DIFFERENCES IN NATURAL INFECTIONS OF TWO MORTALITY-RELATED TREMATODES IN LESSER SCAUP AND AMERICAN COOT

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ABSTRACT: Populations of North American waterbirds, particularly lesser scaup, have been declining due to habitat disturbance, changing food resources, contaminants, bad water quality, and competition. However, epizootic diseases, including parasitism, may also play an important role in further decline. Trematode-associated mortality of migrating waterbirds, mainly American coot and lesser scaup, has been occurring in the Upper Mississippi River National Wildlife and Fish Refuge since 2002. We examined the levels of infective stages of Cyathocotyle bushiensis and Sphaeridiotrema globulus in the invasive, intermediate host snail, Bithynia tentaculata, during the fall of 2005 and compared these to infection levels in moribund or dead bird hosts. Our results show different infection levels of these 2 parasites in the 2 bird species; C. bushiensis is found more frequently in coot, and S. globulus is more common in scaup. This result is interesting because both bird species are presumed to forage on the same snail population and thus should be experiencing the same extent of exposure. These differences in infections could be attributed to differences in resources of gastrointestinal tracts of coot and scaup, or host resistance. Alternatively, differences in feeding behaviors of coot and scaup may also contribute to differential infections of the 2 trematodes.

Declining populations of waterbirds in North America have been a concern for biologists and hunters for some time, especially the marked declines in the lesser scaup (Aythya affinis) population of the Mississippi Flyway (see Beauchamp et al., 1996; Austin et al., 2000; Afton and Anderson, 2001; Costanzo and Hindman, 2007; Moon et al., 2007; Colucey et al., 2008). This decline has been attributed to habitat disturbance, limited food resources during migration, accumulation of contaminants (Afton and Anderson, 2001), poor water quality (Walsh et al., 2006), and/or increased competition with fish (Strand et al., 2008). However, little consideration has been given to epizootic diseases. Since 2002, 28,000–36,000 migrating waterbirds are estimated to have died in Pool 7 of the Upper Mississippi River National Wildlife and Fish Refuge (UMR Refuge) during spring and fall migrations (C. Gehri, pers. comm.). American coot (Fulica americana) and lesser scaup comprise 95% of the die-offs, while they account for only 20–25% of the migrating waterbirds that forage at the UMR refuge.

Moribund and dead individuals are found within days after the migrating birds arrive at UMR Refuge. Cyathocotyle bushiensis (Cyathocotylidae) and Sphaeridiotrema globulus (Psilostomidae) are the 2 trematodes most commonly associated with this recurrent mortality (Sauer et al., 2007). Waterbirds become infected when they ingest second-intermediate hosts, the invasive snail Bithynia tentaculata, containing these parasites. In a river system in southern Quebec, Hoeve and Scott (1988) showed that sentinel dabbling ducks can ingest lethal levels of C. bushiensis and S. globulus from naturally infected snails within 24 hr, and they attributed mortality to a synergistic effect between the infections of both trematodes. Lethally infected birds are unable to fly; as their condition declines, they are unable to dive and typically take refuge along the rocky shore of islands until they die (K. Herrmann, pers. obs.). Similar observations have been made in other studies on these trematodes in North America (Roscoe and Huffman, 1982, 1983; Huffman and Roscoe, 1989; Mucha and Huffman, 1991). Death of lethally infected birds usually occurs within 3 to 10 days (Hoeve and Scott, 1988; Huffman and Roscoe, 1989; Mucha and Huffman, 1991). However, healthy birds harvested by hunters have been shown to have significantly fewer C. bushiensis and S. globulus than birds dying as a result of infections (Hoeve and Scott, 1988). Further, of 9 hunter-shot birds we examined from UMR Refuge, 1 contained a single C. bushiensis, and the other 8 were free of infection by these 2 trematodes (Herrmann and Sorensen, pers. obs.).

Susceptibility of definitive hosts to infection is ultimately affected by their rate of exposure to infective stages and innate compatibility with a particular parasite species (Scott, 1988). Whether a parasite is encountered and the rate at which it is encountered by a potential host are influenced by the behavior and life-history traits of potential hosts (Hoeve and Scott, 1988), spatial overlap of infective stages and hosts (Euzet and Combes, 1980), and density of host populations and infective stages (Scott, 1988). Both coot and scaup are more likely to encounter B. tentaculata snails infected with C. bushiensis and S. globulus than other waterbirds at UMR Refuge because of their preference for foraging on molluscs in deep, open-water habitats with emergent vegetation, whereas other species of waterbirds prefer other invertebrates, vegetation, or shallower habitats (Thompson, 1973). Once a bird has ingested an infective stage, infection success depends upon physiologic/immunologic compatibility with the host (Euzet and Combes, 1980; Scott, 1988).

In the present study, we investigated the abundance of infective stages in B. tentaculata snails and the infection levels in bird hosts. Since both coot and scaup feed upon the same snail population at this site, our null hypothesis was that the 2 species should experience the same rate of exposure to metacercariae, and, thus, we would expect no difference in levels of infection of either C. bushiensis or S. globulus between the 2 bird species.

MATERIALS AND METHODS

Pool 7 (Lake Onalaska) is a 30-km² impoundment in La Crosse County, Wisconsin, created from the backwaters of Lock and Dam 7 on the Mississippi River (Fig. 1). It is part of the 420-km-long UMR Refuge, which is used by almost 300 species of migrating birds of the Mississippi Flyway. Migrating waterbirds are present at Pool 7 from mid-March through late April and mid-September through late November. The average water depth is 1.0–1.3 m.

Snails were collected on 11 October 2005 around Arrowhead and Broken Gun Islands (Fig. 1) at sites established during a previous study (Herrmann and Sorensen, 2009). At 15 shore sites, B. tentaculata snails were randomly collected by hand from the bottom of rocks until a
maximum number of 30 snails was obtained, or a 30-min search limit was reached. Additionally, 3 sites at each island were located 25–100 m from shore with a water depth between 1 and 2 m; snails were collected from the bottom substratum using a benthic grab sampler (Petite Ponar, Wildco, Buffalo, New York). In total, 415 snails were collected and transported on ice to Minnesota State University–Mankato (MSUM), Mankato, Minnesota. Snails were stored at 4°C until examined.

Snail shell length was measured with calipers to the nearest 0.1 mm. Snails were individually crushed, and presence and number of mature metacercariae (infective stage to birds) were enumerated. Metacercariae of *C. bushiensis* and *S. globulus* were identified by relative size and morphological examinations (Khan, 1962; Gibson et al., 1972; Lepitzki et al., 1994).

Immature metacercariae were distinguished from mature metacercariae by the presence of a thin, cyst wall with a translucent center, whereas mature metacercariae of *C. bushiensis* and *S. globulus* were identified by having a thick, translucent cyst wall and a pigmented center (Khan, 1962; Gibson et al., 1972; Lepitzki et al., 1994), and a slightly thicker cyst wall and mostly pigmented center portion (Lepitzki et al., 1994), respectively.

Bird carcasses were collected in conjunction with weekly removal efforts by U.S. Fish and Wildlife Service staff. Moribund birds that were unable to fly or dive were killed by cervical dislocation. Six collections were made between 10 October and 18 November 2005; 9 scaup and 9 coot were collected from each of the islands, for a total of 18 birds per species. All carcasses were either transported on ice or shipped to MSUM and frozen until necropsied. Intestinal contents and tissue were examined for parasites, and pathology was noted.

Specimens of metacercariae and adult worms were deposited in the Harold W. Manter Laboratory of Parasitology collection (accession no. P-2010-009) at the University of Nebraska, Lincoln, Nebraska.

Prevalence and mean abundance (as defined by Bush et al., 1997) of mature metacercariae and adults were quantified. Only mature, and therefore infective, metacercariae were considered in the evaluation of infections in snail hosts for comparison to infections in definitive bird hosts (Khan, 1962; Gibson et al., 1972; Lepitzki et al., 1994). Thus, the metacercariae data used here represent only a proportion of the total metacercariae observed in October. Mean abundance of *C. bushiensis* was divided by mean abundance of *S. globulus* to determine the relative mean abundance ratio of the 2 trematode species for each host.

No differences were found between sexes, or between islands from which the birds were collected, and they were combined for further statistical analyses. First-intermediate host snails were removed from analyses. Differences in prevalence of *C. bushiensis* and *S. globulus* were analyzed with G-tests. Differences in mean abundance between site type (shore and open water) and between *C. bushiensis* and *S. globulus* were assessed with Wilcoxon signed ranks tests. G-tests using the ratio of *C. bushiensis* to *S. globulus* in snails as the expected value and the ratio in each bird host as the observed value were used to assess the relative infections of the 2 trematodes between snail and bird hosts. Relationships between snail shell length and mean abundance were analyzed with a Spearman’s rank correlation. A statistical significance of *P* < 0.05 was used for all analyses.

**RESULTS**

Mean snail size was 8.0 ± 0.09 mm and ranged from 3.0 to 11.0 mm. Snail size did not differ between shore and open-water sites (*Z* = −1.55, *df* = 413, *P* = 0.12). Mean abundance of mature metacercariae of *C. bushiensis* and *S. globulus* was positively correlated with snail shell length (*ρ* = 0.180, *P* < 0.001 and *ρ* = 0.232, *P* < 0.001, respectively).

The proportion of mature metacercariae in snails was 0.27 and 0.17 of the total metacercariae observed for *C. bushiensis* and *S. globulus*, respectively (Herrmann and Sorensen, 2009). Both prevalence (*G* = 118.8, *df* = 1, *P* < 0.001; Table I) and mean abundance (*Z* = −3.52, *df* = 396, *P* < 0.001; Table I) values of mature *C. bushiensis* metacercariae were greater than those of *S. globulus*. Mixed infections of mature metacercariae were found in 40.3% of snails. Mean abundance of mature metacercariae of *C. bushiensis* and *S. globulus* ranged from 0 to 131 and 0 to 74 per snail, respectively.

All birds were adults. Of the 18 coot, 6 were females, and 12 were males; for scaup, 8 were females, and 10 were males. Prevalence of adult *C. bushiensis* individuals did not differ from prevalence of adult *S. globulus* individuals in scaup (*G* = 0.12, *df* = 1, *P* > 0.05; Table I). However, the prevalence of adult *C. bushiensis* individuals was greater than that of *S. globulus* individuals in coot (*G* = 18.50, *df* = 1, *P* < 0.001). Mixed infections of *C. bushiensis* and *S. globulus* were found in 16 of 18 scaup, while only 1 of 18 coot specimens had a mixed infection. The single coot infected with *S. globulus* possessed only 3 worms of this species, none of which contained eggs in utero, whereas all other birds possessed infections of mature parasites.

Mean abundance of adult worms did not differ by island in coot or in scaup and was, therefore, combined for comparison between species. A difference in mean abundance of adult worms between trematode species was found in both coot and scaup; however, the difference showed opposite trends (Table I). Mean abundance of *C. bushiensis* was greater than *S. globulus* in coot (*Z* = −3.724, *df* = 17, *P* < 0.001). Conversely, mean abundance of *S. globulus* was greater than *C. bushiensis* in scaup (*Z* = −3.74, *df* = 17, *P* < 0.001).

Specimens of *C. bushiensis* were found in the ceca of all birds examined. Ceca of coots were hemorrhagic, with ulcerations and whitish caseous plaques, often with a friable core; the rest of the intestine of coot was filled with a firm darkened core. Ceca of scaup were hemorrhagic, with ulcerations and whitish caseous plaques, often with a friable core; the rest of the intestine of scaup was filled with a firm darkened core. Ceca of scaup infected with *C. bushiensis* had ulcerative lesions, but not to the extent of those in coot. *Sphaeridioptera globulus* individuals were located mostly in the jejunum, sometimes spreading throughout the intestines.
entire small intestine in heavy infections. Small intestines of scaup infected with *S. globulus* were distended, with severe ulcerative hemorrhaging. One other adult trematode, an unknown strigeid, was observed occasionally, as were acanthocephalans.

Relative mean abundance of *C. bushiensis* compared to mean abundance of *S. globulus* in snail hosts was different than in coot or scaup hosts (G = 904.48, df = 1, P < 0.001 and G = 920.04, df = 1, P < 0.001, respectively; Table I). The ratio of mean abundance of mature metacercariae of *C. bushiensis* to *S. globulus* was 1.64:1 in snail hosts. In contrast, the ratio of mean abundance of adult worms of *C. bushiensis* to *S. globulus* was 108:1 in coot and 1:100 in scaup. Relative mean metacercariae abundance of *C. bushiensis* to *S. globulus* was not correlated with snail shell length (p = 0.038, P = 0.58).

**DISCUSSION**

Our data show that *C. bushiensis* and *S. globulus* infections in coot and scaup are different from relative infections of *C. bushiensis* and *S. globulus* in snails. Moreover, the change in the relative infections was in opposite directions for coot and scaup. Presumably, coot and scaup experience a similar rate of exposure to metacercariae of *C. bushiensis* and *S. globulus* because these 2 bird species forage in the same area on the same population of snail hosts (data not shown). However, this study found that prevalence, mean abundance, and lesions caused by the 2 trematodes were different between coot and scaup. This conflicts with Hoeve and Scott’s (1988) hypothesis of a synergistic interaction by these 2 trematodes in the mortality of dabbling ducks.

Our results suggest that coot may have low susceptibility to *S. globulus*, since among 18 examined birds, only 1 possessed worms of this species. Further, these 3 *S. globulus* worms were immature and thus may have been unable to establish a successful infection in a coot host. The presence of immature parasites among accidental hosts has been reported previously in many studies. For instance, immature specimens have been widely reported among several taxa of intestinal fish parasites (Trejo, 1994; Pérez-del Olmo et al., 2006, 2007; Randhawa et al., 2007). We acknowledge that experimental infections with coot are needed to support this hypothesis. Experimental infections with each trematode species have been previously conducted with several bird species, but not with coot (Hoeve and Scott, 1988; Huffman and Roscoe, 1989).

The opposite trend was found in scaup hosts and possibly suggests a lowered compatibility with the other trematode, *C. bushiensis*. The change in the relative infections of *C. bushiensis* and *S. globulus* from snail to scaup hosts seems to indicate lowered susceptibility to *C. bushiensis* but greater susceptibility to *S. globulus*. However, we cannot assess whether the lower infection levels of *C. bushiensis* were due to possible incompatibility of physiological/morphological conditions necessary for parasite establishment, or the possibility of host resistance. Macy et al. (1968) showed that intestinal emptying time and enzyme activity affect establishment of *S. globulus* in different bird species. Since the gastrointestinal tracts of coot and scaup are very different, the intestinal emptying time and enzyme activity would also be expected to differ. These factors could result in very different resources available to parasites and may explain the differences found in this study. Alternatively, since acquired immunity to both of these trematodes is possible (Macy, 1973; Huffman and Roscoe, 1986; Hoeve and Scott, 1988), host resistance could also explain the observed patterns. However, this explanation is less likely, since the intermediate host snails that are necessary for these life cycles are not widely distributed in North America, making it unlikely that birds arriving at the refuge will have had previous exposure to these trematodes. Furthermore, birds arriving at UMR Refuge have low fat reserves and use this stopover to replenish those reserves before continuing their migration. Therefore, once a dense patch of these snails is found upon arrival, birds would be expected to forage intensely, leaving little opportunity for their immunity to develop at this site.

There is the possibility of infection levels differing in snails by size (Morley et al., 2004) or with water depth (Jokela and Lively, 1995; Koppel, 2009). Coot and scaup most likely forage intensively upon any patch of snails that they encounter; however, even if the birds did differ in size-selective predation, we found no relationship between snail size and the relative infections of *C. bushiensis* to *S. globulus* metacercariae. Likewise, coot and scaup may be foraging at different depths (Wilson, Hustler et al., 1992) and consequently may be exposed to different concentrations of metacercariae. However, no difference in abundance of metacercariae in snails was found between open-water sites at 1.0–2.0 m and shore sites (Herrmann and Sorensen, 2009). Further, *B. tentaculata* are most abundant at 1.3–2.0 m (Koppel, 2009), and the average depth of the lake is quite shallow (1.0–1.3 m) and within the foraging range for species of both these bird genera (Cronan, 1957; Wilson, Wilson, and Noldeke, 1992). Therefore, both coot and scaup should be capable of feeding on snails at similar depth and encounter the same exposure to infections. Further investigations into foraging depths and infection patterns by depth are recommended.
A concern for the potential spread of these trematodes is that acquired resistance has been shown to be possible after an initial exposure to a low dose (Macy, 1973; Huffman and Roscoe, 1986; Hoeve and Scott, 1988). Thus, for bird species that preferentially feed upon vegetation and may incidentally ingest a sublethal dose, development of acquired resistance is possible if they are compatible with these parasites. Consequently, the birds may leave Pool 7 with a nonlethal infection and be able to deposit parasite eggs elsewhere. This becomes especially important as the invasive B. tentaculara snails spread up and down the river (Sauer et al., 2007).

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LITERATURE CITED


