The impact of infection with the parasite, *Microphallus spp*., on the growth of native and invasive *Orconectes* crayfishes

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Abstract

Invasive species impact aquatic ecosystems by causing ecological disturbances, extirpating native crayfish from their habitat, and competing with them. *O. rusticus* is an example of an invasive species well established in northern Wisconsin and Michigan lakes. *Microphallus spp.* parasites infect the digestive organ of *O. rusticus* as well as other common species of crayfish found in northern lakes (*O. virilis* and *O. propinquus).* *Microphallus spp*. is found in the tissue of the crayfish digestive organ, I hypothesized that it can have an effect on the crayfish growth and its molting time. I collected 40 *O rusticus*, 50 *O. virilis* and 50 *O. propinquus* from different lakes where I knew from previous research, where the parasite was abundant and where the parasite was rare. We monitored all the crayfish growth for four weeks. I found that infected *O. rusticus* grew less than uninfected *O. rusticus*. Smaller *O. rusticus* were affected by the infection, but larger crayfish grew slower than smaller crayfish. For *O. virilis* there was also a significant effect between infection and growth. Infected *O. virilis* grew less than uninfected *O. virilis*. However, my results for *O. propinquus* were different. There was no significant effect between infection and growth, and a trend towards greater growth on *O. virilis*. It is possible that species specific differences in responses to infection are due to different adaptations between the parasite and crayfish species.

Introduction

Invasive species can cause ecological disturbance by extirpating native species from their habitats and competing with them (Olden et al. 2006). Freshwater ecosystems are especially vulnerable to biotic exchange, but the greatest threat to crayfish biodiversity comes from the introduction of non-native crayfishes (Sala et al. 2000; Lodge 2000). The rusty crayfish (*Orconectes rusticus*) is an example of an invasive species which is well established in many northern Wisconsin and Michigan lakes, and was introduced by fishermen in the 1960's (Wilson et al. 2005).

O. rusticus is responsible for declines in other crayfish species (*O. propinquus* and *O. virilis*), macrophytes, snails and macroinvertebrate populations (Wilson et al. 2005). *O. rusticus* has spread slowly but successfully in freshwater ecosystems. *O .virilis* and *O. propinquus* compete with *O. rusticus* for shelter and food, but *O. rusticus* dominates because of its superior aggression and predator avoidance (Garvey et al. 1994). Other *Orconectes* crayfish species do not have the same detrimental impacts on the aquatic community as *O. rusticus*.

Microphallus spp. are a group of closely related trematode parasites that infect the three main *Orconecte*s crayfish species in Wisconsin and Michigan. These parasites are common and widespread in these lakes (Sargent et al. 2012). Other parasites in the *Microphallus* genus can cause necrosis of hepatopancreatic (digestive organ) tissue in other organisms (Robaldo et al.1999). *Microphallus spp.* requires three different hosts to complete their lifecycle. The first secondary host is a snail, and cercariae exit the snail and infect the hepatopancreas of crayfish. The crayfish must be consumed by the definitive host, a vertebrate predator, for the parasite to complete its life cycle.

This parasite changes crayfish behavior, possibly as a mechanism to increase transmission to the definitive host. When infected at low levels, *O. propinquus* and *O. virilis* alter their shelter use and competition behavior which may make them more vulnerable to predation. On the contrary, *O. rusticus* becomes more aggressive and more competitive for shelter (Sargent et al. 2012). At high levels of infection, the parasite reduces the competitiveness of *O. rusticus*.

Parasites can mediate the success of invasive species (Haddaway et al 2012), and this parasite may mediate the success of *O. rusticus*. Rusty crayfish populations have declined in some lakes with high prevalence of *Microphallus spp.,* and lakes with high abundances of crayfish often have low abundances of *Microphallus spp.* (Roesler 2009). In addition, *O. rusticus* populations decline between years at sites with high prevalence of the parasite (Sargent et al. 2012). However, it remains unclear whether the parasite is controlling crayfish populations. Also, the mechanism by which the parasite alters crayfish fitness has not been determined.

Because this parasite affects the digestive organ of a crayfish, my hypothesis is that crayfish infected with *Microphallus spp.* will have hindered digestion compared to uninfected crayfish and therefore grow less. Infection should reduce crayfish growth or result in later molting. If the parasite does affect *O. rusticus* growth, this mechanism may reduce *O. rusticus* populations because larger crayfish produce more offspring and can better escape fish predation. If this parasite does reduce *O. rusticus* growth, it may help reduce this problematic invader and its ecological disturbances.

Materials and Methods

To test whether *Microphallus spp.* alter crayfish growth, I collected *O. rusticus*, *O. propinquus*, and *O. virilis* from the field, and examined their percent growth over four weeks and the timing of their summer molt to breeding form. I collected crayfish from some sites that had high infection levels and some sites that had low infection levels. Infection level at each site was determined beforehand by dissecting 3-6 crayfish (*O. rusticus, O. propinquus* and *O. virilis*) collected from infected and uninfected sites and counting metacercariaes encountered in the liver.

Uninfected *O. propinquus* where collected from Palmer Lake, MI and the infected ones where collected in Tenderfoot Lake, MI. Infected *O. virilis* where collected from Plum Lake, MI and the uninfected where collected at Horsehead Lake, WI. *O. propinquus* and *O. virilis* where housed in the wet lab. We collected 50 *O. propinquus* (25 from a high infection site and 25 from a low infection site site) and 50 *O. virilis* (25 from low infection site and 25 from high infected site). *O.propinquus* and *O. virilis* were each put in a individual container (10cm height, 18cm length and 16cm width) that was perforated to allow the water flow in and out. These containers were placed in large pools with constantly flowing aerated well water. *O. rusticus* was collected and housed in Big Lake. We collected 20 *O.rusticus* from a high infection site and 20 *O.rusticus* from a low infection site with a total of 40 *O. rusticus*. In Big Lake, we put each *O. rusticus* in an individual plastic container (13cm height, 35cm length and 19cm width), with small holes to let the water flow in and out. I used only male crayfish because they molt synchronously in early July. I fed each crayfish 3 to 5 shrimp pallets and 3 spinach leaves once per week. At the end of the experiment, I preserved the crayfish in 70 % ethanol and dissected them to remove the hepatopancreas. To assess infection level, I flattened the hepatopancreas between two glass slides and counted the number of metacercariae present under a dissecting microscope.

All statistical analysis where run using SYSTAT and SAS. I graphically examined the relationship between percent growth and initial carapace length in uninfected crayfish. Because initial carapace length was a strong predictor of percent growth in *O. rusticus*, I ran an ANCOVA comparing percent growth in infected and uninfected *O. rusticus*, using carapace length as the covariate. In addition, I divided *O. rusticus* into two groups, small (35mm or below) and large (above 35mm) and ran t-tests examining percent growth in uninfected and infected crayfish. I also ran two-way t-tests to compare percent growth of infected and uninfected *O.propinquus* and *O.virilis*. To calculate percent growth I used the final carapace length (in mm) minus the initial carapace length divided by the initial carapace length.

For molting timing I examined whether the crayfish were in breeding (I) or non-breeding (II) form each week. I chose a date where roughly half of the crayfish had molted and compared the infection level of form I and form II crayfish using a two-sample t-test.

Results

As I expected, the statistical analysis suggested there was a significant difference in growth between infected and uninfected *O. rusticus*. Infected *O. rusticus* grew less than the uninfected *O. rusticus* ($F_{3,36}$ = 8.05, P = 0.008, Figure 1). In addition, there was a significant effect of carapace length on the percent of growth of infected or uninfected O. *rusticus* ($F_{3,36}$ = 40.47, P < .001, Figure 1) and a significant interaction between infection level and carapace length ($F_{3,36}$ = 7.12, P =0.012, Figure 1).

We did not use the molting time for *O. rusticus* and *O. virilis*. There was not a week where I saw half of the crayfish that had molted and the other half did not molted. *O rusticus* and *O. virilis* molted simultaneously the first week of July; therefore, we did not have enough equally divided data of breeding form I and non- breeding form II to compare the infection level of each one.

Because of the significant interaction between carapace length and infection level, I explored whether the effect of infection level was different between large (36-41 mm carapace length) and small (26-35 mm carapace length) *O. rusticus* and Iran two-sample t-test. Small uninfected *O. rusticus* grew more than small infected *O. rusticus* (t(20)= 3.097, p=0.006, Figure2), but there was no difference in growth between infected and uninfected large *O. rustiucus* (t(14)= -0.799, p= 0.438, Figure 3). The mean of infection level for small *O. rusticus* was 0.093 (± 0.011) while the mean of infection level for large *O. rusticus* was 0.027 (±0.009).

Microphallus spp. did not have an effect on *O.propinquus* growth. There was no significant difference in growth of infected and uninfected *O. propinquus* (t (15)= -0.171, p= 0.866, Figure 4) and also no effect of infection on the molting time (t(13)= 0.676, p=0.511, Figure 5). The mean of infection level in *O. propinquus* was 0.013 (±0.007). The difference was not significant, but on average, infected *O. propinquus* grew more than uninfected *O. propinquus*.

There was no significant interaction between infected and uninfected *O. virilis*. Uninfected *O. virilis* grew more than infected *O. virilis* (t (1.9) = 1.874, p= 0.076, Figure 6). The mean for infection level on *O. virilis* was 0.031 (± 0.014).

As the project went on we had high mortalities of *O. virilis* and *O. propinquus* likely due to low oxygen in their containers.

Discussion

We found a strong relationship between infection, carapace size and percent of growth in *O. rusticus* even though most of the crayfish were only infected at low levels. This suggests that even at very low levels, infection with parasites can reduce rusty crayfish fitness.

Microphallus spp., affect digestive organs in other species, and therefore, their fitness (Robaldo et al.1999). My results suggest that *Microphallus spp*. can also affect fitness of *Orconectes* crayfish, but that they have different effects on different species and sizes of crayfish. It does not appear that the extent of infection made a difference for *O. rusticus*, but that smaller O. rusticus were more affected than large *O. rusticus*.

If the *O. rusticus* is small, one parasite can have a greater effect on its digestive organs than a bigger *O. rusticus* which may be because it has a larger hepatopancreas. For the uninfected *O. rusticus,* small ones grew faster than the big ones, but this may have been due to feeding. Small animals also often have faster growth than large animals. We fed the same amount to small and large *O. rusticus*. If uninfected *O. rusticus* grow faster they may have greater fitness because larger crayfish produce more offspring and can escape fish predation. On other hand small infected *O. rusticus* have a increasing aggressiveness, due to the infection of the parasite as reported on other researches, this can lead to energy cost and therefore and effect of the crayfish fitness. Because its size, it can also be victim of predation.

Large infected crayfish may still be affected by *Microphallus spp*. in ways that were not examined in this paper. In other studies they have found that parasites can alter freshwater pulmonate snail fitness, resulting in reduced reproduction on infected individuals (Bernot 2003).

O. virilis grew more when uninfected than infected. We know that this parasite affects the crayfish digestive organs (Robaldo et al.1999), and therefore it could affect their fitness. One possibility is that this parasite has recently arrived in the lakes where the *O. virilis* was collected.

Opposite findings were observed in *O. propinquus*. There was no difference in growth between infected and uninfected *O. propinquus*. Koskella 2006 found that snails exposed to *Microphallus spp.* parasites developed adaptations to these parasites. It is possible that in the locations where the host of this parasite is abundant, food sources are abundant, resulting in *O. propinquus* fast growth, not mattering if is infected with this parasite, because is possible that *O. propinquus* has adapted to better cope with *Microphallus spp.* parasites.

Overall, we found that *Michrophallus spp.*, mediate *Orconectes* crayfish growth, which suggests that it has the potential to alter crayfish fitness and possibly crayfish populations. This may be why the presence of these parasites (*Microphallus spp*.) is associated with decreases in *O. rusticus* populations (Roesler 2009). Future research should examine the effect of different levels of infection on *O.rusticus*.

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Figures and Tables

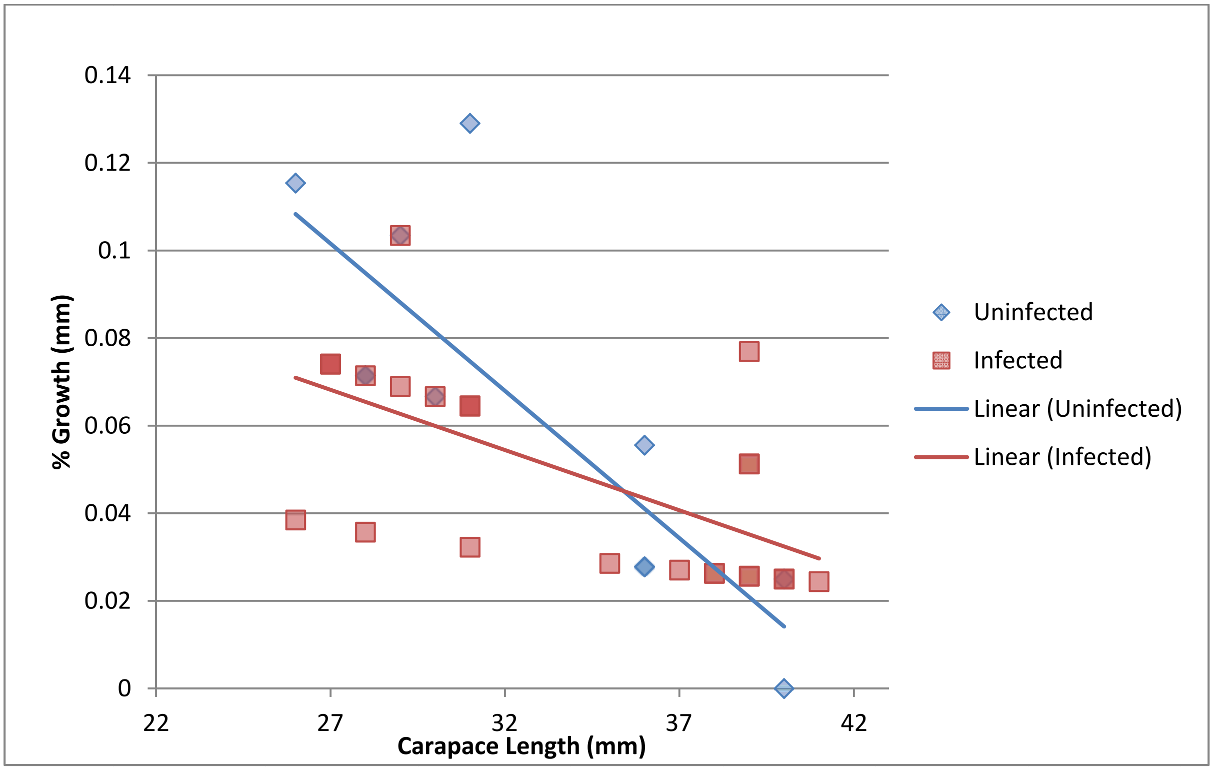

Figure 1. Relationship between growth and carapace length of infected and uninfected *O. rusticus.*

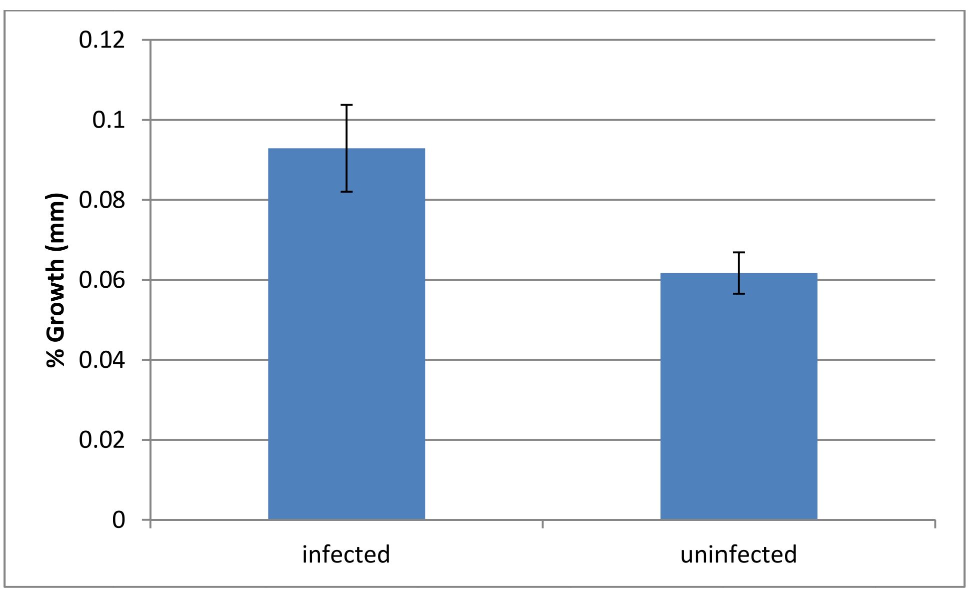

Figure 2. Percent Growth of infected and uninfected *O. rusticus.*

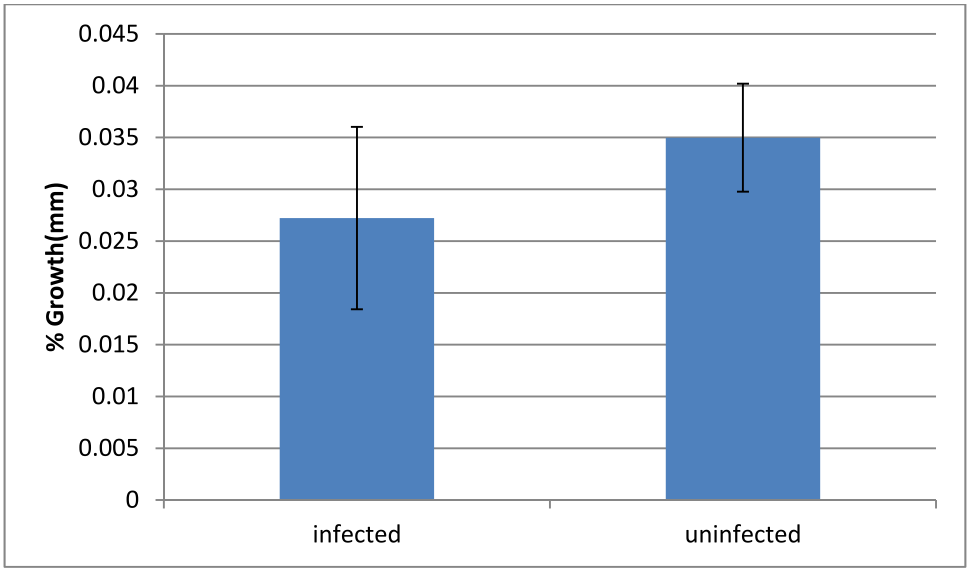

Figure 3. Percent growth of infected and uninfected *O. rusticus*.

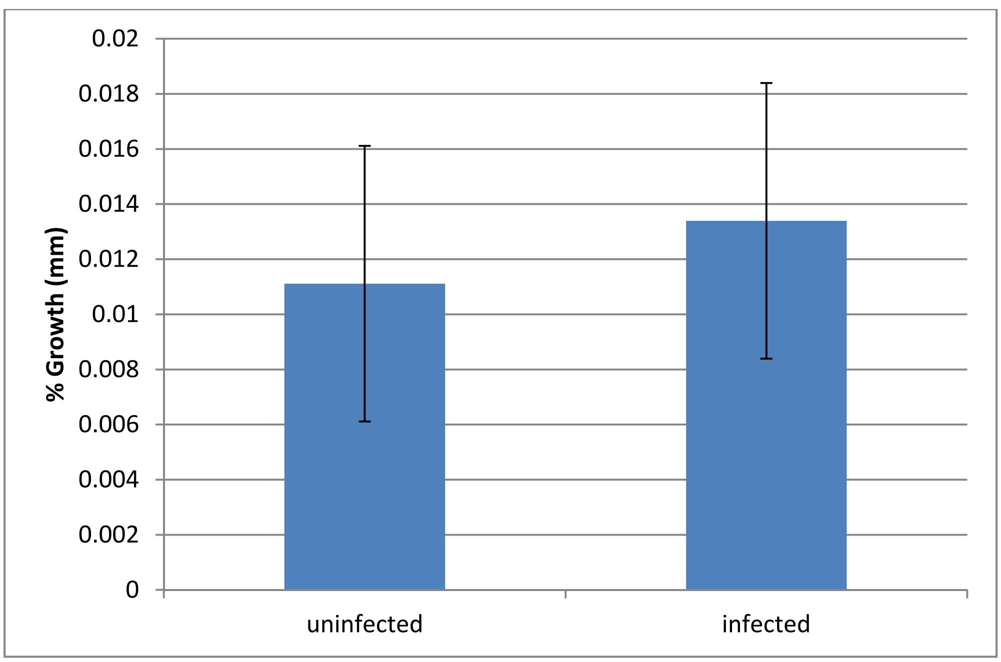

Figure 4. Percent growth of uninfected and infected O. *propinquss*.

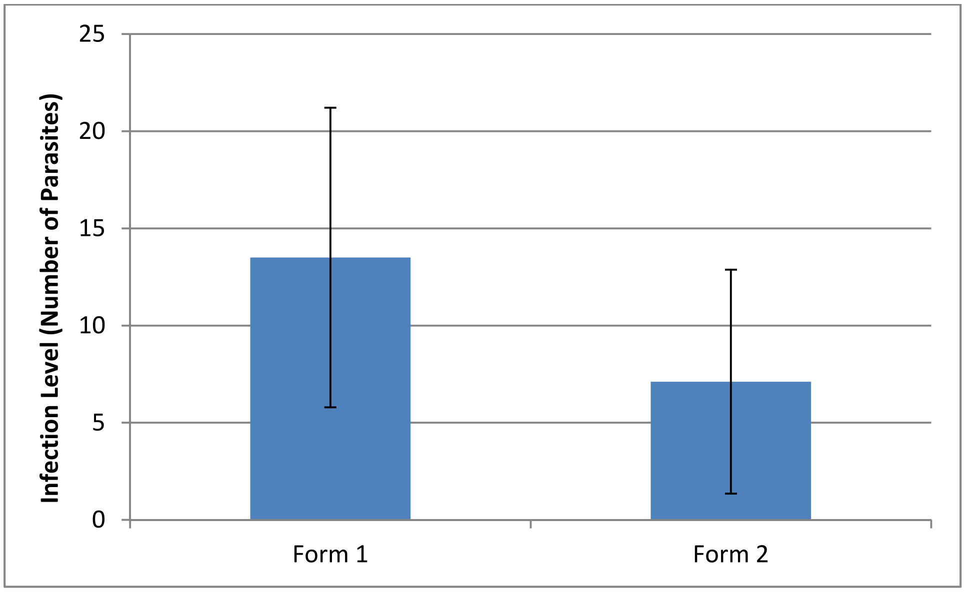

Figure 5. *O.propinquus* forms versus infection level.

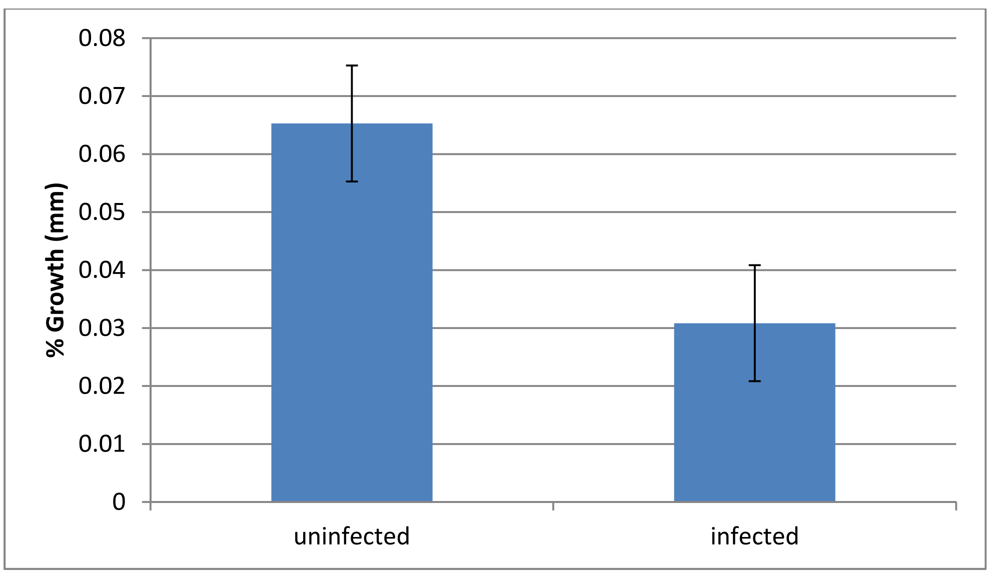

Figure 6. Percent growth of uninfected and infected *O. virilis.*