to be observed in lakes dominated by *O. propinquus* or *O. virilis* because these crayfish species have a lesser impact on snail abundance. Dramatic declines in snail abundance are observed when *O. rusticus* replaces congeners in lakes (Wilson et al. 2004). Our preliminary data and research by Roesler (2009) indicate that *O. propinquus* and *O. virilis* can also harbor a large range of infection intensities; therefore, *O. rusticus* may encounter either high or low *Microphallus* abundance when initially introduced to a lake. Whether *Microphallus* has
different impacts on the fitness of different orconectid crayfish species remains to be
determined, but may be important for competitive outcomes and crayfish species
replacements in lakes.

The differences we observed in crayfish growth are small, but are substantial
evenough to have population level consequences. There is a strong relationship between
female length and egg quantity in crayfish (Larson and Magoullick 2008, Chucholl 2012,
Corey 1988). Projecting our average difference in growth over one year, an infected
female will be 2.6 mm shorter, which will result in 30-35 (or 11%) fewer eggs according
to regressions from Corey (1988). Further, we expect that the effect is more severe than
this projection because reductions in growth are likely to occur over multiple years and
smaller crayfish also experience higher mortality due to predation (Stein et al. 1977).

An alternate possibility is that high densities of the definitive host were present in
areas where snails and parasites were abundant, and that predation from definitive hosts
affects crayfish population growth. Including the definitive host and other predators in
future research could substantially increase our understanding of the dynamics in this
system. Both predators and parasites affect crayfish fitness; therefore, we expect the
inclusion of both of these factors will be important for making accurate predictions about
*O. rusticus* abundance and impacts.

We found that infection status was more important than infection intensity in
predicting crayfish growth and feeding behavior, which suggests that these findings are
the result of behavioral changes associated with infection. *O. rusticus* population
declines associated with *Microphallus* are likely due at least in part to *Microphallus*
reducing crayfish growth. Because reductions in growth did not scale with infection
intensity, we conclude that growth reductions do not result from direct hindrance of
digestion by metacercariae, but are instead more likely due to behavioral changes.
Further, during experimental trials, we observed a significant reduction in feeding
behavior in crayfish that were experimentally infected. In the experiment, the magnitude
of the effect of infection on feeding behavior was similar to that of the presence of
predatory fish. Sublethal effects of predatory fish can substantially reduce the impacts of
*O. rusticus* on lower trophic levels, reduce *O. rusticus* growth, and increase *O. rusticus*
mortality (Hill and Lodge 1995, Hill and Lodge 1999). Therefore, the change in feeding
behavior observed in the experiment may be indicative of a major effect of *Microphallus*
on crayfish fitness and impacts in the field.

Behavioral changes may be achieved when the intermediate host is infected with
only a few parasites, and may not scale with infection intensity (Lefevre et al. 2009). The
observed reduction in feeding may be due to a behavioral response of the crayfish host to
infection (i.e., sickness behavior) or behavioral manipulation of the crayfish host by the
parasite (e.g. Kunz and Pung 2004, Helluy and Thomas 2011). Indirect effects of
parasites such as altering host behavior have community-level impacts in other
ecosystems (Dunn et al. 2012). Other infected animals alter or reduce their foraging
behavior to increase immune function or to improve their tolerance to an infection
(Moore 2002).

*O. rusticus* that were experimentally infected fed less often than uninfected
crayfish, but naturally infected crayfish did not. Because both of these groups were
infected via exposure to hydrobiid snails from Plum Lake (either in the laboratory or in
the field), we do not expect that behavioral differences were due to infection with
different types of parasites. In addition, there was no difference in feeding behavior between uninfected crayfish from these two groups, so it is unlikely that behavioral differences were due to habitat differences between the two lakes or different holding times in the lab, although an interaction between these factors and infection status is possible.

While our experimental design does not allow us to determine which factor is responsible for differences in feeding behavior, we suggest three notable differences between laboratory-infected and lake-infected crayfish that could be important. First, crayfish collected from Plum Lake had 20X higher intensity infections than we achieved in the laboratory, and it is possible that while crayfish infected at low levels reduce feeding, highly infected crayfish increase feeding to compensate for a loss of hepatopancreas function caused by infection. Necrosis of hepatopancreatic tissue has been observed in other decapods infected with trematode metacercariae (Robaldo et al. 1999). Second, laboratory-infected crayfish were likely infected more recently than field-infected crayfish. It could be that the behavioral changes in feeding are stronger in the early stages of infection possibly because of a stronger initial behavioral or immune response to infection. In addition, Microphallus likely cause more tissue damage initially as they travel to the hepatopancreas (Meissner and Bick 1999). Third, our collections of field-infected crayfish were filtered by natural selection within the lake. Those crayfish whose behavior was strongly affected by infection may have experienced higher mortality in the field, and the field-infected crayfish we collected may have been the subset of initially infected individuals that survived (possibly because their behavior was less affected by parasitism). In that case, we should consider that our results from
laboratory-infected crayfish may offer more insight than those from the field-infected individuals.

Our results demonstrate that infection with *Microphallus* reduces *O. rusticus* growth and feeding behavior, but there are other likely mechanisms by which *Microphallus* may limit *O. rusticus* population growth that remain to be tested. Some examples include: *Microphallus* inducing behavioral changes that increase the vulnerability of *O. rusticus* to predation, *Microphallus* increasing the vulnerability of *O. rusticus* to other parasites or pathogens, or *Microphallus* altering the intensity of agonistic encounters between crayfish.

Parasites play an important role in mediating community-level interactions in many ecosystems (Thomas et al. 2000, Lafferty et al. 2008), and we found evidence that this could be the case in Wisconsin lakes invaded by *O. rusticus*. Because *O. rusticus* has major community-level impacts, our results suggest that *Microphallus* may be a major driver of community composition in invaded lakes. Negative impacts of *O. rusticus* on macrophytes, macroinvertebrates, and fish are observed in locations where *O. rusticus* trap catch exceeds nine crayfish per trap, and many impacts scale with *O. rusticus* abundance (Wilson et al. 2004). Long-term studies on *Microphallus* dynamics will help determine whether *Microphallus* is influencing *O. rusticus* abundance over longer time scales. Numerous studies have examined the effects of competition among crayfish species (e.g. Capelli and Munjal 1982, Hill and Lodge 1999) and predation by fishes on crayfishes (e.g. Garvey et al. 1994, Roth et al. 2007, Tetzlaf et al. 2011) in controlling *O. rusticus* abundance and impacts. Predatory fish and parasites may act in concert to keep populations of *O. rusticus* from reaching outbreak densities in some invaded lakes. The
strong relationship between *O. rusticus* population growth and parasite prevalence in locations with low crayfish abundance suggests that parasite prevalence may be an important predictor of whether or not the crayfish population will reach outbreak densities or grow to the extent that crayfish reduce the abundance of predatory fish. Overall, our results demonstrate the need to incorporate parasite dynamics into our understanding of community-level interactions and the ecosystem impacts of invasive species in north temperate lakes.

3.6 Acknowledgments

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3.7 References


CHAPTER 4:

INFECTION WITH A TREMATODE PARASITE DIFFERENTIAL ALTERS COMPETITIVE INTERACTIONS AND ANTIPREDATOR BEHAVIOUR IN NATIVE AND INVASIVE CRAYFISH

4.1 Abstract

Parasites can have profound effects on host behaviour and species interactions, but the consequences of these impacts are inadequately understood. Three common crayfish in northern Wisconsin and Michigan (native *Orconectes virilis*, non-native *O. propinquus* and invasive *O. rusticus*) are intermediate hosts for trematode parasites, *Microphallus* spp. Some other species in the genus *Microphallus* alter host behaviour, increasing their predation risk, but the effects of microphallids on crayfish are unknown. *O. propinquus* replaces *O. virilis* in most lakes where they are introduced, and *O. rusticus* replaces both. These species replacements have major effects on macrophytes, macroinvertebrates and fish. Therefore, differential parasite impacts on crayfish could have community-level effects if competitive outcomes are altered.

We examined the shelter affinity of infected and uninfected individuals of all three species in laboratory experiments in the presence and absence of a conspecific. We

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also observed behavior during agonistic interactions, and measured boldness by quantifying how quickly crayfish emerged from shelter with a predatory fish present.

Infection with *Microphallus* substantially altered crayfish shelter affinity, shelter competition and boldness, though infection affected each species differently. Infection reduced shelter affinity in *O. propinquus* and the ability of *O. virilis* to compete for shelter against uninfected conspecifics. Infected crayfish were bolder in the presence of a predatory fish.

Our results suggest that infection with *Microphallus* alters crayfish behaviour so that all three species are more vulnerable to predation. *O. propinquus* are likely to suffer the greatest increase in predation when infected, due to a reduced affinity for shelter coupled with increased boldness. In lakes where crayfish species coexist, *O. rusticus* will likely be less affected by the parasite than both congeners. Therefore, crayfish parasites could alter crayfish abundance and species composition in north temperate lakes via behavioural modifications.

4.2 Introduction

Parasites can dramatically alter host behaviour and the interactions between hosts and other organisms (Thomas et al. 1998, Wood et al. 2007, Sato et al. 2012). However, whether these trait-mediated effects of parasites are important for shaping freshwater communities has rarely been tested (but see Bernot and Lamberti 2008, Hernandez and Sukhdeo 2008, Sato et al. 2012). Parasites that affect crayfish behaviour are likely to be very important for freshwater communities and ecosystems due to the potentially high population density and biomass of crayfish (Hobbs and Lodge 2010). Further, small
differences in crayfish behaviour among species can cause crayfish species replacements
(Garvey et al. 1994, Soderback 1994, Pintor and Sih 2009) and differences in per capita
impacts on lower trophic levels (Olsen et al. 1991). Crayfish parasites are diverse
(Alderman and Polglase 1989), though ours is one of the first studies to test whether
parasites alter crayfish behaviour (but see Haddaway et al. 2012). Further, we tested
whether parasites have different effects on the behaviour of native and non-native
crayfish.

In northern Wisconsin and Michigan lakes (U.S.A.), trematode parasites
(Microphallus spp.) infect three crayfish (the most common in the region; Olden et al.
2006) that have different introduction histories. The virile crayfish (Orconectes virilis) is
native to northern Wisconsin and Michigan, while the northern clearwater crayfish
(hereinafter the northern crayfish, O. propinquus) was probably introduced by anglers in
the mid-1930s (Capelli and Munjal 1982). The rusty crayfish (O. rusticus) is native to
the Ohio River drainage and was introduced to northern Wisconsin by anglers in the mid-
1960s (Olden et al. 2006). The rusty crayfish is invasive and a major driver of
community composition in northern Wisconsin and Michigan lakes, where it reduces the
abundance and richness of macrophytes and macroinvertebrates (Capelli 1982, Lodge et
al. 1994, Wilson et al. 2004, Olden et al. 2006). Rusty crayfish also reduce the
abundance of some species of fish due to a combination of direct consumption of
substratum-nesting fish eggs (Dorn and Mittelbach 1999, Baldridge and Lodge 2013),
reduction of macrophyte habitat, and competition for invertebrate prey (Wilson et al.
2004). These reductions in fish abundance have major economic consequences in the
region, where recreational fishing is of great value (Keller et al. 2008). The rusty
crayfish is considered invasive because it is nonindigenous, has spread rapidly in its introduced range, and causes ecological and economic harm. Virile and northern crayfish have less severe ecological effects than rusty crayfish, due to their lesser consumption of macrophytes and macroinvertebrates, slower growth and lower population densities (Olsen et al. 1991, Wilson et al. 2004).

Northern and rusty crayfish have spread in northern Wisconsin and Michigan and have displaced other species. The northern crayfish has replaced the virile crayfish in many lakes, while the rusty crayfish has often replaced both and now dominates the crayfish fauna of Wisconsin (Capelli 1982, Capelli and Magnuson 1983, Olden et al. 2006). These species replacements have largely been attributed to the relative vulnerability of each species to fish predation (Didonato and Lodge 1993, Garvey et al. 1994, Hill and Lodge 1999). Crayfish mainly reduce predation risk by using shelter (Garvey et al. 1994, Soderback 1994). In laboratory experiments, the northern crayfish displaces virile crayfish from shelter and the rusty crayfish displaces both (Capelli and Munjal 1982), reflecting the pattern of species replacements in nature. Further, in whole-lake studies, the virile crayfish shifts its distribution from cobbles (where shelter is abundant in interstitial spaces) to macrophytes (where it is more exposed) in lakes with rusty crayfish (Peters and Lodge 2013).

Agonistic interactions between crayfish typically involve a series of predictable, stereotyped behaviours (from threat displays to pushing and/or striking the opponent) that increase in intensity over time until one retreats (Bruski and Dunham 1987). Dominant individuals are more likely to obtain shelter than subordinates (Capelli and Munjal 1982,
Davis and Huber 2007) and changes in traits that alter shelter seeking behaviour or competitive outcomes could alter species replacements and species composition in lakes.

Recently, a group of trematode parasites belonging to the *Microphallus opacus* and *M. fonti* species complex has been identified in all three orconectid crayfish in northern Wisconsin lakes (Roesler 2009, Overstreet 2011). Genetic data indicate that this group of parasites is made up of a number of closely related microphallid species which are indistinguishable morphologically (Overstreet 2011). The most common of these *Microphallus* genotypes has been found frequently to infect all three crayfish (Overstreet 2011). The first intermediate host is a hydrobiid snail (Overstreet 2011), and crayfish are infected by free-swimming trematode cercariae (larval trematodes) that emerge from the snail host. The parasites then encyst in the hepatopancreas (digestive organ) of the crayfish until the crayfish is consumed by a definitive host. The definitive host is currently unknown, but closely related microphallids use one or more birds, fish, turtles or mammals (Bray et al. 2008, Overstreet 2011).

Individual crayfish can be infected with many thousands of metacercariae (encysted larval trematodes) (Roesler 2009, Chapter 3). In northern Wisconsin and Michigan, *Microphallus* prevalence is associated with slower population growth in rusty crayfish, and populations may decline where parasite prevalence is high (Chapter 3). Such declines are probably due partly to reductions in growth and feeding (Chapter 3), but other mechanisms by which *Microphallus* may reduce crayfish fitness, including enhancement of predation, have not previously been tested.

Changes in host behaviour have been observed in a number of parasitized animals (Moore 2002, Lefevre et al. 2009b, Poulin 2010). Some are due to manipulation by
parasites that increase transmission to higher trophic levels, often by releasing hormone-like or regulatory substances that affect the endocrine or nervous system of the host (Helluy and Thomas 2003, Lefevre et al. 2009a). Other microphallid species manipulate the behavior of snail, gammarid and shrimp intermediate hosts (Levri 1999, Kunz and Pung 2004, Helluy and Thomas 2010). Behavioural responses to infection that are beneficial to the host but not the parasite are also often associated with infection. For example, infected hosts may reduce their activity or bask to increase their body temperature (Moore 2002). In addition, some behavioural changes associated with infection benefit neither parasite nor host (Moore 2002, Poulin 2010). Because crayfish must be consumed by a predator for *Microphallus* to complete its lifecycle, we predicted that infection would decrease the antipredator behaviour of orconectid crayfish. Further, because behavioural effects of parasites are often host-specific (e.g. Bauer et al. 2000), we predicted that infection with *Microphallus* would have a different behavioural effect on each crayfish species.

To test our predictions, in laboratory experiments, we compared the behaviour of uninfected crayfish with that of infected individuals. Specifically, we tested how infected and uninfected crayfish differed in their shelter affinity, shelter competition, aggressive behaviour in agonistic encounters between crayfish, and boldness in the presence of a predatory fish.
4.3 Methods

4.3.1 Experimental animals and husbandry

Experimental crayfish were hand-collected from lakes in northern Wisconsin and Michigan known to have infected and uninfected crayfish (Table 4.1). Previous knowledge enabled us to pair individuals likely to be infected and uninfected, but infection status was always confirmed after each experiment through dissection. Only data where we were correct about infection status of pairs were analysed. In experiments where crayfish were not paired, the crayfish was reassigned to the correct infection group. Crayfish used in experiments were between 16 and 36 mm carapace length.

In addition to field collections, we also experimentally infected a subset of rusty crayfish from a lake with low infection levels (Lake Ottawa) via exposure to infected hydrobiid snails. To infect the crayfish, we housed them in three, 38 L aquaria (approximately 30 crayfish per aquarium) filled with aerated, well water and exposed them to roughly 4,000 hydrobiid snails contained within a mesh pouch for 6 weeks. The snails were collected from Plum Lake where crayfish had high intensity infections. Control crayfish (also collected from Lake Ottawa) were housed in three identical aquaria with mesh pouches without snails. All pouches also contained macrophytes (Potamogeton amplifolius) coated with periphyton to provide food for snails, and crayfish were fed shrimp pellets ad libitum. We collected crayfish from many locations and used diverse infection treatments to provide strong evidence that the trends we observed were due to infection and not to differences between lakes or sites. Each crayfish was used only once in each experiment.
### TABLE 4.1

**SUMMARY OF BEHAVIOURAL EXPERIMENTS CONDUCTED, SPECIES USED, NUMBER OF TRIALS AND LAKES IN NORTHERN WISCONSIN AND MICHIGAN WHERE INFECTED AND UNINFECTED CRAYFISH WERE COLLECTED**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Species</th>
<th>N</th>
<th>Sources of infected crayfish</th>
<th>Sources of uninfected crayfish</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Shelter Affinity</td>
<td>V</td>
<td>108</td>
<td>Plum (46°13′N, 89°30′W)</td>
<td>Forest (46°9′N, 89°22′W)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>68</td>
<td>Horsehead (46°14′N, 89°43′W)</td>
<td>Horsehead (46°14′N, 89°43′W)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tenderfoot (46°13′N, 89°31′W)</td>
<td>Big Muskellunge (46°0′N, 89°37′W)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>120</td>
<td>Plum (45°59′N, 89°33′W)</td>
<td>Plum (46°0′N, 89°31′W)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ottawa (46°5′N, 88°45′W)*</td>
<td>Ottawa (46°5′N, 88°45′W)</td>
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<td>2. Shelter Competition</td>
<td>V</td>
<td>30</td>
<td>Plum (46°13′N, 89°30′W)</td>
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</tr>
<tr>
<td>(conspecific)</td>
<td>N</td>
<td>14</td>
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<td>54</td>
<td>Plum (45°59′N, 89°33′W)</td>
<td>Plum (46°0′N, 89°31′W)</td>
</tr>
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<td></td>
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<td>Ottawa (46°5′N, 88°45′W)</td>
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<tr>
<td>3. Shelter Competition</td>
<td>V</td>
<td>16</td>
<td>Plum (46°13′N, 89°30′W)</td>
<td>Forest (46°9′N, 89°22′W)</td>
</tr>
<tr>
<td>(heterospecific R-V)</td>
<td>R</td>
<td>16</td>
<td>Plum (45°59′N, 89°33′W)</td>
<td>Plum (46°0′N, 89°31′W)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plume (46°12′N, 89°26′W)</td>
<td>Big (46°12′N, 89°26′W)</td>
</tr>
<tr>
<td>4. Shelter Competition</td>
<td>N</td>
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<td>Tenderfoot (46°13′N, 89°31′W)</td>
<td>Horsehead (46°14′N, 89°43′W)</td>
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<tr>
<td>(heterospecific R-N)</td>
<td>R</td>
<td>38</td>
<td>Plum (45°59′N, 89°33′W)</td>
<td>Palmer (46°12′N, 89°29′W)</td>
</tr>
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</table>

Species are indicated as V (virile crayfish), N (northern crayfish), or R (rusty crayfish).
TABLE 4.1 CONTINUED

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Species</th>
<th>N</th>
<th>Sources of infected crayfish</th>
<th>Sources of uninfected crayfish</th>
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</thead>
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<tr>
<td>5. Aggression (conspecific)</td>
<td>R</td>
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<td>Plum (45°59’N, 89°33’W)</td>
<td>Ottawa (46°5’N, 88°45’W)*</td>
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</tr>
<tr>
<td>6. Aggression (heterospecific R-V)</td>
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<td>Forest (46°9’N, 89°22’W)</td>
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<td>Plum (45°59’N, 89°33’W)</td>
<td></td>
</tr>
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<td></td>
<td>R</td>
<td>14</td>
<td>Plum (45°59’N, 89°33’W)</td>
<td>Big (46°12’N, 89°26’W)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Palmer (46°12’N, 89°29’W)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>26</td>
<td>Plum (45°59’N, 89°33’W)</td>
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<td>Big (46°12’N, 89°26’W)</td>
</tr>
<tr>
<td>8. Boldness</td>
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<td>50</td>
<td>Plum (46°13’N, 89°30’W)</td>
<td>Forest (46°9’N, 89°22’W)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>50</td>
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<td>Big Muskellunge (46°0’N, 89°37’W)</td>
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<td></td>
<td>R</td>
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<td>Spider (46°7’N, 89°49’W)</td>
<td>Papoose (46°11’N, 89°48’W)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Ottawa (46°54’N, 88°46’W)</td>
<td></td>
</tr>
</tbody>
</table>
After laboratory infection or collection from the field, all species were housed in aquaria with other individuals from the same collection site. Aquaria contained constantly-aerated, well water and polyvinyl chloride (PVC) shelters. Water was changed at least weekly and crayfish were fed shrimp pellets *ad libitum*. Crayfish were exposed to natural light (summer experiments) or to a 12:12 h light dark cycle (autumn experiments). Most crayfish were tested within 12 weeks of collection, but experimentally infected crayfish and associated control crayfish were collected in June and tested in autumn (up to 6 months after collection) in order to provide time for the effects of infection to be expressed.

4.3.2 Shelter affinity and competition experiments

To test the effect of *Microphallus* infection on shelter affinity and competition outcomes within and among species, we used methods similar to those used previously (Capelli and Munjal 1982, Chucholl et al. 2008, Larson and Magoulick 2009). For conspecific trials, we paired crayfish with a conspecific of the same size (within 1 mm carapace length), sex and reproductive form, and attempted to pair infected crayfish with uninfected crayfish based on their collection location (Table 4.1; Experiments 1 - 4). In a small subset of trials, we paired crayfish of different sex or species to determine whether infection effects could overcome differences in competitive ability based on sex or species (Table 4.1; Experiment 2). Previous research indicates that males have a competitive advantage in securing shelter (Martin and Moore 2010, Fero and Moore 2014). For the heterospecific trials, both competitors were either infected or uninfected because we wanted to simulate conditions in natural lakes where we expect all species to be equally susceptible to infection (Table 4.1; Experiments 3 and 4). For these trials, we
focused only on heterospecific interactions involving rusty crayfish (i.e. rusty – virile, rusty – northern) because we were most interested in how infection would affect interactions between invasive rusty crayfish and its congeners.

All trials were conducted between autumn 2011 and summer 2013. Before each trial, individual crayfish were marked on their carapace with a dot of coloured nail varnish for identification. The evening before the experiment, each crayfish was placed alone in a bucket (25 cm diameter) with a single shelter constructed from a section of PVC pipe. The bottom of each bucket was covered with a thin layer of clean sand and filled with constantly-aerated, well water to a depth of 10 cm.

To measure shelter affinity in infected and uninfected crayfish (Table 4.1; Experiment 1), we recorded the location of each crayfish five to seven times the following day between 9:00 and 17:00. Observations were made a minimum of 45 minutes apart. We recorded crayfish as ‘outside the shelter’ if all pereopods (walking legs) were outside the PVC shelter, and ‘inside the shelter’ if some or all of the pereopods were inside. The percent of observations during the day in which the crayfish was inside shelter was recorded as the ‘shelter affinity’ of that individual.

To measure shelter competition outcomes (Table 4.1; Experiment 2 - 4), we removed paired crayfish from their individual buckets and placed them together in a new bucket with a single shelter at the end of the first day. Shelters were scaled to the size of the crayfish so that only one individual could fit within without being in close contact with the other. The location of each crayfish was observed the following day using the same methods described above. The crayfish that was inside the shelter for most observations was deemed the winner. In almost all trials (97%), the losing crayfish was
not observed inside the shelter at any time during the competition. At the end of the experiment, the hepatopancreas of each crayfish was dissected out and flattened between two glass slides (top slide with 8 mm$^2$ grid). We then quantified *Microphallus* metacercariae (the number of encysted larval trematodes) under a dissecting microscope.

The methods differed slightly in the 2011 trials because we were also testing for the effect of predatory fish on shelter affinity and competition (Table 4.1; Experiments 1 and 2). For these trials, we modified buckets of the same diameter used in other trials (25 cm diameter) but cut them to a height of 15 cm with a mesh top and a mesh hole (20 cm x 8 cm) in each side. Each modified bucket was placed inside a 68 L tank filled to a depth of 30 cm with well water, so that crayfish could receive visual and chemical cues from a fish in the outer tank, but could not be consumed by the fish.

To assess the effect of fish presence and infection on shelter affinity, each crayfish was observed with and without a smallmouth bass (*Micropterus dolomieu*) in the outer tank. Smallmouth bass are voracious predators of crayfish and are common in northern Wisconsin and Michigan lakes (Peters 2010). We randomized whether each crayfish received the smallmouth bass treatment first or second. Bass trials were always carried out in the same containers so that bass chemical cues were not present in trials where bass were absent. Bass were placed in the outer tank immediately after crayfish were added to the interior bucket (before the overnight acclimation period). As in the other experiments, shelter competition was assessed following the shelter affinity trials, but in this case a smallmouth bass was always present in the outer tank during each competition trial. Except for the fish and modified setup, all methods for these trials (acclimation period, observations etc.) were identical to the other trials described above.
We observed no significant effect of bass on shelter affinity in these 2011 trials (paired Wilcoxon Signed Rank Test: \( P > 0.1 \)). Therefore, we simplified the experimental methods in 2012 and 2013, to exclude the predatory fish treatment. Because we used similar methods, we pooled the shelter affinity data from the non-fish trials in 2011 with shelter affinity trials in later years for analysis (Table 4.1; Experiment 1). In addition, because there was no indication that fish presence had an effect on the outcome of competition for shelter, we also pooled the results of contests with fish present with those from later trials (Table 4.1; Experiment 2).

To test the effect of infection on shelter affinity, we used general linear models. Shelter affinity data were not normally distributed, but shelter aversion data (the proportion of observations outside of shelter) fit a Poisson distribution. Therefore, we conducted all statistical analyses using the data in the form of ‘shelter aversion’ but report shelter affinity (i.e. 1-shelter aversion) for ease of interpretation. We first ran a general linear model to examine the effects of species, infection and their interaction on shelter aversion. Because all of these variables were important, we then ran separate general linear mixed effects models to examine shelter aversion within each species. In addition to infection, we included sex and the interaction between infection and sex as fixed effects in these models. Because we conducted this research over several years and collected crayfish from different lakes, we also included the set of trials as a random effect in each model. We compared models using likelihood ratio tests. We also used a linear model to examine the effect of infection intensity (the number of parasites in infected individuals) on shelter affinity. The model included the effects of infection intensity, species and their interaction. The number of metacercariae (infection intensity)
was square root transformed to meet the assumption of normality of variance. To assess whether infection status affected the outcome of contests for shelter within and among species, we used $\chi^2$ tests. Finally, we tested the effect of infection intensity on competition outcomes within each species, using logistic regression.

4.3.3 Aggression

In addition to testing whether infection affects the outcome of contests, we were interested in how infection alters the behavioural differences that determine the outcome. To investigate infection effects on competitive behaviour, we recorded crayfish behaviour during the initial encounter between competitors in a subset of contests. We were especially interested in quantifying aggressive behavior, because dominant, aggressive individuals are more likely to win a competition for shelter than subordinates (Capelli and Munjal 1982, Davis and Huber 2007). We did this for contests between rusty crayfish (Table 4.1; Experiment 5) and for heterospecific trials between rusty crayfish and either virile or northern crayfish (Table 4.1; Experiments 6 and 7).

All aggression trials were conducted between 14:00 and 18:00 after crayfish were observed during the day for shelter affinity. At the start of the trial, crayfish were removed from individual buckets and placed on either side of a plexiglass divider in a new bucket. The divider was transparent and perforated so that crayfish could receive visual and chemical cues from competitors during the acclimation period. As in previous experiments, buckets contained a thin layer of clean sand and 10 cm of constantly-aerated, well water. Crayfish were left to acclimate on either side of the divider for 20 min. After the acclimation period, an observer lifted the divider and recorded the behaviour of each crayfish on video for 15 min. Videos were later scored using an
ethogram developed by Bergman and Moore (2003; Table 4.2). Using methods similar to Pintor and colleagues (2008), the most aggressive action of each crayfish was scored every 5 sec, and scores were totalled over the 15 min trial so that we could quantify overall aggression for each individual. After the trial, we placed a shelter in the bucket and proceeded with shelter competition observations the following day as described above.

**TABLE 4.2**

ETHOGRAM CODES USED TO DETERMINE AGGRESSION LEVELS IN CRAYFISH (FROM BERGMAN AND MOORE 2003)

<table>
<thead>
<tr>
<th>Intensity Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2</td>
<td>Tail flip away from opponent or fast retreat</td>
</tr>
<tr>
<td>-1</td>
<td>Slowly back away from opponent</td>
</tr>
<tr>
<td>0</td>
<td>Ignore opponent with no response or threat display</td>
</tr>
<tr>
<td>1</td>
<td>Approach without a threat display</td>
</tr>
<tr>
<td>2</td>
<td>Approach with threat display using meral spread and/or antennal whip</td>
</tr>
<tr>
<td>3</td>
<td>Initial claw use by boxing, pushing or touching with closed claws</td>
</tr>
<tr>
<td>4</td>
<td>Active claw use by grabbing opponent with open claws</td>
</tr>
<tr>
<td>5</td>
<td>Unrestrained fighting by grasping and pulling opponent’s claws or appendages</td>
</tr>
</tbody>
</table>
We examined the effect of infection on aggression scores using ANOVA. Data from males ($N = 26$) and females ($N = 34$) were pooled because we found no effect of sex on aggression score ($F_{1, 60} = 1.02$, $P = 0.316$). For conspecific trials, we compared aggression scores of infected crayfish (from trials against uninfected conspecifics), uninfected crayfish (from trials against infected conspecifics), and control crayfish (from trials where uninfected crayfish interacted with other uninfected conspecifics). For heterospecific trials, we compared aggression scores of infected and uninfected crayfish for each species within rusty-virile crayfish trials and rusty-northern crayfish trials.

4.3.4 Boldness

To assess how infection altered the behaviour of crayfish in the presence of a predatory fish, in summer 2013 we conducted an emergence ‘boldness’ assay that tested how infection status altered the short-term shelter use of crayfish in the presence of a common predatory fish, the rock bass *Ambloplites rupestris* (Peters 2010; Table 4.1; Experiment 8). Our methods were similar to those used in many ‘fish boldness’ assays (Wilson and Godin 2009, Lopez et al. 2012). Trials were conducted between 9:00 and 18:00 under natural light. Trials were carried out in two replicate tanks (60cm length x 50cm width x 35cm height; Figure 4.1), which were filled with constantly-aerated, well water. The bottom of each tank was covered with a thin layer of clean sand. The top and bottom halves of the tank were divided by a removable mesh divider. A crayfish was placed in the bottom half of each tank within a shelter constructed from a PVC pipe (6 cm diameter, 17 cm length). The back of the pipe was capped and the front of the pipe had a plexiglass insert which could be removed to allow the crayfish to leave the shelter.
The plexiglass insert was opaque but perforated so that the crayfish could receive chemical cues from the predator. After the crayfish was placed in the shelter, the mesh divider was secured in the tank, and a rock bass was added to the top section of the tank. The crayfish then acclimated in the tank with the fish present for 15 minutes. After the acclimation period, the plexiglass insert was gently removed from the shelter through a small slit in the mesh divider. The rock bass remained in the top half of the tank above the divider so that crayfish could receive visual and chemical cues from the fish, but could not be consumed (Figure 4.1). We then observed the tank for a period of 30 min and recorded the time (the latency) that the crayfish emerged from the shelter. Latency was used as an index of boldness; crayfish that emerged quicker were considered bolder than those that took longer to emerge. Two trials were run simultaneously with conspecifics of different infection status. After the trials, we dissected crayfish and counted the number of *Microphallus* metacercariae (using the same methods as described above).

To determine whether infection with *Microphallus* altered the latency of crayfish, we used a Cox Proportional Hazards Model. The full model included the effects of species, sex, carapace length, infection and all interactions between these variables on latency. We used a stepwise model selection procedure using AIC to simplify the model. Infection, species, sex, and the interactions between infection and species and between infection and sex, were included in the simplified model. We used likelihood ratio tests to test the importance of each of these variables. All analyses were conducted in R, version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria). We used the survival package (Therneau and Grambsch 2000) for Cox Proportional Hazards Models.
Figure 4.1 Setup for boldness experiment.
(a) The crayfish was initially placed within a PVC pipe shelter in the bottom half of the tank. (b) A removable mesh divider ensured that the rock bass could not approach or consume the crayfish. (c) A perforated plexiglass insert was removed from the shelter after the acclimation period to allow the crayfish to leave.
4.4 Results

4.4.1 Shelter affinity

Infection altered crayfish shelter affinity, and had a different effect on each species ($\chi^2 = 768.47, P < 0.001, N = 296$; Figure 4.2). Within virile crayfish, infection reduced shelter affinity by roughly 15% ($\chi^2 = 180.32, P < 0.001, N = 108$; Figure 4.2), with uninfected virile crayfish spending 88 ± 4% (mean ± SE) of the day in shelter and infected virile crayfish spending 75 ± 4% of the day in shelter. Male virile crayfish had a greater affinity for shelter than females ($\chi^2 = 57.58, P < 0.001, N = 108$), although infection had a similar effect on the affinity of both sexes ($\chi^2 = 0.61, P = 0.437, N = 108$).

In northern crayfish, infection reduced shelter affinity by roughly 40% ($\chi^2 = 797.96, P < 0.001, N = 68$), with uninfected and infected individuals spending 89 ± 4% and 53 ± 6% of the day in shelter, respectively. Northern crayfish were collected from different locations in different years (Table 1), but trials from both years showed the same pattern, in which shelter affinity was reduced in infected individuals (Figure 4.2). Female northern crayfish were more likely to take shelter than males ($\chi^2 = 99.62, P < 0.001, N = 68$), while infection reduced affinity for shelter in males more than in females ($\chi^2 = 9.58, P = 0.002, N = 68$).

In contrast to the other congeners, infection increased shelter affinity within rusty crayfish by about 11% ($\chi^2 = 13.76, P < 0.001, N = 120$); uninfected and infected rusty crayfish spent 73 ± 4% and 81 ± 4% of the day in shelter, respectively. Experimentally infected rusty crayfish behaved similarly to infected rusty crayfish collected from the field (Figure 4.2). Male rusty crayfish had greater affinity for shelter than females ($\chi^2 = 118$).
99.58, \( P < 0.001, N = 120 \), while infection had a similar impact on the affinity of both sexes \((\chi^2_1 = 1.74, P = 0.188, N = 120)\).

Infection intensity had no effect on shelter affinity overall \((F_{1, 177} = 0.61, P = 0.436)\), and there was no interaction between infection intensity and species \((F_{2, 176} = 0.65, P = 0.521)\). Overall, infection had the greatest impact on shelter affinity in northern crayfish, which had a high affinity when uninfected, but the lowest shelter affinity among species when infected (Figure 4.2).

![Figure 4.2](image)

Figure 4.2 Shelter affinity (percent of observations in shelter) ± SE for (a) virile, (b) northern, and (c) rusty crayfish collected from different northern Wisconsin and Michigan lakes. White bars indicate uninfected individuals and grey bars indicate infected individuals. Data from the same species are depicted separately in cases where infected or uninfected crayfish were collected from different lakes (northern crayfish) or were infected using different techniques (rusty crayfish). Rusty crayfish were either collected from Plum Lake where a subset were already infected (Field Infected) or collected from Lake Ottawa where a subset were experimentally infected via exposure to infected snails (Exp Infected).
4.4.2 Competition for shelter

Infection had different effect on shelter competition within each species ($\chi^2_2 = 9.65, P = 0.008, N = 99$; Table 4.3). Infected virile crayfish were likely to lose a contest for shelter to an uninfected individual or to one with fewer metacercariae ($\chi^2_1 = 6.53, P = 0.011, N = 30$; Table 4.3). This was also true of male-female trials, where uninfected females defeated infected males in all five instances and uninfected males defeated infected females in all three instances. For virile crayfish, we did not have a large range of infection intensities within our data (metacercariae range = 1 to 46), so we were unable to assess whether infection intensity had an effect on shelter competition.

<table>
<thead>
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<th>Species</th>
<th>Less Infected Wins</th>
<th>More Infected Wins</th>
</tr>
</thead>
<tbody>
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<td>Virile crayfish</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Northern</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Rusty crayfish</td>
<td>26</td>
<td>28</td>
</tr>
</tbody>
</table>
In contrast to our findings for virile crayfish, infection did not alter competitive ability significantly in northern crayfish, although there was a trend in which infected northern crayfish won most shelter competitions against uninfected individuals ($\chi^2_1 = 3.27, P = 0.071, N = 15$; Table 4.3). In the subset of trials in which we paired males with females, the infected crayfish also won most often. Males are generally expected to win contests with females, but in the three trials in which an infected female competed with an uninfected male of similar size, the female won. This is further evidence that infection increased competitive ability in northern crayfish. If these trials are included in the analysis, infected northern crayfish are significantly more likely to win a competition against an uninfected conspecific ($\chi^2_1 = 5.56, P = 0.018, N = 18$). When infected males were paired with uninfected females, the males won most of the trials (five out of six), as expected. Logistic regression did not reveal any effect of infection intensity on the probability that an infected northern crayfish would win a competition with an uninfected conspecific ($P > 0.7$); however, we also had a relatively small range of infection intensities for this species (metacercariae range = 1 to 87). Even though infected northern crayfish won most trials, they still spent a substantial amount of time out of shelter during the day with an uninfected conspecific present (% of observations in shelter $\pm$ SE = 60 $\pm$ 11%).

Infection status (infected/uninfected) did not alter the mean competitive outcome in rusty crayfish; however, logistic regression revealed that infection intensity affected competition for shelter (likelihood ratio test: $\chi^2_1 = 4.33, P = 0.037, N = 43$). Overall, infected and uninfected rusty crayfish had similar competitive abilities ($P > 0.7$; Table 4.3). However, rusty crayfish with a low intensity infection were somewhat more likely
to win a contest with an uninfected individual, although competitive ability declined as infection intensity increased (Figure 4.3). In competition experiments, experimentally infected rusty crayfish behaved similarly to infected crayfish from the field (Figure 4.3). Rusty crayfish infection intensity ranged from 1 to 1,329 metacercariae. Overall, infection reduced competitive ability in native virile crayfish, increased it in northern crayfish, and had less impact on competitive ability in invasive rusty crayfish.

Figure 4.3 Logistic regression examining the effect of infection intensity on rusty crayfish shelter competition outcomes. Dashed lines indicate 95% confidence intervals. All trials represent the outcome of a contest between one infected and one uninfected rusty crayfish. All outcomes are either 1 (infected crayfish won) or 0 (uninfected crayfish won), but points are jittered in the figure so that all data can be visualized. Grey points represent trials with experimentally-infected crayfish and black points represent field-infected crayfish.
Heterospecific trials suggested that infection is unlikely to alter the outcome of competition for shelter between rusty crayfish and the other two species. Previous research indicates that the rusty crayfish is more competitive for shelter than either virile or northern crayfish (Capelli and Munjal 1982), so we predicted that uninfected rusty crayfish would win contests against size- and sex-matched congeners. Rusty crayfish did win all trials against congeners when both crayfish were uninfected (against virile crayfish: \( N = 6 \), against northern crayfish: \( N = 10 \)). Rusty crayfish won almost all trials against virile crayfish when both crayfish were infected (8 out of 10 trials). Infected rusty crayfish won slightly fewer trials when paired with infected northern crayfish (15 out of 19); however, this was not significantly different from the outcome when both were uninfected (\( \chi^2 = 2.40, P = 0.118, N = 28 \)). In summary, while infection increases competitive ability in northern crayfish, this was not sufficient to overcome the competitive advantage of rusty crayfish.

While we observed no differences in heterospecific trials where crayfish were uninfected and infected, power analysis revealed that we would need more replicates to detect small differences in competitiveness. For example, if northern crayfish lose 100% of trials against rusty crayfish when both species are uninfected, and 80% of trials against rusty crayfish when both species are infected, we would need 34 replicates of both uninfected and infected trials to detect this difference. Therefore, we cannot rule out small differences in competitiveness.

4.4.3 Aggression

Infection altered aggressive behaviour in rusty crayfish in conspecific trials. In contests between infected and uninfected rusty crayfish, the former were significantly
more aggressive (mean score ± SE, 41±11) than the latter (1±9; \(F_{2, 60} = 3.84, P = 0.027\); Tukey’s HSD: \(P = 0.020\); Figure 4.4a). In control trials (in which neither contestant was infected), aggression scores were intermediate (21±15), and there was no difference in aggression scores between crayfish that won the competition for shelter the following day and those that lost (22±18 and 20±19, respectively; \(P > 0.9\)). Aggression scores of control crayfish were not significantly different from those of infected or uninfected rusty crayfish (Tukey’s HSD: \(P > 0.4\); Figure 4.4a).

We found no significant differences in aggression between infected and uninfected crayfish in heterospecific interactions. Uninfected virile crayfish had very low aggression scores when paired with uninfected rusty crayfish, but they may be marginally more aggressive in trials where both competitors were infected (\(F_{1, 13} = 4.46, P = 0.055\); Figure 4.4b). Rusty crayfish were also marginally more aggressive in trials with virile crayfish when both competitors were infected (\(F_{1,13} = 3.50, P = 0.084\); Figure 4.4b). In rusty-northern crayfish contests, there was no significant effect of infection on aggression in either northern (\(F_{1, 25} = 0.75, P = 0.393\)) or rusty crayfish (\(F_{1, 25} = 0.25, P = 0.8767\); Figure 4.4c).
Figure 4.4 Aggression scores of infected and uninfected crayfish ± SE in conspecific (a) and heterospecific trials (b, c). White bars indicate uninfected crayfish and grey bars indicate infected crayfish. (a) Aggression scores of control crayfish (in agonistic encounters with other uninfected individuals) compared to infected and uninfected *O. rusticus* (in agonistic encounters with one another). (b) Aggression scores of virile crayfish and rusty crayfish in heterospecific encounters. (c) Aggression scores of northern crayfish and rusty crayfish in heterospecific encounters. Species are indicated as V (virile crayfish), N (northern crayfish), or R (rusty crayfish).
4.4.4 Boldness

In the presence of a predator, infection significantly reduced the time it took for crayfish to emerge from shelter ($\chi^2_1 = 31.02, P < 0.001, N = 142$; Figure 4.5). This latency period differed between species ($\chi^2_1 = 47.17, P < 0.001, N = 142$; Figure 4.5). Virile crayfish were relatively slow to emerge from the shelter (mean ± SE, 19 ± 2 min and 18 ± 3 min for uninfected and infected individuals, respectively; Figure 4.5a,d). Northern crayfish emerged from shelter more quickly (uninfected, 12 ± 2 min; infected, 4 ± 1 min; Figure 4.5b,e). Rusty crayfish were the boldest overall (uninfected, 9 ± 2 min; infected, 4 ± 2 min; Figure 4.5c,f). Males were also significantly bolder than females ($\chi^2_1 = 12.01, P = 0.003, N = 142$; Figure 4.5), and there was an interaction between infection and sex, with infection altering the behaviour of males more than that of females ($\chi^2_1 = 9.75, P = 0.002, N = 142$; Figure 4.5). The interaction between the effects of infection and species was not significant ($P > 0.1$), indicating that infection increased boldness in each species to a similar extent. Overall, infection increased boldness in all three species, but northern and rusty crayfish were bolder overall than virile crayfish (Figure 4.5).
Figure 4.5 Cox regression estimates of the probability that a crayfish will emerge from shelter throughout a 30 minute trial with a rock bass predator present in the aquarium. Grey lines indicate estimates for uninfected crayfish and black lines indicate estimates for infected crayfish.
4.5 Discussion

4.5.1 Effects of infection in lakes with one crayfish species

The changes in crayfish behaviour associated with *Microphallus* infection are likely to have consequences for lake communities. In lakes with a single crayfish species, we expect infection to increase predation on crayfish and, therefore, to reduce crayfish populations. Many north temperate lakes contain only one crayfish species after northern crayfish have replaced virile crayfish or rusty crayfish have replaced one or both (Capelli 1982). Infection with *Microphallus* either reduced shelter affinity or predator avoidance in all three species. Therefore, our results suggest that infection will make all three species more vulnerable to predation, but the species will be affected to a different extent.

Infection is likely to increase predation on northern crayfish to the greatest extent. When infected, this species was bold in the presence of predators and was less likely to take shelter. This combination of responses would make northern crayfish more vulnerable to predation (Stein and Magnuson 1976, Stein 1977, Garvey et al. 1994). Rusty crayfish were also bold when infected, which may lead to greater vulnerability to predation. However, because infected rusty crayfish had a higher affinity for shelter when infected, it is unlikely that this species would be affected by *Microphallus* to the same extent as northern crayfish. Infected virile crayfish had a reduced affinity for shelter, but this effect was less than that in northern crayfish. Therefore, we expect most virile crayfish to occupy available shelters when infected. Further, due to their typically shy behaviour, most infected virile crayfish would probably hide if fish predators were close. Thus, infection would probably not increase predation on virile crayfish to the
same extent as on northern crayfish. Previous research indicates that population growth in rusty crayfish declines with increasing *Microphallus* prevalence (Chapter 3), perhaps due in part to increased boldness. We expect this effect to be more pronounced in northern crayfish than in rusty or virile crayfish.

Infection with *Microphallus* increased the intensity of competitive interactions, which may result in increased crayfish mortality and injury in lakes with high infection prevalence. Injury reduces the growth and survival of crayfish (Figiel and Miller 1995). The aggressive behaviour displayed by crayfish in agonistic encounters, such as boxing or grabbing with claws, is likely to lead to injury, and infected individuals engaged in those behaviours more frequently. Rusty crayfish infected with *Microphallus* were more aggressive than uninfected competitors, but not significantly more aggressive than control rusty crayfish (those within uninfected-uninfected pairs). This may have been because infected crayfish were paired with an uninfected individual, and the uninfected competitor tended to submit or retreat in response to aggressive displays. If we had instead paired two infected crayfish, we suspect that interactions would have been more intense than those between two uninfected crayfish.

4.5.2 Effects of infection in lakes with more than one crayfish species

While infection altered competitive outcomes within species, infection is unlikely to alter contests for shelter between species. Within species, infection decreased the likelihood that a virile crayfish would win, increased the likelihood that a northern crayfish would win, and depending on infection intensity either slightly increased or decreased the likelihood that a rusty crayfish would win an intraspecific contest. Previous research indicates that virile crayfish are less competitive for shelter than either
congeners (Capelli and Munjal 1982, Garvey et al. 1994, Hill and Lodge 1999). Thus, the virile crayfish is likely to remain subordinate to northern and rusty crayfish when infected; virile crayfish lost almost all competitions with rusty crayfish regardless of infection status. We did not test how infection alters contests between virile and northern crayfish, but we expect that infection is likely to increase the dominance of northern crayfish because infection increased competitiveness in northern crayfish and but decreased it in virile crayfish. Even though infection did not substantially alter the outcomes of interspecific competitions between size- and sex-matched northern and rusty crayfish, an increase in competitive ability could benefit individual northern crayfish in other contests such as those between crayfish of different sizes or sexes. Even though infection increased the ability of northern crayfish to compete for shelter, other behavioral effects of infection are likely to have the greatest impact on this species, so we expect that rusty crayfish are likely to rapidly displace infected northern crayfish from lakes due to apparent competition.

In lakes with several competing crayfish species, Microphallus may increase the ability of rusty crayfish to replace congeners, especially northern crayfish, via apparent competition. Some lakes in northern Wisconsin and Michigan contain several coexisting crayfish species or have crayfish assemblages in transition (rusty or northern crayfish were recently introduced and are in the process of spreading around the littoral zone). Microphallus could alter apparent competition between crayfish species in these assemblages. Because the northern crayfish is probably most vulnerable to predation when infected, we expect that more northern crayfish, and fewer rusty or virile crayfish, would be consumed by predators in a lake with high infection prevalence. Infection may
allow rusty crayfish to replace northern crayfish more rapidly or it may slow the spread of northern crayfish in a lake with virile crayfish present. While there is a general pattern in north temperate lakes in which rusty crayfish replace northern crayfish and northern crayfish replace virile crayfish, the time that it takes for one species to replace another varies greatly between lakes, and species coexist in some lakes (Capelli 1982, Lodge et al. 1986, Peters and Lodge 2013). Microphallus infection has not been taken into account in previous studies and may be partially responsible for these variable outcomes.

Taking into account additional factors, such as differences in body-size and habitat use between species, rusty crayfish will probably benefit from a high prevalence of Microphallus in lakes with more than one crayfish species. Larger crayfish are more likely to win a competition and, in the field, virile crayfish are the largest on average followed by rusty and then northern crayfish (Olsen et al. 1991). The competitive advantage of virile crayfish due to size may be diminished in a lake with high infection prevalence because virile crayfish are less competitive for shelter when infected. Further, at the whole lake scale, changes in habitat use by northern and virile crayfish after invasion by rusty crayfish are likely to expose these congeners to more cercariae than rusty crayfish. Both northern and virile crayfish shift from cobbles to macrophytes (where there are more snails and, therefore, more cercariae) when rusty crayfish are present (Hill and Lodge 1994, Peters and Lodge 2013). Further, because of the greater effect of rusty crayfish on snail abundance, rusty crayfish may locally reduce snail populations and, therefore, reduce Microphallus prevalence in locations where they are abundant (Chapter 3). Overall, the prevalence of Microphallus is likely to benefit rusty crayfish in lakes where it competes with northern and virile crayfish.
4.5.3 Evolutionary history between *Microphallus* and crayfish

Because virile, northern and rusty crayfish are native to different regions, one of these species may share a longer coevolutionary history with *Microphallus*, and parasites may have spread to the other crayfish hosts when they came into contact in north temperate lakes. These crayfish-parasite interactions, therefore, could improve our understanding of the effects of coevolution between parasites and hosts. Growing evidence suggests that parasites can alter interactions between pairs of invasive and native species (e.g. Bauer et al. 2000, Tompkins et al. 2003, Georgiev et al. 2007, Dunn et al. 2012). The numerous introduced parasites or pathogens that have catastrophic effects on naïve, native populations indicate that a lack of coevolutionary history between hosts and pathogens can greatly reduce host populations (Vogt 1999, Orwig et al. 2002, Fisher, Garner and Walker 2009). However, in other cases, parasites and pathogens may be more detrimental to the host with which they share the longest coevolutionary history (Bauer et al. 2000, Dunn et al. 2012).

It is currently unclear which, if any, of these crayfish species shares the longest coevolutionary history with *Microphallus*. Caveny and Etges (1971) found that northern crayfish collected from Muchinippi Creek in central Ohio were infected with *Microphallus* metacercariae. Whether or not this same species of *Microphallus* is present in northern Wisconsin and Michigan crayfishes is unclear. *Microphallus* does have the greatest behavioural impact on northern crayfish, and this could be due to successful behavioural manipulation by the parasite which has developed over a long shared coevolutionary history. Future research using genetic tools to identify parasites in both
the native and invasive ranges of each of these crayfishes could shed light on the length of coevolutionary history among these species.

4.5.4 Mechanism for behavioural changes

Some other microphallids manipulate the behavior of their intermediate hosts (Levri 1999, Kunz and Pung 2004, Helluy and Thomas 2010), and our findings are consistent with such manipulation of crayfish by Microphallus. Increased boldness and reduced use of shelter would make crayfish more vulnerable to a variety of potential definitive hosts such as fish, birds, mammals or turtles. Other closely related microphallids are able to infect a variety of definitive hosts (Overstreet 2011), so it is possible that there is more than one suitable definitive host for Microphallus, and thus generally increasing the susceptibility of an infected crayfish to predation may increase Microphallus fitness. In addition, because there are multiple closely related species present, it is possible that some of these species have different definitive hosts. We cannot rule out other potential causes of behavioural changes in infected individuals, such as those that benefit the host (i.e. sickness behaviour) or those that are a byproduct of infection. However, our results are most consistent with behavioural manipulation by the parasite to increase transmission to higher trophic levels.

4.5.5 Parasite-induced changes to lake communities

Regardless of the cause of the behavioural changes observed, they are likely to have consequences for lake communities. The negative effects of rusty crayfish on macrophytes, macroinvertebrates and fish scale with crayfish density, and a simple way in which infection is likely to influence lake communities is by reducing crayfish
abundance (Wilson et al. 2004). Infection may also alter the likelihood that an introduction of rusty or northern crayfish will succeed in a lake. Further, *Microphallus* is likely to alter interactions between species, favouring rusty crayfish over northern and virile crayfish, both of which have lesser ecological effects than rusty crayfish (Olsen et al. 1991, Wilson et al. 2004). Another aspect of infection with Microphallus is the altered response of crayfish to predatory fish associated with infection. Nonconsumptive effects of predatory fish lead to increased macrophyte and macroinvertebrate biomass and richness (Hill and Lodge 1995), and if those effects are lost and crayfish continue to forage with predators present, crayfish may have greater impacts on lower trophic levels. Our results suggest that microphallid parasites can affect crayfish behavior and, because of the widespread ecological effects of crayfish in fresh waters, the presence of these parasites and their effects warrants further attention.

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CHAPTER 5:
PARASITES ALTER FRESHWATER COMMUNITIES BY MODIFYING BEHAVIOR OF INVASIVE CRAYFISH HOSTS

5.1 Abstract

Parasites can alter communities by reducing densities of keystone hosts, but few studies have examined how trait-mediated indirect effects of parasites on hosts can alter ecological communities. We test how trematode parasites (*Microphallus* spp.) that affect the behavior of an invasive crayfish host (*Orconectes rusticus*) alter the per capita impacts of this host on lake littoral communities. *O. rusticus* are major drivers of community composition in north temperate lakes, and predatory fish can reduce the impacts of *O. rusticus* by reducing crayfish activity and feeding behavior. In laboratory studies, *Microphallus* parasites also alter *O. rusticus* behavior: infected *O. rusticus* consume fewer macroinvertebrates and are bolder in the presence of predatory fish than uninfected individuals. We used a 2 X 2 factorial experiment to test the combined effects of predatory fish and parasites on *O. rusticus* impacts in large mesocosms over four weeks. We predicted (1) that when predators were absent, infected crayfish would have lower impacts than uninfected crayfish on macrophytes and macroinvertebrates (as well as reduced growth). However, (2) when predators were present but unable to consume

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crayfish, infected crayfish would have greater impacts than uninfected crayfish (as well as greater growth) because of increased boldness. Because of its effect on crayfish feeding behavior, we also predicted that (3) infection would alter macrophyte and macroinvertebrate community composition. In contrast to our first hypothesis, we found that infected crayfish were more likely to be found outside of shelter and had greater growth than uninfected crayfish across all treatments, suggesting that the reduction in feeding behavior observed in short term experiments does not occur over longer timescales. However, in support of second hypothesis, when predatory fish were present, infected crayfish consumed a greater number of macroinvertebrates than uninfected crayfish, likely due to increased boldness. We also observed a trend for greater macrophyte consumption associated with infection in mesocosms with predatory fish. In addition, as we expected, we observed a trend indicating infection may alter the composition of the macroinvertebrate community. Our results suggest that parasites can substantially alter aquatic communities merely by modifying host behavior.

5.2 Introduction

Parasites can alter interspecies interactions and community composition (Minchella and Scott 1991, Lafferty et al. 2008), and in most ecosystems in which parasites are recognized as being important, their impact is assumed to result from changes in the density of keystone hosts (Mouritsen and Poulin 2005). However, parasites also alter host traits, which could in turn impact communities and ecosystems (Mouritsen and Poulin 2005, Lefevre et al. 2009). Such trait-mediated community impacts of parasites are less studied, but potentially strong (e.g. Thomas et al. 1998,
Wood et al. 2007, Sato et al. 2012). For example, trematode parasites reduce the ability of cockles to bury into sediment; therefore, different organisms colonize the shells of infected and uninfected cockles (Thomas et al. 1998). In addition, nematomorph parasites manipulate crickets to enter streams where the parasites reproduce. These crickets provide an important subsidy for trout, which therefore, have a lesser impact on benthic macroinvertebrates (Sato et al. 2012). We test whether parasite-induced behavioral changes in an invasive crayfish host alter the impacts of this host on lake littoral communities.

Crayfish often have large impacts on aquatic ecosystems because of their large size compared to other aquatic invertebrates, their omnivorous feeding habits, and their ability to reach high population densities (Hobbs and Lodge 2009). In addition, small differences in crayfish behavior among species can result in large differences in crayfish impacts on lower trophic levels (Olsen et al. 1991). Therefore, parasites that alter crayfish behavior are especially likely to affect the ecological community. Invasive rusty crayfish (Orconectes rusticus) are a major driver of community composition in northern Wisconsin and Michigan lakes. O. rusticus are native to streams in the Ohio River drainage and were introduced by anglers to northern Wisconsin and Michigan in the 1960s (Olden et al. 2006). After they are introduced to a lake, O. rusticus often replace other crayfishes and reduce the abundance and richness of macrophytes and benthic macroinvertebrates (Capelli 1982, Lodge et al. 1994, Wilson et al. 2004, Olden et al. 2006). O. rusticus also reduce the abundance of some species of fish due to a combination of direct consumption of substrate-nesting fish eggs, reduction of macrophyte habitat, and competition for macroinvertebrate prey (Dorn and Mittelbach
Trematode parasites (*Microphallus* spp.) are widespread in northern Wisconsin and Michigan lakes (Roesler 2009, Overstreet 2011, Chapter 3). Genetic data indicate that the microphallids that infect *O. rusticus* are a group of closely related species in the same species complex as *M. opacus* and *M. fonti* (Overstreet 2011). The first intermediate host of *Microphallus* is a hydrobiid snail (Overstreet 2011), and *O. rusticus* are infected with *Microphallus* after free-swimming cercariae (larval trematodes) emerge from the snail. The parasites encyst in the hepatopancreas (digestive organ) of the crayfish and remain there until the crayfish is consumed by an unknown definitive host. Closely related trematodes use a number of bird, fish, turtle, and mammal species as definitive hosts (Bray et al. 2008, Overstreet 2011). *Microphallus* prevalence (% of crayfish infected) and infection intensity (number of parasites within infected individuals) varies substantially within and between lakes (Chapter 3). In some locations, parasite prevalence reaches 100% and some crayfish harbor thousands of metacercariae (encysted larval trematodes). In other locations, nearly every crayfish is uninfected. Because of these striking differences in *Microphallus* prevalence and abundance, there is the potential for different ecological impacts of *O. rusticus* if infected and uninfected individuals have different traits.

Laboratory studies indicate that *O. rusticus* infected with *Microphallus* behave differently than uninfected individuals. Specifically, infected *O. rusticus* are bolder (emerge more quickly from shelter) than uninfected *O. rusticus* in mesocosms containing
predatory fish (Chapter 4). In addition, in short-term experiments, infected crayfish consume fewer macroinvertebrates (Chapter 3, Reisinger unpublished data). Further, when provided with the same amount of food, infected *O. rusticus* grow more slowly than uninfected individuals, potentially because of reduced feeding (Chapter 3). These changes in behavior are likely to alter the impact of *O. rusticus* on lake communities.

We tested the combined effects of predatory fish and *Microphallus* parasites on the ecological impacts of *O. rusticus* using large mesocosms stocked with prey organisms from north temperate lakes. When predators are absent, we predicted that infected crayfish would have lower impacts on macrophytes and macroinvertebrates than uninfected crayfish due to reduced feeding. In addition, we expected infected crayfish to have reduced growth and higher mortality compared to uninfected crayfish when predators are absent. In contrast, when predators are present but unable to consume crayfish, we predicted that infected crayfish would have greater impacts (as well as greater growth and lower mortality) than uninfected crayfish because of increased boldness. Specifically, we predicted that uninfected crayfish would spend more time hiding and less time feeding when predatory fish are present, but this effect would be reduced in infected crayfish. We also examined whether differences in feeding behavior between infected and uninfected crayfish produced differences in macrophyte or macroinvertebrate community composition. Finally, our experimental design allowed us to test whether trait-mediated effects of parasites were similar in magnitude to previously appreciated (e.g. Hill and Lodge 1995) trait-mediated effects of predators.
5.3 Methods

5.3.1 Mesocosms

To test whether *Microphallus* alters crayfish impacts, we conducted an experiment over 4 wks (early June-early July, 2013) in 12 large mesocosms (2 m diameter, 0.6 m depth) near the shore of Trout Lake, WI (46° 0' N, 89° 40' W). In order to test the effect of infection on crayfish impacts, we used a 2 X 2 factorial design. Half of the mesocosms were stocked with infected crayfish and half were stocked with uninfected crayfish. Half of the mesocosms also contained predatory fish.

Water from the epilimnion of Trout Lake continuously flowed through each mesocosm, so that the total volume was replaced at least once every 12 h. Each mesocosm contained two airstones to maintain high dissolved oxygen levels. We stocked mesocosms with materials collected from Trout Lake including 250 L of sand, which we spread evenly over the bottom of each mesocosm. We also spread 10 (13L) buckets of cobble over 1/3 of each mesocosm to provide cover for crayfish. At the start of the experiment, we stocked each mesocosm with 5 L of detritus collected with a dip net from Tenderfoot Lake. Any macroinvertebrates associated with this detritus were added to mesocosms along with the detritus, but the macroinvertebrates were not quantified. We measured temperature in each mesocosm using temperature loggers (Onset Hobo Data Loggers) that took measurements every 2 h for the duration of the experiment. Mean temperature during the experiment was 18.9°C in the coolest mesocosm and 20.7°C in the warmest mesocosm (Figure 5.1).
Figure 5.1 Temperature (°C) in (a) mesocosms containing uninfected crayfish and (b) mesocosms containing infected crayfish for selected dates (June 18th through July 5th). Mesocosms with infected and uninfected crayfish were separated for ease of visualization. Mesocosms containing fish are represented in blue and mesocosms without fish are represented in orange. The legend contains the mesocosm number and mean temperature (°C) over the experiment. On average, the warmest mesocosms were 2°C warmer than the coolest mesocosms, but at times, they were warmer by approximately 4°C.
The experiment was originally designed to be repeated later in the summer to increase replication. We initiated this second experiment, but were unsuccessful in creating the fish treatment. During the second experiment, we observed signs of disease and a number of fish mortalities. The fish in the first experiment gained weight, but almost all fish lost weight in the second experiment, suggesting that they were not behaving normally. Therefore, we discuss only the results of the first experiment in this manuscript. Results from the second experiment (fish and crayfish growth and mortality, and crayfish shelter use) can be found in Appendix B (Figures B.1 though B.3). Thus, the experiment we successfully completed has 3 replicates of each treatment combination, and therefore low statistical power. Given low power, we highlight in the Results some apparent trends where the $P$-value is between 0.05 and 0.10, in addition to emphasizing significant results ($P < 0.05$).

5.3.2 Crayfish

We collected crayfish for the experiment from northern Wisconsin and Michigan lakes. Previous to collection, we surveyed locations to identify sites where infection status was highly consistent. Crayfish were predictably uninfected within areas of Star Lake (46°1’N, 89°28’W; 19/20 crayfish uninfected) and Papoose Lake (46°11’N, 89°48’W; 20/20 crayfish uninfected). Crayfish were predictably infected with *Microphallus* within areas of Plum Lake (46°0’N, 89°29’W; 13/14 crayfish infected) and Lake Ottawa (46°54’N, 88°46’W; 14/15 crayfish infected). Mean infection intensity was 17.7 ± 3.8 (SE) in Plum Lake and 13.0 ± 6.1 in Lake Ottawa. Before the start of the experiment, crayfish were housed in aquaria containing constantly aerated lake water and
shelters constructed from polyvinyl chloride pipe. During this time, crayfish were fed shrimp pellets *ad libitum*.

Forty crayfish (22 males and 18 females) were placed in each mesocosm at the start of the experiment. Each mesocosm contained either uninfected crayfish (approximately 50% from Star Lake and 50% from Papoose Lake) or infected crayfish (approximately 50% from Lake Ottawa and 50% from Plum Lake). Crayfish ranged in size from 23 to 37 mm carapace length (CL) (mean = 28 mm ± 0.1 SE), with the same size distribution in each mesocosm. In order to identify individuals, we marked each crayfish by injecting two colors of visual implant elastomer (Northwest Marine Technologies, Inc.) into the muscle in the ventral abdomen prior to the experiment.

During the experiment, we recorded crayfish shelter use in each mesocosm. We made six daytime observations (between 9:30 and 15:00) and four night observations (between 21:30 and 23:00), and each observation was made on a different day. To record shelter use, an observer peered into each mesocosm and recorded the total number of crayfish that were exposed (not underneath cobble or detritus). At night, this was accomplished using a headlamp with a red, light-emitting diode bulb. To calculate the proportion of crayfish exposed, we estimated the total number of crayfish in the mesocosm at the time of each observation by assuming that crayfish mortality rate was constant over the duration of the experiment.

At the end of the experiment, we captured and froze all remaining crayfish. Crayfish were later thawed, identified via their elastomer tag, and measured to the nearest 0.25 mm CL to determine growth over the course of the experiment. To assess infection, we removed the hepatopancreas of each crayfish, flattened it between two glass slides,
and examined it under a dissecting microscope. We recorded the number of *Microphallus* metacercariae present in each individual.

5.3.3 Fish

Our goal was to create conditions where crayfish would respond behaviorally to fish without fish predation. Therefore, we selected predatory fish that were slightly too small to consume the crayfish, but would orient to crayfish and appear as a threat. We added two rock bass (*Ambloplites rupestris*) to half of the mesocosms at the beginning of the experiment. Rock bass are common predators of *O. rusticus* in northern Wisconsin and Michigan lakes (Peters 2010) and are present in all lakes where crayfish were collected.

We conducted a preliminary experiment in 38 L tanks to determine which sizes of crayfish and rock bass to use in the experiment. Rock bass were placed in a tank with a single crayfish for 24 hours. We found that rock bass under 18 cm total length did not eat crayfish over 22 mm CL, but often consumed smaller crayfish. Therefore, the largest fish we used in the mesocosm experiment were 17.0 cm. We added a smaller fish (12-14 cm) and a larger fish (15.5-17 cm) to each mesocosm. The smallest crayfish used in the experiment were 23 mm CL, so we expected them to be too large for the rock bass to consume. We did observe two instances of fish consuming crayfish during the experiment.

5.3.4 Macrophytes

We collected six common macrophyte species from Tenderfoot Lake. At the beginning of the experiment, in each mesocosm, we planted 11 shoots of *Potamogeton*
zosteriformis, 10 shoots of P. gramineus, 5 shoots of P. robbinsii, 5 shoots of P. praelongus, 20 shoots of Myriophyllum sibericum, and 7 shoots of Ceratophyllum demersum. After 2 wks, in each mesocosm, we also added 11 shoots of P. gramineus, 4 shoots of P. praelongus, and 11 shoots of M. sibericum. Before planting, we blotted shoots with paper towels and weighed them. After 2 wks, we periodically removed floating shoots to quantify crayfish destruction. At the end of the experiment, we removed remaining macrophytes, counted clipped and intact shoots, and obtained blotted wet weight. Finally, we placed shoots and fragments in separate paper bags, dried them for 48 hours at 60˚ C, and weighed them. Using the wet:dry weight ratio at the end of the experiment, we estimated the dry mass of plants stocked at the beginning of the experiment.

5.3.5 Macroinvertebrates

In addition to any macroinvertebrates added incidentally with the detritus described earlier, we also hand-collected macroinvertebrates from nearby lakes (Tenderfoot Lake, High Lake, and Forest Lake) added them to mesocosms at the start of the experiment and once each week. Throughout the experiment, we added a total of approximately 180 gastropods (primarily Viviparidae, Physidae, Hydrobiidae, and Lymnaeidae), 30 odonates (Aeshnidae and Corduliidae), 165 ephemeropterans (Heptageniidae, Caenidae, and Baetidae), 50 trichopterans (Limnephilidae), 6 isopods (Asellidae), and 30 amphipods (Dogielinotidae) to each mesocosm. After 2 wks, we carefully lifted 20 cobbles from each mesocosm and recorded any macroinvertebrates we observed. At the end of the experiment, we removed all cobbles and collected associated macroinvertebrates, and collected gastropods from the walls of each mesocosm and the
upper 5 cm of sand. In addition, we collected 300 g of detritus (wet weight) from each mesocosm using a dip net, divided the sample into white trays, and collected all macroinvertebrates we observed. Macroinvertebrates were preserved in 70% ethanol. Gastropods, insects, amphipods, isopods and bivalves were later identified to family. Leeches and oligochaetes were identified to class. To obtain dry mass, we measured macroinvertebrates to the nearest 0.1 mm, and used published size-mass equations (Benke et al. 1999, Edwards et al. 2009, Methot et al. 2012). We dried (60 °C for 48 h) and weighed taxa for which there was no published equation. For the most common macroinvertebrates (chironomids, amphipods, isopods, and heptageniid ephemeropterans) we obtained an average length by measuring 120 individuals (10 per mesocosm) instead of measuring each individual.

5.3.6 Statistical procedures

Because the fish treatment was applied at the mesocosm level, we first used ANOVA to examine mortality (% of crayfish that died) at the mesocosm level (to avoid a pseudoreplicated design) and included the effects of infection, fish, and their interaction in the model. We also included temperature as a covariate. We then examined the within-mesocosm effects of crayfish size and sex on mortality because the effects of predatory fish on crayfish behavior depend on crayfish size and sex (Stein and Magnuson 1976). Within fish and non-fish mesocosms, we used logistic regression to test the effects of sex and initial length on whether an individual survived or died over the course of the experiment. We also included mesocosm as a block in this model. We did not include infection in the within-mesocosm analysis because we found that it did not significantly affect mortality in the previous model. We also used ANOVA to assess crayfish growth.
at the mesocosm level and included the effects of infection, fish, and their interaction in the model. Again, we included temperature as a covariate. To examine individual growth rates within fish and non-fish mesocosms, we used ANOVA with the effects of infection, sex, and their interaction. We also included initial length as a covariate and mesocosm as a block. In addition, we used ANOVA to examine the effects of infection and fish on crayfish shelter use. In this model, we also included mesocosm as a block and temperature at the time of the observation as a covariate.

Finally, we examined the effects of crayfish on macrophytes and macroinvertebrates. We used ANOVA to test whether infection, fish, and their interaction affected the macrophyte biomass consumed by crayfish (initial dry mass - remaining dry mass + fragment dry mass), and average temperature was included as a covariate. We also conducted an identical analysis with macrophyte destruction (initial dry mass - remaining dry mass) as the dependent variable. We conducted the destruction and consumption analyses on total and per capita (biomass lost/average number of crayfish in the mesocosm) crayfish impacts. We also used PERMANOVA with Bray Curtis distances (Anderson 2001) to examine the effects of infection, fish, and their interaction on macrophyte community composition. In addition, we used ANOVA to test the effects of infection, fish, and their interaction on macroinvertebrate abundance after 2 wks and at the end of the experiment, as well as biomass at the end of the experiment. Again, we included average temperature as a covariate. We analyzed end biomass for gastropod and non-gastropod taxa separately because gastropods dominated the biomass. We also used PERMANOVA with Bray Curtis distances to examine the effects of infection, fish, and their interaction on macroinvertebrate community composition. All analyses were
conducted in R, version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria). We used the vegan package (Oksanen et al. 2010) for PERMANOVA.

5.4 Results

5.4.1 Infection

Dissection of the crayfish remaining at the end of the experiment indicated that our infection treatments were successful. Of the 301 surviving crayfish, only 5 were incorrectly assigned to an infection treatment. These crayfish were uninfected but were assigned to infected mesocosms. Therefore, there were most likely a few (0 - 4) uninfected crayfish in each infected crayfish mesocosm, but the large majority of crayfish (40 - 36) in these mesocosms were infected. In uninfected mesocosms, we expect that all crayfish were uninfected because we did not find any *Microphallus* present in the remaining crayfish. The mean infection intensity within crayfish at the end of the experiment was $32 \pm 4$ SE parasites.

5.4.2 Temperature

Although it was our goal to have temperature equal in all mesocosms, the variation in exposure to sunlight caused differences among mesocosms. Among mesocosms, mean temperature ranged from 18.9 to 20.7°C (Figure 5.1). Rather than assume that those temperature differences were biological insignificant, our inclusion of temperature as a covariate in our statistical models allowed us to discover that such small differences in temperature had significant biological effects as described in the sections below. However, because the infection and fish treatments were spread across the range
of temperatures (Figure 5.1), temperature did not confound our ability to detect
differences in impacts caused by infection and fish.

5.4.3 Mortality

Infection did not have a significant impact on crayfish mortality ($P > 0.7$). Fewer
crayfish survived in mesocosms with predatory fish present ($F_{1,11} = 25.82$, $P = 0.001$): a
mean of $76 \pm 3$ (SE) % of crayfish survived in mesocosms without fish, while $50 \pm 5$% of
crayfish survived in mesocosms containing predatory fish. Temperature ($P > 0.1$) and the
interaction between infection and fish ($P > 0.2$) did not have a significant effect on
mortality. Within mesocosms, there was no effect of sex or initial length on crayfish
mortality in either mesocosms with or without fish ($P > 0.1$).

5.4.4 Growth

Infected crayfish grew approximately 20% more than uninfected crayfish across
treatments ($F_{1,11} = 14.51$, $P < 0.007$; Figure 5.2). However, there was no effect of fish ($P
> 0.4$), nor an interaction between fish and infection ($P > 0.5$) on growth. Temperature
also did not significantly affect growth ($P > 0.4$). Within non-fish mesocosms, male
crayfish grew approximately 30% more than female crayfish ($F_{1,170} = 17.49$, $P < 0.001$).
In addition, within non-fish mesocosms, there was a significant negative relationship
between initial length and growth ($F_{1,170} = 9.09$ $P = 0.003$), but initial length controlled
only 4% of the variance in growth. Finally, in non-fish mesocosms, there was no
significant interaction between infection and growth ($P > 0.3$). In contrast, in fish
mesocosms, there was a significant interaction between infection and sex on growth ($F_{1,
109} = 6.96$, $P = 0.010$). We observed the greatest difference in growth between infected
and uninfected female crayfish when fish were present, with infected females growing approximately 50% more than uninfected females (Figure 5.2). In addition, in fish mesocosms, male crayfish grew approximately 20% more than female crayfish ($F_{1, 109} = 5.63, P = 0.020$). There was no significant effect of initial length on growth in mesocosms with fish ($P > 0.5$).

Figure 5.2 Increase in crayfish carapace length over the four week experiment ± SE for (A) females and (B) males.
5.4.5 Shelter Use

Approximately 20% more infected crayfish were found outside shelter than uninfected crayfish during daytime observations ($F_{1, 70} = 4.39, P = 0.041$; Figure 5.3). In addition, during the day, approximately 28% more crayfish were exposed in mesocosms without fish present ($F_{1, 70} = 7.59, P = 0.008$), and crayfish were more exposed in mesocosms with cooler temperatures ($F_{1, 70} = 6.99, P = 0.011, R^2 = 0.08$). Crayfish were also generally more exposed during night observations (72% of crayfish exposed) compared to day observations (32% of crayfish exposed), and infection did not affect crayfish exposure at night ($P > 0.1$; Figure 5.3). Crayfish in mesocosms without fish present were 10% more exposed at night than those in mesocosms containing fish ($F_{1, 47} = 5.21, P = 0.029$). We also observed a non-significant trend in which crayfish may have been more exposed at night in warmer tanks ($F_{1, 47} = 3.08, P = 0.088$).

5.4.6 Macrophytes

We observed a non-significant trend in which infected crayfish may have consumed more macrophytes than uninfected crayfish (overall: $F_{1, 11} = 4.43, P = 0.073$; per capita: $F_{1, 11} = 4.46, P = 0.073$), especially when fish were present (infection X fish overall: $F_{1, 11} = 4.56, P = 0.070$; per capita: $F_{1, 11} = 4.99, P = 0.061$). Infected crayfish reduced their consumption by approximately 7% when fish were present, but uninfected crayfish reduced their consumption by approximately 35% (Figure 5.4). In addition, there was a positive relationship between average temperature and macrophytes consumed (overall: $F_{1, 11} = 9.88, P = 0.016, R^2 = 0.31$; per capita: $F_{1, 11} = 9.63, P = 0.017, R^2 = 0.34$).
Figure 5.3 The percent ± SE of crayfish remaining in mesocosms that were exposed (active) during (A) daytime observations or (B) night observations.
Figure 5.4 The dry mass of macrophytes consumed ± SE during the experiment (A) within mesocosms or (B) per crayfish.
While infection may have affected macrophyte consumption, we did not observe an effect of infection on macrophyte destruction \((P > 0.1)\). However, fish presence reduced macrophyte destruction (overall: \(F_{1,11} = 5.63, P = 0.050\); per capita: \(F_{1,11} = 11.37, P = 0.012\)). Approximately 18% more destruction occurred in mesocosms without fish, and destruction was positively related to average temperature (overall: \(F_{1,11} = 5.68, P = 0.049\), \(R^2 = 0.31\); per capita: \(F_{1,11} = 20.62, P = 0.003\), \(R^2 = 0.37\)). Approximately 30% more macrophyte biomass was destroyed in the warmest mesocosms compared to the coolest mesocosms (Figure 5.5). We found no effect of infection, fish, or their interaction on the composition of the macrophyte community at the end of the experiment \((P > 0.3)\).

5.4.7 Macroinvertebrates

After two weeks, there were fewer macroinvertebrates observed in infected mesocosms than uninfected mesocosms \((F_{1,11} = 15.26, P = 0.006\); Figure 5.6). There was no overall effect of fish \((P > 0.9)\), but there was an interaction between infection and fish on macroinvertebrate abundance \((F_{1,11} = 12.89, P = 0.009)\). Macroinvertebrate abundances were similar between infected and uninfected mesocosms when fish were absent; however, when fish were present, macroinvertebrates were approximately 70% less abundant in infected mesocosms than uninfected mesocosms. There was no effect of temperature on macroinvertebrate abundance after two weeks \((P > 0.5)\).
Figure 5.5 The relationship between mean temperature in mesocosms (°C) and macrophyte destruction (g of macrophytes lost) over the course of the experiment. Temperature controlled approximately 30% of the variance in macrophyte destruction.
Figure 5.6 The number of macroinvertebrates observed ± SE (A) on 20 cobbles after 2 wks of the experiment and (B) within tanks at the end of the experiment.
In contrast, the end of the experiment, we did not find an effect of infection on macroinvertebrate abundance ($P > 0.3$), but approximately 59% more macroinvertebrates remained in mesocosms without fish ($F_{1, 11} = 17.74, P = 0.004$). There was also no interaction between fish and infection ($P > 0.3$) or effect of temperature ($P > 0.1$). For gastropod biomass, there was no effect of infection, fish, or their interaction ($P > 0.2$), but there was a significant negative relationship between gastropod biomass and temperature ($F_{1, 11} = 10.72, P = 0.014, R^2 = 0.42$; Figure 5.7). Approximately 19% more gastropod biomass was present in the coolest mesocosms than the warmest mesocosms. For non-gastropod macroinvertebrates, approximately 53% more biomass was present in mesocosms without fish ($F_{1, 11} = 7.66, P = 0.028$), but no other variable (infection, infection x fish, or temperature) had a significant effect ($P > 0.8$).

We also observed a non-significant trend in which macroinvertebrate community composition may have been affected by infection ($F_{1, 11} = 2.45, P = 0.065$; Figure 5.8). Mesocosms with infected crayfish tended to have fewer gastropods, but more ephemeroptera, odonates, amphipods, isopods, and chironomids. In addition, the macroinvertebrate community differed between mesocosms with and without fish present ($F_{1, 11} = 4.86, P = 0.006$), but there was no interaction between infection and fish on the macroinvertebrate community ($P > 0.1$). Mesocosms with fish tended to have fewer amphipods, isopods and chironomids.
Figure 5.7 The relationship between mean temperature in mesocosms (°C) and gastropod biomass remaining at the end of the experiment. Temperature controlled approximately 40% of the variance in gastropod biomass.
Figure 5.8 Visualization of macroinvertebrate community composition using nonmetric multidimensional scaling (NMDS) ordination. Circles indicate 95% confidence intervals around the macroinvertebrate community for mesocosms with infected or uninfected crayfish.
5.5 Discussion

In contrast to our initial prediction, when fish were absent, we found similar effects of infected and uninfected crayfish on lower trophic levels. However, when fish were present, we found greater impacts of infected crayfish on lower trophic levels as we expected. These data suggest that parasites can have strong effects on per capita impacts of crayfish. Below we elaborate on the details behind these conclusions.

5.5.1 Infection

The infection intensity of the crayfish included in this study (32 ± 4 SE parasites) is common in north temperate lakes and relatively low compared to many sites surveyed previously. For example, a survey of 16 northern Wisconsin and Michigan lakes indicated that the mean parasite abundance in crayfish (including both infected and uninfected individuals) was 80 (Chapter 3). In the same survey, parasite prevalence was 63% (Chapter 3). Therefore, we expect that effects of parasites at least as strong as those that we observed are common in this region.

5.5.2 Temperature

Our results also revealed an important effect of temperature on crayfish impacts. Mean temperature in the coolest mesocosm was 18.9°C, and mean temperature in the warmest mesocosm was 20.7°C. Temperature had a significant effect on macrophyte consumption and destruction as well as gastropod biomass. For example, crayfish in the warmest tanks destroyed approximately 30% more macrophyte biomass and 16% more gastropod biomass than crayfish in the coolest tanks. Temperature did not affect crayfish growth; thus, the increase in consumption may have allowed crayfish to cope with greater
metabolic costs when temperatures were warmer. Therefore, *O. rusticus* effects in lakes may differ substantially between years with different mean temperatures and will likely increase with climate change.

5.5.3 Mortality

We found no effect of infection on crayfish mortality in our experiment; however, increased boldness would most likely lead to increased crayfish mortality in lakes due to predation. Crayfish that are more exposed are more vulnerable to predation by fish (Stein and Magnuson 1976, Stein 1977, Garvey et al. 1994), and in our experiment, the shelter use data indicate that a greater proportion of infected crayfish were exposed across fish treatments. The increased mortality we observed in the fish treatments was not solely due to predation from fish. Previous similar experiments have found an increase in crayfish mortality associated with the presence of predatory fish that were too small to consume the crayfish present (Hill and Lodge 1995), and we expect that most crayfish in our experiment were outside the gape limit of the rock bass. Further, there was no effect of initial length on mortality in mesocosms containing fish, and if fish were consuming many individuals, we would expect small crayfish to have the highest mortality. In addition to predation, the increase in mortality associated with fish may be due to increased competition for food resources (fish also consumed macroinvertebrates) or more intense agonistic encounters between crayfish for limited shelter. Crayfish may also consume conspecifics, especially those that are molting (Taugbol and Skurdal 1992).
5.5.4 Growth and feeding

The greater growth observed in infected individuals indicates that infected crayfish were likely consuming more food than uninfected crayfish. When infected and uninfected *O. rusticus* are provided with an equal amount of food, infected crayfish grow less (Chapter 3), potentially due to damage to the hepatopancreas caused by metacercariae. Therefore, the greater growth in infected individuals was most likely due to increased feeding. It is also possible that the greater growth we observed in infected crayfish could be due to those individuals allocating more resources to somatic growth and less to reproduction. The macrophyte and macroinvertebrate data, however, offer further support that infected crayfish were consuming more than uninfected crayfish when fish were present. Greater macrophyte biomass was missing in mesocosms with fish present and infected crayfish, and after two weeks, the fewest macroinvertebrates were present in these mesocosms. The greater percent of crayfish exposed in mesocosms containing infected crayfish also suggests that infected crayfish spent more time foraging than uninfected crayfish.

The significant interaction between sex and infection on growth in mesocosms containing fish may be due to the differential response of males and females to predators. Females are more vulnerable to fish predation than males because of their smaller chelae, and thus, females modify their behavior to a greater extent than males when fish are present (Stein and Magnuson 1976). This may be why we observed a greater difference in female growth between infected and uninfected individuals when fish were present, and less of a difference in male growth. Females, therefore, may be more important drivers of the community impacts of *Microphallus* than males.
5.5.5 Community effects

In contrast to our initial hypothesis, when fish were absent, we did not find evidence that infected crayfish had lesser impacts on lower trophic levels than uninfected crayfish. Therefore, although it is unclear why, the effects of infection on feeding observed in previous laboratory experiments was not observed over longer time scales and in more natural conditions. However, in support of our second hypothesis, when fish were present, we did find that infected crayfish had greater impacts than uninfected crayfish, probably due to increased boldness. Macrophyte destruction and macrophyte community composition was similar between infected and uninfected mesocosms, but we observed a trend in which infected crayfish consumed more macrophyte biomass than uninfected crayfish when fish were present. Therefore, if the crayfish in a lake are infected, our results suggest that more macrophyte biomass may be utilized by crayfish and less may contribute to the detritus. In addition, some macrophyte species propagate vegetatively, and clipped shoots may colonize new locations if they are not consumed. Thus, our results indicate that there may be less colonization from clipped shoots in lakes where crayfish are infected.

We also observed fewer macroinvertebrates after two weeks of the experiment in mesocosms with infected crayfish and fish present, probably because both crayfish and fish were actively consuming macroinvertebrates in these mesocosms. In mesocosms containing fish and uninfected crayfish, more macroinvertebrates were present, probably because of reduced consumption from crayfish that were altering their behavior in response to predators. We did not observe this same effect at the end of the experiment, and instead found either no difference between treatments (gastropods) or a reduction in
abundance and biomass associated with fish but not infection (non-gastropods). These results may be due to acclimation of crayfish to the fish treatment by the end of the experiment. Few crayfish were consumed by fish, so crayfish may have reduced their behavioral response to fish after a few weeks, and unlike macrophytes, new macroinvertebrates were added weekly. In addition, as the experiment progressed, we observed an increase in fish consuming macroinvertebrates as we added them to the mesocosms. Finally, there was a trend suggesting that macroinvertebrate community composition may have differed between mesocosms with infected and uninfected crayfish. This result indicates that slowly moving species such as gastropods may be especially vulnerable to predation by infected crayfish, while faster moving species such as ephemeropterans, odonates, amphipods, isopods, and chironomids may be more vulnerable to predation by uninfected crayfish.

Per capita impacts of crayfish in north temperate lakes are likely to be most similar to those in the fish treatment of our experiment. In natural lakes, predatory fish would be less dense than in the experiment, but crayfish consumption by fish is likely to be more frequent because larger fish and smaller crayfish are present. Therefore, crayfish are less likely to acclimate to fish in lakes than in our experiment. The greater impacts of infected crayfish on gastropods may reduce future parasite abundance in lakes because the first secondary host of Microphallus is a hydrobiid snail.

In north temperate lakes, Microphallus prevalence is associated with reduced O. rusticus population growth (Chapter 3). Therefore, while the behavioral effects of Microphallus increase the per capita impacts of O. rusticus on lower trophic levels the overall impacts may also be affected by declines in O. rusticus density associated with
infection. Whether behavior or density effects of *Microphallus* are more important for freshwater communities remains to be determined; however, previous research suggests that the relative importance of these effects may be dependent on crayfish population size. Where crayfish populations are initially low, *Microphallus* prevalence has a strong relationship with crayfish population growth (Chapter 3). Therefore, if the observed decline in population growth is due *Microphallus* infection, the effects of parasites on crayfish density are likely to be more important for lake communities than their trait-mediated effects. However, when crayfish are initially abundant, there is not a strong relationship between parasite prevalence and crayfish population growth (Chapter 3), potentially because the population is near carrying capacity and density dependent effects compensate for increased predation where parasites are prevalent. In these conditions, the trait-mediated effects of *Microphallus* are likely to be more important for the community than density effects.

5.5.6 Conclusions

Our results suggest that trematode parasites can affect north temperate lake communities merely by modifying host behavior. The behavioral effects of parasites that we observed were on par with the previously appreciated behavioral effects of predators. For example, infection increased crayfish growth by 20%, but removing fish only increased uninfected crayfish growth by 9%. In addition, infection increased crayfish exposure by 20%, and removing fish increased crayfish exposure by 28%. Further, the behavioral effects of parasites negated the behavioral effects of fish on crayfish macrophyte consumption. Trematode parasites are common in many freshwater and coastal marine ecosystems (Hechinger et al. 2008, Kuris et al. 2008), so understanding
their effects on host behavior may be broadly important for understanding parasite and host impacts on communities.

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5.7 References


CHAPTER 6:

ASSESSING HUMAN BEHAVIOR TO ANTICIPATE CONSEQUENCES OF
POLICY CHANGE: HOW BANNING CRAYFISH FROM THE BAIT TRADE
WOULD IMPACT THE MISSOURI FISHING INDUSTRY⁵

6.1 Abstract

Changes in natural resources policy may have unintended consequences because
of unanticipated behavioral responses of resource users. Therefore, combining ecological
and social science research may improve predictions about the outcome of policy
changes. We applied this perspective to the live bait trade, which is a major introduction
pathway for nonindigenous species, especially crayfish. Nonindigenous crayfish have
causéd major impacts in some aquatic ecosystems including reduced growth,
reproduction, and/or survival of fish. In some locations, these impacts on fishes have
reduced the economic value generated by the fishing industry. If reduced bait availability
causes anglers to fish less often, then policies or practices that limit the bait trade could
also reduce the revenue generated by the fishing industry. To investigate whether
banning crayfish from the bait trade would affect fishing industry revenue, we conducted
a mail survey of anglers in Missouri (where policymakers have recently considered
prohibiting the sale of crayfish as bait). Survey results revealed that if crayfish were

⁵ In prep for submission to Fisheries with coauthor D. M. Lodge.
unavailable from bait shops, most anglers would be likely to purchase another type of bait from a bait shop or to collect crayfish directly from the environment. If directly collecting crayfish increases in response to banning crayfish from the bait trade, this behavior could be an additional source of crayfish introductions, which could be an unintended consequence of this policy change. On the other hand, anglers reported that banning crayfish from the bait trade would not affect the frequency with which they go fishing. Therefore, banning crayfish from the bait trade is unlikely to reduce angler spending on equipment, travel, or trip-related expenses, but instead could increase the revenue generated by the fishing industry by protecting game fish populations from future crayfish introductions.

6.2 Introduction

Changes in policy or practices are often necessary to improve resource management and increase social welfare, but altering policy can also create perverse outcomes when incentives for resource users cause unanticipated changes in human behavior (Bunnefeld et al. 2011, Fulton et al. 2011). There are numerous examples from fisheries management of unintended consequences of policy changes (Fulton et al. 2011). For example, spatial closures intended to reduce catch can lead to increased effort in other locations or impacts on unprotected species (Dinmore et al. 2003). In addition, in mixed fisheries, total allowable catch quotas have not been successful at limiting catch because fishers discard overquota catch to continue fishing for other species (Kraak et al. 2013). Combining social science research with ecological research may help managers better predict how policy changes will alter human behavior and, therefore, avoid
unintended consequences. We used this approach to evaluate how banning crayfish from the live bait trade to reduce introductions of nonindigenous crayfishes would affect human behavior and the fishing industry in Missouri.

Nonindigenous species can substantially alter communities and ecosystems, sometimes causing ecological and economic harm (Keller et al. 2009, Strayer 2012, Paolucci et al. 2013, Simberloff et al. 2013). In the United States (U. S.), the impacts and control of invasive species costs at least $120 billion per year (Pimentel et al. 2005). Freshwater ecosystems may be especially vulnerable to invasive species (Sala et al. 2000), and nonindigenous fishes and mollusks cost the U. S. over 7 billion dollars annually (Pimentel et al. 2005). Many invasive species are difficult or impossible to eradicate after they become established. Therefore, preventing introductions is the most effective strategy for avoiding the costs associated with invasions (Kolar and Lodge 2001, Finnoff et al. 2007). Freshwater nonindigenous species may be introduced unintentionally, for example, through ballast water discharge (Ruiz et al. 1997, Bobeldyk et al. 2015). Here we consider commerce in live bait species, which is also an important introduction pathway for many nonindigenous organisms (Keller and Lodge 2007).

Moving crayfish to new drainages within North America has had major ecological and economic consequences. Crayfish often have large impacts on ecosystems and communities because of their large size relative to other aquatic invertebrates, extremely omnivorous feeding behavior, and ability to reach high biomass (Hobbs and Lodge 2009). Invasive crayfish can reduce the abundance of some game fish through destruction of macrophyte habitat, competition for food resources, or direct consumption of substrate nesting fish eggs (Dorn and Mittelbach 1999, Dorn and Wojdak 2004,
Wilson et al. 2004, Baldridge and Lodge 2013). In addition, invasive crayfish can reduce growth or abundance of benthic fish through competition for shelter (Guan and Wiles 1997, Light 2005).

Invasive crayfish also often alter aquatic communities by causing declines in the abundance and richness of macrophytes and macroinvertebrates (Lodge et al. 1994, Nystrom et al. 1999, Wilson et al. 2004) as well as amphibians (Gamradt et al. 1997). In addition, transferring crayfish between basins is a major threat to crayfish biodiversity because the introduction of nonindigenous crayfishes has caused the extirpation or extinction of native crayfishes (Lodge et al. 2000a). Many nonindigenous crustaceans that have been introduced to new locations within the U. S. are native to other locations within this region (Perry et al. 2002). Therefore, if preventing the spread of these nonindigenous species and their impacts is a management goal, then new practices or policies may be needed at the state level.

Although introductions of invasive crayfish from the bait trade threaten communities and ecosystems, practices and policies aimed at reducing these introductions vary substantially between U. S. states. Some states, such as Wisconsin, have banned the use of all crayfish species as live bait to prevent new introductions (Peters and Lodge 2009). In addition, many other states (Arizona, Idaho, Minnesota, Utah, Virginia, Washington, and Wyoming) have prohibited the sale of live crayfish as bait either directly or by prohibiting live transport of crayfish (DiStefano et al. 2009). Minnesota and New York prohibit the use of crayfish in some waters (DiStefano et al. 2009). Finally, some states (Arizona, Idaho, Minnesota, Ohio, Utah, Washington, and Wyoming) allow the use of live crayfish as bait only in the body of water in which they
were collected (DiStefano et al. 2009, Peters and Lodge 2009). In addition, some states prohibit the use (Illinois and Pennsylvania) or sale (Michigan, Illinois, Ohio, and Pennsylvania) of specific, harmful crayfish species such as rusty crayfish, *Orconectes rusticus* (Peters and Lodge 2009). In addition to policy approaches, some states have implemented educational outreach to the bait industry to discourage crayfish introductions (DiStefano et al. 2009).

Because anglers often release unused bait, the bait trade is a major introduction pathway for nonindigenous species such as fishes, earthworms, and crayfishes (Litvak and Mandrak 1993, Keller et al. 2007, DiStefano et al. 2009, Kilian et al. 2012). For example, 69% of anglers fishing with live crayfish in Maryland reported releasing unused crayfish into aquatic ecosystems (Kilian et al. 2012). Further, 40% of Missouri anglers reported releasing live bait (Banek and Colatskie 2011). In addition, half of fishing agencies surveyed in the U. S. and Canada reported a freshwater crayfish problem in their state, province, or territory in which the suspected cause was a bait-bucket introduction (DiStefano et al. 2009).

Many states have not addressed crayfish in the bait industry until after substantial damages to ecosystems have already occurred. In Missouri, banning live crayfish from the bait trade was recently proposed as a measure to prevent potential harm from future crayfish introductions. This measure was passed in August 2011 as a regulation amendment to the Wildlife Code of Missouri (Missouri Department of Conservation 2012), but was rescinded in 2012. The current regulation allows bait shops to sell only one species, the virile crayfish (*Orconectes virilis*; Missouri Department of Conservation 2014). In addition, anglers possessing a valid Missouri fishing license may harvest up to
150 crayfish (of any species) per day for use as bait. However, these crayfishes may not be released into waters from which they did not originate (DiStefano et al. 2009).

*O. virilis* is native to portions of Missouri (Pflieger 1996), but it has been introduced and become established in at least one new Missouri drainage (DiStefano personal communication), and many additional watersheds within the state are vulnerable to introduction. Its current distribution may have already been enlarged due to releases from anglers as this species has previously been common in the bait industry (DiStefano et al. 2009). While *O. virilis* is native to many Missouri drainages, data suggest that this species has the potential to cause economic harm in other watersheds, particularly to the fishing industry. *O. virilis* is invasive in the western U. S. where it has caused reduced growth in rainbow trout (Hepworth and Duffield 1987) and small fishes (Carpenter 2005). *O. virilis* also readily consumes benthic nesting fish eggs (Morse et al. 2013). Therefore, if *O. virilis* is introduced to new waters, game fish populations within these waters might decline. Further, evolution of crayfish within the introduced range or hybridization with native crayfishes can alter the traits of crayfish and may enhance the impacts of introduced crayfish populations (Arcella et al. 2014, Chapter 2). Thus, *O. virilis* may have unpredictable impacts as it is introduced to new waters.

Policy surrounding crayfish in the bait trade has the potential to alter angler behavior, and angler behavior is likely to be important for the outcome of such a policy change. If crayfish in the bait trade continue to be introduced to new waters, introductions of *O. virilis* could impact the fishing industry by reducing game fish populations. Reductions in fish abundances due to the introduction of nonindigenous crayfishes may alter angler behavior by reducing fishing frequency. Some anglers are
motivated primarily by non-catch experiences (e.g. spending time outdoors) and others are motivated primarily by catch (Fedler and Ditton 1986). However, even among anglers that are motivated primarily by non-catch experiences, angler satisfaction (the difference between the outcomes an angler desires and the perceived fulfillment of them) is primarily catch-dependent (Arlinghaus 2006, McCormick and Porter 2014), and catch may influence where and how often anglers fish (Fenichel et al. 2013). Anglers from other states that fished in Missouri in 2011 spent a total of $199,040,000 on equipment and fishing trip related expenditures (U.S. Department of the Interior et al. 2013). If crayfish introductions reduce fish abundance, then the fishing industry in Missouri is likely to suffer. If catch declines, anglers may choose to fish less often or to fish in other states.

Alternatively, banning crayfish from the bait trade could also affect the fishing industry if this policy causes anglers to fish less often. However, little is known about how Missouri anglers use crayfish as bait and how their behavior would change if crayfish were not available from bait shops. We conducted a survey of Missouri anglers to inform this topic. Specifically, we investigated how anglers use crayfish as bait to determine where crayfish introductions from the bait trade are likely and how fishing with crayfish contributes to the revenue generated by the fishing industry. In addition, we examined how angler behavior would change if crayfish were unavailable from bait shops to determine to what extent the revenue generated by the fishing industry would be affected and whether other behaviors (such as direct collection of crayfish) would be an additional source of crayfish introductions.
6.3 Methods

To examine the behavior of Missouri anglers, we conducted a mail survey in July, 2014. We obtained the names and addresses of all Missouri residents who purchased fishing licenses in 2013 from the Missouri Department of Conservation’s permit buyer database. We removed all individuals less than 18 years of age (a requirement of our human subjects protocol) and randomly selected 1,004 adult Missouri anglers to receive surveys. Ninety-two of these surveys were returned as undeliverable. Therefore, 912 surveys were delivered to Missouri fishing license holders.

The questions in the survey were aimed at determining how Missouri anglers currently use crayfish as bait and how banning crayfish from the bait trade would influence angler behavior and the fishing industry (Appendix A). To determine to what extent fishing with crayfish contributes to the fishing industry, we used $t$-tests to test for differences in the behavior of anglers that reported fishing with crayfish in the past year and anglers that did not. Specifically, we tested for differences in total days that anglers fished (trips * typical trip length in days), total amount spent on food and lodging per year on fishing trips, and typical travel time to fishing locations. In addition, for anglers that reported using crayfish, we used paired $t$-tests to compare behavior between fishing trips in which crayfish were used as bait and trips in which crayfish were not used.

While our survey asked respondents to report the number of days that they typically spent on each fishing trip, we believe that some anglers reported the total number of days that they spent fishing over the year because some responses were impossible (i.e. added up to more days than are present in a year). Therefore, if the number of days reported multiplied by the number of trips was greater than half of the
year (183 days), we assumed that the respondent was most likely reporting the total days, and we divided the days reported by the number of trips to get the average length of each trip. Therefore, the number of days spent fishing that we report in this study may be overestimated because of other respondents making this error. However, because we used the same criteria to adjust the responses of all anglers, this should not affect our ability to detect differences between anglers that use crayfish as bait and those that do not.

6.4 Results

6.4.1 Sample

Our sample included 188 surveys that were returned, for a response rate of approximately 21%. Surveys in which people reported that they had not been fishing in the past 12 months \( N = 4 \) were removed from the sample. We also did not include surveys that were returned without a signed informed consent page \( N = 6 \). Therefore, we used data from the responses of 178 anglers. Twenty percent of anglers \( N = 36 \) indicated that they had fished with crayfish as bait within the past year. The surveys in our sample were from counties distributed throughout Missouri (Figure 6.1), and were most common from counties near cities (Saint Louis, Kansas City, and Springfield). Surveys in which people reported fishing with crayfish were also from counties distributed throughout the state (Figure 6.1).
6.4.2 Bait preferences

To evaluate whether anglers would fish with bait other than crayfish, we assessed their bait preferences. Considering all respondents, crayfish ranked as the bait used fourth most often (Table 6.1). Artificial bait, live worms, live minnows, and insects were ranked higher on average than crayfish (Table 6.1). Anglers who fished with crayfish also commonly used other baits. Anglers who fished with crayfish ranked worms as the bait they used most often (mean rank = 1.9 ± 0.1 (SE)), followed by artificial bait (2.3 ± 0.2) and minnows (2.5 ± 0.2). Anglers who fished with crayfish typically ranked crayfish as the bait they used third most often (mean rank = 3.3 ± 0.2; range = 1 - 6).
6.4.3 Where crayfish are used

To determine which habitat types are vulnerable to crayfish introductions, we examined which habitats were most often fished with crayfish. Seventy-eight percent of anglers that used crayfish as bait reported typically using crayfish in a river or stream, 50% reported typically using crayfish to fish in a lake or reservoir, and 30% reported typically using crayfish to fish in a pond. Most anglers that fished with crayfish were fishing for bass (36.1%), catfish (22.2%), or both (38.9%). Twenty five percent of anglers also reported using crayfish to fish for sunfish, but these individuals also reported fishing for bass or bass and catfish. Seven anglers also reported using crayfish to fish for crappie (11.1%), walleye (5.6%), or drum (2.8%), but again these individuals also reported fishing for bass and/or catfish.

### TABLE 6.1
BAIT TYPES RANKED ACCORDING TO THE FREQUENCY WITH WHICH MISSOURI ANGLERS USED THEM

<table>
<thead>
<tr>
<th>Bait Type</th>
<th>Mean Rank</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial</td>
<td>1.76</td>
<td>0.08</td>
</tr>
<tr>
<td>Live Worms</td>
<td>1.88</td>
<td>0.07</td>
</tr>
<tr>
<td>Live Minnows</td>
<td>2.28</td>
<td>0.09</td>
</tr>
<tr>
<td>Insects</td>
<td>3.48</td>
<td>0.15</td>
</tr>
<tr>
<td>Crayfish</td>
<td>4.13</td>
<td>0.17</td>
</tr>
<tr>
<td>Fish Eggs</td>
<td>5.10</td>
<td>0.19</td>
</tr>
</tbody>
</table>
6.4.4 Trips and days

To assess whether fishing with crayfish contributes a similar amount to the fishing industry as other bait types, we compared trips between anglers that used crayfish and those that did not. Anglers that reported fishing with crayfish in the past year (hereinafter crayfish anglers) fished for approximately 60% more days that those that did not fish with crayfish (hereinafter non-crayfish anglers; $T = 2.56$, $P = 0.013$, $df = 63.06$; Figure 6.2a). Crayfish anglers went on a mean of 24.0 ± 3.6 (SE) fishing trips, and each trip lasted a mean of 2.5 ± 0.5 days. Non-crayfish anglers went on a mean of 17.0 ± 3.3 fishing trips, and each trip lasted a mean of 2.1 ± 0.2 days.

Even though crayfish anglers went on more trips, they often used other bait types. Crayfish anglers only used crayfish during approximately 40% of fishing trips, and they went on trips in which they used other bait more often than they went on trips in which they used crayfish ($T = 2.92$, $P = 0.006$, $df = 34$; Figure 6.2b). The mean number of trips these anglers took to fish with crayfish was 7.5 ± 1.4, and these trips lasted a mean of 2.2 ± 0.3 days. Overall, trips in which anglers used crayfish as bait made up 8.2% of total fishing trips, and crayfish were used during 10.7% of total days anglers in our sample spent fishing.
To assess whether fishing with crayfish contributes a similar amount to the fishing industry as other bait types, we compared spending on trips between anglers that used crayfish and those that did not. Crayfish anglers and non-crayfish anglers spent a similar amount on food and lodging per trip ($T = -0.43, P = 0.667, df = 63.29$; Figure 6.3). Per angler, over the year, crayfish anglers spent $265 \pm 97$ (SE) on lodging and $280 \pm 56$ on food while on fishing trips. Per angler, over the year, non-crayfish anglers spent $148 \pm 30$ on lodging and $165 \pm 23$ on food.

In addition, no significant difference existed in the amount that crayfish anglers spent (per trip) on trips in which they used crayfish and trips in which they used other bait ($T = -1.69, P = 0.101, df = 31$; Figure 6.3). Per angler, when using crayfish, they...
spent $116 \pm 42$ on lodging and $126 \pm 24$ on food during the year. Per angler, they also spent $142 \pm 64$ on lodging and $147 \pm 3$ on food during the year while fishing with non-crayfish bait.

We also wanted to know which equipment anglers used to fish with crayfish in order to assess whether they were contributing to equipment purchases. Anglers used a variety of equipment to fish with crayfish. Ninety-one percent reported using a rod and reel, and 37% reported using a trot line (or jug line, bank line, throw line or limb line). Only anglers fishing for catfish reported using a trot line. In addition, 37% of anglers that used crayfish as bait typically fished using a motorized boat, and 26% used a non-motorized boat such as a canoe or kayak. Overall, crayfish were used in different types of fishing, and anglers that fish with crayfish contributed to equipment purchases.

![Figure 6.3 Dollars that anglers spent per fishing trip ± SE (a) for anglers that used crayfish and anglers that did not and (b) within anglers that used crayfish for trips in which they used crayfish and trips in which they used other baits.](image)
6.4.6 Travel

As an index of travel costs, we asked how far anglers traveled to go fishing. Crayfish anglers and non-crayfish anglers traveled for a similar amount of time to go fishing ($T = 0.86, P = 0.396, df = 46.88$; Figure 6.4a). In addition, crayfish anglers traveled for a similar amount of time on trips in which they used crayfish as bait and on trips in which they used other baits ($T = 0.90, P = 0.374, df = 33$; Figure 6.4b).

![Figure 6.4 Minutes that anglers traveled per fishing trip ± SE (a) for anglers that used crayfish and anglers that did not and (b) within anglers that used crayfish for trips in which they used crayfish and trips in which they used other baits](image-url)
6.4.7 Angler behavior

To determine how banning crayfish from the bait trade in Missouri would affect the fishing industry, we investigated how this policy change would affect angler behavior. All respondents that fished with crayfish reported that they would take the same number of fishing trips if crayfish were unavailable from bait shops. If crayfish were unavailable from bait shops, many crayfish anglers (approximately 60%) would be likely to purchase a different live bait or artificial bait from a bait shop instead of crayfish (Figure 6.5). In addition, approximately 85% of anglers would be likely to collect crayfish directly from the location where they fish, and approximately 50% of anglers would be likely to collect crayfish from one water body and bring them to a different location to fish. Anglers wrote that the need to switch baits would be very unlikely to cause them to fish less. Therefore, our data indicate that anglers would switch bait types and would fish with the same frequency if crayfish became unavailable from bait shops.
Figure 6.5 Likelihood of changed behavior reported by anglers that use live crayfish as bait if crayfish were no longer available from Missouri bait shops. Respondents reported the likelihood of that they would do each of the following behaviors: (a) purchase another type of live bait from a bait shop, (b) purchase an artificial bait form a bait shop, (c) collect crayfish directly from the water body in which they will fish, (d) collect crayfish directly from a water body other than where they will fish, and (e) not switch bait types and thus would fish less often. Each selection was made independently (i.e. anglers could report that they were likely to do multiple things). The numbers within each section of the chart represent the number of responses for each category.
6.5 Discussion

Anglers indicated they would continue to fish with the same frequency regardless of whether crayfish are available from bait shops, so it is unlikely that banning crayfish from the bait trade would reduce fishing industry revenue generated from equipment, travel, or trip-related expenses. On the other hand, our results suggest that many anglers would directly collect crayfish to use as bait and may introduce crayfish to new drainages if these crayfish are released. Therefore, this policy change may have unintended consequences by causing crayfish introductions from non-bait trade sources.

Crayfish were used frequently as bait (on approximately 8% of trips in Missouri), but other bait types including artificial bait, worms, minnows, and insects were used more frequently. These data suggest that other baits are more important to Missouri anglers, and we expect that anglers will use these other bait types more frequently if crayfish are unavailable. The responses from the angler behavior section of our survey offer additional support for this expectation. Approximately 60% of anglers wrote that they would be very likely or likely to purchase another type of live or artificial bait from a bait shop if crayfish were unavailable. Therefore, the loss of crayfish sales in bait shops would be mitigated to some extent by anglers purchasing other baits. We had no question in our survey to determine whether these anglers already typically collect their bait and, thus, do not contribute to current bait shop sales. However, four respondents wrote on the survey that they always collect crayfish and do not purchase them. Thus, the 40% of respondents who said they were neutral, unlikely, or very unlikely to purchase another type of bait from a shop if crayfish were unavailable includes some individuals that already do not purchase their crayfish from bait shops. Finally, it might be possible to
reduce some loss of revenue to bait shops while still reducing introductions by banning only sales of live crayfish and allowing the sale of preserved crayfish (Litvak and Mandrak 1993).

Our data, combined with previous research that indicates that anglers release unwanted bait into the water (Banek and Colatskie 2011), indicate that crayfish introductions from the bait trade are likely to occur across the state and across all types of water bodies. Anglers reported fishing with crayfish across all regions of Missouri. Anglers traveled approximately 1.5 h on average to go fishing. There are a number of crayfish species in Missouri that have very small distributions, less than 100 km² (Pflieger 1996), so if crayfish are introduced to new waters after anglers travel for 1.5 h, they could be introduced to a new drainage. In addition, anglers reported most commonly fishing with crayfish in rivers and streams, but also often fishing with crayfish in reservoirs and ponds. Therefore, all of these habitat types are vulnerable to crayfish introductions from the bait trade. Invasive crayfish may impact fish abundance in any of these waters, but they may be especially likely to reduce fish abundance in reservoirs and ponds where the abundance of fish is likely to be positively related to macrophyte abundance (Dibble et al. 1996). Crayfish often have major impacts on macrophytes due to both direct consumption and destruction (Nystrom and Strand 1996, Wilson et al. 2004). In addition, anglers most commonly reported fishing for catfish (likely channel catfish (Ictalurus punctatus) blue catfish (I. furcatus) or flathead catfish (Pylodictis olivaris)), bass (likely smallmouth bass (Micropterus dolomieu), largemouth bass (M. salmoides), spotted bass (M. punctulatus), rock bass (Ambloplites rupestris) or white bass (Morone chrysops)), and panfish (Lepomis spp.). All of these fishes lay their eggs in the
substrate where they may be consumed by crayfish such as *O. virilis* (Morse et al. 2013). Further, previous research indicates that introduced crayfish can increase rates of nest abandonment by nest-guarding fish (Baldrige and Lodge 2013). These fish taxa are well-distributed throughout Missouri waters (Pflieger 1975), again indicating the vulnerability of many aquatic ecosystems to crayfish introduced from the bait trade.

Anglers that reported fishing with crayfish within the past year fished more often than anglers that did not fish with crayfish. This result may be due to crayfish being less-preferred than other baits. Therefore, only anglers who fished often used this less common bait type, perhaps to increase variety in their fishing.

Our data on spending and travel suggest that anglers using crayfish as bait are contributing a similar amount per trip to the fishing industry as anglers using other bait types. Although crayfish anglers took more trips than non-crayfish anglers, crayfish anglers were most often fishing only with non-crayfish bait. Crayfish anglers also commonly reported fishing from motorized and non-motorized boats, suggesting that they are spending a substantial amount on equipment for fishing. Overall, these data indicate that fishing with crayfish contributes a substantial amount to the revenue generated by the fishing industry.

Anglers spent $657,000,000 on fishing in Missouri in 2011 (U.S. Department of the Interior et al. 2013). The anglers in our survey used crayfish on 8.2% of trips or 10.7% of fishing days, so based on the 2011 data, we estimate that fishing with crayfish contributes to between $53,874,000 and $70,299,000 of annual fishing industry revenue in Missouri. However, this is likely an overestimate because anglers that fished with
crayfish typically ranked crayfish as the bait they used third most often, so they were most likely fishing with other baits on those trips as well.

To cause economic impacts to the fishing industry that are equal to their benefits, crayfish introduced from the bait trade would have to reduce fish populations in 8.2% to 10.7% (the overall percent of trips and days that anglers reported fishing with crayfish) of water bodies to the extent that people no longer fish in these locations. Data from nearby states suggest that invasive crayfish impacts of this magnitude are possible. In one Wisconsin county (Vilas County), crayfish cause at least an annual loss of $1,505,205 to the fishing industry due to the negative impacts of crayfish on panfish populations (Keller et al. 2008). In addition, crayfish invasions can spread rapidly once they are established. In Wisconsin, rusty crayfish were detected in 7% of locations surveyed between 1965 and 1984 (Olden et al. 2006). In later surveys (1984-2004), rusty crayfish were detected in 36% of locations, indicating that they spread rapidly within this time period (Olden et al. 2006). In addition, one Missouri crayfish invasion spread approximately 650 km, from a single stream to eleven streams, within 24 years (DiStefano and Westhoff 2011). Thus, even if there are not current adverse effects of invasive crayfish on Missouri’s fisheries, the effects of an introduction could occur rapidly in the future. While invasive crayfish costs to the fishing industry in Missouri could be equal in magnitude to their benefits, our data indicate that because anglers will change their behavior, the revenue generated by the fishing industry is unlikely to be negatively affected by banning crayfish from the bait trade.

Responses throughout the survey indicated that anglers would continue to fish as often as before if crayfish were not available from bait shops. First, the bait preference
data indicated that even among anglers that fished with crayfish, crayfish were not the most important bait. Thus, it is likely that anglers would still fish if crayfish were not available. In addition, the fishing behavior data indicate that 90% of respondents were either unlikely or very unlikely to fish less often if crayfish were unavailable. The remaining 10% were neutral about whether they would fish less often. Finally, every angler that fished with crayfish told us that they would still have taken the same number of fishing trips if crayfish bait had not been available from bait shops. Overall, our results suggest strongly that banning live crayfish from the bait trade would not reduce fishing frequency and thus would not negatively affect the fishing industry.

Banning live crayfish from the Missouri bait trade could reduce the likelihood of or rate at which _O. virilis_ spreads to new drainages; however, it could also cause the spread of bait crayfish via collection by anglers directly from the environment and introductions to different drainages. Approximately 50% of respondents indicated that if crayfish were unavailable from bait shops they would be likely to collect crayfish directly from one body of water and transport them to a different location to fish. Some of these anglers already obtain their bait this way, but it is possible that this behavior would increase if crayfish were unavailable from bait shops. Missouri regulations state that anglers collecting crayfish for bait are prohibited from releasing that bait to waters of the state (Missouri Department of Conservation 2014). However, a recent survey of anglers in Maryland indicated that anglers were unaware of a similar Maryland regulation that prohibited the release of live bait into new waters (Kilian et al. 2012). Therefore, many anglers that collect crayfish in one location may release crayfish into a new water body without being aware that this behavior is prohibited. Thus any perverse impacts of a
policy banning the use of live crayfish might be at least partly mitigated by an education campaign to increase angler awareness about the prohibition of releasing crayfish.

The Missouri department of Conservation has implemented an education campaign by producing an educational brochure for bait shop owners, publishing articles in the *Missouri Conservationist*, and presenting information to public groups (DiStefano personal communication). Even if it is not the intention of anglers to release their crayfish bait into new waters, transporting crayfish to new locations for fishing increases the chance of an introduction from an accidental release. Allowing anglers to only use crayfish as bait in the water body in which they were collected (as has been implemented in Arizona, Idaho, Minnesota, Ohio, Utah, Washington, and Wyoming) would likely help reduce this source of introductions.

Our results demonstrate that understanding how policy decisions will affect human behavior is important for predicting their ecological and economic consequences. Our survey of Missouri anglers suggests that fishing with crayfish makes up a substantial portion of the revenue generated by the fishing industry. However, prohibiting the sale of crayfish as bait would not affect the frequency with which people go fishing and is unlikely to alter angler spending on equipment, travel, or trip-related expenses. In addition, the loss of crayfish sales in bait shops is likely to be mitigated to some extent by anglers purchasing other baits. In contrast, invasive crayfish impacts in nearby states suggest that crayfish introductions could reduce the revenue generated by the fishing industry by reducing game fish populations. Our survey also identified angler behavior that could potentially lead to unintended consequences of banning crayfish from the bait
trade: anglers collecting crayfish directly from the environment. However, this perverse effect could be partially mitigated by an educational campaign.

6.6 Acknowledgments

We would like to thank R DiStefano for providing information about the policy surrounding crayfish in the bait trade in Missouri as well as providing information about crayfish invasions in this state. In addition, we appreciate assistance from S Berry and R Reitz on survey design. Funding for this research was provided by NSF IGERT grant award #0504495 to the GLOBES graduate training program at the University of Notre Dame, NOAA CSCOR funding, and a gift from G and L Anderson. This is a publication of the Notre Dame Environmental Change Initiative.

6.7 References


dispersal patterns and community change in a north temperate lake. Canadian Journal of Fisheries and Aquatic Sciences 61:2255-2266.
CHAPTER 7:

CONCLUSION

7.1 General overview

Understanding the factors that make species successful invaders is crucial for managing current invasions and predicting which species are likely to cause ecological or economic harm when introduced to new locations. My dissertation research focused on three factors which may be important in controlling invasions, contemporary evolution, parasitism within the introduced range, and human behavior. While some researchers have addressed the roles of contemporary evolution and parasitism in invasions in other ecosystems, there are few examples, and invasion biologists lack a general understanding of how often these factors are ecologically important. Further, understanding the effects of contemporary evolution and parasitism in invasions will provide insight into their role in controlling native populations and structuring native communities. In addition, I examined the consequences of policy aimed at reducing crayfish introductions by changing angler behavior. To determine whether a policy change will be effective, it is essential to examine whether humans will respond in the desired way or whether perverse effects of policy might occur.
7.2 New insights into evolution during invasions

In Chapter 2, I investigated whether evolution during the *O. rusticus* invasion contributes to the strong impacts of *O. rusticus* in the invaded range. I tested for differences in growth rate, survival, and response to predators in native and invaded range populations of *O. rusticus*. I hypothesized that low conspecific densities during introductions into lakes would select for increased investment in growth and reproduction in invasive populations. In both lake and mesocosm common gardens, *O. rusticus* from invasive populations had significantly faster growth rates and higher survival than individuals from the native range, especially in mesocosms where fish were present. However, there was no influence of within-range collection location on growth rate. In addition, egg size was similar between ranges and did not affect crayfish growth. My results, therefore, suggest that growth rate has diverged in *O. rusticus* since it was introduced to the invaded range. These data are consistent with my hypothesis that *r*-selected traits would evolve in the invaded range due to low densities during the initial stages of crayfish introductions into lakes.

Faster growth rates in the invaded range contribute to the strong impacts of *O. rusticus*. *O. rusticus* has a greater impact on the ecological community than congeners, *O. virilis* and *O. propinquus* (Wilson et al. 2004), and the ability of *O. rusticus* to replace *O. propinquus* has been attributed in part to its faster growth rate and ability to outcompete smaller individuals for shelter (Hill et al. 1993, Garvey et al. 1994, Hill and Lodge 1994). Faster growth also allows *O. rusticus* to escape predation from gape-limited fish more rapidly (Stein 1977).
These findings suggest that including evolutionary potential in risk assessments may enhance our ability to predict invasion success. Even though a species may not be problematic in its native range, or may be unproblematic initially in a new location, traits such as rapid growth and high reproductive output that may increase ecological impacts can evolve within the invaded range. Hybridization may increase the likelihood for $r$-selected traits to evolve within the invaded range because of increased additive genetic variance in hybrids. Especially when introduced to new locations within North America, crayfish are often exposed to closely-related, native species with which they are likely to hybridize (Perry et al. 2002). Understanding the likelihood of the evolution of invasive traits in nonindigenous populations is crucial for weighing the costs and benefits of moving species to new locations.

7.3 The effects of crayfish parasites

In addition to elucidating the role of parasites in controlling the success and impacts of an invasive species, my dissertation research provides one of the first studies to test whether parasites alter crayfish behavior (but see Haddaway et al. 2012). Because crayfish are important ecologically (Hobbs and Lodge 2009), parasite effects on crayfish behavior are likely to have large impacts on aquatic communities and ecosystems. Further, impacts of parasites are typically assumed to be due to their effects host density (Mouritsen and Poulin 2005), but my research indicates that the trait-mediated indirect effects of parasites are important for the per capita impacts of crayfish. The impacts of trait-mediated effects of parasites are not well understood (Mouritsen and Poulin 2005, Lefevre et al. 2009). Therefore, my dissertation research not only contributes to our
understanding of invasion biology, but is also an important contribution to our general understanding of the role of parasites in communities.

In Chapter 3, I investigated the distribution of *Microphallus* parasites in *O. rusticus* in northern Wisconsin and Michigan Lakes and examined whether these parasites could contribute to previously-documented, alternate states in the abundance of *O. rusticus*. From samples collected from 109 sites in 16 lakes, I found a positive relationship between crayfish infection intensity and hydrobiid snail abundance, a negative relationship between parasite prevalence and crayfish abundance, and a negative relationship between parasite prevalence and crayfish population growth. My results were consistent with the hypothesis that *Microphallus* contributes to alternate states in the abundance and impacts of *O. rusticus*.

I also conducted experiments on the effect of parasites on *O. rusticus* feeding and growth in Chapter 3. I found that when infected and uninfected crayfish were fed a standard amount of food, uninfected crayfish had greater growth. Further, I observed reduced feeding in infected crayfish. Therefore, in this chapter, I concluded that reductions in population growth associated with *Microphallus* may be due to reduced feeding and growth. However, later experiments (in Chapter 5) indicate that infected crayfish have greater feeding and growth than uninfected crayfish over longer timescales. Thus, differences in growth are unlikely to be responsible for the negative relationship I observed between crayfish population growth and parasite prevalence. Behavioral experiments from Chapter 4 revealed a different potential mechanism for this relationship: infected crayfish were bolder and likely more susceptible to predation.
Overall, my results suggest that predatory fish and parasites may act in concert to prevent *O. rusticus* from reaching ‘outbreak’ densities in some lakes.

In Chapter 4, I expanded my focus to include *Microphallus* effects on three common orconectid crayfish (*O. rusticus*, *O. propinquus*, and *O. virilis*), and investigated whether infection altered crayfish behavior. I found that infection substantially altered crayfish shelter affinity, shelter competition, and boldness, though infection affected each species differently. Infection reduced shelter affinity to the greatest extent in *O. propinquus*. All three species were also bolder in the presence of a predatory fish when infected, which is likely to increase their vulnerability to predation. This behavioral modification may be an adaptation of *Microphallus* to increase transmission to higher trophic levels. My results suggest that of the three crayfish, *O. propinquus* are likely to suffer the greatest increase in predation when infected, due to a substantially reduced affinity for shelter coupled with increased boldness. The negative effects of *O. rusticus* on macrophytes, macroinvertebrates and fish scale with crayfish density, so my findings indicate that infection is likely to influence lake communities by reducing crayfish abundance. Infection may also alter the likelihood that an introduction of *O. rusticus* or *O. propinquus* will succeed in a lake. On the other hand, *Microphallus* is likely to alter interactions between species in lakes where crayfish species coexist, favoring *O. rusticus* over congeners, both of which have lesser effects on macrophytes, macroinvertebrates, and fish than *O. rusticus* (Olsen et al. 1991, Wilson et al. 2004).

In Chapter 5, I tested whether the behavioral changes associated with infection could alter the per capita impacts of *O. rusticus* on lower trophic levels. Results from Chapter 3 and Chapter 4 indicated that infected *O. rusticus* consumed fewer
macroinvertebrates and were bolder in the presence of predatory fish than uninfected individuals. Therefore, I predicted that when predators were absent, infected crayfish would have reduced impacts on macrophytes and macroinvertebrates compared to uninfected crayfish. However, when predators were present, I expected infected crayfish to have the greatest impact on lower trophic levels because of increased boldness. Infected crayfish were more active and had greater growth than uninfected crayfish across treatments, suggesting that the reduction in feeding behavior observed in short term experiments (in Chapter 3) does not occur over longer timescales. Further, as I expected, when predatory fish were present, I observed fewer macroinvertebrates and a trend for reduced macrophyte consumption within tanks containing infected crayfish. My results from this chapter suggest that parasites can substantially alter the per capita impacts of hosts merely by modifying host behavior.

Overall, my parasite research indicates that Microphallus increases per capita impacts of O. rusticus by increasing boldness and foraging, but may also decrease O. rusticus populations in lakes by increasing their vulnerability to predation. The overall impacts of Microphallus on the lake community will likely be dependent on initial crayfish population size. For example, I found (in Chapter 3) that when crayfish population size was initially low, Microphallus prevalence had a strong negative relationship with crayfish population growth. Crayfish populations grew by an average of approximately 170% between years when Microphallus was absent, but did not grow on average when parasite prevalence reached 100%. (Figure 3.3). Therefore, when crayfish densities are low, the effects of parasites on crayfish density are likely to be more important for lake communities than their trait-mediated effects. However, when
crayfish were initially abundant, I did not observe a strong relationship between parasite prevalence and crayfish population growth, potentially because the population was near carrying capacity and density dependent effects compensated for increased predation when parasites were prevalent (Figure 3.3). In these conditions, the trait-mediated effects of *Microphallus* are likely to be more important for the community than density effects.

7.4 Assessing human behavior to anticipate consequences of policy change

In Chapter 6, I investigated whether a change in Missouri policy to prevent crayfish introductions and protect the fishing industry from crayfish impacts would have the intended consequences. Approximately twenty percent of Missouri anglers reported fishing with crayfish, and if crayfish were unavailable from bait shops, anglers reported that they would be most likely to purchase another type of bait from a bait shop or to collect crayfish directly from the environment. If directly collecting crayfish increases in response to banning crayfish from the bait trade, this behavior could be a substantial new source of crayfish introductions. Anglers reported that banning crayfish from the bait trade would not affect the frequency with which they go fishing. Therefore, while crayfish introductions could reduce fishing frequency in Missouri by reducing game fish populations, banning crayfish from the bait trade is unlikely to affect angler spending on equipment, travel, or trip-related expenses. Banning crayfish from the bait trade may allow states to avoid ecological and economic impacts from invasive crayfish without impacting the revenue generated by the fishing industry. However, examining how angler behavior will change in response to policy may be necessary to reduce the
likelihood of unintended consequences such as an increase in introductions from anglers collecting crayfish directly from the environment.

7.5 Concluding remarks

Overall, my dissertation investigated three understudied mechanisms contributing to invasion success: contemporary evolution, parasitism, and human behavior. I found that the response of anglers to a policy change that would ban crayfish from the bait trade could alter its effectiveness at protecting game fish populations. I also found that both contemporary evolution and parasitism were important for *O. rusticus* invasion success and impacts. Further, my research indicates that behavioral changes associated with infection may be important for interspecies interactions and the ecological impacts of hosts. These mechanisms may be broadly important in communities, and research that investigates the density and trait-mediated effects of parasites as well as the effects of contemporary evolution will allow ecologists to more fully incorporate these effects into the field of community ecology.

7.6 References


APPENDIX A:
SURVEY QUESTIONS SENT TO MISSOURI ANGLERS

1. How many fishing trips did you take in Missouri during the last 12 months? Please include any outings involving fishing. A trip may last an hour, a day, or a number of days.
   ____________ Trips

2. How many days did each typical Missouri fishing trip last? Please include all days in which you fished for any part of the day.
   ____________ Days

3. Please rank the following bait types by how often you used them when fishing in Missouri during the last 12 months. Rank the bait you used most often as 1, the second most often as 2, etc. If you did not use a particular type of bait, please leave the space blank.
   ___ live minnows
   ___ live worms
   ___ live insects (such as crickets)
   ___ fish eggs
   ___ artificial bait (such as lures or artificial worms)
   ___ crayfish/crawfish

4. Have you fished in Missouri using crayfish/crawfish as bait in the last 12 months? Please choose one.
   ○ No, I have not fished with crayfish/crawfish ➔ Skip to question 15
   ○ Yes, I fished with crayfish/crawfish ➔ Continue to question 5

5. For which types of fish are you typically fishing when using crayfish/crawfish as bait? Please check all that apply.
   □ Panfish (such as bluegill, sunfish, or perch)
   □ Bass
   □ Catfish or bullheads
   □ Trout
   □ Crappie
   □ Walleye
   □ Turtles
   □ Other ________________________________

6. Where do you typically go fishing when using crayfish/crawfish as bait? Please check all that apply.
   □ Lake or reservoir
   □ Pond
   □ River or stream
7. If crayfish/crawfish were no longer available from bait shops, how would your behavior change? Please fill in the bubble that most closely matches how likely you are to do each of the following.

<table>
<thead>
<tr>
<th>I would:</th>
<th>Very Unlikely</th>
<th>Unlikely</th>
<th>Neutral</th>
<th>Likely</th>
<th>Very Likely</th>
</tr>
</thead>
<tbody>
<tr>
<td>purchase another type of live bait such as minnows, worms, fish eggs, or insects</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>purchase artificial bait such as lures or artificial worms</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>collect crayfish/crawfish from the river, lake, or pond where I fish</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>collect crayfish/crawfish from a river, lake, or pond (other than where I will fish)</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>not switch to any other bait type, and thus I would fish less often</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

8. Which types of equipment do you typically use when you are using crayfish/crawfish as bait? Please check all that apply.

- □ Rod and reel
- □ Trot line, jug line, throw line, bank line, or limb line
- □ Motor boat
- □ Non-motorized boat such as a canoe or kayak

For Questions 9-14, please think of all the times you went fishing in Missouri and used crayfish/crawfish bait (for any part of the trip) during the last 12 months:

9. During the last 12 months, in how many fishing trips did you use crayfish/crawfish as bait? Please include all trips where you fished with crayfish/crawfish as bait for any part of the trip.

_____________ Trips

10. How many days did each trip typically last? Please include all days in which you fished for any part of the day.

_____________ Days

11. How long does it typically take you to travel (one way) on these trips?

_____________ Hours ___________ Minutes
12. If you were not able to purchase crayfish/crawfish from a bait shop, would you still have taken all of your previous fishing trips during the last 12 months? Please choose one.
   O No, I would have taken fewer trips
   O Yes, I would still have taken all of the trips

13. If you answered NO to question 12, please think about the trips you took in the last 12 months in which you used crayfish/crawfish as bait (in question 9). How many of these trips would you have taken if you had NOT been able to purchase crayfish/crawfish from a bait shop? Please choose one.
   O I would not have taken any of these trips
   O I would have taken less than half of these trips
   O I would have taken roughly half of these trips
   O I would have taken more than half of these trips

14. During the last 12 months, how much did you spend in total on lodging and food while on fishing trips in Missouri when you used crayfish/crawfish as bait?
   Lodging ............ $ ____________
   Food ................. $ ____________

For questions 15-16, please think of all the times you went fishing in Missouri and did NOT use crayfish/crawfish as bait in the last 12 months.

15. How long did it typically take you to travel (one way) on a fishing trip when you were using bait other than crayfish/crawfish in the last 12 months?
   __________ Hours ________ Minutes

16. During the last 12 months, how much did you spend in total on lodging and food while on fishing trips in Missouri when you did NOT use crayfish/crawfish as bait?
   Lodging ............ $ ____________
   Food ................. $ ____________

17. Which county do you live in? We will use this information to determine whether the responses we receive represent all areas within the state.
   ________________________________ (write in county name)
Figure B.1 Fish weight gain per day ± SE in the first and second experiments. While fish in the first experiment gained weight throughout the four weeks, fish in the second experiment lost weight. In addition, there were 7 fish mortalities during the second experiment, but no mortalities occurred within the first experiment. Even though we replaced mortalities, we observed signs of disease and abnormal behavior (fungus and listlessness) in fish in the mesocosms during the second experiment.
Figure B.2 Increase in crayfish carapace length during the second experiment ± SE for (A) females and (B) males. In the second experiment, there was no significant difference in growth between crayfish in tanks with and without fish ($P > 0.9$). In addition, we did not observe a significant effect of infection ($P > 0.1$), temperature ($P > 0.4$), or interaction between fish and infection ($P > 0.5$) on crayfish growth.
Figure B.3 The percent ± SE of crayfish remaining in mesocosms that were outside of shelter (active) during (A) daytime observations or (B) night observations made during the second experiment. We did not observe a significant effect of fish ($P > 0.08$) or infection ($P > 0.8$) on shelter use in the experiment.