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Author(s) :Miranda D. Redmond, Richard B. Hartson, Jason T. Hoverman, Christina N. De Jesús-Villanueva, and Pieter T. J. Johnson

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EXPERIMENTAL EXPOSURE OF *HELISOMA TRIVOLVIS* AND *BIOMPHALARIA GLABRATA* (GASTROPODA) TO *RIBEIROIA ONDATRAE* (TREMATODA)

Miranda D. Redmond, Richard B. Hartson*, Jason T. Hoverman, Christina N. De Jesús-Villanueva†, and Pieter T. J. Johnson
Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado 80309. *e-mail: mirandaredmond@gmail.com*

ABSTRACT: Experimental infections provide an important foundation for understanding host responses to parasites. While infections with *Ribeiroia ondatrae* cause mortality and malformations in a wide range of amphibian second intermediate host species, little is known about how the parasite affects its snail first intermediate hosts or even what species can support infection. In this study, we experimentally exposed *Helisoma trivolvis,* a commonly reported host of *R. ondatrae,* and *Biomphalaria glabrata*, a confamilial snail known to host *Ribeiroia marini*, to increasing concentrations of embryonated eggs of *R. ondatrae* obtained from surrogate definitive hosts. Over the course of 8 wk, we examined the effect of parasite exposure on infection status, time-to-cercariae release, host size, and mortality of both snail species. *Helisoma trivolvis* was a highly competent host for *R. ondatrae* infection, with over 93% infection in all exposed snails, regardless of egg exposure level. However, no infections were detected among exposed *B. glabrata*, despite previous accounts of this snail hosting a congener parasite. Among exposed *H. trivolvis*, high parasite exposure reduced growth, decreased time-to-cercariae release, and caused marginally significant increases in mortality. Interestingly, while *B. glabrata* snails did not become infected with *R. ondatrae,* individuals exposed to 650 *R. ondatrae* eggs grew less rapidly than unexposed snails, suggesting a sub-lethal energetic cost associated with parasite exposure. Our results highlight the importance of using experimental infections to understand the effects of parasite exposure on host- and non-host species, each of which can be affected by exposure.

Ribeiroia ondatrae is a trematode found in wetland habitats across much of North America (Johnson and McKenzie, 2008). This parasite uses rams horn snails (Planorbidae) as first intermediate hosts, fishes and amphibians as second intermediate hosts, and birds or mammals as definitive hosts (Price, 1931; Beaver, 1939; Johnson et al., 2004). *Ribeiroia ondatrae* can have deleterious effects on amphibian intermediate hosts, in which infections cause increased mortality and severe limb malformations, ranging from missing limbs to multiple supernumerary limbs (Johnson et al., 1999, 2001; Szuroczki and Richardson, 2009; Johnson et al., 2010; Rohr et al., 2010). These malformations are hypothesized to increase the susceptibility of infected amphibians to definitive host predators (Rohr et al., 2009). In avian definitive hosts, *R. ondatrae* infection can produce necrotic lesions in the mucosa of the proventriculus (Newsom and Stout, 1933; Beaver, 1939; Leibovitz, 1961; Dyer et al., 2002), sometimes leading to increased mortality (Newsom and Stout, 1933; Leibovitz, 1961; Basch and Sturrock, 1969).

Although *R. ondatrae* can infect a wide range of amphibians and birds (Johnson et al. 2004), less is known about its use of first intermediate hosts, including what species are susceptible and how infection influences host fitness. As with many trematode species (Baudoin, 1975; Crews and Esch, 1987; Negovetich and Esch, 2008), *Reibeiroia* spp. infections cause complete castration in snail hosts (Harry, 1965; Huizinga, 1973), which can reduce population size. For instance, in a large-scale field experiment, Nassi et al. (1979) found that the introduction of 7–9 million *Ribeiroia marini* eggs caused a temporary collapse in a *Biomphalaria glabrata* population, thereby reducing available hosts for the human blood fluke *Schistosoma mansoni*. These results highlight the potential consequences of *Ribeiroia* spp. infection on snail host population dynamics. However, because few studies have experimentally examined the effects of different *Ribeiroia* sp. exposure levels on snail hosts, it is unclear how many miracidia are required to induce infection successfully and what other effects occur in suitable first intermediate host species. Given the potential negative effects of *R. ondatrae* on its hosts, including amphibians, birds, and snails, there is a need for more mechanistic studies that examine the effects of infection on host fitness.

Ribeiroia ondatrae has been reported in the United States and Mexico, where its first intermediate hosts are planorbid snails (Beaver, 1939; Kuntz, 1951; Malek, 1977; Fried et al., 1998; Johnson et al., 2004; Peterson, 2007). Species of *Helisoma* (=*Planorbella*) are among the most commonly reported first intermediate hosts of *R. ondatrae* (9 of 12 records in Johnson et al., 2004). In the southern United States, the Caribbean, and South America, species of *Biomphalaria* have also been recorded to support different species of *Ribeiroia*, e.g., *B. glabrata* is the only known snail host of *R. marini* (Basch and Sturrock, 1969; Huizinga, 1973; Johnson et al., 2004). Native to the Neotropics (Campbell et al., 2000), *B. glabrata* also serves as a host of the human parasite *S. mansoni* (Loker, 1982; Théron and Moné, 1986). *Ribeiroia marini* is antagonistic with *S. mansoni*, such that rediae of *R. marini* will actively consume schistosome sporocysts within infected snails (Page and Huizinga, 1976). Indeed, *R. marini* has historically been used as a control agent against *B. glabrata* and *S. mansoni* (Nassi et al., 1979; Paraense, 1987; Pointier, 1989). In the southern United States, *Biomphalaria obstructa* has also been recorded as a host for *R. ondatrae* (Malek, 1977). Despite these observations, there is no information about whether *B. glabrata* can host *R. ondatrae*, which could provide a useful experimental system for further studies into the ecology and pathogenesis surrounding *Ribeiroia* spp. infections.

In the current study, we experimentally exposed *Helisoma trivolvis* and *B. glabrata* to a range of *R. ondatrae* exposure levels to compare host competency for the 2 species under controlled conditions and quantify the effects of exposure on snail survival, growth, and the maturation of infection. In 2 separate experiments, we quantified the effects of *R. ondatrae* exposure on the survival, growth, and maturation of infection within *H. trivolvis* (Experiment 1) and *B. glabrata* (Experiment 2). In Experiment 2, which was conducted a separate year from Experiment 1, we also included a small number of *H. trivolvis* to confirm *R. ondatrae* egg viability and to test susceptibility to *R. ondatrae* among 2 different

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* Department of Water Resources, University of Idaho, Moscow, Idaho 83843.
† Department of Biology, University of Puerto Rico, Humacao, Puerto Rico.
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H. trivolvis populations (California [Experiment 1] and Wisconsin [Experiment 2]). By exposing individual snails to embryonated eggs obtained from surrogate definitive hosts under controlled conditions, we aimed to quantify the dose-dependent effects of *R. ondatrae* on multiple dimensions of first intermediate host fitness. Given the importance of first intermediate hosts in determining trematode distributions and the noteworthy pathology caused by *R. ondatrae* infections in amphibians and select bird species, these results have the potential to help further our understanding of the ecology of this parasite.

MATERIALS AND METHODS

Parasite culturing

We obtained embryonated eggs of *R. ondatrae* from experimentally infected Holzman rats (75–124 g). Male rats (n = 10–15) were raised in the laboratory and exposed to *R. ondatrae* metacercariae (n = 50–75) isolated from infected amphibians (*Lithobates catesbeianus* and *Pseudacris regilla*). After 2 wk, rat fecal matter was collected on wet paper towels, soaked in spring water for 24 hr at 5 C, and then filtered through a sieve series (largest to smallest: 2 mm, 425 μm, 180 μm, 106 μm, 45 μm). The remaining rat fecal matter was placed in 2 mason jars, 2 L each, filled with 1.9 L commercial spring water and incubated at 23–27 C in the dark for 3 wk. During this time, spring water was changed weekly and aerated continuously to prevent bacterial buildup. Eggs were re-filtered through the 45-μm sieve during each water change. After 3 wk, we determined *R. ondatrae* egg density by quantifying the number of viable eggs in at least 10 sub-samples, 10 μl each, examined at ×200 magnification. Only eggs that appeared viable were included in this count; any eggs that had already hatched or no longer contained a developing miracidium were excluded from the count. We used these *R. ondatrae* egg density estimates to determine the amount of rat fecal material each snail should be administered based on their treatment.

Experiment 1: *Helisoma trivolvis*

Helisoma trivolvis were obtained from the Art Oehmnke Fish Hatchery in Woodruff, Wisconsin, and maintained for several generations under laboratory conditions. We placed 80 uninfected adult *H. trivolvis* individually into 1-L tubs filled with 0.75 L of filtered lake water. We measured the shell width of each snail before placing it into the tub (mean shell width ± 1 SE = 11.4 ± 0.1 mm). Tubs each contained 1 snail and were randomly assigned to 1 of 4 treatments (0, 50 [±4], 400 [±30], or 1,000 [±74] *R. ondatrae* eggs). Each treatment was replicated 20 times. We administered the treatments by slowly lowering a 60 × 15 mm–Petri dish filled with the appropriate amount of filtered material based on the sub-sample examinations into the bottom of each tub. A comparable amount of fecal material from uninfected rats was added to snails in the control treatment.

Snail water was changed weekly, and any snails that died were necropsied to determine infection status. During the first 2 wk, snails received a partial (50%) water change to prevent the loss of unhatched *R. ondatrae* eggs or miracidia. Thereafter, snails received complete (100%) water changes. Throughout the experiment, we fed snails romaine lettuce and a 1:1 mixture of ground TetraMin tropical flakes and ground TetraVeggie Spirulina Enhanced Flakes ad libitum. During the experiment, air temperature was 28 ± 2 C, and the photoperiod followed a 16:8 light:dark cycle. On days 32, 38, and 43 post-exposure, we placed each snail into a 50-ml centrifuge tube filled with 40 ml treated water to determine whether snails were releasing cercariae and confirm patent infections. Given that *R. ondatrae* releases cercariae at night (Beaver, 1939), snails were left in a dark room overnight, and in the morning the centrifuge tube water was examined for cercariae. The experiment was terminated on day 44 post-exposure, and final shell width was measured. All snails that did not release cercariae on day 32, 38, or 43 were necropsied to determine infection status.

Experiment 2: *Biomphalaria glabrata*

We obtained *B. glabrata* that were known to be susceptible to *S. mansoni* from the National Institutes of Health and raised them through multiple generations in the laboratory. For this experiment, *H. trivolvis* snails were raised through multiple generations in the laboratory and originally obtained from a wetland in Santa Clara County, California. We placed 60 uninfected juvenile *B. glabrata* (mean shell width ± 1 SE = 2.98 ± 0.09 mm) and 15 uninfected juvenile *H. trivolvis* (mean shell width ± 1 SE = 7.10 ± 0.28 mm) individually into 1-L tubs filled with 0.75 L of dechlorinated tap water. Owing to a limited number of available *R. ondatrae* eggs, in this experiment each tub containing 1 snail was randomly assigned to 1 of 3 treatments (0, 65 [±11], or 650 [±108] *R. ondatrae* embryonated eggs). Each treatment was replicated 20 times for *B. glabrata* and 5 times for *H. trivolvis*. We administered the treatments by slowly lowering a 60 × 15 mm–Petri dish filled with the allocated amount of infected rat feces (0, 0.273, and 2.73 ml of infected rat feces for the 0-, 65-, and 650-egg treatment, respectively) into the bottom of each tub. Snails assigned to the control treatment, i.e., 0 eggs, were given 1.5 ml of uninfected rat feces.

Because differences in snail sizes can influence infection likelihood (Huizinga, 1973), we placed 20 *B. glabrata* and 10 *H. trivolvis* snails of varying sizes (3–12 mm shell width) as a group within a 11.3 L–container filled with dechlorinated tap water and exposed them to a large quantity of infected rat feces (>1,000 eggs). This group exposure treatment was used to test infection results when varying sizes of *B. glabrata* and *H. trivolvis* were exposed concurrently to large concentrations of *R. ondatrae* eggs.

We changed snail water weekly and dissected all snails that died to determine *R. ondatrae* infection. During the first 2 wk, snails received a partial (50%) water change to prevent the loss of unhatched *R. ondatrae* eggs or miracidia. Thereafter, snails received complete (100%) water changes. Every 3–4 days during the experiment, all snails were fed a small (~125 mm^3) cube containing ground TetraMin tropical flakes solidified with agar. The food was prepared by mixing 40 ml of treated tap water, 0.5 g of agar, and 3 g of ground TetraMin tropical flakes. Throughout the experiment air temperature was 23 ± 2 C, and the photoperiod followed a 14:10 light:dark cycle. We accidentally damaged 2 *B. glabrata* (1 snail each from the 65 and 650 treatments) and 1 control *H. triovlvis* during the first water change, and their data were omitted from the analysis. Shell width was measured 23 and 50 days post-exposure to estimate growth in all *B. glabrata* snails, except for those snails in the group exposure treatment. On days 29 and 50 post-exposure, we placed each *B. glabrata* snail into an individually labeled 50-ml centrifuge tube filled with 40 ml of water in a dark room overnight to determine whether snails were releasing cercariae and confirm patent infections. After 51 days, all snails that had not released cercariae were dissected to determine infection status.

Analyses

We evaluated the effects of different *R. ondatrae* exposure levels on the survival, growth, infection prevalence, and time-to-cercariae release of *H. trivolvis* (Experiment 1) and *B. glabrata* (Experiment 2). We used logistic regression to assess the relationship between *R. ondatrae* exposure level and the infection status, time-to-cercariae release, and mortality of *H. trivolvis* and *B. glabrata*. For time-to-cercariae release, a logistic regression was performed for each sampling date. For all logistic regression tests, we used a Wald's chi-square test to compare among treatments. Because *Ribeiroia* sp. requires 1–2 wk to achieve detectable infection in snails after exposure (Huizinga, 1973), snails that died earlier than 3-wk post-exposure were not included in the infection analysis. To examine the effects of the parasite exposure on snail growth, we used analysis of variance (ANOVA) for *H. trivolvis* and repeated-measures ANOVA for *B. glabrata* with treatment as a fixed effect. We used repeated-measures ANOVA for *B. glabrata* because growth was assessed twice during the experiment (1–3-wk post-exposure and 4–7-wk post-exposure). We used Tukey's pairwise comparisons to identify significant differences among groups. All analyses were conducted using SPSS 19 (SPSS, Chicago, Illinois).

RESULTS

Experiment 1: *Helisoma trivolvis*

Exposure to *R. ondatrae* did not significantly affect *H. trivolvis* survival (logistic regression, $\chi^2 = 3.3$, df = 3, $P = 0.352$). However, mortality tended to be higher in the 1,000-egg treatment

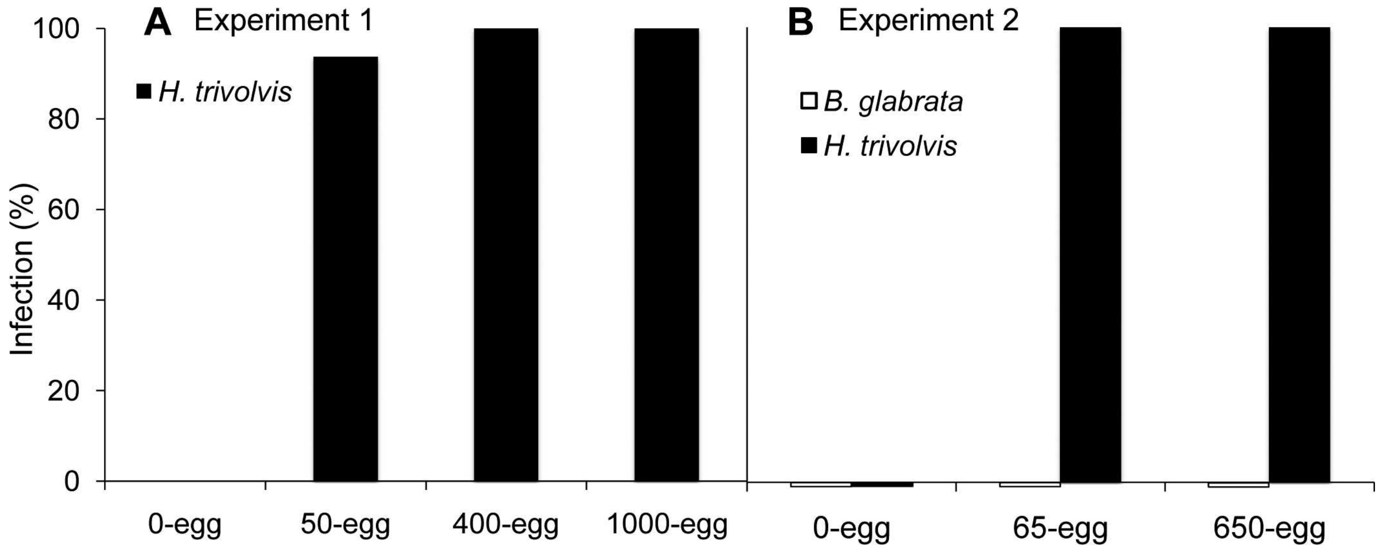

FIGURE 1. Percentage of *Helisoma trivolvis* in Experiment 1 (**A**) and *H. trivolvis* and *Biomphalaria glabrata* in Experiment 2 (**B**) that became infected with *Ribeiroia ondatrae* and lived for at least 3-wk post-exposure in each treatment.

(45%) compared with the 0-, 50-, and 400-egg treatments (25%, 30%, 20% mortality, respectively). For *H. trivolvis* that lived for at least 3-wk post-exposure, *R. ondatrae* exposure significantly affected infection status (logistic regression, $\chi^2 = 72.4$, df = 3, $P < 0.001$, Fig. 1A). While none of the control snails (n = 18) became infected with *R. ondatrae*, 93.8%, 100%, and 100% of the snails in the 50-egg (n = 16), 400-egg (n = 16), and 1,000-egg treatment (n = 17) became infected, respectively (Fig. 1A). There were no differences in infection status among the egg exposure treatments ($P \geq 0.236$; Fig. 1A). Among snails that died before wk 3 in the 50-, 400-, and 1,000-egg treatments (n = 4, 4, and 3 respectively), none was infected with *R. ondatrae.*

Ribeiroia ondatrae exposure significantly affected the proportion of *H. trivolvis* that released cercariae on each sample date (logistic regression, $\chi^2 \geq 12.9$, df = 3, $P \leq 0.005$; Fig. 2). On all sample dates, exposure to parasite eggs significantly increased time-to-cercariae release compared with the control treatment ($P \leq 0.004$). Differences between *R. ondatrae* exposure levels varied by days post-exposure with significantly more *H. trivolvis* releasing cercariae day 43 post-exposure than day 32 post-exposure ($P < 0.003$, Fig. 2). On day 32 post-exposure, 31.9% of all exposed snails released cercariae, but there were no differences among the 3 different egg exposure treatments (Fig. 2). However, by day 38 post-exposure, the proportion of snails releasing cercariae increased monotonically with egg exposure level, with a significantly greater proportion of 1,000-egg snails releasing cercariae than 50-egg snails ($P < 0.003$, Fig. 2). On the final sampling date (43 days post-exposure), 100% of surviving exposed snails released cercariae (Fig. 2), at which point the experiment was terminated.

Helisoma trivolvis growth exhibited a concave relationship with exposure, such that growth tended to be greatest in the 50- and 400-egg exposure treatment but lower in the control and 1,000-egg treatments ($F = 3.619$, df = 3, $P = 0.019$, Fig. 3). Snails in the 1,000-egg treatment grew significantly less than snails in the 50-egg treatment ($P = 0.032$, Tukey's test, Fig. 3), while there were no significant growth differences among the other treatments ($P \geq 0.0956$, Tukey's test, Fig. 3). Host egg mass data were not collected in this study because *H. trivolvis* tend to produce few eggs when isolated into individual containers (Escobar et al., 2011).

Experiment 2: *Biomphalaria glabrata*

Biomphalaria glabrata exhibited high survival (>89%), with no significant differences in mortality among treatments (logistic regression, $\chi^2 = 2.9$, df = 2, $P = 0.236$). None of the 58 surviving *B. glabrata* became infected with *R. ondatrae*, regardless of egg exposure level (Fig. 1B). Correspondingly, no *B. glabrata* released cercariae and, upon dissection, no evidence of trematode rediae was noted. However, and in support of the results of Experiment 1, all *H. trivolvis* exposed to *R. ondatrae* in Experiment 2 that lived for at least 17 days were infected (n = 9), as determined from necropsies and cercariae release (Fig. 1B). Furthermore, within the group exposure treatment involving 20 *B. glabrata* and 11

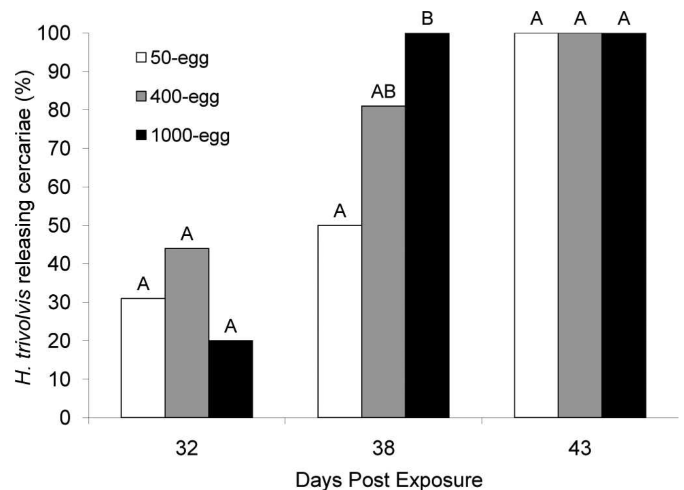

FIGURE 2. Percentage of *Helisoma trivolvis* within each treatment that released cercariae within each sampling event. Treatments at each sampling event sharing letters are not significantly different based on based on Wald's chi-square tests ($P > 0.05$).

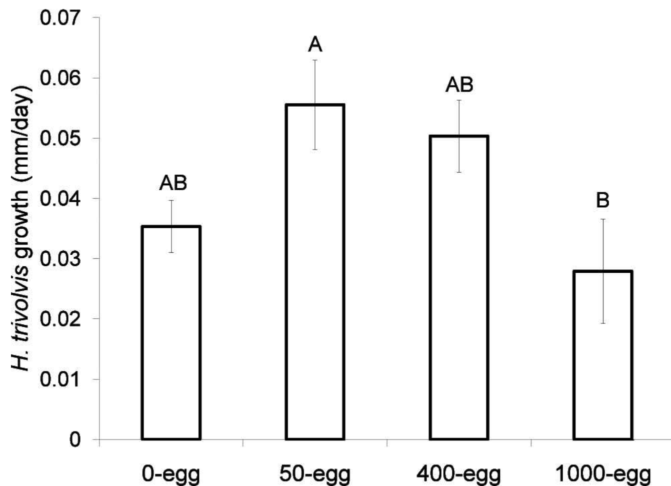

FIGURE 3. Daily growth rate (mm/day) of *Helisoma trivolvis* exposed to increasing numbers of *Ribeiroia ondatrae* eggs. Growth rate was assessed at wk 6 post-exposure. Treatments sharing letters are not significantly different based on Tukey's test ($P > 0.05$). Average initial sizes of *H. trivolvis* in each treatment were all within 1 standard error of each other. Data are means ± 1 SE.

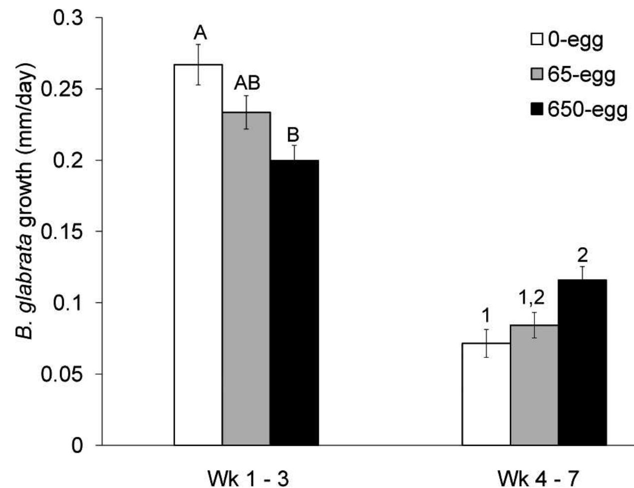

FIGURE 4. Daily growth rate (mm/day) of *Biomphalaria glabrata* exposed to increasing numbers of *Ribeiroia ondatrae* eggs. Growth rate was assessed at weeks 3 and 7 post-exposure. Within the same growth period, treatments sharing letters within weeks 1–3 and numbers within weeks 4–7 are not significantly different based on Tukey's test ($P > 0.05$). Data are means ± 1 SE.

H. trivolvis exposed to >1,000 eggs concurrently, all of the *H. trivolvis* were infected, whereas none of the *B. glabrata* was infected.

The repeated-measures ANOVA on snail growth exhibited significant effects of time ($F_{1,52} = 196.4$, $P < 0.001$) and the time × exposure treatment interaction ($F_{2,52} = 10.2$, $P < 0.001$), but not the main effect of exposure treatment ($F_{2,52} = 0.7$, $P = 0.524$). Given the significant interaction, we examined the effects of the parasite exposure treatment on growth for each observation date. *Biomphalaria glabrata* growth was significantly different among exposure treatments during the first 3-wk post-exposure ($F = 7.210$, df = 2, $P = 0.002$, Fig. 4) and from wk 4–7 post-exposure ($F = 6.025$, df = 2, $P = 0.004$, Fig. 4). During the first 3-wk post-exposure, snails in the control treatment grew significantly more than snails in the 650-egg treatment ($P < 0.001$, Tukey's test, Fig. 4). There were no differences in growth of *B. glabrata* between the 0-egg and 65-egg or between the 650-egg treatment (all $P > 0.14$, Tukey's test, Fig. 4). From wk 4 through 7, growth of *B. glabrata* in the 650-egg treatment was greater than the 0-egg treatment ($P = 0.0039$, Tukey's test, Fig. 4) and marginally greater than the 65-egg treatment ($P = 0.0591$, Tukey's test, Fig. 4). However, there was no difference in growth between the 0-egg and 65-egg treatments ($P = 0.62$, Tukey's test, Fig. 4).

DISCUSSION

Growing attention has focused on the ecology and pathology of *R. ondatrae,* which causes severe malformations in amphibian hosts. However, little is known about its infection patterns or pathology in its first intermediate host. Results of our experimental exposure study revealed clear differences in the susceptibility of snail species to infection by *R. ondatrae*. The high infection prevalence (>93%) of *H. trivolvis* exposed to 50–1,000 *R. ondatrae* embryonated eggs demonstrates that both *H. trivolvis* from California and Wisconsin are susceptible to *R. ondatrae.* In contrast, none of the 58 surviving *B. glabrata* exposed to *R. ondatrae* became infected. Moreover, when *H. trivolvis* and *B. glabrata* representing a range of sizes (3–12 mm) were exposed concurrently to >1,000 eggs ('group exposure treatment'), all of the *H. trivolvis* became infected, while none of the *B. glabrata* became infected. Taken together, these findings provide compelling evidence of variation in competency among planorbid snail species, with *H. trivolvis* functioning as a highly competent host for *R. ondatrae* and *B. glabrata* being resistant to infection.

Given that *B. glabrata* has been recorded as a host for *R. marini* and *B. obstructa* as a host for *R. ondatrae,* the lack of susceptibility of *B. glabrata* to *R. ondatrae* is rather surprising. Native to the Neotropics (Campbell et al., 2000), *B. glabrata* is the only snail host of *R. marini* and *R. marini guadeloupensis* (Harry, 1965; Huizinga, 1973). Moreover, in Louisiana, Malek (1977) reported *B. obstructa* supported what was identified as *R. ondatrae. Ribeiroia ondatrae* and *R. marini* are closely related species (Wilson et al., 2005), such that apparent differences in usage of snail hosts, even within the same family, is striking. Considering that we exposed 60 *B. glabrata* to viable *R. ondatrae* embryonated eggs, which included a range of snail sizes, and the majority were the size typically most susceptible to infection (Richards, 1977), we are confident that the strain of *B. glabrata* used was not susceptible to infection. However, we cannot rule out different strains of *B. glabrata*, and we emphasize future work using field-collected strains. These results highlight the importance of host-by-parasite compatibility (Combes, 2001; Théron and Coustau, 2005). As one of the first studies to examine the effects of *R. ondatrae* infection on snail hosts under controlled laboratory conditions, this experiment furthers our understanding of the life cycle of *R. ondatrae*, including both time to infection maturation and amount of exposure needed to cause infection, as well as the consequences of infection on snail fitness of both host and non-host species.

Results of our experimental infections involving *H. trivolvis* revealed that mature infections can occur within 32 days of exposure to embryonated *R. ondatrae* eggs, as evidenced by the 32% of exposed *H. trivolvis* that released cercariae. Furthermore, within 43 days of exposure, all exposed *H. trivolvis* had released

cercariae. This time course of infection is similar to other snail–parasite interactions (Basch and Sturrock, 1969; Gérard and Théron, 1997). For instance, in an experimental study, Basch and Sturrock (1969) found *B. glabrata* began to release *R. marini* cercariae 25–27 days after exposure to miracidia. Interestingly, while all surviving *H. trivolvis* exposed to *R. ondatrae* released cercariae by day 43 post-exposure (Fig. 2), a greater proportion of *H. trivolvis* exposed to higher dosages, e.g., 1,000-egg, released cercariae on day 38 post-exposure than those exposed to lower dosages, e.g., 50-egg (Fig. 2). These results suggest that initial exposure levels influenced the time-to-cercariae release, but did not affect the final infection prevalence.

Although not significant, high *R. ondatrae* exposure, e.g., 1,000-egg, potentially increased mortality in *H. trivolvis*. Increased mortality of infected snails compared with control snails has been observed in other snail–parasite systems (Nassi, 1978; Kuris, 1980; Krist et al., 2004; Blair and Webster, 2007). For example, Nassi (1978) found that *B. glabrata* exposed to *R. marini* experienced higher mortality relative to uninfected individuals. Additional research that uses larger sample sizes and longer experimental durations will help to determine whether parasite-induced mortality occurs in *H. trivolvis.*

Among *H. trivolvis, R. ondatrae* had mixed effects on growth; high dosages (1,000-egg) had no effect on growth, while low to moderate dosages (50- and 400-egg) increased growth (Fig. 2). Parasite-induced growth responses have been known to vary depending on when snails become infected (Sousa, 1983; Krist and Lively, 1988). Trematode infections in juvenile snails generally stunt or have no affect on growth, while infections in adult snails can enhance growth (Sousa, 1983; Krist and Lively, 1988). These growth differences in response to trematode infections are the result of differences in the investment to reproductive tissue, which is the target site for infection. Given that juvenile snails lack reproductive tissue, parasites draw their resources from the maintenance energy of the snail, resulting in reduced snail growth. In contrast, parasites are able to use the reproductive tissue of adult snails, thereby enabling the snail to allocate resources to growth rather than reproduction following infection. In addition to snail maturity level at time of exposure, previous research has found that trematode induced snail growth, known as 'snail gigantism', is also influenced by parasite pathogenicity (Gorbushin, 1997), snail food availability (Eisenberg, 1970; Fernandez and Esch, 1991), and snail life history strategy (Sousa, 1983; Minchella, 1985; Gorbushin, 1997). However, no study to our knowledge has reported threshold effects in snail growth, with increases up to a certain level of trematode exposure, followed by decreases in growth. Our growth results suggest that low levels of infection, i.e., stress, may enhance growth, i.e., hormesis, while higher levels suppress, or have no effect on, growth in adult *H. trivolvis*. Further experimentation is needed to fully explain this trend.

Interestingly, while *R. ondatrae* did not establish in *B. glabrata* successfully or increase mortality, *B. glabrata* did show an initial reduction in growth with increased exposure to *R. ondatrae*. This finding suggests that juvenile *B. glabrata* may be allocating energy toward preventing, or responding to, attempted infections, which resulted in reduced growth. Previous snail–trematode studies have found that *B. glabrata* and *Lymnaea elodes* experience reduced growth when exposed to *Echinostoma* spp. (Kuris, 1980; Sandland and Minchella, 2003). However, in these studies, the snails were suitable hosts for the trematode, as opposed to our findings in which even non-host species were affected by trematode exposure. The increase in growth of exposed compared with unexposed *B. glabrata* during the second half of the study (weeks 4–7) is likely due to compensatory growth leading to no final size difference among treatments. The initial stunted growth of exposed *B. glabrata* snails highlights the fact that while species may not be a host of a parasite, they can still be affected by exposure.

The high susceptibility of *H. trivolvis* to *R. ondatrae*, as well as the concave growth response of *H. trivolvis*, has potential implications for the effects of *R. ondatrae* on snail populations in the wild. Given that *R. ondatrae* causes complete castration in its snail hosts (Harry, 1965; Huizinga, 1973), the high susceptibility of *H. trivolvis* to *R. ondatrae* infection when exposed to $\geq$50 eggs indicates the potential of *R. ondatrae* to influence population dynamics when comparable amounts of *R. ondatrae* miracidia are introduced to the system. Similarly, Nassi et al. (1979) found the addition of 7–9 million *R. marini* eggs resulted in a temporary collapse in a *B. glabrata* population. However, little is known about the number of *R. ondatrae* eggs and miracidia present in natural systems, and we, therefore, have no information on how our experimentally administered egg levels compare to what snails experience in nature. Our findings of *H. trivolvis* having a concave growth response, with mid-exposure treatment levels having the maximum growth rate, suggest that exposure, especially the quantity of exposure, could have important effects in natural systems. In natural populations, snail size is an important determinant of susceptibility to predators, both vertebrate and invertebrate (Osenberg and Mittelbach, 1989; Chase, 2003). Thus, parasitism and predation could interact in important ways to influence snail population indirect effects (Bernot and Lamberti, 2008).

Our results underscore the importance of using experimental infections to better understand the effects of parasites on potential host species. Through experimental exposure, these findings demonstrate the susceptibility of *H. trivolvis* collected from 2 different locations to *R. ondatrae* infection. In contrast, *B. glabrata* was not a suitable host for *R. ondatrae*, despite reports of susceptibility to similar trematode species (*R. marini*) and 1 account of *R. ondatrae* infection in a congener (*B. obstructa*). Furthermore, we found that high parasite exposure reduced growth in juvenile snails in both species, decreased time-to-cercariae release in *H. trivolvis*, and may increase *H. trivolvis* mortality. Additional exposure studies that include smaller exposure levels (<10 eggs) are needed to determine whether infection patterns are linearly related to exposure or display thresholds. Such data will provide a more comprehensive understanding of host–parasite interactions within natural communities in which exposure is highly heterogeneous. Furthermore, studies that examine the effects of *R. ondatrae* exposure and infection on snail reproduction would aid in understanding the effects of *R. ondatrae* on snail populations. While laboratory infection experiments such as this one provide a good foundation in understanding parasite life cycles and the effects of infection on hosts, it is important to follow up these experiments with field studies.

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