

## Research Article

## Assessing infection patterns in Chinese mystery snails from Wisconsin, USA using field and laboratory approaches

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### Abstract

The success of invasive species establishment in new habitats depends, in part, on interactions with other members of the invaded community. *Cipangopaludina chinensis*, the Chinese mystery snail (CMS), is an exotic, invasive snail in North America. Since its introduction in the 1890s, CMS have spread to a number of watersheds across the United States and parts of Canada. This invasion has generated questions about the snail's capacity to serve as a host for parasites within local habitats, including parasites with conservation implications. To begin addressing these questions, we necropsied 147 wild-caught snails from 22 lakes across Wisconsin, finding only two CMS individuals harboring trematode (flatworm) parasites. We also conducted experimental exposures using a trematode (*Sphaeriodotremum pseudoglobulus*) implicated in waterfowl die-offs and found that CMS infection levels were significantly lower than those in co-occurring snail species. Furthermore, the parasites that did successfully infect CMS were often found encased in the shells of the snails in a non-viable state. Together these results 1) provide insight into the importance of CMS as a host for parasites in the region, and 2) may help to explain the wide distribution of CMS across WI and the Midwest.

**Key words:** *Cipangopaludina (Bellamya) chinensis*, invasive species, trematode infection, *Sphaeriodotremum pseudoglobulus*, Viviparidae

### Introduction

Species introductions are well-recognized as key threats to the integrity of native communities worldwide (Pyšek and Richardson 2010; Kumschick and Richardson 2013; Sandland et al. 2013) which, in turn, has important implications for local biodiversity, conservation policy, and ultimately, regional and national economics. Introductions may be particularly problematic in aquatic ecosystems where a combination of natural connections among water bodies and anthropogenic activities can facilitate the spread and proliferation of invaders (Dudgeon et al. 2006; Johnson et al. 2006; Lodge et al. 2006; Epifanio 2013). The degree to which the occurrence of aquatic invasive species alters local communities depends, in part, on their biotic interactions with native species (Human and Gordon 1996; Hawkes et al. 2010). A substantial

amount of evidence now exists to support the idea that invader-induced alterations to local predator-prey and competitive interactions can both enhance invader success and disrupt community structure (Zukowski and Walker 2009; for a review see Weis 2011). Although less-recognized, other biotic interactions, such as parasitism, can also contribute to the establishment of invasive species and their impacts on native species (Kelly et al. 2009; Sandland et al. 2013).

Recent work has started to elucidate the role that parasites can play in modulating aquatic invasions (for a review see Poulin et al. 2011). This can occur via differences in infection competencies between invasive and native hosts (Bachelet et al. 2004). For example, Genner et al. (2008) reported the absence of flatworm (trematode) infections in an invasive gastropod population (*Melanoides tuberculata* [Müller,

1774]) and used this observation to help explain the success of this invader relative to the native population which was more heavily parasitized. Although a handful of studies have documented relative infection competencies between native and introduced hosts (Kelly et al. 2009; Thielges et al. 2009; Sandland et al. 2013, Glodosky and Sandland 2014), the vast majority of interactions remain unknown, restricting our ability to predict invasive and native host responses to infection, and the capacity of parasites to transmit within local habitats invaded by exotic species.

The Chinese mystery snail (*Cipangopaludina chinensis* [Gray, 1834], CMS) is a viviparous, operculate snail that was first observed in North America at Asian-food markets in San Francisco in the early 1890s (Wood 1892; Jokinen 1982). Since its introduction, CMS have established populations in freshwater ecosystems throughout the United States and southern Canada (Jokinen 1982; Mastitsky et al. 2013). Adults are olive green to brown in color, live for 3–5 years, and can reach a large size, up to 62 mm in total length (Jokinen 1982; Solomon et al. 2010). They are typically found in lakes and slow-moving lentic systems with sandy or muddy substrates and require rooted aquatic vegetation (Jokinen 1982). Within these invaded habitats, CMS can be found at relatively high densities (Stanczykowska et al. 1971; Solomon et al. 2010) leading researchers to implicate this snail in the disruption of native aquatic communities (Unstad et al. 2013).

The effects that CMS have on native species within their invaded range have been recently investigated by a handful of authors. Johnson et al. (2009) found that in outdoor mesocosms, the indigenous gastropods, *Lymnaea stagnalis* (Linnaeus, 1758) and *Physa gyrina* (Say, 1821), exhibited reduced wet mass in the presence of CMS. Moreover, *L. stagnalis* abundance was reduced when it co-occurred with CMS, although follow-up field assessments did not fully support this finding (Solomon et al. 2010). Johnson et al. (2009) also suggested that CMS could influence the structure of native algal communities. Unfortunately, little else is known about CMS interactions with other members of the native aquatic community, which severely limits our ability to understand the potential impacts of the snail on invaded ecosystems (Chaine et al. 2012).

In its native range of eastern and southeastern parts of Asia, CMS are well-documented hosts for a number of different parasites, some of which are responsible for human disease (Bury et

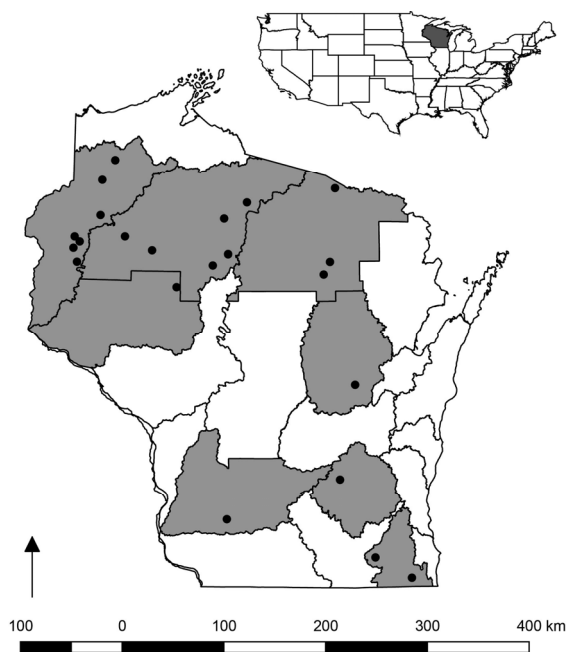
al. 2007; Karatayev et al. 2012). In contrast, there are only a few reported cases of parasitism in North American CMS populations, all involving *Aspidogaster conchicola* (Baer, 1827), a native aspidogastreaan trematode that typically infects bivalves (Michelson 1970; Jokinen 1982). One explanation for why infection patterns differ between invaded and native areas is that North American CMS populations have reduced infection competency compared to Asian populations. However, it is important to recognize that few studies have actually investigated parasitic infections in CMS from its introduced range, suggesting that infection differences may be simply due to under-sampling.

Here we assess the potential for CMS to harbor and transmit parasites in its invaded range. We address this question by necropsying wild-caught snails from Wisconsin lakes and by performing experimental infections using laboratory-raised snails. Working on snails from Wisconsin provides us with a unique opportunity to better understand CMS infection patterns as native and invasive freshwater snail species within this state have been documented to harbor a number of trematode species (Yoder and Coggins 1998; Krist 2006; Sandland et al. 2014). In addition, a number of these parasite species (such as *Cyathocotyle bushiensis* and *Sphaerioditrema pseudoglobulus*) have been implicated in waterfowl die-offs in the region (Sauer et al. 2007; Herrmann and Sorensen 2009; Sandland et al. 2013) making CMS compatibility assessments both necessary and timely.

## Materials and methods

### Field collections

Chinese mystery snails were first reported from Wisconsin in the 1950s (Teskey 1954) and currently occur throughout waterbodies in the three major drainages in this region: the Lake Superior, Lake Michigan, and Mississippi River drainages (Jass 2004; Johnson et al. 2009; Solomon et al. 2010). Twenty-two lakes in Wisconsin (Figure 1) were sampled for CMS by the Wisconsin Department of Natural Resources Aquatic Invasive Species task force in 2012. Each lake was sampled at five sites that were deemed most likely to support CMS based on bathymetric maps. Each site was explored via snorkeling over a 10 minute period. Fifty haphazard sites were also sampled at each lake using standardized rakes and dip nets. These protocols ensured a broad sampling of different



**Figure 1.** Inset: Map of the United States with study area, Wisconsin, indicated by filled polygon. Larger map: State of Wisconsin with major drainage basins outlined. Drainages sampled are shown in gray; the location of each lake with populations of CMS examined in this study indicated by filled black circle.

habitats within each lake. Collected snails were stored in 95% ethanol and shipped to our laboratory at the University of Wisconsin-La Crosse where they were processed.

In total, 147 individual CMS from 22 lakes were necropsied for parasites. Each necropsy was carried out by first crushing the snail shell with a hammer and then dissecting the tissues using forceps and a dissecting microscope ( $\sim 0.67\times - 5\times$  magnification). Soft tissues as well as shell cavities were examined for the presence of trematode larvae. Any suspected larvae were mounted on a microscope slide and examined at higher magnification ( $40\times$ ) for species-level identification.

#### Laboratory exposures

To acquire uninfected juvenile CMS for our parasite exposure experiments, adult snails were first collected from a single population, East Horsehead Lake in Oneida County, and kept in 19-liter aquariums containing well water. All CMS juveniles born in the laboratory were

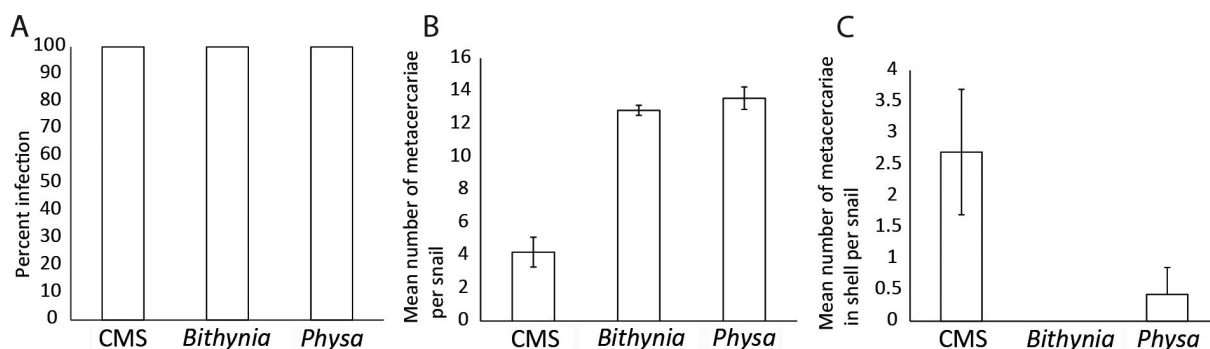
removed from the adult aquariums within 24 hours of birth and placed into a 9-liter nursery container. Juveniles were maintained separately from adults to eliminate the potential for parasite transmission between the field-collected snails and their uninfected, laboratory-reared offspring. Three weeks after birth, juveniles ( $n=10$ ) were removed from their nursery container and each individual was placed into a 10 ml plastic well containing well-water. Snails were then exposed to 15 *Sphaeriodotremia pseudoglobulus* (McLaughlin et al. 1993) cercariae which reflect parasite numbers found in other species of field-collected gastropods. Cercariae were acquired from laboratory-reared *Bithynia tentaculata* (Linnaeus, 1758) that had been experimentally infected with an earlier stage of the parasite; details regarding those infection protocols can be referred to in Sandland et al. (2013). Simultaneous exposures were also conducted using laboratory-reared *B. tentaculata* (invasive;  $n=6$ ) and *Physa gyrina* (native;  $n=7$ ) which are known hosts for *S. pseudoglobulus* (Sandland et al. 2014). *Sphaeriodotremia pseudoglobulus* was used in these exposures for 2 reasons: it is an important waterfowl parasite in the Midwest and it has broad host specificity at the cercarial stage (Sandland et al. 2014). After the parasites were added, juvenile snails remained in the wells for two to four days (to allow for metacercarial development) before being crushed and necropsied. A total of 15 juvenile CMS from our laboratory colony were also necropsied to ensure that all metacercariae were the result of experimental exposures and not *in utero* infections acquired by field-collected adults. Diagnostics (normality and homogeneity of variance tests) and statistical analyses were performed on the experimental data using SPSS. Diagnostic analyses revealed that intensity data met the homogeneity of variance assumption but violated normality. Therefore, intensity data were assessed using a Kruskal-Wallis non-parametric test with post-hoc pairwise comparisons.

#### Results

Of the 147 snails necropsied, 2 individuals from Little Chelsea Lake in the Jump River drainage were infected with trematodes. One snail contained a single metacercaria of *Cyathocotyle bushiensis* (Kahn, 1962) and a different individual harbored nine aspidogastrea flatworms (*Aspidogaster conchicola*; Table 1). No other parasites were observed in the necropsied CMS.

**Table 1.** Wisconsin drainage basins with number of lakes surveyed and number (*n*) of Chinese mystery snails (CMS) necropsied per basin. Number of infected CMS is also indicated. (\* Indicates the watershed where one individual was infected with *Aspidogaster conchicola* and a second individual was infected with *Cyathocotyle bushiensis*).

Lake	<i>n</i> snails necropsied	<i>n</i> infections	Parasite species
Lake Poygan	1	0	
Sauntry's Pocket	1	0	
Ward Lake	14	0	
Antler Lake	15	0	
Big Blake Lake	1	0	
Camelia Lake	9	0	
Amacoy Lake	3	0	
Round Lake	15	0	
Sailor Lake	10	0	
Little Chelsea Lake*	4	2	<i>A. conchicola</i> , <i>C. bushiensis</i>
Mondeaux Flowage	16	0	
Hulls Lake	17	0	
Big Sand Lake	1	0	
Red Cedar	13	0	
Blackhawk Lake	1	0	
Lazy Lake	2	0	
Paddock Lake	3	0	
Powers Lake	4	0	
Kimbal Lake	1	0	
South Neva Lake	1	0	
Big Bass Lake	3	0	
Lincoln Lake	12	0	
	<i>n</i> = 147	<i>n</i> = 2	



**Figure 2.** Experimental infections of three snail species with *Sphaeridiotrema pseudoglobulus* cercariae. For each panel, CMS: *n*=10, *Bithynia*: *n*=6, *Physa*: *n*=7. When appropriate standard error above and below the mean is indicated. A. Percent of individuals infected. B. Mean number of *S. pseudoglobulus* metacercariae encysted for each snail species. C. Mean number of *S. pseudoglobulus* metacercariae found in the shell (rather than tissue) of each snail species.

All 3 snail species (*P. gyrina*, *B. tentaculata*, and *C. chinensis*) experimentally exposed to *S. pseudoglobulus* harbored metacercariae (=100% prevalence; Figure 2A). However, there were significant differences in the overall numbers of metacercariae found across each snail species ( $\chi^2=15.80$ ,  $df=2$ ,  $P<0.001$ ) with CMS containing fewer metacercariae than either *B. tentaculata*

( $P=0.010$ ) or *P. gyrina* ( $P=0.001$ ). There was no difference in metacercariae intensity between *P. gyrina* and *B. tentaculata* ( $P=1.00$ ). On average CMS harbored 4.2 cysts per host whereas *B. tentaculata* and *P. gyrina* were infected with an average of 12.8 and 13.6 cysts per host respectively (Figure 2B). During necropsies, we also noticed that some of these metacercariae

were encased in the shell of infected snails rather than loosely attached. This was most common in CMS where on average 2.7 metacercariae were encased per snail (Figure 2C). Very low numbers of metacercariae were encased in *P. gyrina* (mean of 0.43 parasites per snail) and none were found encased in the shells of *B. tentaculata*. It should be noted here that metacercariae encased within the shells of CMS were extremely degraded and likely non-viable whereas those from the shells of *P. gyrina* were intact with worms moving inside their cysts.

## Discussion

Chinese mystery snails are a well-recognized invasive species in North America, yet their interactions with other members of the invaded community remain relatively unexplored (Bury et al. 2007, but see Richardson et al. 2009 and Solomon et al. 2010). Little knowledge of these biotic interactions makes it difficult to elucidate the potential mechanisms underlying invasion success. We attempted to begin addressing this shortcoming by investigating infection patterns in field-collected CMS and in laboratory exposed individuals. These approaches were undertaken to gain baseline information about parasitism in CMS and whether infection patterns may help to explain the spread of CMS throughout North American freshwater habitats.

Although information on CMS infections in North America is relatively sparse, work has shown that the snail can serve as a host for trematodes, specifically aspidogastreae (Michelson 1970; Huehner and Etes 1977). That being said, studies on CMS rarely assess the snails for parasites, suggesting that the low number of documented infection cases may reflect under-sampling rather than a true biological pattern. Results from our necropsies of field-collected snails support the idea that trematode infections are rare in CMS populations from Wisconsin. Interestingly, this observation reflects those reported for CMS from other areas of North America (Karatayev et al. 2012) and highlights an infection pattern oftentimes seen in invaders, including other snail species (Torchin et al. 2003; Mastitsky et al. 2010). Taken together, these results suggest that low trematode infection levels may be typical of CMS populations within their invaded ranges. This is a very different pattern compared to Asian CMS populations, which are often infected by a number of trematode species (Bury et al. 2007).

A number of factors may explain the differing infection levels reported for CMS collected from introduced and native habitats. First, it could be that CMS are inherently refractory to the parasite species cycling through the invaded aquatic community. This idea was at least partially supported by our experimental results showing that CMS harbored fewer metacercariae after exposure to a generalist trematode compared to other snail species. A second possibility is that habitat preferences differ between CMS and members of the aquatic community (i.e. other gastropods) that transmit parasites, thereby reducing overlap between CMS and trematodes. In terms of metacercarial infections, differences in host-habitat preference seem unlikely as previous research has reported spatial overlap between CMS and other gastropod species (Solomon et al. 2010), many of which can harbor trematodes that transmit among snails. Whether CMS co-occur with other hosts in the life cycles of these parasites (i.e. waterfowl) requires further investigation. Lastly, it is possible that the collection protocols undersampled infected CMS subpopulations. Although this may be possible for some water bodies (such as Amacoy Lake, n=3), it seems less likely for other sampled areas where larger numbers of CMS were necropsied.

The general lack of infections in CMS across Wisconsin lakes may have consequences for the establishment and spread of the snail in the region. Because trematode infections can reduce snail reproduction and survival (Sandland and Minchella 2003; Lafferty and Kuris 2009), lower parasite numbers in CMS relative to other members of the gastropod assemblage may lead to the invader having a fitness advantage relative to infected snail species. Higher reproductive output in CMS through reduced infection levels may help to explain this species' relatively wide range across Wisconsin. It should be recognized however, that CMS did exhibit infections (albeit at relatively low intensities) in our experiment which could actually dilute the parasite pool that could otherwise infect other snails in the community. Expanding necropsies to include co-occurring snail species should help to resolve this idea and improve our understanding of natural infection rates across different hosts.

Although trematode infections were rare in necropsied CMS, the two species that we did discover may be of potential concern within Wisconsin. *Aspidogaster conchicola* is a trematode flatworm that is typically found in bivalves;

however it also has been reported in CMS collected from field sites in Massachusetts and from experimentally exposed snails (Michelson 1970; Huehner and Etges 1977). The fact that CMS can serve as an additional viable host for these parasites may facilitate their transmission to species of native Wisconsin mussels, many of which are threatened or endangered. The second parasite reported, *Cyathocotyle bushiensis*, is one of 4 trematode species associated with waterfowl mortality in the Upper Mississippi River region of Wisconsin. This observation along with the fact that we were able to experimentally infect CMS with another parasite associated with Wisconsin waterfowl die-offs (*S. pseudoglobulus*) means that CMS could elevate parasite transmission to migrating waterfowl. However, the likelihood of this scenario is tempered by our experiments showing reduced infection competency and increased parasite encasement within CMS, which could limit its ability to transmit to waterfowl in the region.

Interactions between invasive species and parasites can play a crucial role in ecosystem dynamics. Both field and experimental research suggest that CMS have low infection levels that may help to explain the species' success in Wisconsin. Additional experiments looking at snail responses to different parasite species and life history stages will provide a more complete view of CMS compatibility. Furthermore, necropsies of CMS and native species from other watersheds will allow us to more-broadly assess trematode infections in CMS.

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