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ASSESSING TREMATODE INFECTION PATTERNS IN AQUATIC ECOSYSTEMS INVADED BY THE FAUCET SNAIL (*BITHYNIA TENTACULATA*)

A Chapter Style Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

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ASSESSING TREMATODE INFECTION PATTERNS IN AQUATIC ECOSYSTEMS

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ABSTRACT

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Species introductions have profound impacts on global ecosystem dynamics leading to billions of dollars being spent on their control annually. Once introduced, invasive species can spread rapidly within their new environments via natural and anthropogenic means. Although more recent studies have begun investigating the mechanisms underlying the establishment and spread of invaders, there is still very little known about the factors dictating the success of invasive species in new environments. This knowledge gap becomes even more pronounced when invasive organisms are considered in conjunction with symbiotic species such as parasites. In 2002, the invasive faucet snail (*Bithynia tentaculata*) was discovered in the upper Mississippi River (UMR) and has been implicated in transmitting four novel parasite species to migrating waterfowl. Since the snail's introduction, over 100,000 waterfowl have succumbed to trematodiasis during subsequent spring and fall migrations. In addition, new evidence suggests that the distribution of *B. tentaculata* has spread as far west as Georgetown Lake (Butte, MT) where waterfowl mortality has also been reported. The purpose of my thesis research was to address distinct questions regarding 1) the patterns of infection in *B. tentaculata* in two areas across the USA (Chapter I) and 2) the potential for *B. tentaculata* to interact with parasites native to one of the specific regions (the UMR) (Chapter II). To address the first question, field collections were performed at Georgetown Lake and the UMR to compare parasite populations residing in *B. tentaculata*. A deceased waterfowl was also collected from Georgetown Lake to compare infection patterns in definitive hosts to those found in *B. tentaculata*. The second question was addressed using both field and laboratory methods to assess the competency of *B. tentaculata* for the native digenetic trematode, *Echinostoma revolutum*. Results from Chapter I indicate the presence of one of the waterfowl-killing trematodes present in Georgetown Lake at a significantly higher mean intensity than those observed in *B. tentaculata* from the UMR. Chapter II reports *B. tentaculata* to be a competent host for the native parasite, *Echinostoma revolutum*. In addition, no difference in life history traits between *B. tentaculata* and a native snail (*Physa gyrina*) commonly serving as host for *E. revolutum* were observed. Together, the results from these two studies highlight the fact that broad spatial differences in the location of invasive species can equate to variability in the parasites found within these (as well as other) hosts. Furthermore, these results provide more insight into the ecological mechanisms determining the success of invasive species in new areas which may help to better predict the occurrence of waterfowl disease in areas where *B. tentaculata* and its parasites have the potential to occur. Research stemming from that reported in this thesis may uncover potential life cycle vulnerabilities in *B. tentaculata* and/or its parasites to attempt to alleviate waterfowl mortality in regions where it is found.

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ABSTRACT

Invasive species have the capacity to rapidly alter the native ecosystems in which they establish, resulting in billions of dollars spent on their control annually. The faucet snail (*Bithynia tentaculata*) was introduced into the Great Lakes in the 1880s and has spread to numerous waterbodies throughout North America. This snail is associated with four species of digenetic trematodes which have been implicated in mass die-offs of waterfowl each migratory season. The fact that *B. tentaculata* is found at a number of widely-separated sites across North America provides a unique opportunity to assess how patterns of infection change within hosts among these different locations. To begin to assess this question, field collections were conducted at two geographically distinct sites where the presence of *B. tentaculata* and waterfowl mortality have been reported. Host necropsies were performed to quantify and compare the parasite species and numbers found within these spatially distinct populations of *B. tentaculata*. My results indicate the presence of two waterfowl-killing trematodes (*Sphaeridiotrema pseudoglobulus* and *Cyathocotyle bushiensis*) in the upper Mississippi River and one waterfowl-killing trematode (*C. bushiensis*) in Georgetown Lake (Butte, MT). Moreover, *C. bushiensis* intensities were much higher at Georgetown Lake than the UMR. Together, these results suggest that local/regional factors (such as definitive host densities, the timing of parasite introductions, etc.) may dictate patterns of infection in invasive hosts. These results have important ramifications for the control/migration of invaders as they spread across large geographic regions.

INTRODUCTION

Invasive species can have significant impacts on ecosystem dynamics resulting in billions of dollars in control expenditures each year (Pimentel et al., 2005). In addition to their spread through local habitats at the point of invasion, invaders can rapidly disseminate into new environments through anthropogenic mechanisms. For example, the failure to properly decontaminate personal watercraft has played an influential role in the dissemination of several invasive plant and animal species (Kelly et al., 2013). In contrast, the non-anthropogenic factors allowing for the continued success of these species within their invaded range are often poorly understood. In cases where invasive species serve as vectors for novel diseases (Karatayev et al., 2012), it is even more unclear as to the factors that allow both the invader and its parasite(s) to establish and maintain their life cycles in a new environment (Dunn et al., 2012). In past work, most studies have investigated invader/parasite interactions from a small area within a broadly invaded region which may limit a more complete understanding of how invasive hosts and parasites vary across geographic landscapes. To better understand the process of species invasions, future work must expand upon the study of just one area within an invaded range. To do this, it is important to compare interactions across the invaded range in order to gain better insight into invader and/or parasite patterns that may facilitate or inhibit the establishment and spread of an invasive species and its potential parasites.

In the 1880s, the aquatic faucet snail (*Bithynia tentaculata*) was introduced into the Great Lakes (Karatayev et al., 2012), and has since expanded its distribution considerably throughout North America (Hoeve and Scott, 1988; Cole, 2001; Sauer et al., 2007). The snail appeared in the St. Lawrence River (Quebec, Canada) in the 1960s (Gibson et al., 1972), and in 2002, was discovered in the upper Mississippi River (UMR) where it remains a prominent species within the gastropod assemblage (Flessas et al., 2000; Sauer et al., 2007). To date, there is evidence that the snail has spread to a number of other waterbodies in the Midwest (such as Lake Winnibigoshish (Minnesota)) and occurs as far west as Montana, USA (Henningsen et al., 2010; Montana Field Guide, 2014). While there are numerous potential impacts of *B. tentaculata* on competing species (Mills et al., 2004), perhaps the most disconcerting and widely-recognized impact of the snail is its ability to transmit disease-causing trematode parasites to a number of species of migrating waterfowl (Hoeve and Scott, 1988; Cole, 2001; Sauer et al., 2007; Herrmann and Sorensen, 2011).

Throughout North America, four species of digenetic trematodes (*Cyathocotyle bushiensis*, *Leyogonimus polyoon*, *Sphaeridiotrema globulus*, and *Sphaeridiotrema pseudoglobulus*) have established and maintained their life cycles in migrating waterfowl populations since the arrival of *B. tentaculata* (Hoeve and Scott, 1988; Cole, 2001; Sauer et al., 2007). In the UMR alone, the death of > 100,000 birds has occurred over the last 12 years with *B. tentaculata* and its parasites being implicated as the cause (Cole, 2001; Sauer et al., 2007; Bergmame et al., 2011; Sandland et al., 2013). These deaths are disconcerting from a conservation perspective because of the high degree of pathology generated within infected waterfowl. Recent work has shown that a bird can consume a

lethal dose of parasites in as little as 24 hours (Hoeve and Scott, 1988), with subsequent mortality occurring within 6 days due to hemorrhagic enteritis and hypovolemic shock (Sauer et al., 2007). The infective stage of the parasite most commonly resides in *B. tentaculata*, which are heavily foraged upon by migrating waterfowl (Sauer et al., 2007). The most affected species of waterfowl are the American Coot (*Fulica americana*) and the Lesser Scaup (*Aythya affinis*), which serve as definitive hosts primarily for *C. bushiensis* and *Sphaeridiotrema* spp., respectively (Hoeve and Scott, 1988).

The generalized life cycles of *C. bushiensis* and *Sphaeridiotrema* spp. begin when eggs are voided in the feces of the definitive host (Figure 1). Eggs enter the water column and undergo a period of development prior to hatching into free-swimming miracidia. Each miracidium seeks out and infects a snail first-intermediate host (*B. tentaculata*) and after undergoing further development, gives rise to the next larval form (sporocysts in the case of *C. bushiensis* or rediae in *Sphaeridiotrema* spp.). Sporocysts and rediae asexually generate an additional free-swimming larval form called cercariae. Each cercaria infects a snail as its second-intermediate host, which can either be *B. tentaculata* or one of several native species in the gastropod assemblage (Sandland et al., 2014). Each cercaria will further develop into an encysted form of the parasite called a metacercaria. Once consumed by the definitive host, each metacercaria will excyst and mature into an ovigerous adult worm. Through repeated consumption of metacercariae-infected snails, parasites can quickly accumulate in a bird's gastrointestinal tract and generate extreme pathology and subsequent mortality (Huffman et al., 1984; Sauer et al., 2007).

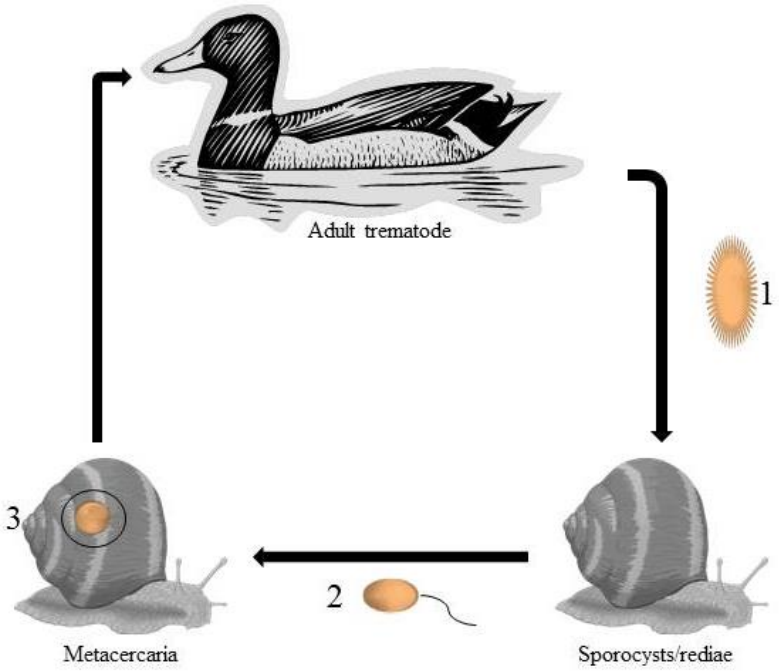

Figure 1. Generalized life cycle for the waterfowl-killing parasites *Sphaeridiotrema* spp. and *Cyathocotyle bushiensis*. Numbers correspond to 1) miracidium, 2) cercaria, and 3) metacercaria.

While annual waterfowl die-offs have been widely associated with *B. tentaculata* and its associated parasites in areas of the Midwest, much less is known about the emergence of *Bithynia*-related waterfowl disease along the Western flyway (Missoulian, 2006). Relatively recently, American Coot have experienced annual die-offs on Georgetown Lake (GTL) in Montana, and this has occurred in association with the presence of *B. tentaculata.* Having populations of *B. tentaculata* that are from two widely separated sites in North America (Wisconsin and Montana) provides a novel opportunity to investigate whether infection patterns vary spatially. If such differences exist, attempts can be made to elucidate the factors underlying the difference in parasite populations between these two sites. Identifying these factors could help to better predict

the conditions responsible for the establishment of *B. tentaculata* and its associated parasites.

The purpose of this study was to obtain samples of *B. tentaculata* and deceased waterfowl from GTL in order to compare potential spatial differences in parasite prevalence and intensity to those collected during the same timeframe from the UMR. Additional host metrics such as sex and size were also considered as potential predictors of *B. tentaculata* infection at the two sites.

METHODS

Snails were collected from two widely separated sites where the presence of *B. tentaculata* and waterfowl mortality has been reported. The first collection site was a rocky outcropping in Navigation Pool 8 of the upper Mississippi River near Stoddard, WI (43°40’39”N; 91°13’18”W). Snails were collected on 07 June 2013 from individual rocks removed from the substrate. Each rock was lifted from the substrate in the orientation in which it was found and snails were collected from each side of the rock and placed into labeled gallon-sized plastic bags. Bags were transported back to the laboratory at the University of Wisconsin-La Crosse and stored in a freezer at -20°C for subsequent necropsy. The second sample site was Comers Point of Georgetown Lake near Butte, MT (46.18°N, -113.29°W). Snails were collected haphazardly from the substrate on 25 July 2013 and placed into 20 mL scintillation vials in 70% ethanol prior to being transported back to the laboratory at the University of Wisconsin-La Crosse (K. Hoar pers. comm.). The fact that snails from each site were stored using different methods was unlikely to influence my ability to detect parasites as both freezing and ethanol maintain the integrity of larval forms (Lepitzki et al., 1994).

Prior to dissection, each snail was identified as *Bithynia tentaculata* before being measured with digital calipers from the base of the aperture to the tip of the spire. Each snail (n=50 per site) was then placed into the bottom half of a petri dish under a dissecting microscope and gently crushed with a glass plate. Snail sex was determined after careful removal of the shell, and tissues were teased apart with fine-tipped forceps.

All snail tissues were then thoroughly examined for the presence of parasite stages (sporocysts/rediae and/or metacercariae) at 30X magnification.

In addition to snail collections, I was fortunate enough to acquire one deceased American Coot which was collected from the shoreline of Georgetown Lake on 21 October 2013 and subsequently mailed to the laboratory at the University of Wisconsin-La Crosse (Mike Haggerty pers. comm.). The bird was stored in a freezer at -20°C until time of necropsy. I necropsied the bird by first making a midline incision from the anus to the base of the sternum. The entirety of the gastrointestinal tract was then removed by carefully cutting the intestines from the remaining viscera with scissors. Once removed, the intestines were separated from the ceca and cut into approximately 10 cm sections. I then longitudinally cut each section and examined it under a dissecting microscope to enumerate adult trematodes. All parasites were identified using standard trematode keys (Schell, 1985).

Statistical analyses were performed using SPSS Statistics (v. 20) and interpreted at a 0.05 level of significance. I assessed potential differences in mean infection intensity between the two sites and sexes with a two-way analysis of variance (ANOVA).

RESULTS

The most overwhelmingly prevalent parasite infecting snails from both sites was metacercariae of the digenetic trematode *Cyathocotyle bushiensis*. Snails from the UMR also contained metacercariae of the digenetic trematode *Sphaeridiotrema* spp. However, this parasite was only observed in 4% of necropsied snails. Since this species was found exclusively in the UMR and at such low numbers, the analysis between sites was restricted to *C. bushiensis.* Overall, 78% (n=50) of snails from the UMR and 96% (n=50) of snails from GTL were infected with *C. bushiensis*. The mean (±SE) number of *C. bushiensis* metacercariae was also significantly greater in snails collected from GTL (28.4±1.24) than from the UMR (2.82±0.911) ($F_{1,96} = 35.657$, $P < 0.001$). In addition, there was a significant difference in mean intensity between male (19.69±5.66) and female (13.00±2.81) snails ($F_{1,96} = 5.281$, $P = 0.024$) as well as an interaction effect of gender and site on intensity ($F_{1,96} = 5.420$, $P = 0.022$). The mean size difference of necropsied snails was minimal (0.72 mm) between the two sites.

The deceased American Coot exhibited several indications of trematodiasis; blood was expelled from the mouth of the bird, indicating significant blood loss within the intestinal tract. In addition, significant hemorrhaging was visible along the length of the intestinal tract, and plaque-like lesions containing adult trematodes were readily visible upon external inspection of the ceca. Internal inspection revealed one unidentified species of acanthocephalan located approximately halfway through the intestinal tract. The remaining worms were identified as *C. bushiensis* and resided in each of the two

ceca. The first cecum contained 44 adults and exhibited many plaque-like lesions and blood occupying the lumen, whereas the second cecum contained 3 adults and exhibited significantly less pathology. There were no worms observed posterior to the ceca, however, thousands of trematode eggs resided along the remaining length of the intestinal tract in addition to further hemorrhaging.

DISCUSSION

The distribution of *B. tentaculata* has widened considerably since its introduction into the Great Lakes in the 1880s. Evidence for the presence of *B. tentaculata* and its associated waterfowl mortality now includes areas such as the St. Lawrence River (Gibson et al., 1972; Hoeve and Scott, 1988), upper Mississippi River (Sauer et al., 2007; Herrmann and Sorensen, 2011), Lake Winnibigoshish (Minnesota Department of Natural Resources, 2013), and Shawano Lake (Cole, 2001). While the UMR has been considered one of the more "recent" sites of establishment, there is additional evidence to indicate that *B. tentaculata* has established in locations as far west as Montana (Henningsen et al., 2010), including Georgetown Lake (Butte, MT) (Montana Field Guide, 2014). While the mechanisms allowing *B. tentaculata* to disseminate and establish elsewhere remain largely unknown, there is growing evidence that its spread can result in additional waterfowl mortality, even when locations are widely separated across different migratory flyways.

While waterfowl deaths have been occurring in Montana for a number of years, the cause of these mortality events have been speculative (Missoulian, 2006). Hypotheses have ranged from outbreaks of high-pathogenicity avian influenza (Dusek et al., 2009) to trematodiasis from infection with *Sphaeridiotrema globulus* (Missoulian, 2006). The trematodiasis hypothesis is now further supported by that fact that 1) *C. bushiensis* has been observed in its intermediate and definitive hosts in GTL and 2) the fact that the American Coot examined as part of this study contained an adult *C.*

bushiensis number sufficient to kill waterfowl (Hoeve and Scott, 1988). Together, these results identify *C. bushiensis* as a contributor to coot mortality on GTL. The presence of *C. bushiensis* in these samples also provides the first report of this parasite in *B. tentaculata* from GTL and Montana in general.

In the UMR, *B. tentaculata* is known to harbor four trematode parasites that have been implicated in waterfowl mortality since 2002 (Sauer et al., 2007). Three of these species (*C. bushiensis*, *S. globulus*, and *S. pseudoglobulus*) are able to transmit to waterfowl using *B. tentaculata* as both first- and second-intermediate hosts (Sandland et al., 2013 and 2014). In contrast, *C. bushiensis* alone was seen in *B. tentaculata* from GTL where its numbers were significantly higher than those in the UMR. This variation in parasite infection pattern between these spatially distinct sites may have occurred for a number of reasons. One possibility is that the absence of interspecific competition with other trematode species such as *Sphaeridiotrema* may have enhanced *C. bushiensis* infections. Within the first intermediate host, *C. bushiensis* and *Sphaeridiotrema* spp. differ in their larval stage that generates cercariae. The sporocyst generated by *C. bushiensis* (Herrmann and Sorensen, 2009) chemically castrates the snail host, whereas the rediae of *Sphaeridiotrema* spp. (Herrmann and Sorensen, 2009; Sandland et al., 2013) possess a rudimentary mouthpart with which they mechanically castrate their host. Previous work has shown that the establishment of sporocyst-generating trematodes is greatly hindered by the presence of a trematode that generates a redial stage (Joe, 1966; Basch and DiConza, 1975; Leung and Poulin, 2011). Perhaps the potential absence of *Sphaeridiotrema* spp. in Georgetown Lake is allowing *C. bushiensis* to proliferate; hence the significantly higher mean intensity of metacercariae compared to that observed in the

UMR. Additional factors, such as 1) varying parasite tolerance to abiotic conditions (Thieltges et al., 2009), 2) genetic differences in *B. tentaculata* resulting in resistance to certain parasite species (such as *Sphaeridiotrema* spp.) (Henningsen et al., 2010), or 3) lower densities of Lesser Scaup (Ryder et al., 2007) on GTL may also help to explain the potential absence of *Sphaeridiotrema* spp. as this waterfowl species tends to carry the highest intensities of these parasites relative to other birds. Regardless of the mechanism(s) responsible for higher *C. bushiensis* infections in GTL, this pattern is concerning given that it takes relatively few adult *C. bushiensis* to cause waterfowl mortality (Herrmann and Sorensen, 2011). This means that large numbers of foraging birds (American Coot) could potentially ingest lethal doses of the parasite in a short period of time on Georgetown Lake.

While *Sphaeridotrema* spp. has not been documented in GTL, the potential for its arrival (due to *B. tentaculata* presence) should be of concern for conservation and wildlife managers in the region. To date, reports of waterfowl mortality on GTL have only reported die-offs of American Coot (Missoulian, 2006). This is not overly surprising due to the fact that: 1) American Coot appear to be relatively susceptible to *C. bushiensis* (Herrmann and Sorensen, 2011), and 2) *C. bushiensis* was the only species that I found infecting *Bithynia.* However, other species of waterfowl inhabit GTL including Lesser Scaup (Montana Ecological Systems/Landcover Report, 2011), which as mentioned above, tend to be susceptible to *Sphaeridiotrema* spp.. An introduction of *Sphaeridiotrema* spp. into the GTL system could have major impacts on the species of waterfowl experiencing die-offs and the period over which mortality events occur. Parasite introduction could occur via a variety of mechanisms such as 1) the introduction

of parasites through more resistant species such as Wood Ducks (Hoeve and Scott, 1988), or 2) increasing densities of Lesser Scaup populations along the Western flyway due to changes in their population distribution (Holm et al., 2011). Moreover, despite the current absence of other waterfowl-killing trematode species in GTL, a single egg deposition event via waterfowl could be, hypothetically, sufficient to initiate the life cycle of *Sphaeridiotrema* spp. leading to increased mortality in additional waterfowl species (such as Lesser Scaup).

The ability to study spatially distinct regions that have been invaded by *B. tentaculata* at different time points can provide unique insight into invasion biology. This is especially important when considering changes in parasite communities as an invader expands its geographic range. For example, these changes may include the emergence of novel parasites that are associated with waterfowl mortality as was observed in this chapter, or an invader's interaction with parasites native to the environment in which it has established (Chapter II). While my results are preliminary, the work does suggest that parasite community make-up differs between the Midwest and Montana. Continuing comparative studies aimed at assessing invaders and their parasites from different geographic locations will allow future work to gain insight into 1) the factors facilitating/restricting parasite transmission, 2) the differential impacts that parasite communities may have on local ecosystems, and 3) how waterfowl communities may respond if *B. tentaculata* and its associated parasites continue to spread throughout other areas of North America. Moreover, future research can seek to identify and exploit either attributes of the snail and/or parasite life cycle to mitigate the impact that these species are having on migrating waterfowl populations.

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CHAPTER II

ASSESSING HOST COMPETENCE AMONG NATIVE AND INVASIVE SNAIL SPECIES EXPOSED TO THE NATIVE PARASITE *ECHINOSTOMA REVOLUTUM*

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Research Article

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 Sacra-mento 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

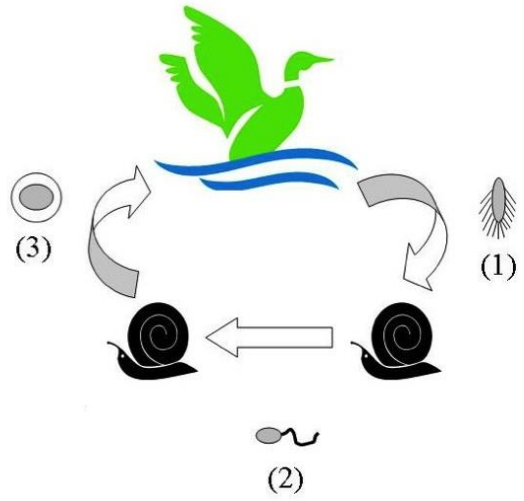

Figure 1. Generalized life cycle for *E. revolutum* and the waterfowl-killing parasites. Numbers correspond to 1) miracidium, 2) cercaria, and 3) metacercaria.

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 degree 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

(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.

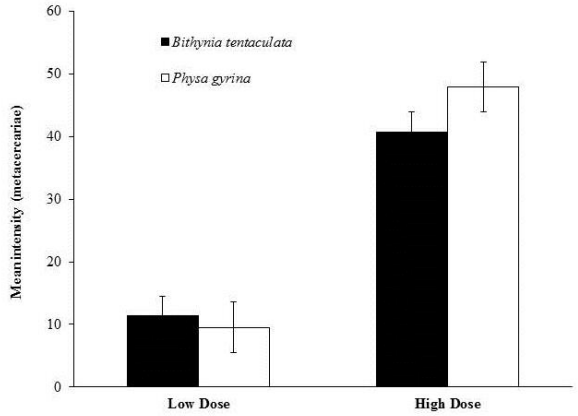

Figure 2. Mean intensity (metacercariae ± SE) for *Bithynia tentaculata* and *Physa gyrina* snails exposed to low (25) and high (125) doses of *Echinostoma revolutum* cercariae.

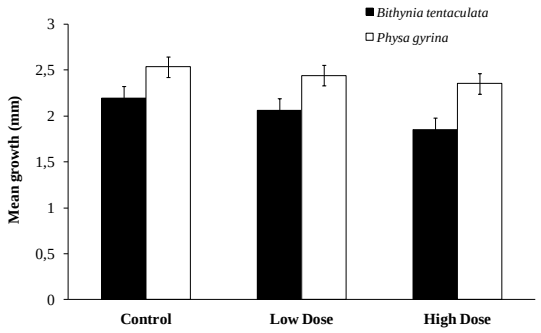

Figure 3. Mean overall growth (mm ± SE) for *Bithynia tentaculata* and *Physa gyrina* snails in control, low dose, and high dose groups over 4-week experimental duration. Snails were measured weekly using digital calipers.

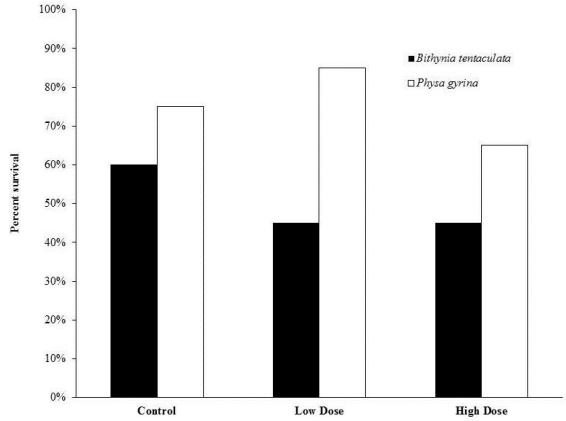

Figure 4. Percent survival of *Bithynia tentaculata* and *Physa gyrina* snails in control (0), low dose (25), and high dose (125) groups exposed to *Echinostoma revolutum* cercariae. Graph shows the percent of snails from each group (n=20) surviving the duration of the 4-week experiment.

Growth rates were compared between host species across exposure doses using a two-way ANOVA with repeated measures. Because these data did not adhere to the sphericity assumptions, a more conservative metric (Greenhouse-Geisser) was used to assess significance.

To determine whether snail survival at the end of the study was influenced by host species and/or infection dose, binary logistic regression was performed on the data. To do this, snails were first coded as "0" for dead or "1" for alive prior to analysis.

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 ($F_{1,71}$ = 148.565, P < 0.001). There was no effect of host species ($F_{1,71}$ = 0.242, P = 0.624) or the interaction between exposure dose and snail species on *E. revolutum* intensities ($F_{1,71}$ = 2.246, P = 0.138) (Figure 2).

There were no significant differences in the proportion of metacercariae establishing between species ($F_{1,71}$ = 0.002, P = 0.969). Moreover, there was no difference in the proportion of parasites establishing between doses ($F_{1,71}$ = 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 ($F_{1,71}$ = 1.555, P = 0.217).

Two-way ANOVA with repeated measures (Greenhouse-Geisser) indicated a significant difference in snail size across time ($F_{2.910}$ = 338.842, P < 0.001). There was also a significant interaction between time and species ($F_{2.910}$ = 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 ($F_{5.821}$ = 1.395, P = 0.220), or between time, dose, and species ($F_{5.821}$ = 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 *Pseudechinoparyphium 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 (Matitsky 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 *Echinoparyhpium 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 *E. revolutum* did not impact life history expression in native versus invasive snails suggests that this parasite is not contributing to *B. tentaculata*'s spread in the UMR. However, this lack of life history impact may actually further enhance *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 *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 *E. revolutum* in *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 *B. tentaculata* in the region.

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