Assessing host competency between native and invasive snail species exposed to the native parasite *Echinostoma revolutum*

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**Abstract**

Invasive species have the ability to rapidly and extensively alter native ecosystems, and there is accumulating evidence to suggest that the introduction of invasive hosts can have influences on parasite transmission in native communities. In 2002, the aquatic snail *Bithynia tentaculata* was discovered in the Upper Mississippi River (UMR) where it now co-occurs with several native snails and their parasites. The goal of this study was to determine the competencies of a native snail (*Physa gyrina*) and an invasive snail (*B. tentaculata*) after controlled exposure to a native parasite species (*Echinostoma revolutum*). Results of our laboratory experiment indicated no difference in either the prevalence or intensity of infection between native and invasive snails, which was unexpected given past work on *B. tentaculata*. In addition, infection had no discernible influence on host life-history traits such as growth and survival. Together, these results may have a number of consequences for hosts and parasites within the UMR region. First, the presence of an additional competent host in the snail assemblage may reduce infection risk for native snail species through parasite dilution. Second, the occurrence of a competent invasive host may increase the transmission of *E. revolutum* to native definitive host species such as waterfowl and mammals. Ultimately, a better understanding of how native parasites cycle through the UMR snail assemblage could allow us to better predict: 1) transmission/invasion outcomes in the UMR and 2) the potential alterations that may occur in ecosystems at high risk of *B. tentaculata* invasion.

**Key words:** *Bithynia tentaculata*, faucet snail *Physa gyrina*, invasive species, Mississippi River, parasite

**Introduction**

Species introductions have profound impacts on global ecosystem dynamics leading to billions of dollars being spent on their control annually (Pimentel et al. 2005). Once introduced, invasive species can spread rapidly within their new environments via natural and anthropogenic means (Frisch et al. 2007; Meyerson and Mooney 2007). For example, Holway (1998) observed that natural variation in stream flow modulated the invasion rates of Argentine ants (*Linepithema humile*; Mayr, 1868) in various areas of the Sacramento River Valley. Moreover, the anthropogenic spread of invasive organisms can be facilitated through unconscious practices such as the placement of watercraft into multiple water bodies over short periods of time without proper decontamination (Kelly et al. 2013). Although studies have assessed the mechanisms underlying the dissemination of invaders from their points of origin, there is still very little known about the factors that facilitate invader success once these organisms arrive in new environments (Lodge et al. 2006). This lack of knowledge hinders the application of control measures to mitigate the extent to which these organisms spread. This becomes even more important when invasive organisms serve as hosts for parasites within native habitats.

Invading organisms have been shown to participate in a number of symbioses including parasitism, which can influence both host and parasite dynamics within local systems (Karatayev et al. 2012; Sandland et al. 2013). One way in which this can occur is if invaders interact with native parasites (Krakau et al. 2006). Under this scenario, parasite transmission rates may increase within the system due to higher densities of available competent hosts (Brown et al. 2012). Furthermore, the presence of these additional hosts may actually lessen the infection risk and...
parasite burden in native host species through the “dilution effect” (Keesing et al. 2006). While these ideas are viewed as important in invasion biology, they are only rarely considered and are often overlooked in empirical studies. Unfortunately, this reduces our ability to fully understand the dynamics of invasive species, native hosts, and parasites in local habitats.

The aquatic snail *Bithynia tentaculata* (Linnaeus, 1758) was first discovered in the Upper Mississippi River (UMR) system in 2002. This has been of major concern in the region as these snails harbor a number of parasitic flatworm species that kill migrating waterfowl after they consume infected snails (Sauer et al. 2007). Currently, it is estimated that over 70,000 birds have died along the UMR since 2002 which is disconcerting given the region’s importance as a critical stopover and foraging site during the spring and fall migrations (U.S. Fish and Wildlife Service: Sandland pers. comm.).

Although research has investigated interactions between these parasites and *B. tentaculata* (Herrmann and Sorensen 2009; Sandland et al. 2013), little is known about the interactions between this invasive snail and documented native parasite species cycling through native hosts in the UMR. One of the most prevalent native parasites in the region is the digenetic trematode *Echinostoma revolutum* (Frölich, 1802), which utilizes three hosts to complete its life cycle (Figure 1). The life cycle of *E. revolutum* begins when eggs are voided in the feces of the definitive host, which can be a number of vertebrate species including ducks and muskrat. The eggs then mature within the environment and eventually hatch releasing larvae known as miracidia. Miracidia subsequently seek out and infect their snail first-intermediate host, which is commonly the marsh pondsnail, *Stagnicola elodes* (Say, 1821). After a period of approximately 5 weeks, snails begin to release (“shed”) a second free-living form of the parasite called cercariae. Once in the environment, the swimming cercariae seek out and penetrate snails as second-intermediate hosts where they encyst as infective metacercariae. Cercariae can infect *S. elodes* as a second-intermediate host along with a number of other snail species including *Physa gyrina* (Say, 1821) (Beaver 1937). The life cycle is continued when definitive hosts consume snails harboring *E. revolutum* metacercariae. Once in the gut, the larvae excyst and mature into adult worms (Johnson 1920; Sandland and Minchella 2003). The broad host-specificity of *E. revolutum* at several points in its life cycle may allow several species (including invasive species) to serve as hosts.

The host to which *B. tentaculata* serves as a competent host for native parasites likely has ramifications for its dissemination as well as native parasite transmission in the UMR. Previous work has reported that *B. tentaculata* is relatively refractory to echinostome infection (Evans and Gordon 1983; McCarthy and Kanev 1990) in other geographic regions. If this is also occurring in the UMR, *B. tentaculata* may have a fitness advantage over co-occurring competent native species such as physids and stagnicolids (Anderson and Fried 1987; Sandland and Minchella 2003) which may help to explain *B. tentaculata*’s success in the region. To begin to address this question, we used controlled laboratory experiments to determine whether *B. tentaculata* could serve as a competent second-intermediate host for *E. revolutum*. Additionally, we expanded the experiment to include a known competent second-intermediate host (*P. gyrina*) that commonly co-occurs with *B. tentaculata* to discern similarities and/or differences in host responses to infection and parasite establishment.

**Methods**

**Host material**

Adult *B. tentaculata* and *P. gyrina* were collected from a rocky breakwater in Pool 8 of the UMR.
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(43°40'39"N; 91°13'18"W) on 28 June 2012 and transported to the laboratory at the University of Wisconsin–La Crosse. Snails were separated by species, placed into 250 ml plastic cups filled with well water (n=10 snails/cup), and fed lettuce ad libitum. Cups were checked every 48 hrs for egg masses. Once large numbers of egg masses were observed (after 96 hrs), adult snails were removed and eggs were maintained until hatching (approximately 8 days for *P. gyrina* and 18 days for *B. tentaculata*). Juvenile snails were fed ground lettuce and water was refreshed every 48 hrs. Snails were reared for approximately 34 day to a size of approximately 3–5 mm, after which they were separated into labeled individual cups for a 7-day acclimation period prior to parasite exposure.

**Parasite material**

Snails (*S. elodes*) were collected from a number of ponds in Myrick Marsh (43°49'45"N; 91°13'43"W) on 13 August 2012 and transported back to UW–La Crosse. Snails were placed into 6-well plates (16 ml/well) filled with 10 ml of well water and exposed to incandescent light for a minimum of 2 hrs prior to being observed for cercarial emergence. Wet-mounts were made of cercariae from each infected snail and identified to the species level using standard keys (Schell 1985). Snails identified as shedding *E. revolutum* were collectively placed into a 500 ml plastic jar and the larvae from these snails were used in our experimental exposures.

**Snail exposures**

Prior to exposure, laboratory-reared snails of each species (*B. tentaculata* (n=60) and *P. gyrina* (n=60)) were size-matched to reduce infection variability arising through differences in extraneous factors such as host volume and/or age. Snails were then placed individually into 16 ml plastic wells (6 wells/plate) containing 5 ml of well water/well. Each row of wells within a plate was then randomly allocated to one of three exposure treatments (control, low parasite dose, and high parasite dose). Individual snails from the low-dose and high-dose wells were each exposed to 25 and 125 cercariae, respectively. Hosts from control wells did not receive a parasite dose, but were otherwise treated in the same manner as exposed individuals.

Cercariae (1–3 hrs old) shed from three field-infected *S. elodes* snails were pooled in a standard Petri plate containing 25 ml of well water. Larvae were then distributed in appropriate numbers (25 or 125) to specified wells (low-dose or high-dose) via Pasteur pipette. After exposures, water was then added to achieve consistent volumes (10 ml) across all wells and treatments. Snails were then left in these wells overnight. Because cercarial quantities limited the number of snails that could be exposed each day, this procedure was repeated daily (over a 6-day period) until there were 20 replicates per treatment (control, low, and high) for each snail species. After each overnight exposure, snails were returned to their original labeled plastic cups and fed lettuce ad libitum. Partial water changes, which involved replacing one-third of the water volume, were performed every 2 days.

Over the following 28 days, snails were measured weekly from the base of the aperture to the tip of the spire using digital calipers and survival was assessed every 2–3 days. Snails failing to move after 5 min were considered dead and were immediately necropsied. At the end of the study, all surviving snails were measured and necropsied. Hosts were necropsied by first carefully crushing the shell using glass plates. All internal tissues were then teased apart using forceps and a dissecting microscope at 30X magnification to enumerate metacercariae.

**Statistical analyses**

All statistical analyses were performed using SPSS Statistics (v. 20) at a 0.05 level of significance. Prior to analyses, all data were first assessed for adherence to parametric assumptions. Any data failing to meet these assumptions were first transformed and then rechecked to ensure that assumptions were met. All data were back-transformed for use in figures.

To determine whether snails were appropriately size-matched, an independent t-test was performed. This allowed us to ensure that the mean size of each snail species did not differ significantly at the start of the experiment.

To assess whether metacercarial intensities differed between species at the two experimental doses, a two-way analysis of variance (ANOVA) was performed on the data after square-root transformation. To compare the proportion of parasites establishing across dose and host species, the number of establishing metacercariae was divided by the original cercarial dose and the data were then arcsine square-root transformed prior to running a two-way ANOVA.
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Figure 2. Mean intensity (metacercariae ± SE) for Bithynia tentaculata and Physa gyrina snails exposed to low (25) and high (125) doses of Echinostoma revolutum cercariae.

Figure 3. Mean overall growth (mm ± SE) for Bithynia tentaculata and Physa gyrina snails in control, low dose, and high dose groups over 4-week experimental duration. Snails were measured weekly using digital calipers.

Figure 4. Percent survival of Bithynia tentaculata and Physa gyrina snails in control (0), low dose (25), and high dose (125) groups exposed to Echinostoma revolutum cercariae. Graph shows the percent of snails from each group (n=20) surviving the duration of the 4-week experiment.

Results

In five cases, decomposition of dead snails made metacercarial counts unreliable; these snails were excluded from any statistical analysis. Among the remaining exposed snails, 100% (35 of 35) of P. gyrina and 97.5% (39 of 40) of B. tentaculata were infected by E. revolutum. Two-way ANOVA on parasites from the remaining snails demonstrated a significant effect of dose on parasite intensity with snails exposed to more cercariae exhibiting higher metacercarial numbers (F1,71 = 148.565, P < 0.001). There was no effect of host species (F1,71 = 0.242, P = 0.624) or the interaction between exposure dose and snail species on E. revolutum intensities (F1,71 = 2.246, P = 0.138) (Figure 2).

There were no significant differences in the proportion of metacercariae establishing between species (F1,71 = 0.002, P = 0.969). Moreover, there was no difference in the proportion of parasites establishing between doses (F1,71 = 1.260, P = 0.265): low and high dose snails exhibited mean infection proportions of 42.2% and 35.3% respectively. There was also no interaction between host species and exposure dose (F1,71 = 1.555, P = 0.217).

Two-way ANOVA with repeated measures (Greenhouse-Geisser) indicated a significant difference in snail size across time (F2.910 = 338.842, P < 0.001). There was also a significant interaction between time and species (F2.910 = 5.803, P = 0.001) with P. gyrina growing at a faster rate than B. tentaculata. There was no significant interaction between time and dose (F5.821 = 1.395, P = 0.220), or between time, dose, and species (F5.821 = 1.177, P = 0.320), indicating that parasite dose did not have an effect on the growth rate of snails in this study (Figure 3).
Binary logistic regression indicated that species (Wald = 6.303, df = 1, P = 0.012) was the only variable that added predictive value to survivorship with *P. gyrina* exhibiting higher survival than *B. tentaculata*. Neither parasite dose (Wald = 2.049, df = 2, P = 0.359) nor the interaction between dose and species (Wald = 1.707, df = 2, P = 0.426), were significant predictors of snail survivorship (Figure 4).

**Discussion**

The distribution of *Bithynia tentaculata* has widened considerably since its introduction to the Great Lakes in the 1880s and it now includes the UMR (Sauer et al. 2007). While the exact mechanisms underlying the successful expansion of this species remain unknown, it is possible that interactions with native species, including parasites, may be modulating its success (Strayer 1999; Mills et al. 2004; Sandland et al. 2013). Our study sought to assess the competency of *B. tentaculata* as a second-intermediate host for a common native trematode species (*Echinostoma revolutum*) found in native snails. In addition, we compared its competency and life-history responses to that of a known native second-intermediate host (*Physa gyrina*) which commonly co-occurs with *B. tentaculata*.

While past studies have documented the ability for invasive mollusks to serve as hosts for native parasites (Aguirre-Macedo and Kennedy 1999; Krakau et al. 2006; Thieltges et al. 2006), very few (Krakau and Thieltges 2004; Kopp and Jokela 2007) have compared the competency of invasive mollusks relative to known natural hosts of native parasites. Our work demonstrates that both an invasive and native snail can serve as suitable hosts for *E. revolutum*. This result was not entirely unexpected given the broad specificity of *E. revolutum* for second-intermediate hosts (Beaver 1937). However, the similarity in infection intensities between snail species was surprising given that past work has shown *B. tentaculata* to be relatively refractory to echinostome infections (Evans and Gordon 1983; McCarthy and Kanev 1990). One potential reason for this discrepancy stems from the fact that previous research studied *B. tentaculata*’s interaction with echinostomes in its native range (Europe) whereas we focused on this interaction within the snail’s introduced habitat. Differences in the expression of host traits associated with infection (i.e. behavior, immunology, etc) between native and introduced populations could be generated through processes such as local adaptation and/or founder effects and may help to explain the variability in metacercarial intensities observed across studies. Another possibility is that infectivity varies inherently across trematode species with *E. revolutum* possessing a greater capacity to infect *B. tentaculata* than either of the other echinostomes (*Echinoparyphium recurvatum* and *Pseudochinoparyphium echinatum*) used in previous work. Lastly, it should be noted that the methodologies used to enumerate metacercariae differed among studies. For example, we thoroughly teased host tissues apart using forceps and located the metacercariae adjacent to the gill tissue whereas McCarthy and Kanev (1990) used two glass microscope slides to crush the snail before subsequently enumerating metacercariae. If variability in these procedures led to variability in metacercarial identification, this too could have contributed to the broad differences in echinostome infectivity seen in these studies.

The fact that *E. revolutum* can infect both native and invasive snail species may have important implications for transmission dynamics within the UMR. For example, *E. revolutum*’s capacity to “spillback” into an invasive host may actually enhance transmission rates to definitive hosts (Mastitsky and Veres 2010). Spillback occurs when parasites in their native habitat are able to utilize invasive species as hosts. Spillback has been reported in other systems involving echinostomes. For example, the invasive zebra mussel (*Dreissena polymorpha*; Pallas, 1771) has been implicated in enhancing the transmission of *Echinoparyphium recurvatum* (Dietz, 1909) to waterfowl definitive hosts (Matitsky and Veres 2010). If a similar pattern is at play in the UMR, foraging waterfowl may be consuming more echinostome-infected snails which could enhance *E. revolutum*’s overall occurrence in the region and its pathological impact on birds (Mullican et al. 2001).

The ability for *E. revolutum* to infect *B. tentaculata* as its second-intermediate host may actually lessen the parasite burden on native snail hosts. This is commonly referred to as parasite dilution. This pattern is highlighted in work by Kopp and Jokela (2007) who reported that native snails exhibited reduced infection prevalence when exposed to native parasites in the presence of an invasive snail. While native snails were not exposed to *E. revolutum* in the presence of the invasive snail in this study, the
similar parasite intensities observed between the two species adds plausibility to the dilution effect occurring in this system. Additionally, the fact that \textit{E. revolutum} did not impact life history expression in native versus invasive snails suggests that this parasite is not contributing to \textit{B. tentaculata}’s spread in the UMR. However, this lack of life history impact may actually further enhance \textit{E. revolutum}’s transmission in the region. Since neither host may be at an advantage when infected, the combination of spillback resulting in a dilution effect may increase the probability of definitive host species consuming a snail infected with \textit{E. revolutum}. It must also be acknowledged that the life history traits we chose to observe in this study were not exhaustive and may not have captured more subtle fitness differences between the snail species (Levri et al. 2005). Future studies should consider using additional fitness endpoints in order to better resolve where infection costs occur in this system and if they vary between snail species.

Results from our study provide insight into the implications for parasite transmission dynamics following the establishment of an invasive species. Additionally, our study highlights the importance of considering simultaneous infection comparisons between known native and potentially invasive hosts to help better predict the possible outcomes of invasion events in areas at risk of species introductions. For our system, more robust complementary field studies are necessary for more thoroughly understanding host and parasite interactions in the UMR, as we currently have only anecdotal field evidence for the presence of \textit{E. revolutum} in \textit{B. tentaculata}. Results from this and future experimentation will provide greater insight into host-parasite dynamics following an invasion event and may eventually lead to the development of control strategies aimed at reducing the impact of \textit{B. tentaculata} in the region.

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Interactions between an invasive snail and a native parasite


