Determining the Effects of a Trematode Parasite, *Microphallus* spp., on Rusty Crayfish (*Orconectes rusticus*) Fecundity

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Abstract

Rusty crayfish (*Orconectes rusticus*), native to the Ohio River Drainage, have been able to successfully invade lakes of Wisconsin and the Upper Peninsula of Michigan by displacing other crayfish species, including the northern crayfish (*O. propinquus*) and the virile crayfish (*O. virilis*). Rusty crayfish also have a significant impact on community composition, especially on lake macrophytes and other macroinvertebrates, which decrease as crayfish abundance increases. Recent research has discovered a trematode parasite, *Microphallus* spp., is infecting crayfish species, both native and invasive. This study investigates the effect of the parasite on the fecundity of rusty crayfish by testing the hypotheses that infected crayfish would be less fecund than uninfected individuals and crayfish with higher infection intensity would be less fecund than crayfish with lower infection intensity. Fecundity of crayfish from ten different sites in Wisconsin was recorded by measuring the abundance and weight of eggs from each crayfish. Results showed that infected crayfish had a greater number of eggs, but the eggs were also significantly smaller. Crayfish with high infection intensity also had a significantly greater number of smaller eggs than crayfish with low infection intensity. Smaller eggs are likely to produce smaller, less competitive young, and these juveniles may have higher mortality than those from larger eggs. Therefore, these results indicate a possible decrease in the rusty crayfish population, which would cause alterations across many trophic levels and substantially change lake communities if realized. On the other hand, the more numerous eggs infected crayfish produce could make up for the lower quality of the eggs.

Introduction

Making up majority of crayfish diversity, the North American crayfish species also represent one of the continent's most threatened taxonomic groups (Larson 2008). Also the largest and longest lived freshwater crustaceans in North America (Momot et al 1978), many of these crayfish have been introduced to new drainages, and are now invasive species in the ecosystems they thrive in. They are introduced to new ecosystems through activities surrounding aquaculture, angling, and the live bait trade. Of these species, one of the best known is the rusty crayfish (*Orconectes rusticus*).

Rusty crayfish have been studied in lakes and streams of northern Wisconsin and the Upper Peninsula of Michigan, but this crayfish is native to the Ohio River drainage (Olden et al 2006). Rusty crayfish, like other invasive species, are strong indicators of freshwater systems and have large impacts on many invaded systems (Wilson et al 2004). The large impacts that crayfish can have on local aquatic ecosystems are attributed to their large size compared to other invertebrates, omnivorous feeding habits, and ability to reach high population densities (Sargent et al 2014). Although there were already orconectid crayfish present in north temperate lakes, rusty crayfish have unique characteristics that affect the ecological community. For example, rusty crayfish lay eggs at a lower temperature than northern and virile crayfish, which allows neonates to be born sooner, reach a larger size faster, and better insure replacement as they are able to outcompete smaller, younger crayfish of different species (Momont 1984). In fact, in the lakes and streams of Northern Wisconsin and Michigan, previous research has shown that the rusty crayfish displaces congeners such as the northern crayfish (*O. propinquus*) and the virile crayfish (*O. virilis*) causing shifts in species dominance (Olden et al 2006).

Not only does the introduction of the rusty crayfish cause other species of crayfish to decline, but increases in rusty crayfish populations are also connected to declines in the abundance of other macroinvertebrates including snails and macrophytes. Macroinvertebrates decline because they experience increased predation, habitat loss, and competition for food resources when rusty crayfish are present (Wilson et al 2004). Rusty crayfish have also been shown to consume more and cause greater damage to snails and macrophytes than other crayfish on a weight-specific basis (Olsen et al 1991). Rusty crayfish are able to affect many different trophic levels, as they can simultaneously act as decomposers, primary consumers, and carnivores and have been described as keystone predators (Momot et al 1978).

Overall, it is clear that *O. rusticus* can have a great effect on local aquatic ecosystems. However, there has been a new found factor at play in rusty crayfish ecosystems in Northern Wisconsin and Michigan. A trematode parasite, *Microphallus* spp., has been infecting all species of crayfish, native and invasive. The parasite has potential to alter community composition if it alters the life history of a host species with large impacts, such as the rusty crayfish (Sargent et al 2014). The first intermediate host of the parasite is the hydrobiid snail through which the crayfish are infected by free-swimming larvae from the host snails. The parasite then remains in the crayfish's hepatopancreas, its digestive organ, until the crayfish is consumed by a definitive host, which remains unknown (Sargent et al unpublished). Previous research has shown that there is a negative relationship between the abundance of rusty crayfish and the prevalence of crayfish infected with *Microphallus*. Research has been conducted on how infection alters behaviors in crayfish, resulting in evidence that crayfish are more vulnerable to predation when infected, due to increased boldness and aggression (Sargent et al unpublished).

It is also possible that the trematode parasite alters crayfish fecundity and that this could contribute to the crayfish population declines observed in the infected populations. My research is the first to examine whether there is an effect of this parasite on crayfish fecundity. Because the parasite infects the hepatopancreas (the digestive organ of the crayfish) it could influence digestion and therefore affect the resources that females can allocate to their eggs. When infected with the trematode parasite, crayfish growth is reduced, and uninfected crayfish experience greater growth (Sargent et al 2014). This provides further evidence that infection may reduce egg size and abundance. Larson et al (2008) has also shown that invasive species usually have higher individual fecundity; therefore higher fecundity may allow rusty crayfish to outcompete native species, but infection with *Microphallus* could alter this relationship. There is also a positive relationship between size and fecundity in female crayfish (with fecundity measured in abundance of abdominal eggs) (Corey 1988). Therefore, the effect of female size was taken into account in my analysis. I hypothesized that after female length is taken into account, crayfish infected with *Microphallus* will be less fecund than crayfish that are uninfected, meaning they will have smaller (measured using dry weights) and less abundant eggs. I also hypothesized that within crayfish which are infected, those with a greater number of parasites would also be less fecund than crayfish with fewer parasites. If these hypotheses are supported, implications could be additional alterations in community structure as rusty crayfish populations decrease due to lessened fecundity with cascading effects through different trophic levels.

Materials and Methods

Female rusty crayfish, both infected and uninfected, were hand collected from ten sites in eight lakes in the region of the Upper Peninsula of Michigan and Wisconsin. Locations were off

UNDERC property and included Ottawa, Plum, Star, High, Spider, Papoose, Presque Isle, and Big lakes (Table 1). Lakes exhibited varying temperatures, but all collections were completed within the same week in early June. Based on information from previous surveys, I alternated collecting from locations where crayfish were likely to be infected with locations where crayfish were likely to be uninfected throughout the week. Once crayfish were hand collected, they were euthanized with 95% ethanol before being transported to UNDERC property for analysis. In order to determine the fecundity of the crayfish, I counted the total number of eggs and measured the carapace length of each crayfish. Crayfish were then dissected to count the number of *Microphallus* parasites present. To view parasites, each crayfish's hepatopancreas was flattened between glass slides and examined under a microscope. Eggs were then placed in a drying oven at 60 degrees Celsius for a minimum of 48 hours. After drying, the weights of five eggs from each crayfish were recorded.

Two ANCOVA statistical tests in were used to determine whether there were significant differences in 1) abundance of eggs 2) weight of eggs between infected and uninfected crayfish. I included carapace length in each analysis as a covariate in order to take size into account. Multiple regression was used to determine whether the there was a relationship between infection intensity (the number of parasites present in infected individuals) and egg abundance or egg mass. Again, I included carapace length as a covariate in the analysis to account for its influence.

Results

The results are based on data collected from 10 different sites (Table 1). The ANCOVA revealed that infected crayfish had eggs that were significantly lighter than those of uninfected crayfish ($F_{1, 219}$=7.43, p= 0 .0069; Figure 1). The analysis also showed that carapace length was significantly positively related to dry weight of crayfish eggs ($F_{1, 219}$= 96.45, $p < 0.0001$; Figure 1), so the larger the crayfish, the larger its eggs were. Additionally, there was a significant interaction between carapace length and infection status on egg weight ($F_{1, 219}$= 5.34, p=0.0218). At small carapace lengths, infection level did not have a great effect on egg weight, but as carapace length increased, infection had a greater negative effect on the dry weight of crayfish (Figure 1).

The second ANCOVA investigated whether there was a significant difference in number of eggs between infected and uninfected crayfish of various carapace lengths. The results indicated that infected crayfish have significantly more eggs than uninfected crayfish ($F_{1, 233}$= 17.53, $p < 0.0001$; Figure 2). Carapace length also had a significant positive relationship with the number of eggs crayfish carried ($F_{1, 233}$= 183.72, $p < 0.0001$), but there was no significant interaction between these two variables on the number of eggs ($F_{1, 233}$= 1.49, p= 0.2234; Figure 2).

Further analysis examined only infected crayfish. First, I tested whether the weight of eggs was related to infection intensity. It found that parasite abundance was significantly negatively related to egg weights ($F_{1, 96}$= 14.83, p =0.002; Figure 3). The covariate, carapace length, still had a significant effect on the egg weight ($F_{1, 96}$= 74.80, $p <0.0001$; Figure 3), with larger crayfish exhibiting heavier weights. There was also a significant interaction between parasite abundance and carapace length ($F_{1, 96}$= 4.57, p =0.0357), where the infection intensity had a greater influence as carapace length increased.

Secondly, I investigated whether crayfish infection intensity affected the number of eggs it carried, while taking into account carapace length. The number of eggs was significantly affected by infection intensity, as the number of eggs increased with increasing parasite abundances ($F_{1, 109}$= 47.82, $p <0.0001$; Figure 4). Again, there was a positive relationship between carapace length and egg number ($F_{1, 109}$= 58.86, $p < 0.001$; Figure 4). However, in this case there was no interaction between carapace length and infection intensity ($F_{1, 109}$= 2.47, $p =0.1190$; Figure 4), so that the two variables affected the number of eggs in infected crayfish independently.

Discussion

The hypothesis that infection by a trematode parasite (*Microphallus* spp.) would cause egg size in rusty crayfish to decrease was supported by the data collected. Data collected from infected females also supported the hypothesis that with a greater abundance of parasites within the crayfish, egg size would decrease. However, data showing that infected female crayfish had significantly more eggs, and still more eggs as the level of parasitism increased, goes against original hypotheses. The importance of carapace length is also noteworthy when discussing dry egg weights. Interaction among carapace and infection status on dry egg weights shows that when crayfish are small, infection status is not as important as when carapace length increases. However, this does not seem to be the case for egg abundance, where infected crayfish have significantly more eggs independently of carapace length, though larger crayfish are also seen to have greater abundance. In summary, infected female rusty crayfish had significantly more, smaller eggs than those that were uninfected.

Knowing that the parasite is affecting female crayfish by causing them to have more abundant but smaller eggs, can help predict how infection would have an effect on the future population. Despite having a greater amount of eggs, having smaller eggs is equivalent to having eggs of a lesser quality and in turn a lower probability of survival for offspring. Having smaller eggs could be related to infection taking place in the hepatopancreas, and therefore causing nutrient deficits or other nutritional alterations, as there is evidence that infection causes rusty crayfish to eat less (Sargent et al 2014). Energy reserves are present in the post-embryonic yolk (PEY) of eggs on which neonates of many species are dependent upon for survival during early stages of life (Goulden et al 1987). Larger eggs have an advantage in that they have the ability to contain a greater amount of PEY than smaller eggs. Past studies have shown that there is a correlation between egg size, the amount of energy reserve present in neonates, and survivorship, where it is argued that animals should increase egg size to increase survivorship (Goulden et al 1987). Size of eggs is also a characteristic known to affect a female's fecundity and ability to increase population size (Goulden et al 1987). This information supports the idea that although parasitized individuals may produce more young, the young will also be less likely to survive, perhaps leading to a decline in the overall population.

From this study, it remains unclear as to why infection would cause crayfish to produce a greater amount of eggs. Perhaps it is a way that crayfish are trying to increase their fitness under circumstances of stress, in this case infection. When under stress or facing alterations in population size, many organisms will change aspects of their reproductive cycles. Three common strategies include quickening the rate of maturity and shortening life span, as well as altering age-specific fecundity and mortality rates. When under stress many organisms tend to experience high juvenile mortality and reduced mortality for those that reach adulthood (Momot

1984). Rusty crayfish producing more eggs with the tradeoff of having high juvenile mortality for more successful adults would be supported here. Additionally, Sargent et al (unpublished) discussed how infected crayfish become increasingly bold and aggressive, thereby increasing their susceptibility to predation. Under these circumstances having more eggs of a lesser quality, rather than a few eggs of high quality, may be advantageous to the rusty crayfish under stress as there are better chances that one egg may survive. Because infection takes place in the hepatopancreas, nutrition can be affected, which is an important factor affecting fecundity by changing the distribution of resources an organism can use in reproduction (Schreck et al 2000). Sargent et al (2014) also discusses how infected crayfish grow more slowly, so it is likely that between an infected and uninfected crayfish of the same size, the infected crayfish would be older. Taking this into consideration, even if the infected crayfish produced more eggs than the uninfected individual, it may not produce a greater amount in its lifetime. Therefore, the increase in eggs could be due to a combination of factors relating back to how the parasite affects crayfish nutrition, behavior, and adaptations to increase fitness under the stress of infection.

Using this information to predict alterations in rusty crayfish populations is important because this species is polytrophic and plays many different roles in aquatic ecosystems. Being decomposers, primary consumers, and carnivores, a change in the abundance of rusty crayfish would not only alter the abundance of organisms in the trophic levels on which crayfish feed, but also the abundance of organisms which feed on crayfish (Momot et al 1978). For example, fish that feed on crayfish are affected by changes in habitat caused by crayfish and egg predation by crayfish (Peters et al 2008). Crayfish can also reduce habitat heterogeneity, most notably by negatively affecting macrophyte populations (Wilson et al 2004). A reduction of macrophytes can have a significant effect on the structure of an ecosystem as they significantly affect physical, chemical, and biotic components as well as temperature, pH, dissolved oxygen, and nutrient dynamics of lakes (Peters et al 2008).

Although the data support that infection of rusty crayfish could lead to a decline in the population, the effects of the species will still be felt. In lakes where the parasite is absent, it can be reasoned that crayfish populations will remain abundant. This is due to the fact that snail populations, which act as the first intermediate host for *Microphallus,* will remain low because they are an important food source to crayfish. Even if declines occur in rusty crayfish populations, it is likely that portions of rusty crayfish will persist. In order for their effects on communities to be eliminated, rusty crayfish populations would have to dramatically decrease. Research by Peters et al (2008) demonstrates how even at low densities crayfish can significantly alter community composition.

Being able to predict changes in ecosystems with relation to invasive rusty crayfish populations can help influence management techniques for aquatic environments. With a reduction of rusty crayfish, other crayfish (northern and virile) may be able to maintain a greater population size due to reduced competition. If these populations have been overwhelmed and are too small to rebound, that niche may not be filled causing imbalances at all trophic levels. As for effects on flora, Peters et al (2008) mentions that even after reductions of rusty crayfish populations, macrophytes are unable to naturally repopulate an area. Restoration is uncommon in areas with crayfish because of the knowledge that they will be reduced. One possible management technique for maintaining a balanced ecosystem deals with algae-detritus pathways, in which food availability is controlled from the bottom-up. Crayfish would likely be controlled as detritus and algae comprise significant portions of their diet. Also, a top-down proposed management technique is to regulate the amount of fish present in lakes (Momot et al 1978).

Since *Microphallus* parasites can also control crayfish populations, likely in part by reducing crayfish egg quality, these techniques may be especially effective in lakes with high prevalence of *Microphallus* parasites.

While this study was successful in determining that infection with *Microphallus* spp. is associated with more abundant, but smaller eggs, future investigations can lead to a better understanding of why this occurs and what effects it will have on communities. One aspect to look into is how lake composition can affect infection levels and fecundity of rusty crayfish. Factors such as temperature, vegetation, and location could help to determine under what conditions crayfish are being most affected. It would also be helpful to include more large rusty crayfish that were uninfected in future research, which is a more challenging task because as crayfish grow older and larger, the more likely they are to be exposed to the trematode parasite. With increased investigations, more successful management strategies to help restore natural environments and prevent the extermination of native species can be implemented.

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Tables

Table 1. Displayed below are the ten different sites from which crayfish were collected in northern Wisconsin and Michigan's Upper Peninsula, along with the date and temperature of the surface water upon collection.

Date	Lake	Temperature
6/3/2014	Lake Ottawa- Site 1	17°C
6/3/2014	Lake Ottawa- Site	17°C
6/3/2014	Big Lake	18°C
6/4/2014	Plum Lake- Site 1	17.5°C
6/4/2014	Plum Lake- Site 2	19°C
6/4/2014	Star Lake	19°C
6/5/2014	Spider Lake	20°C
6/5/2014	High Lake	20°C
6/6/2014	Presque Isle Lake	17.5°C
6/6/2014	Papoose Lake	20.5°C

Figures

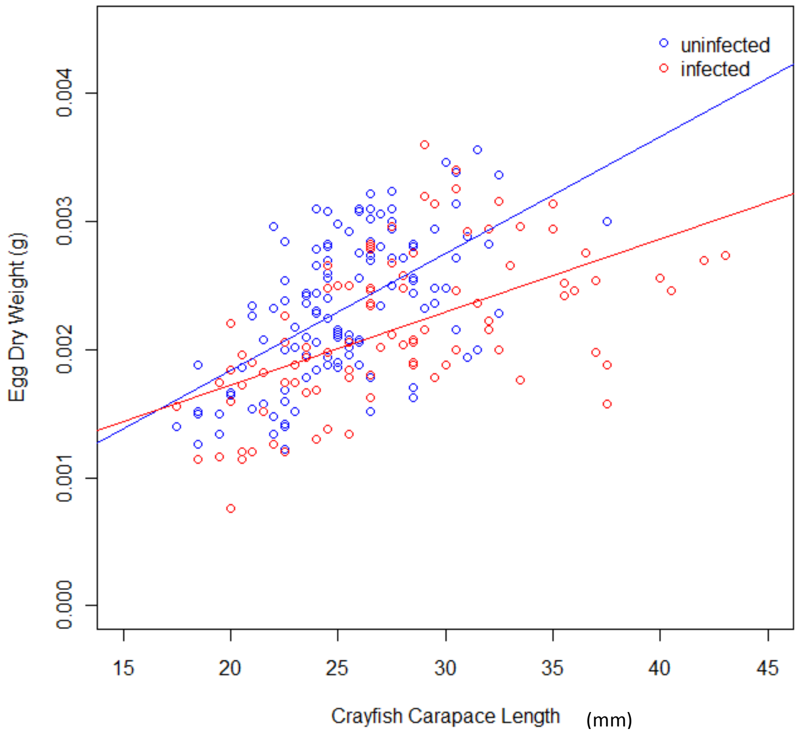

Figure 1. The relationship between carapace length (mm) and egg dry weight (g) for infected and uninfected rusty crayfish.

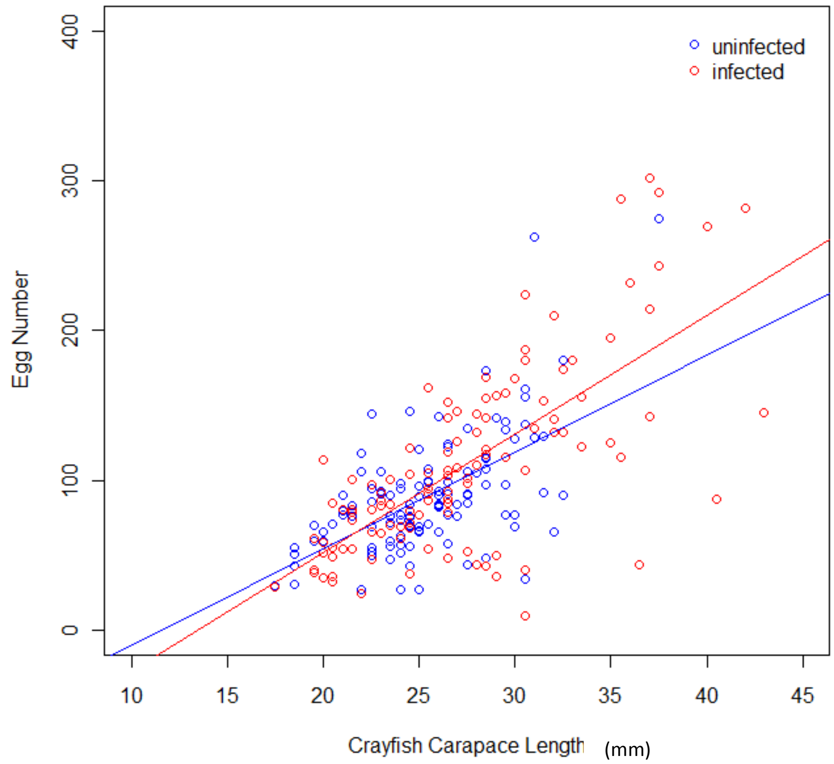

Figure 2. The relationship between carapace length (mm) and egg number for infected and uninfected rusty crayfish

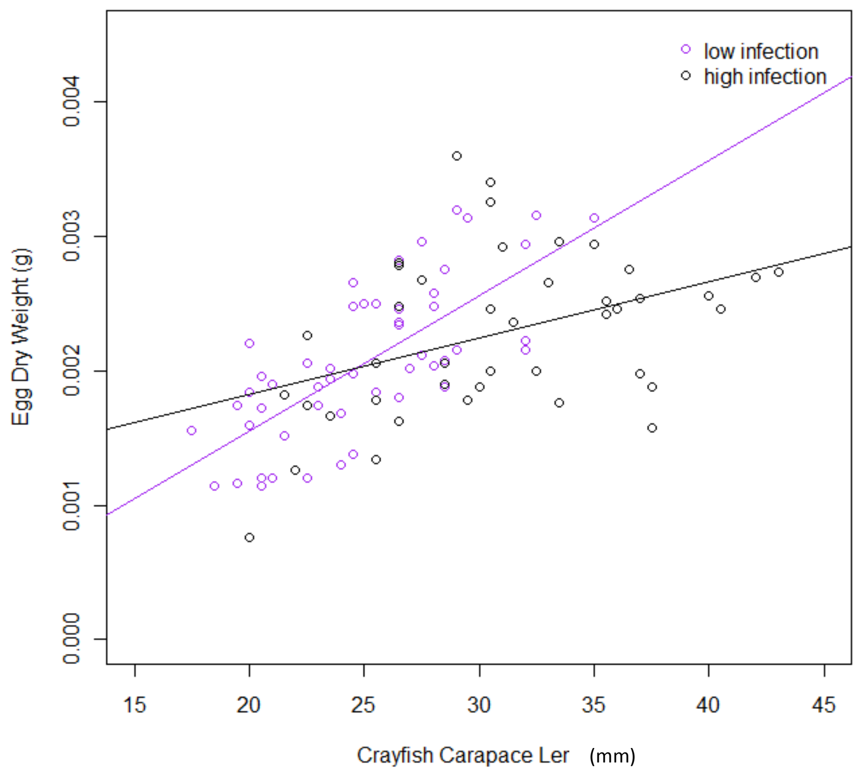

Figure 3. The multiple regression showing the relationship between carapace length (mm) and egg dry weight (g) for crayfish with high and low infection intensities. In the regression analysis, infection intensity was used as a continuous variable, but for visualization was broken down into two categories, low (50 or less parasites) and high (more than 50 parasites) infection.

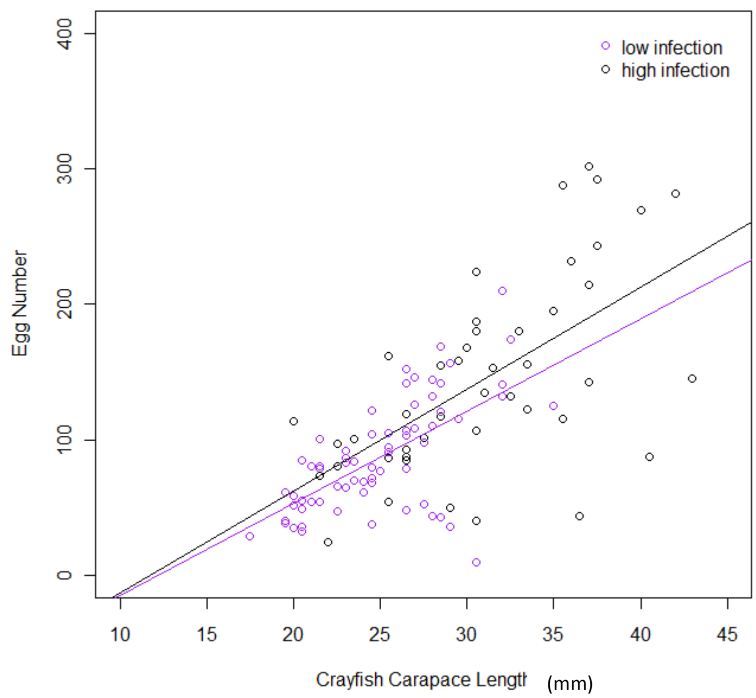

Figure 4. The multiple regression showing the relationship between carapace length (mm) and egg number for crayfish with high and low infection intensities. During analysis, infection intensity was used as a continuous variable, but for visualization was broken down into two categories, low (50 or less parasites) and high (over 50 parasites) infection.