

Infection Patterns in Invasive and Native Snail Hosts Exposed to a Parasite Associated with Waterfowl Mortality in the Upper Mississippi River, USA

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ABSTRACT: *Bithynia tentaculata* is an aquatic invasive snail first detected in the upper Mississippi River (UMR) in 2002. The snail harbors a number of parasitic trematode species, including *Sphaeridiotrema pseudoglobulus*, that have been implicated in waterfowl mortality in the region. We assessed the capacity of *S. pseudoglobulus* cercariae to infect *B. tentaculata* and native snails found in the UMR. Four snail species (one invasive and three native) were individually exposed to *S. pseudoglobulus* larvae and all were successfully infected. A subsequent experiment examining infection patterns in invasive and native hosts exposed singly or in mixed treatments revealed no difference in parasite establishment among snail species. Our results add to our understanding of *S. pseudoglobulus* transmission and provide insight into processes underlying waterfowl disease in the UMR.

Key words: *Bithynia*, host competency, species invasions, *Sphaeridiotrema*, waterfowl disease.

Invasive species are key threats to the integrity of terrestrial and aquatic ecosystems around the world (Clavero and Garcia-Berthou 2005). Detrimental effects of invaders on native systems can occur through several mechanisms, including their capacity to harbor and transmit parasites within the local community (Poulin et al. 2011). Unfortunately, in many of these systems, little is known about relative infection competencies of native and invasive hosts (Thieltges et al. 2009). This knowledge gap can reduce our ability to predict parasite success within local environments and subsequent disease events associated with such infections.

Bithynia tentaculata is an aquatic snail native to European freshwater systems. It was introduced into the Great Lakes in the late 1800s and has spread throughout several North American freshwater

environments (Mills et al. 1993), including the upper Mississippi River (UMR; Sauer et al. 2007; Wood et al. 2011). Geographic expansion of *B. tentaculata* has generated concern because of its potential effects on native aquatic species, particularly gastropods (Strayer 1999). Community-level influences are exacerbated by the fact that *B. tentaculata* harbors trematode parasites (including *Cyathocotyle bushiensis*, *Sphaeridiotrema globulus*, and *Sphaeridiotrema pseudoglobulus*) that cause mortality in waterfowl after the birds consume infected snails (McLaughlin et al. 1993; Sauer et al. 2007; Bergmame et al. 2011). These parasite species have three-host life cycles involving waterfowl as definitive hosts and snails as first- and second intermediate hosts (Fig. 1).

In the UMR, it is estimated that more than 70,000 waterfowl (of 13 species) have succumbed to trematodiasis since 2002 (US Fish and Wildlife Service, unpubl.), yet the factors that influence parasite persistence and outbreaks of waterfowl disease in this region remain poorly understood. Recent research has shown that host competency may have important consequences for disease transmission and host infection risk in the UMR (Sandland et al. 2013). However, this work focused exclusively on interactions between snails and parasites (*Sphaeridiotrema* spp.) at one point in the parasite's life cycle (first intermediate host stage; Fig. 1). The degree to which subsequent stages of *Sphaeridiotrema* (specifically, *S. pseudoglobulus*) interact with invasive and native snails of the UMR has not been explored, yet this question requires attention

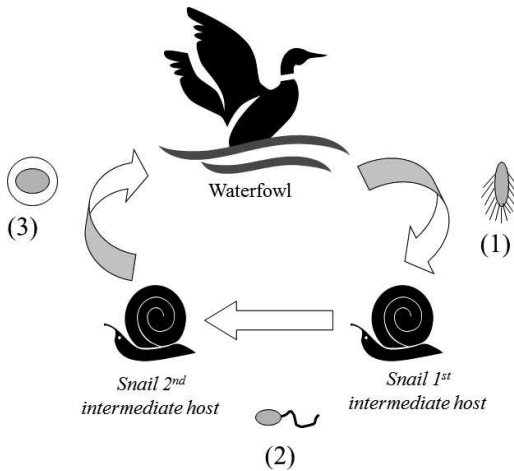


FIGURE 1. Life cycle of *Sphaeridiotrema pseudoglobulus* involves a snail first intermediate host, a snail second intermediate host, and a bird-definitive host. First intermediate host snails become infected by swimming miracidia (1) which hatch from eggs. After a period of development in snails (months), cercariae (2) are released and encyst as metacercariae (3) within snail second intermediate hosts. After waterfowl consume snails containing metacercariae, the parasites develop to maturity in the gut and begin producing eggs that are subsequently released into the aquatic environment.

because these stages transmit directly to waterfowl (Fig. 1).

In June 2010, we collected adult *B. tentaculata* from a rocky breakwater in pool 8 of the upper Mississippi River (43°40'39"N; 91°13'18"W). Snails were transported to the laboratory at the University of Wisconsin–La Crosse and placed into 150-mL plastic cups in groups of 10. Within 24 hr, snails had laid large numbers of egg clutches within these cups. Adults were removed, and eggs were reared to hatching. These juvenile snails served as the initial founders for our laboratory population of *B. tentaculata*.

In April 2011, adult native snails (*Physa gyrina*, *Stagnicola elodes*, and *Helisoma trivolvis*) were collected from Myrick Marsh (43°49'35"N, 91°13'46"W), which is part of a larger wetland in the Mississippi River floodplain. Each of these species has also been observed in pools 7 and 8 of the UMR (Wood et al. 2011;

Sandland et al. 2013). Snails were placed under incandescent light for 4 hr to determine infection status for all hosts. Uninfected individuals within a species were placed into 4-L plastic trays and fed lettuce ad libitum. Styrofoam pieces were added to each tray to facilitate egg deposition. Eggs laid on the styrofoam were then transferred to smaller “nursery” containers where they hatched; juveniles were reared for use in our experiments.

Sphaeridiotrema pseudoglobulus cercariae were acquired from laboratory-infected *B. tentaculata* snails that had been experimentally exposed to 10 parasite miracidia per snail in April 2011. Specific details regarding the protocols used to infect and maintain these snails can be referred to in Sandland et al. (2013). To facilitate parasite release from these infected hosts, snails were first individually transferred into 16-mL plastic wells with ~14 mL of well water and then placed under incandescent light from 9:00 AM to 6:00 PM. Because *S. pseudoglobulus* larvae did not emerge from snails until 3:00 PM each day, collections took place from 2:00 PM to 6:00 PM. Cercariae were pooled from all infected snails before host exposures. Exposures occurred immediately after parasite collections.

For the first experiment, *B. tentaculata* ($n=11$), *P. gyrina* ($n=11$), *H. trivolvis* ($n=13$), and *S. elodes* ($n=12$) (all <1 yr old) were size matched and placed individually into 3-mL plastic wells with 2 mL of well water. Each snail was exposed to 25 *S. pseudoglobulus* cercariae. Exposures were performed across species to accommodate any temporal variability in larval infectivity. After exposure, snails were maintained in wells overnight before being moved to 250-mL plastic cups filled with 150 mL of well water. After 48 hr, exposed snails were carefully crushed and examined postmortem to assess metacercarial establishment.

For the second experiment, size-matched, laboratory-reared *B. tentaculata*

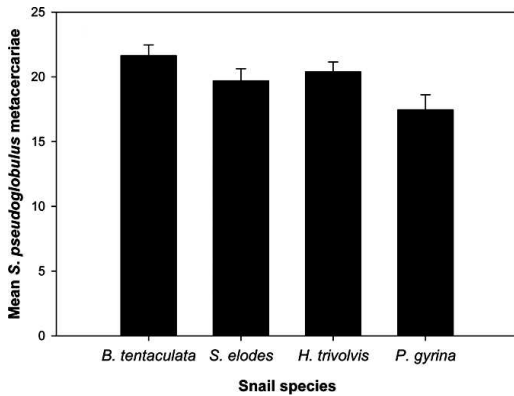


FIGURE 2. Mean intensity (\pm SE) of *Sphaeridiotrema pseudoglobulus* metacercariae recovered from experimentally exposed invasive (*Bithynia tentaculata*) and native (*Stagnicola elodes*, *Helisoma trivolvis*, *Physa gyrina*) snail species.

($n=17$) and *P. gyrina* ($n=18$) (all <1 yr old) were placed into 16-mL plastic wells (containing ~ 14 mL of well water) either individually (single treatment) or with a size-matched member of the other species (mixed treatment). Twenty-five *S. pseudoglobulus* cercariae were then added to each well. After exposures, snails were maintained and subsequently examined postmortem following the same protocols used in the first experiment.

Because of similarities in prevalence among treatment groups, data were compared among hosts using qualitative statistics (%). Intensity data were compared across host species using analysis of variance (ANOVA) followed by pair-wise comparisons (Scheffe's post hoc test). Two-way ANOVA was used to investigate differences in *S. pseudoglobulus* intensities in *B. tentaculata* and *P. gyrina* exposed individually (single-host treatment) or in combination with a member of the other species (mixed treatment).

Sphaeridiotrema pseudoglobulus cercariae infected all of the snails from the first experiment regardless of whether they were invasive or native species. Although *S. pseudoglobulus* prevalence was 100%, intensities varied significantly across host species ($F_{3,43}=3.38$, $P=0.027$),

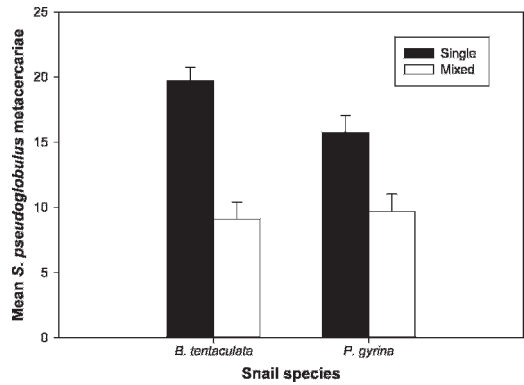


FIGURE 3. Mean (\pm SE) *Sphaeridiotrema pseudoglobulus* metacercariae recovered from invasive (*Bithynia tentaculata*) and native (*Physa gyrina*) snails experimentally exposed singly or in mixed-species treatments.

with *B. tentaculata* exhibiting higher parasite intensities than *P. gyrina* ($P=0.034$) (Fig. 2). There were no other significant differences in intensity between the remaining snail species (all $P>0.05$).

For the second experiment, *S. pseudoglobulus* prevalence was again 100% in *B. tentaculata* and *P. gyrina* across both exposure treatments (single vs. mixed). There was a significant difference in parasite intensity between exposure treatments, with individually exposed snails harboring approximately twice as many metacercariae as hosts from the mixed treatment ($F_{1,31}=40.36$, $P<0.001$). However, neither snail species ($F_{1,31}=1.65$, $P=0.209$) nor the interaction between snail species and exposure treatment ($F_{1,31}=3.03$, $P=0.092$) significantly influenced *S. pseudoglobulus* intensities (Fig. 3).

Since its initial discovery in the UMR in 2002, *B. tentaculata* has been implicated in thousands of annual waterfowl deaths through its transmission of trematode parasites to foraging birds (Sauer et al. 2007). Yet the mechanisms underlying parasite transmission and outbreaks of waterfowl disease in this region remain unclear (Herrmann and Sorensen 2009; Sandland et al. 2013). Our goal was to better understand the potential role that native members of the gastropod assemblage plays

in transmitting *S. pseudoglobulus* to waterfowl within the UMR.

Results suggest that the narrow host competency expressed by *S. pseudoglobulus* at the first stage of its life cycle (Sandland et al. 2013) broadens at the next stage. This complements anecdotal field observations reporting *Sphaeriodiotrema* spp. metacercariae in snail hosts other than *B. tentaculata* (Lepizki 1993). Broadening specificity across life cycle stages is a relatively common trait in trematodes and likely benefits these organisms by increasing the densities of compatible hosts in the surrounding environment (Evans and Gordon 1983). In the UMR, increasing competency across second intermediate hosts may have two important implications for species invasion and disease transmission in the region. First, the ability of *S. pseudoglobulus* to infect numerous additional species may increase transmission to waterfowl in the region. Second, the fact that additional snail species can be infected by *S. pseudoglobulus* larvae at this life cycle stage may actually reduce *B. tentaculata*'s infection risk through parasite dilution. Whether this provides the invader with a fitness advantage in the UMR relative to native snails remains to be tested, but parasite dilution has been shown to modulate the success or failure of invasive species in other systems (Kelly et al. 2009).

Although individual exposures are important for understanding infection risk across host species, coexposing hosts may better represent natural infection processes. Past work has shown that infection outcomes can vary dramatically when parasites have the opportunity to select among host species (McCarthy and Kanev 1990; Muñoz-Antoli et al. 2008). This did not appear to be the case for *S. pseudoglobulus*, in that native and invasive snails exhibited similar intensities in each exposure treatment (single vs. mixed). This suggests that, at least under our experimental conditions, *S. pseudoglobulus* does not discriminate between invasive and

native hosts, providing further evidence that parasite dilution could be occurring in the system.

Our work herein, in combination with past research (Sandland et al. 2013), demonstrates that host competency varies dramatically across *S. pseudoglobulus* life cycle stages. Broadening host competency at the second intermediate host stage may help to offset potential transmission constraints associated with narrow specificity documented earlier in the life cycle (Sandland et al. 2013), resulting in enhanced transmission of metacercariae to waterfowl during spring and fall migrations. Future studies should attempt to assess host competency in additional parasites associated with waterfowl mortality in the region and experimentally determine how well these parasites transmit from invasive and native snails to waterfowl hosts.

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