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Graduate Studies

THE SPATIAL AND TEMPORAL DISTRIBUTION OF *BITHYNIA TENTACULATA* AND THREE PARASITE SPECIES IMPLICATED IN WATERFOWL MORTALITY IN THE UPPER MISSISSIPPI RIVER NATIONAL WILDLIFE AND FISH REFUGE

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THE SPATIAL AND TEMPORAL DISTRIBUTION OF *BITHYNIA TENTACULATA* AND THREE PARASITE SPECIES IMPLICATED IN WATERFOWL MORTALITY IN THE UPPER MISSISSIPPI RIVER NATIONAL WILDLIFE AND FISH REFUGE

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ABSTRACT

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The Upper Mississippi River National Wildlife and Fish Refuge (UMRNW&FR) is a 418 km long refuge that lies within the Mississippi Flyway. Over 40% of the nation's waterbirds use the Mississippi Flyway to navigate to and from their breeding grounds in the spring and the fall. Since 2002, large waterbird mortality events caused by the parasitic trematodes, *Cyathocotyle bushiensis* (Digenea: Cyathocotylidae) and *Sphaeridiotrema* spp. (Digenea: Psilostomidae), have been occurring within the Refuge's boundaries. The parasites use the prosobranch snail, *Bithynia tentaculata* as first and second intermediate hosts. Although *B. tentaculata* and its parasites have caused seasonal waterbird mortality events, little is known about the spatial or temporal distributions of these snails and their trematodes in the UMRNW&FR. The objectives of this study were to quantitatively sample both *B. tentaculata* densities and parasites intensities in waterbird stopover areas across space (Chapter 1) and time (Chapter 2), and to map disease risk with the use of a Geographic Information System (GIS) (Chapter 3). Snails with primary and secondary infections were found to vary in infection prevalence and intensity across 2 navigation pools of the UMRNW&FR. Much of this variability was likely driven by the presence or absence of vegetation and water velocity. Parasite infection prevalence either decreased or remained stable from summer 2010-spring 2011, suggesting that overwintering results in a reduction in parasite transmission. GIS risk maps based on sampling data identified transmission hotspots in one of the UMRNW&FR navigation pools. In the future, this work will aid in development of conservation strategies and the design of UMRNW&FR rehabilitation projects.

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CHAPTER I

THE SPATIAL DISTRIBUTION AND PARASITE INFECTION STATUS IN TWO CRITICAL CLOSED AREAS OF THE UPPER MISSISSIPPI RIVER NATIONAL WILDLIFE AND FISH REFUGE (UMRNW&FR)

ABSTRACT

Bithynia tentaculata (Gastropoda: Prosobranchia) is an invasive aquatic snail that was first detected in the Upper Mississippi National Wildlife and Fish Refuge (UMRNW&FR) in 2002. Since that time the species has become a major concern in the region because it harbors the trematode species; *Cyathocotyle bushiensis* (Digenea: Cyathocotylidae) and *Sphaeridiotrema* spp. (Digenea: Psilostomidae) that cause high levels of waterfowl mortality after birds consume infected hosts. Although *B. tentaculata* and associated parasites are having significant biotic and economic impacts in the region, little is known about the spatial distributions of these snails and their trematodes in the UMRNW&FR. The objectives of this study were to investigate both *B. tentaculata* densities and parasites' intensities at offshore sites that are critical habitats for bird migration through Navigation Pools 7 and 8 of the upper Mississippi River. Areas of high waterfowl use were identified in these two pools from weekly aerial surveys and a 23.9 hectare cell size grid was overlaid across the area to identify sampling locations. Three replicate samples were taken using a standard ponar grab at each sampling location on the grid. Snails within each replicate sample were counted and then necropsied to assess infection. Results demonstrate that host densities and parasite intensities differ between

pools and among sampling locations within a pool. Substrate type, vegetation presence, and water velocity appear to be important predictors of snail densities across sites. This suggests that habitat rehabilitation programs aimed at developing suitable habitat for waterfowl may also be facilitating the establishment and population growth of *B. tentaculata* and its parasites. The results from this work will help managers understand the spatial dynamics of the system, which will aid in the development of rehabilitation projects.

INTRODUCTION

The Upper Mississippi River Wildlife and Fish Refuge (UMRNW&FR) consists of 418 km of Mississippi River floodplain from Wabasha, MN to Rock Island, IL (U.S. Fish and Wildlife Service, 2006). A significant proportion of the UMRNW&FR contains nutrient-rich waters and shallow marshes, which provide excellent habitat for waterfowl moving to and from their breeding grounds in the Yukon and Alaska (Reid *et al.*, 1989; U.S. Fish and Wildlife Service, 2006). This flyway serves as a primary migration corridor for over 40% of North American waterfowl (Afton and Anderson, 2001). The diversity of waterfowl species within the UMRNW&FR generates both ecological and recreational interest making it one of the most widely visited and used refuges in the nation (U.S. Fish and Wildlife Service, 2006).

The Mississippi Flyway is a critical route for a number of waterbird species on the U.S. Fish and Wildlife Service Region 3's conservation priority list, including: wood duck (*Aix sponsa*), mallard (*Anas platyrhynchos*), blue-wing teal (*Anas discors*), canvasback (*Aythya valisineria*), and lesser scaup (*Aythya affinis*) (U.S. Fish and Wildlife Service, 2006). Before the installation of the lock and dam system, lesser scaup were the most abundant duck that used the Mississippi Flyway (Green, 1970). The lock and dam



Figure 1. Location of the Upper Mississippi River National Wildlife and Fish Refuge. The refuge is highlighted with the darker line. This image was obtained from the U.S. Fish and Wildlife Service.

system stabilized water levels and provided better habitat for puddle ducks as lesser scaup populations slowly decreased (U.S. Fish and Wildlife Service, 2006). Populations of lesser scaup have continued to decline over the last two decades, with an average loss of more than 150,000 individuals every year (Austin *et al.*, 1999). Due to these mortality levels, scaup populations have remained well below the North American Waterfowl Management's population goal of 6.3 million birds since 1985 (Afton and Anderson, 2001). Environmental problems such as contaminant introductions, habitat alterations, and decreased food resources have been identified as likely variables influencing lesser scaup mortality (Austin *et al.* 1999). Because of this, the UMRNW&FR's Comprehensive Conservation Plan (CCP) has listed lesser scaup as a conservation priority, stating that further research is needed to better understand the habitat requirements of the birds and the quality of stopover sites along their migration routes.

In 2002, large numbers of lesser scaup and American coot (*Fulica americana*) began dying within the UMRNW&FR's boundaries (Sauer *et al.* 2007). These deaths coincided with the discovery of an aquatic invasive snail, *Bithynia tentaculata* which serves as an important link in the life cycle of parasites implicated in waterfowl mortality. Although this snail was only recently discovered in the Upper Mississippi River (UMR), it has been in the Great Lakes region since the late 1880s when it was introduced from Europe (Baker, 1928). Currently, the North-American range of this snail is believed to extend from the Mississippi River to the east coast. In addition, it has also been recently documented in a select number of lakes in Minnesota and Montana (Baker, 1928; Berry, 1943; Clarke, 1981; Jokinen, 1992; Sauer pers. comm.; Cole pers. comm.).

Bithynia tentaculata has been shown to be restricted to low-flow areas where it can successfully attach to a variety of substrates including gravel, the underside of rocks, silt, clay, and on submerged macrophytes (Vincent *et al.*, 1981; Richter, 2001). This fact may contribute to the observation that the snail is typically found in shallow waters from 1-3 m (Vincent *et al.,* 1981). Another factor restricting *Bithynia tentaculata* distribution is oxygen. Work by Richter (2001) showed that *B. tentaculata* is intolerant of anoxic waters, which may explain the lack of snail establishment in high-productivity environments. Although *B. tentaculata* has a rather narrow tolerance for flow and oxygen concentration (Richter, 2001), it can tolerate a wide range of other aquatic variables such as pH, temperature, and salinity, which likely facilitates it success in the UMRNW&FR (Mitchell and Cole, 2008).

Most life-history information for *Bithynia tentaculata* has been documented from European or Canadian populations; little information exists on the upper Mississippi River population. For example, Richter (2001) found that *Bithynia tentaculata* is relatively inactive from November to March in populations from Germany. During this time snails

will migrate to deeper depths to overwinter as the water temperature starts to decrease (Richter, 2001). In March, reproduction begins when water temperatures rise above 12˚C in the spring. Female *B. tentaculata* reach sexual maturity at 6 mm and eggs are deposited on any hard substrate including vegetation, word, rock and even the shells of other mollusks (Vincent and Gaucher, 1983; Richter, 2001). Eggs begin to hatch when the developing embryos reach 0.8 mm in length (Vincent and Gaucher, 1983). During the first year, juveniles grow rapidly at a rate of 0.5 mm a week during the months with warm water temperature (Pinel-Alloul and Magnin, 1971). Most snails will not grow during the winter months when the water temperature drops below 20˚C (Vincent *et al.,* 1981). Mature individuals will reach up to 12 mm in length (Baker, 1928a, Berry, 1943, Fretter and Graham, 1962) and survive, on average, for 18 months although data suggest that some can live for over 32 months (Pinel-Alloul and Magnin, 1971; Mattice, 1972; Dussart, 1979).

Bithynia tentaculata has a potential advantage over other co-occurring gastropods, as the snail is able to filter feed, unlike many native species (Meier-brook and Kim, 1977; Jokinen, 1992). This advantage allows them to 1) exploit new areas that may not be colonized by natives and 2) survive in areas with high gastropod densities (Meier-Brook and Kim, 1977). For example, researchers have reported densities of 80 *B. tentaculata* per m^2 in Oneida Lake in New York under favorable water conditions (Jokinen, 1992). This feature along with its life-history and abiotic tolerances may explain why *B. tentaculata* makes up a large proportion of the mollusk community in large, slow moving water bodies (Vincent, 1981; Jokinen, 1992; Sandland pers. obs). These advantages likely make *B. tentaculata* a strong competitor with native snails, which may result in a reduction in native snail densities (Strayer, 1999).

Along with consequences to native snails, *B. tentaculata* invasion of the UMR has also disrupted local biota by transferring parasites to mollusciverous waterfowl (Sauer *et al.*, 2007). Once ingested, the parasites mature and attach to the intestine of the bird, causing massive hemorrhaging and ultimately death of the host. Avian mortality was originally linked to two species of intestinal trematodes, *Cyathocotyle bushiensis* (Khan, 1962), and *Sphaeridiotrema globulus* (Rudolphi, 1814). Since that time however, two species of *Sphaeridiotrema* have been recognized to infect *B. tentaculata* in the UMRNW&FR – *S. globulus* and *S. pseudoglobulus* (Bergamme *et al.*, 2011; Sandland *et al.*, 2011). All of these trematode species complete their life cycles by using *Bithynia tentaculata* as both first-intermediate (rediae/sporocyst) and second intermediate (metacercariae) hosts in the UMR (Sauer *et al.*, 2007, Sandland, pers. comm., Cole, pers. comm.) (Figure 2). Since 2002, it is estimated that these parasites have contributed to waterbird mortality exceeding 60,000 individuals. Much of this die-off has continued to be made up of lesser scaup and American coot.

Lesser scaup and American coot both rely on nutrient rich feeding areas in the UMRNW&FR during migration to replenish lost fat stores (Afton and Ankley, 1991; U.S. Fish and Wildlife Service, 2006). Therefore nutrient requirements and nutritional status during migration can be a driving force behind this disease. A waterbird in good physical condition will have a lower probability of infection due to shorter residency in the UMRNW&FR and less aggressive feeding. The abundance of high-quality food sources for lesser scaup and American coot may decrease the chance of birds feeding on *Bithynia tentaculata*. Unfortunately, *Bithynia tentaculata* along with native snails are an important food items for migrating waterfowl along the Upper Mississippi River. This diet of widely

available prey like *B. tentaculata* in important feeding areas, is likely contributing to the high waterbird mortality reported annually (Sauer *et al.*, 2007).

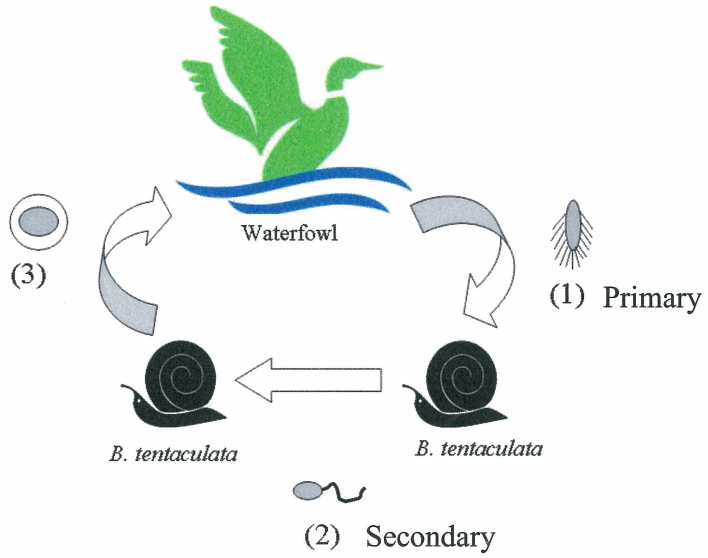


Figure 2. Life cycle of *C. bushiensis* and *Sphaeridiotrema* spp. In this case the parasite has three hosts: a snail first-intermediate host, a snail second-intermediate host, and a bird definitive host. Snails become infected by swimming miracidia (1), which hatch from eggs that are released from adult worms in the intestines of birds. Once in the snail, the parasites transition through a number of stages (sporocysts, rediae, or both) before releasing cercariae (2). Cercariae then encyst as metacercariae (3) within the snail 2nd intermediate host and remain encysted until the snail is eaten by a bird. Parasites then develop to maturity in the bird and begin generating eggs which are released into the environment.

The UMRNW&FR's Comprehensive Conservation Plan (CCP) has established two major closed areas in Pools 7 and 8 where waterfowl hunting is not allowed. These regions provide areas so the birds can rest and feed during migration. Unfortunately, these areas also contain habitat suitable for *B. tentaculata* and its waterfowl killing parasites. However, the spatial relationships among the waterbirds, *B. tentaculata* and its parasites have not been investigated across these areas in Navigation Pools 7 and 8 of the UMRNW&FR. This

study provides the first comprehensive information on the transmission dynamics of the parasites where the birds rest and feed in the Upper Mississippi River National Wildlife and Fish Refuge.

Study Objectives

This research expands upon previous work conducted in the Pool 7-closed area of the UMRNW&FR. In a previous study, sampling occurred in the shallow areas surrounding constructed islands in Pool 7 (Hermann and Sorensen, 2009). The authors found infection patterns to be quite variable across sites and concluded that these patterns were driven primarily by water temperature. However, how these patterns apply to deep-water sites in Pool 7 (where lesser scaup preferentially feed) and across pools themselves remains unknown.

Based on a lack of information on the spatial distributions of *B. tentaculata* and its parasites across deep-water sites in Pool 7 and across other navigation pools, I undertook a study to investigate the spatial distributions of hosts and parasites in areas where waterbirds are known to forage and rest (closed areas). Within this study, I addressed 2 specific objectives. First, I used a quantitative sampling protocol to assess snail distributions in off-shore areas that are used by lesser scaup populations in Pools 7 and 8 the UMRNW&FR. Second, I determined the occurrence of three *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. in *B. tentaculata* collected from areas of heavy scaup use in these pools.

METHODS

Study Sites

This study focused on two main study locations, the Pool 7 Lake Onalaska closed area (43°54'13.52" N, 91°17'41.80" W) and the Pool 8 Wisconsin Islands closed area (43°36'47.10" N, 91°14'44.92" W) due to high waterfowl use days, high waterfowl densities, and the high levels of seasonally occurring waterbird mortality (J. Nissen, pers. comm.). Pool 7 extends 18 km from Trempealeau, WI to Dresbach, MN and consists of a main navigation channel, marshes, bottom-land forests, backwater lakes, and sloughs. Pool 7 contains Lake Onalaska, a 2,983 hectare backwater lake which is a critical stopover area for migrating waterbird (Figure 3). Lake Onalaska is closed to all migratory waterfowl hunting and a voluntary avoidance program is in effect throughout the waterbird migration. The closed area is sheltered from the main channel, has an average depth of 1-2 meters, and has a relatively uniform flow pattern. In the late 1980s, three islands were constructed to aid in habitat rehabilitation in the Lake Onalaska Closed area. The islands were armored with rock reinforcements ("rip-rap") for protection from wave action; sampling in previous studies was focused on these islands (Hermann and Sorensen, 2009; Sandland, pers. comm.).

Pool 8 of the Upper Mississippi River is 39 kilometers long and stretches from La Crescent, MN to Genoa, WI. Pool 8 consists of the main navigation channel, floodplain forests, and an extensive backwater slough system. In the southern half of Pool 8, a 2,635 hectare closed area was established (Figure 4). Because river currents and wave action

eroded away many of the islands that were present after lock and dam installation, a habitat rehabilitation project was initiated in 1989 to begin rebuilding these areas as a means of enhancing habitat for waterfowl. The habitat rehabilitation project rebuilt 22 islands and was completed in the summer of 2011.

To develop a strategy to sample these large closed areas, a standard square grid with a cell area of 23.9 hectare was overlaid on top of aerial photographs and scaup congregation areas. A 23.9 hectare grid allowed maximization of coverage while minimizing sampling time and the amount of resources required (Figure 5). This was done for both Pool 7 where 55 sites were sampled and Pool 8 where 66 sites were sampled. Each cell intersection represented a single sampling point. Coordinates of each sampling location were extracted from ArcMap (ArcGIS 9.3, ESRI, Redlands, CA, USA) and uploaded onto a handheld global position system (GPS) to aid in navigation to each site and to ensure correct spatial location.

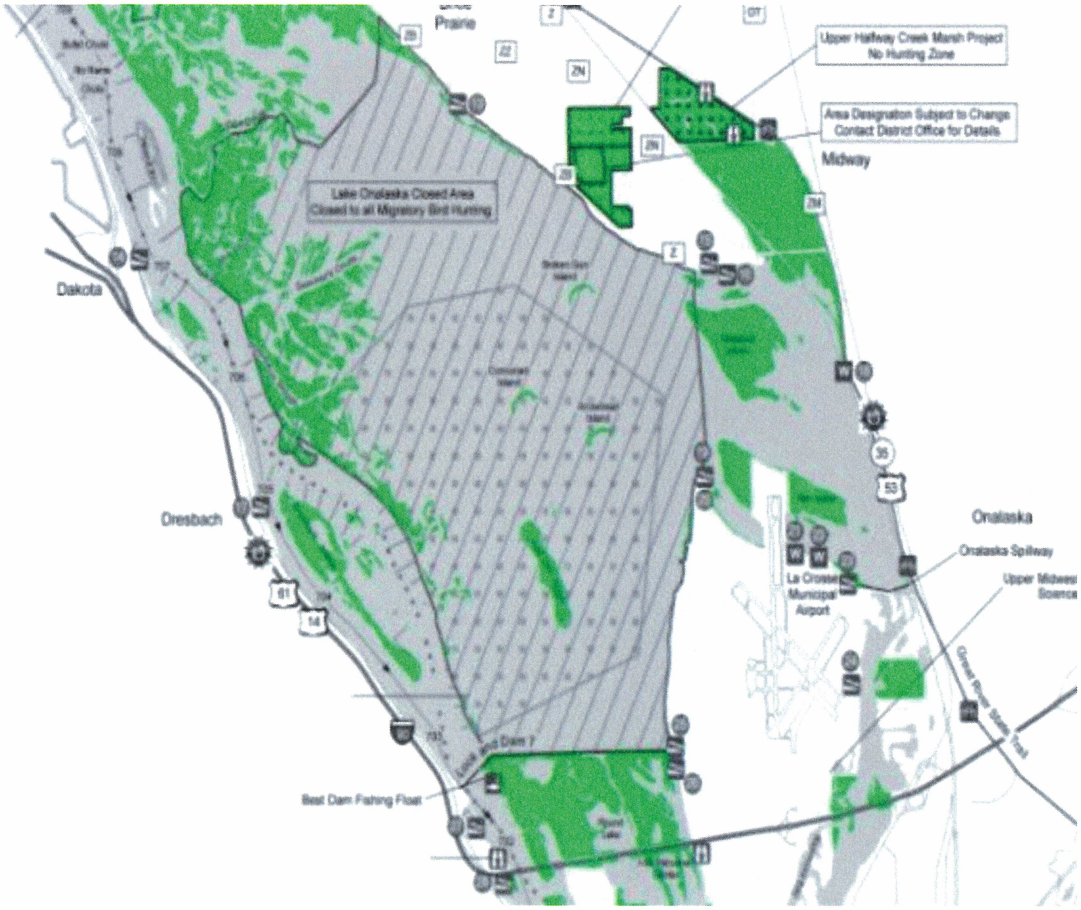


Figure 3. Lower Pool 7 with emphasis on the Lake Onalaska Closed Area shown in the hatched area. This map was obtained from the Upper Mississippi River National Wildlife and Fish refuge.

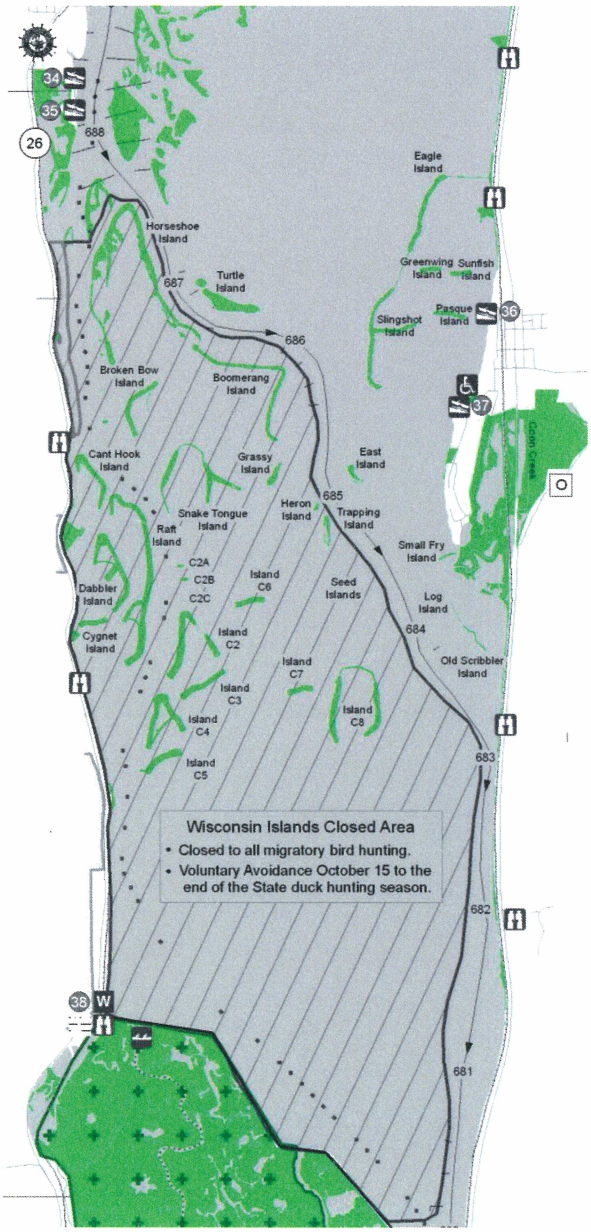


Figure 4. The Lower Pool 8 Wisconsin Islands closed area where recent habitat rehabilitation work that has been completed. This map was obtained from the Upper Mississippi River National Wildlife and Fish Refuge.

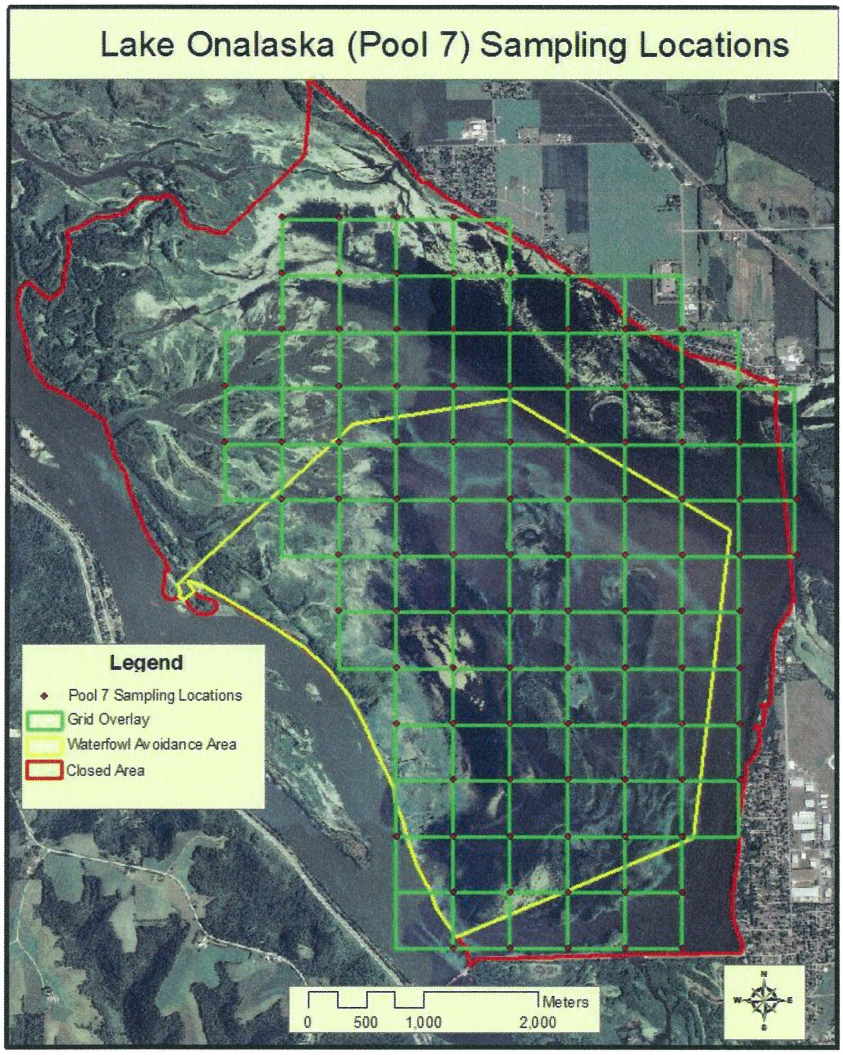


Figure 5. The Pool 7 Lake Onalaska closed area sampling locations showing the 23.9 hectare grid in green. The yellow outline represents the voluntary avoidance area in effect during migration. The red line represents the closed area, which does not permit migratory waterfowl hunting. The closed areas throughout the UMRNW&FR allow migrating waterbirds to feed and rest without disturbance. A similar protocol was also used for establishing sample sites in Pool 8.

Collection methods

Quantitative benthic sampling was conducted in Pool 7 from July 1, 2010 to July 12, 2010 and in Pool 8 from July 12, 2010 to July 25, 2010. Sampling took place in July due to closed areas restrictions minimizing waterfowl disturbance during migration. Although the July samples do not overlap with bird migrations, they do provide initial (and important) snapshots of where snail densities and parasitic infections are highest relative to the areas of heavy scaup use. Sites that were not accessible by boat were not sampled, as this study focused on investigating deep water areas where waterbirds such as lesser scaup forage.

All sites were sampled using a ponar dredge deployed from a boat in open water. This device had a sampling area of 229 mm by 229 mm, and contained a volume of 8.2 L. Sampling was aided by a crane-arm and a hand winch, which was used to raise and lower the dredge. The boat was positioned at the sampling location by a handheld GPS and benthic sampling took place within 10 m of the designated site. This distance was calculated by adding the distance away from the waypoint to the accuracy of GPS. If the distance was greater than 10 m, the boat was repositioned until the location was within 10 m. Drift was controlled for by two anchors, one deployed from the bow and another from the stern of the boat. The GPS location was verified after sampling at each point to ensure that all samples were taken in the appropriate area. Three replicate samples were collected at each sampling location and each replicate was taken from a different side of the boat (starboard, bow, and port sides) to ensure representative sampling. Depth was measured at each sampling location to provide site-specific bathymetry due to variable water levels. Any vegetation that was outside of the ponar scoops was discarded to ensure consistency among samples.

Sample Processing

Samples were partially sorted on the boat to remove any large materials (including native mussels) using a large aluminum trough containing wash frames. The wash frame had a 500 μm stainless steel mesh bottom that was used to sort out the fine material. Buckets of water from the river were used to wash the sample; each bucket was checked for additional snails to prevent them from being accidentally added to the sample. Live snails from each replicate were placed into a 7.5 L plastic bag labeled with the corresponding site and replicate numbers. River water was added to the bags and samples were transported back to the laboratory at UW-La Crosse and processed within 48 hours. In addition, the presence or absence of aquatic-vegetation species was noted for each sample as was the number of *Vallisneria americana* (wild celery) stems. *Vallisneria americana* was the only vegetation species quantified due to its importance as a food source for some waterbird species, and the availability of large scale vegetation distribution data from the U.S. Geological Survey's Long Term Resource Monitoring Program (LTRMP).

Necropsies

In the laboratory, snail species were identified using standard snail keys (Burch, 1989) and the shell length of each snail was determined using digital calipers. Snails were necropsied using a dissecting microscope to assess parasite infections. Glass plates were used to carefully crush the snail shell before tissues were teased apart using fine forceps. Parasite species were identified and recorded using trematode keys (Yamaguti, 1972; Schell, 1985) and descriptions published in previous work (McLaughlin *et al.*, 1993). Primary (sporocysts/rediae) and secondary (metacercariae) infections were recorded as presence or absence. In addition, metacercariae were enumerated for each snail to further investigate spatial variability in the parasite stage responsible for disease in waterfowl. Because *S.*

globulus and *S. pseudoglobulus* are morphological very similar and difficult to identify without observing key lifecycle points or obtaining DNA confirmation, infected snails were only identified to *Sphaeridiotrema* spp. (McLaughlin *et al.*, 1993, Mckindsey and Mclaughlin, 1994, Sandland *et al.*, 2011).

Supplemental data acquisition

Waterbird use in both sampling areas were verified by aerial surveys conducted by the USFWS which are performed weekly during the fall and every other spring using a fixed wing aircraft (U.S. Fish and Wildlife Service, 2006; B. Thrune, pers. comm.). Waterbird species and population sizes are estimated while flying predetermined transects throughout the Refuge. This information was obtained from the La Crosse District of the Upper Mississippi River National Wildlife and Fish Refuge.

Statistical Analyses

To assess the spatial variability in *B. tentaculata* densities and infection metrics within a pool, replicates were used as observations in the analyses. To compare differences between pools, I took the mean of the replicates and used these means as observations in all analyses. In all cases, data were initially assessed for parametric assumptions; in situations where the assumptions failed, I analyzed the data using non-parametric tests. Due to the fact that the homogeneity of variance assumption was often violated within this data set, non-parametric tests (Mann-Whitney U-test; Kruskal Wallis tests) were used to compare snail density, snail size, primary prevalence, secondary prevalence, and larval intensity (as defined by Bush *et al.*, 1997). Snail sizes of individuals infected with a primary parasite stage were compared to snails without infection using an independent sample t-test. Hosts infected with two parasite life cycle stages were excluded from analyses to avoid any

confounding associated with dependent interactions among stages. Data were analyzed using SPSS statistical software.

To assess whether snail densities and/or parasite metrics varied based on depth, I generated depth categories based on the optimal and suboptimal foraging depths of lesser scaup. Optimal foraging depth was defined as greater than 1.14 m but less than 2.13 m as found by White and James (1978).

To assess whether parasite metacercariae were overdispersed (aggregated), I calculated an aggregation index (*k)* which is commonly used by researchers to better understand parasite distributions (Southwood, 1966; Elliot, 1977, Hudson *et al.*, 2001). Large *k* values (> 20) indicate a random distribution, whereas low values indicate high aggregation (Hudson *et al.* 2001). This index was reinforced by calculating variance to mean ratios for metacercariae (Hudson *et al.*, 2001) where a random distribution would have a variance equal to the mean. Conversely, the variance is greater than the mean if parasite distributions are aggregated (Hudson *et al.* 2001).

RESULTS

Bithynia tentaculata density

In total, 2,524 snails were collected and assessed for parasitic infection. *Bithynia tentaculata* was the most abundant snail species found: in Pool 7, it made up 71.8% of the gastropod community and in Pool 8 it comprised 83.7% of the gastropod community (Figure 6 and Figure 7). *Bithynia tentaculata* presence did not differ between pools as the species was present in 39 of the 55 (71%) sites sampled in Pool 7 and 46 out of the 66 (70%) sites in Pool 8 (Mann-Whitney U-test, $P > 0.05$) (Figure 8 and Figure 9). However, *B. tentaculata* densities did differ between pools (Mann-Whitney U-test, $P < 0.05$) with Pool 8 having significantly greater *B. tentaculata* densities (36 snails/site) than Pool 7 (9.6 snails/site) (Figure 10).

Snail densities also varied among sites within each of the pools (both pools - Kruskal-Wallis test, $P < 0.001$). The greatest single site density was found in Pool 8 where 579 total snails were collected. Differences in snail densities were not observed between optimal and suboptimal foraging depths of lesser scaup (Mann-Whitney U-test, $P > 0.05$).

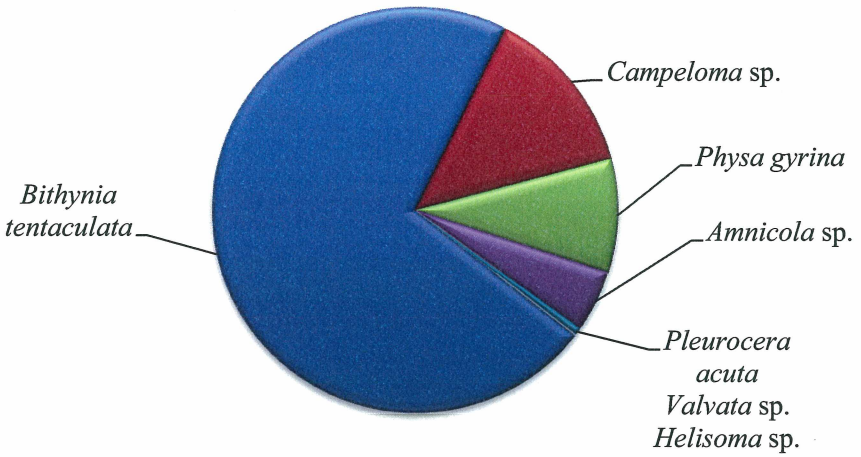


Figure 6. Gastropod composition found in the Pool 7 closed area in Lake Onalaska. *Bithynia tentaculata* made up 71.8% of the gastropod community that was collected.

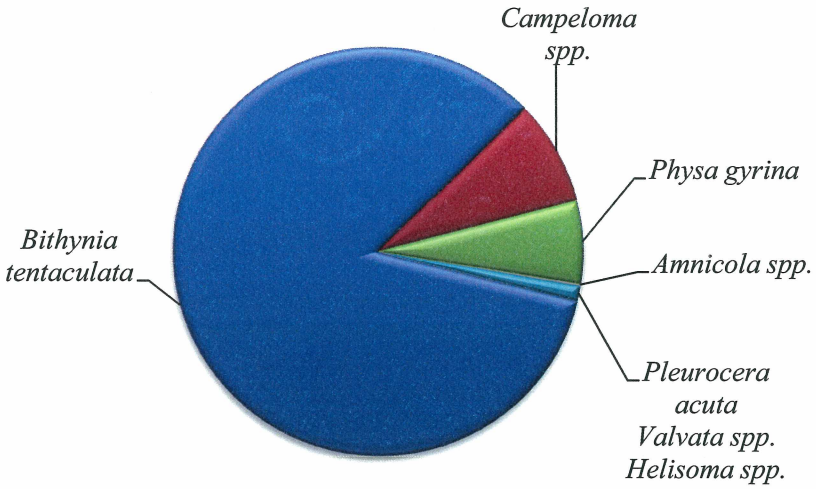


Figure 7. Gastropod composition found in the Pool 8 closed area near the Wisconsin Islands. *Bithynia tentaculata* made up 83.7% of the gastropod community that was collected.

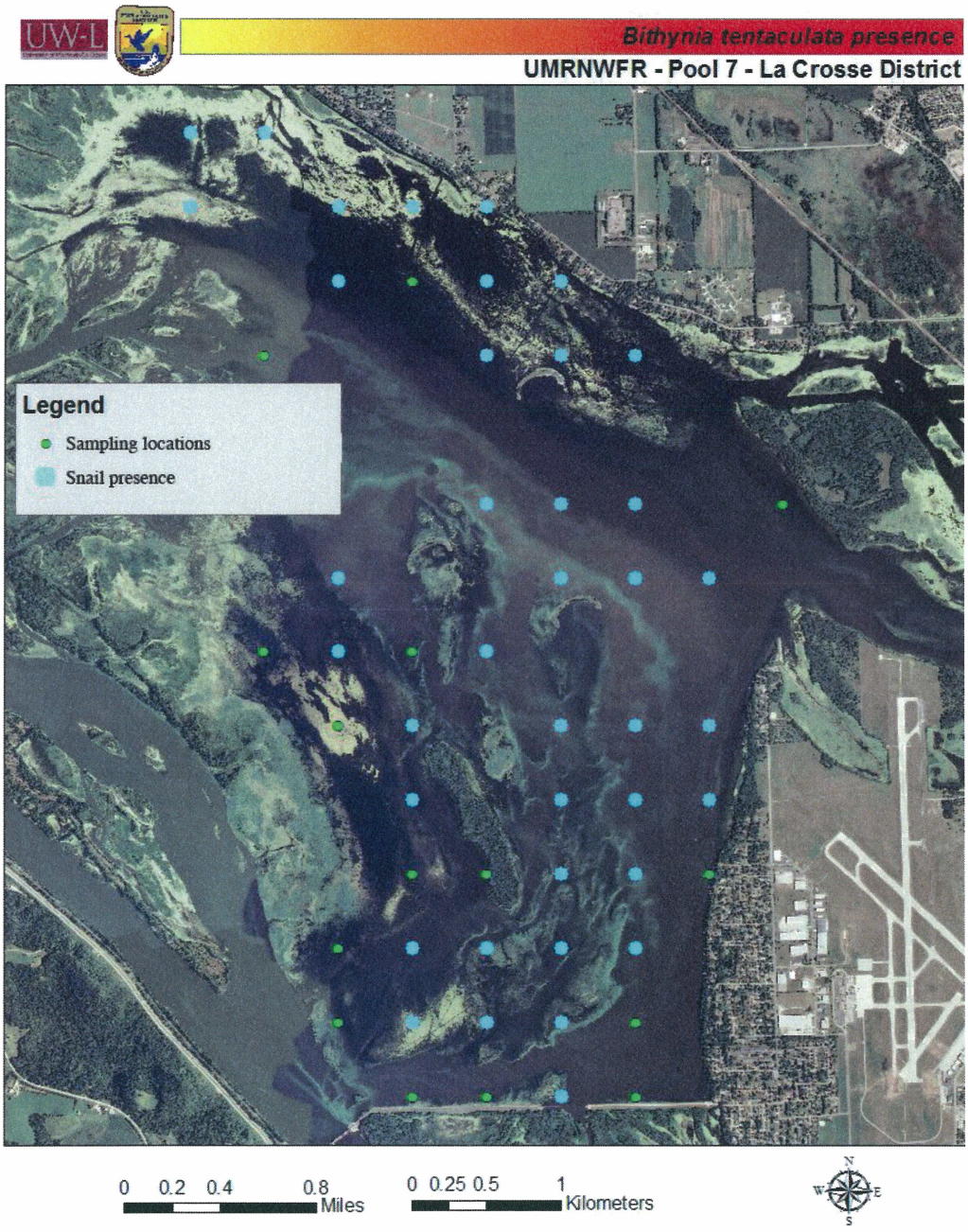


Figure 8. Sampling locations in the Pool 7 closed area of the Upper Mississippi River. Small circles (green) indicate sampling points and larger circles (blue) indicate sampling points where *B. tentaculata* was found. Samples were collected in July 2010.

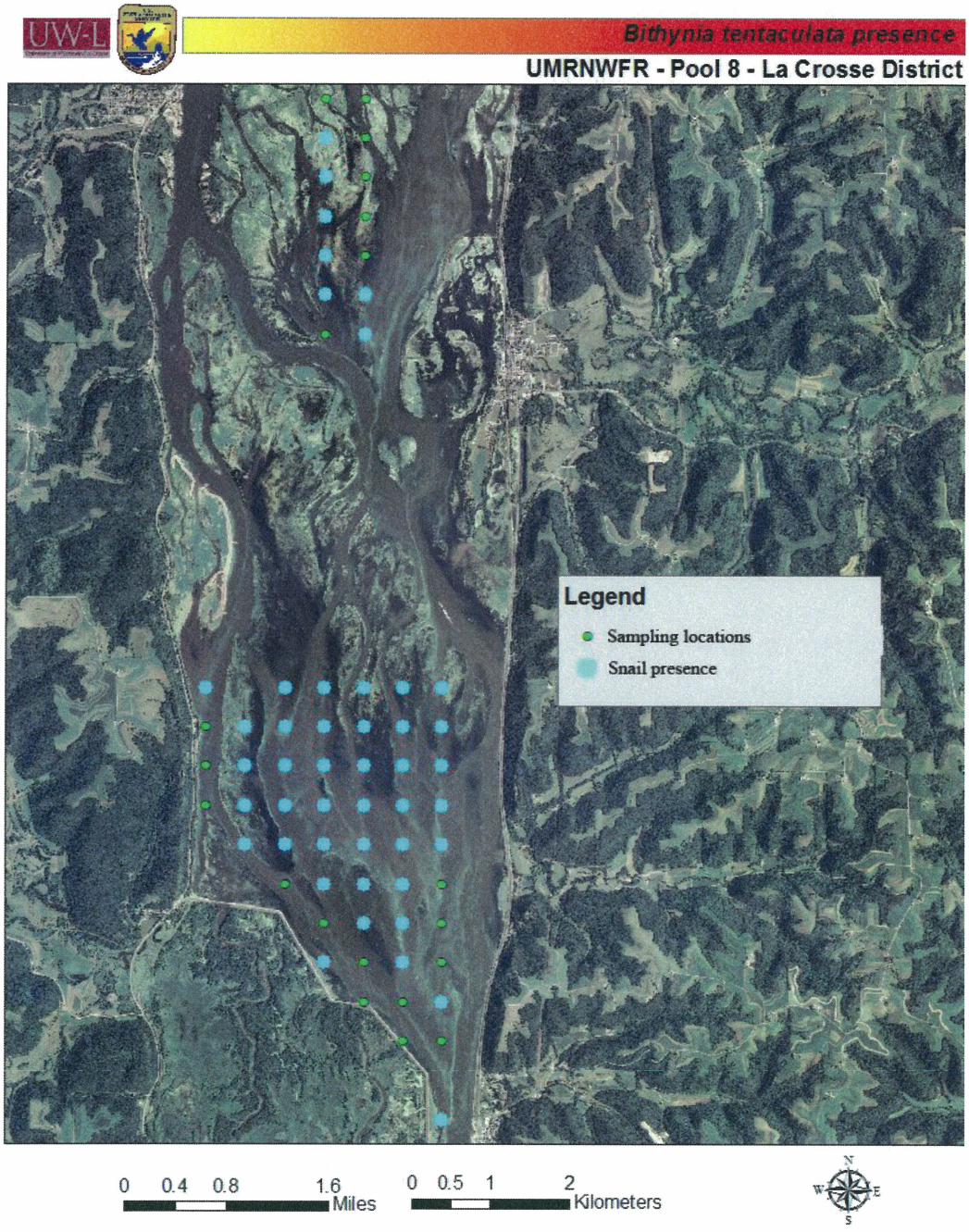


Figure 9. Sampling locations in the Pool 8 closed area of the Upper Mississippi River. Small circles (green) indicate sampling points and larger circles (blue) indicate sampling points where *B. tentaculata* was found. Samples were collected in July 2010.

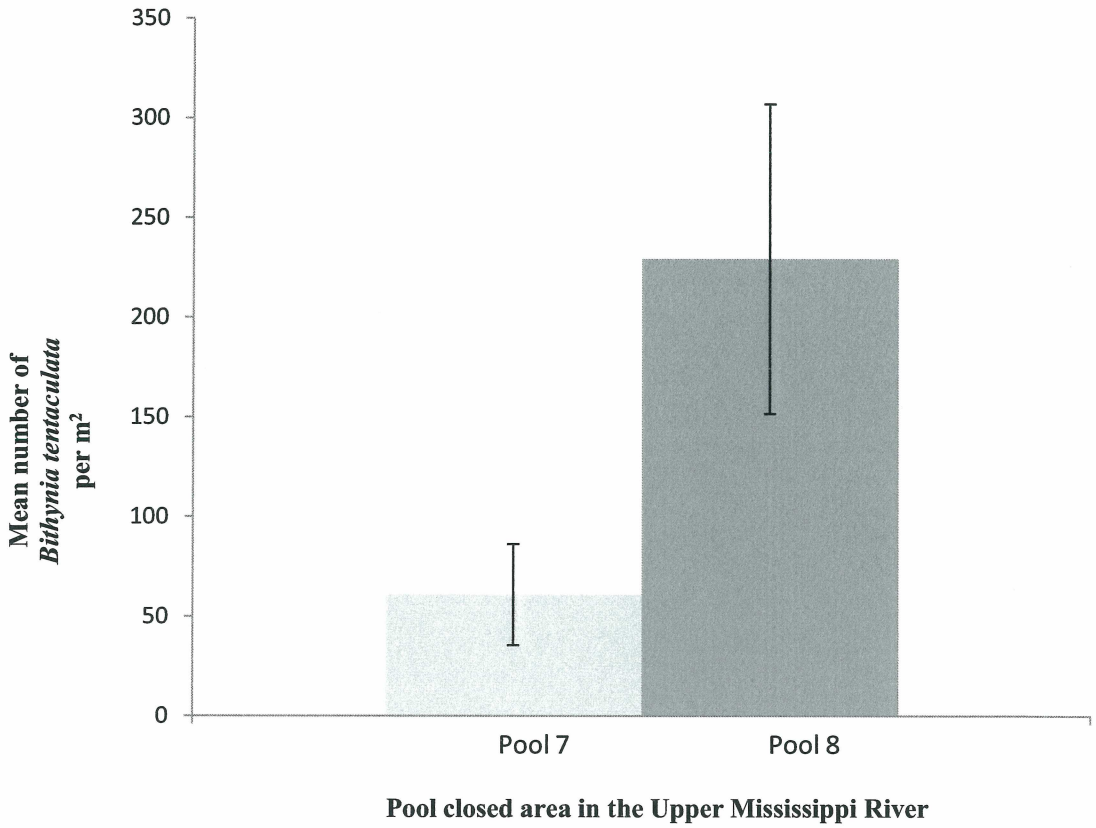


Figure 10. Mean number of *Bithynia tentaculata* per m^2 (± SE) collected from Navigation Pools 7 and 8 from in the Upper Mississippi River in July 2010.

Overall patterns

Snails with primary infections were larger, reaching a mean length of 9.74 mm whereas uninfected snails had a mean length of 7.91 mm (Mann-Whitney U-test, $P < 0.001$) (Figure 11). Snail size was positively correlated with metacercarial intensity ($R^2 = 0.2513$) (Figure 12). In terms of overall distribution of the parasites in the snails metacercariae were highly aggregated as demonstrated by the high variance/mean ratio (11.6) and low *k* value (0.885); many of the snails were uninfected or had relatively low numbers of metacercariae, whereas a small number of others had very high larval intensities (Figure 13).

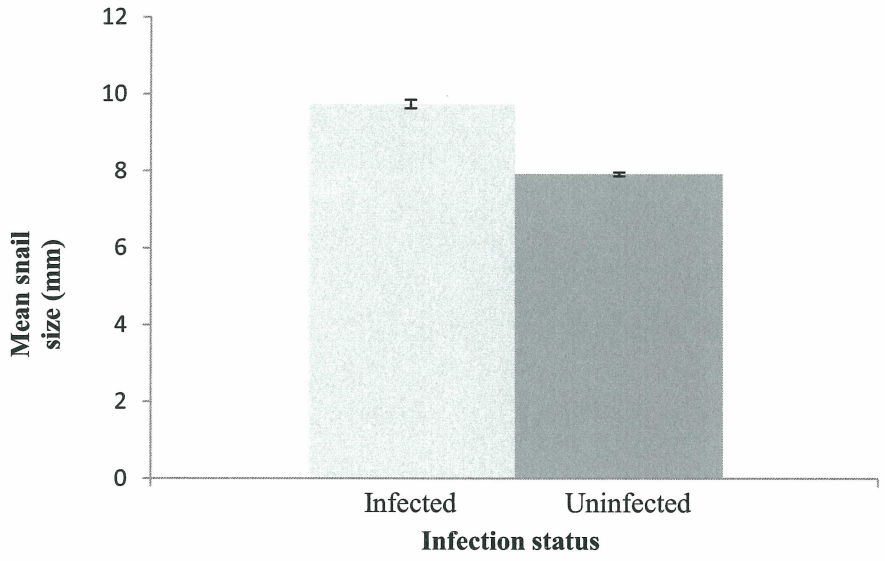


Figure 11. Mean sizes (±SE) of infected and uninfected *Bithynia tentaculata* collected from Pools 7 and 8 in the UMR (July 2010).

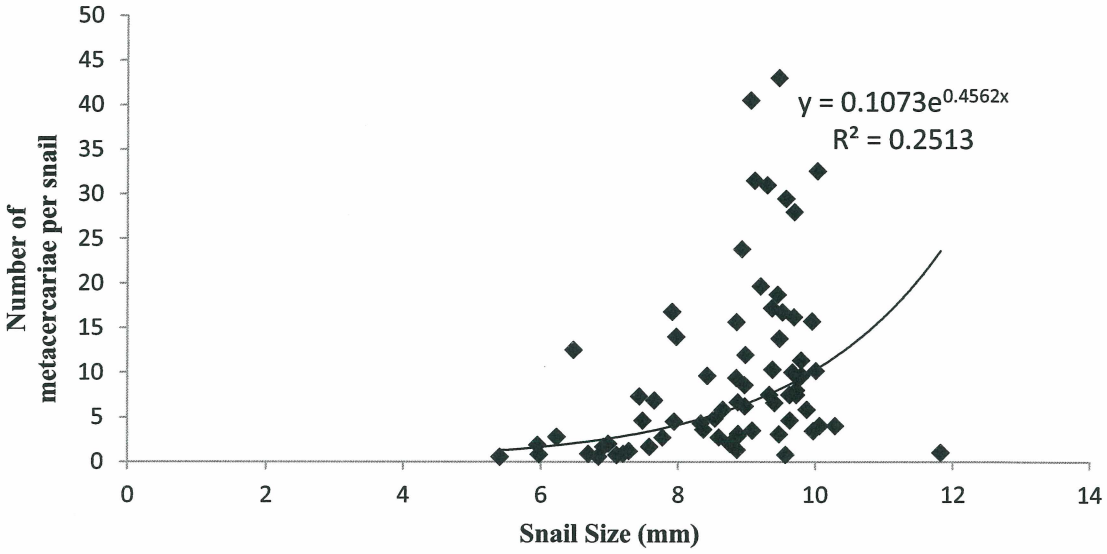


Figure 12. Correlation between metacercarial intensity and *Bithynia tentaculata* size. Snails were collected from Pools 7 and 8 of the Upper Mississippi River. An exponential regression line was added in which $R^2 = 0.2513$.

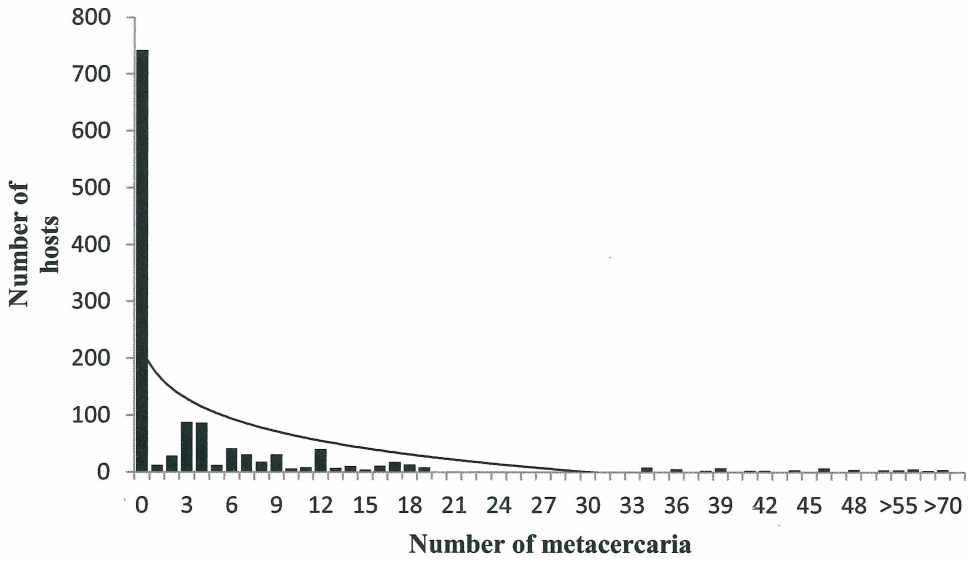


Figure 13. Aggregated distribution of metacercariae (pooled across species and pools) within the intermediate host *Bithynia tentaculata*.

Cyathocotlye bushiensis infections

Primary infections were detected at 4 out of 55 (7%) sites in the Pool 7 closed area and 22 out of the 66 (33%) sites in the Pool 8 closed area (Figure 14 and Figure 15). The prevalence of *Cyathocotyle bushiensis* primary infections in *B. tentaculata* did not differ among sites within either the Pool 7 or Pool 8 closed areas (Mann-Whitney U-test, $P > 0.05$). However, the Pool 8 closed area did have a higher prevalence of *C. bushiensis* primary infections (6%) than Pool 7 (3%) (Mann-Whitney U-test, $P < 0.05$).

Secondary metacercarial infections were detected in 30 out of 55 (55%) sites in the Pool 7 closed area whereas 40 out of 66 (61%) sites in the Pool 8 closed area contained infected snails (Figure 16 and Figure 17). Metacercarial prevalence in *B. tentaculata* differed among sites within each pool. This was driven by the fact that some sites had uninfected snails whereas infection reached 100% at other sites (Kruskal-Wallis test, $P < 0.05$). A difference in metacercarial prevalence was not found between pools (Mann-Whitney U-test, $P > 0.05$). Similarly, *Cyathocotyle bushiensis* intensities differed among sites within a pool (Kruskal-Wallis test, $P < 0.05$) but did not differ between pools (Mann-Whitney U-test, $P > 0.05$).

Sphaeridiotrema spp. infections

Primary infections were detected at 8 out of the 55 (15%) sites in the Pool 7 closed area and 34 out of the 66 (52%) sites in the Pool 8 closed area (Figure 18 and Figure 19). *Sphaeridiotrema* spp. primary infection prevalence differed among sites, as the highest mean was 43% at one site compared to other sites without primary infections (Kruskal-Wallis test, $P < 0.05$). The prevalence of primary infections with *Sphaeridiotrema* spp. did not differ between pools (Mann-Whitney U-test, $P > 0.05$).

Sphaeridiotrema spp. metacercarial infections were detected in 19 out of 55 (35%) sites in the Pool 7 closed area whereas 38 out of 66 (58%) sites in the Pool 8 closed area had metacercarial infections (Figure 20 and Figure 21). Metacercarial prevalence differed among sites within a pool as snails from some sites expressed 100% infection while others were absent of *Sphaeridiotrema* spp. parasites (Kruskal-Wallis test, $P < 0.001$). Metacercarial prevalence differed between pools where 16% of the snails were infected in the Pool 8 closed area while only 13% of the snails were infected in the Pool 7 closed area (Mann-Whitney U-test, $P < 0.05$). Average intensities of *Sphaeridiotrema* spp. metacercariae differed between pools as well, with snails from Pool 8 exhibiting almost twice as many larvae (6.1 metacercariae/snail) as hosts from Pool 7 (3.1 metacercariae/snail) (Mann-Whitney U-test, $P < 0.05$). Metacercarial intensity also differed among sites within a pool with the highest site average being 41 metacercariae in the Pool 7 closed area (Kruskal-Wallis test, $P < 0.001$).

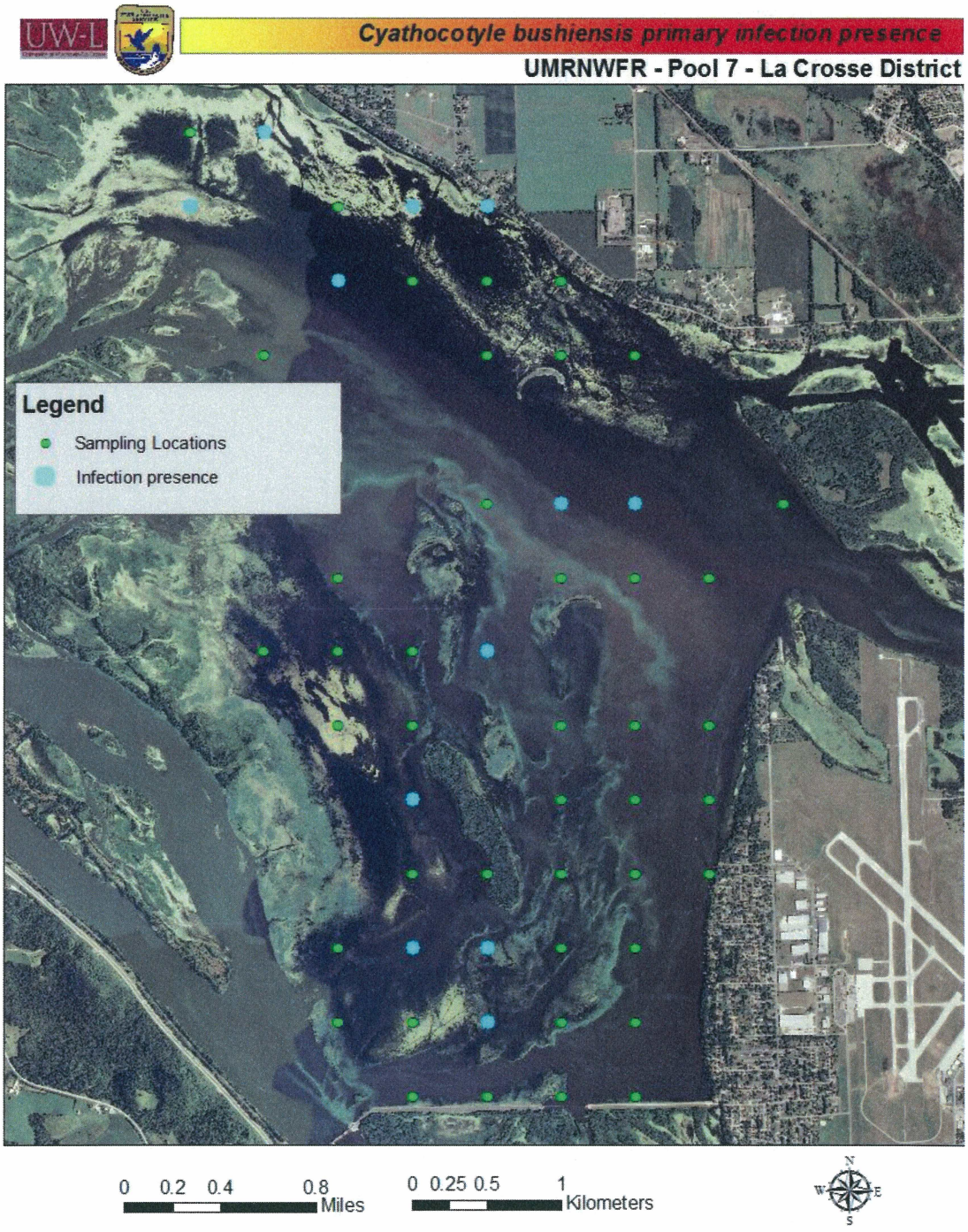


Figure 14. The sampling locations in the Pool 7 closed area of the Upper Mississippi River. The small circles (green) indicate sampling points and the larger highlighted circles (blue) indicate the presence of snails infected with the primary stage of *Cyathocotyle bushiensis*.

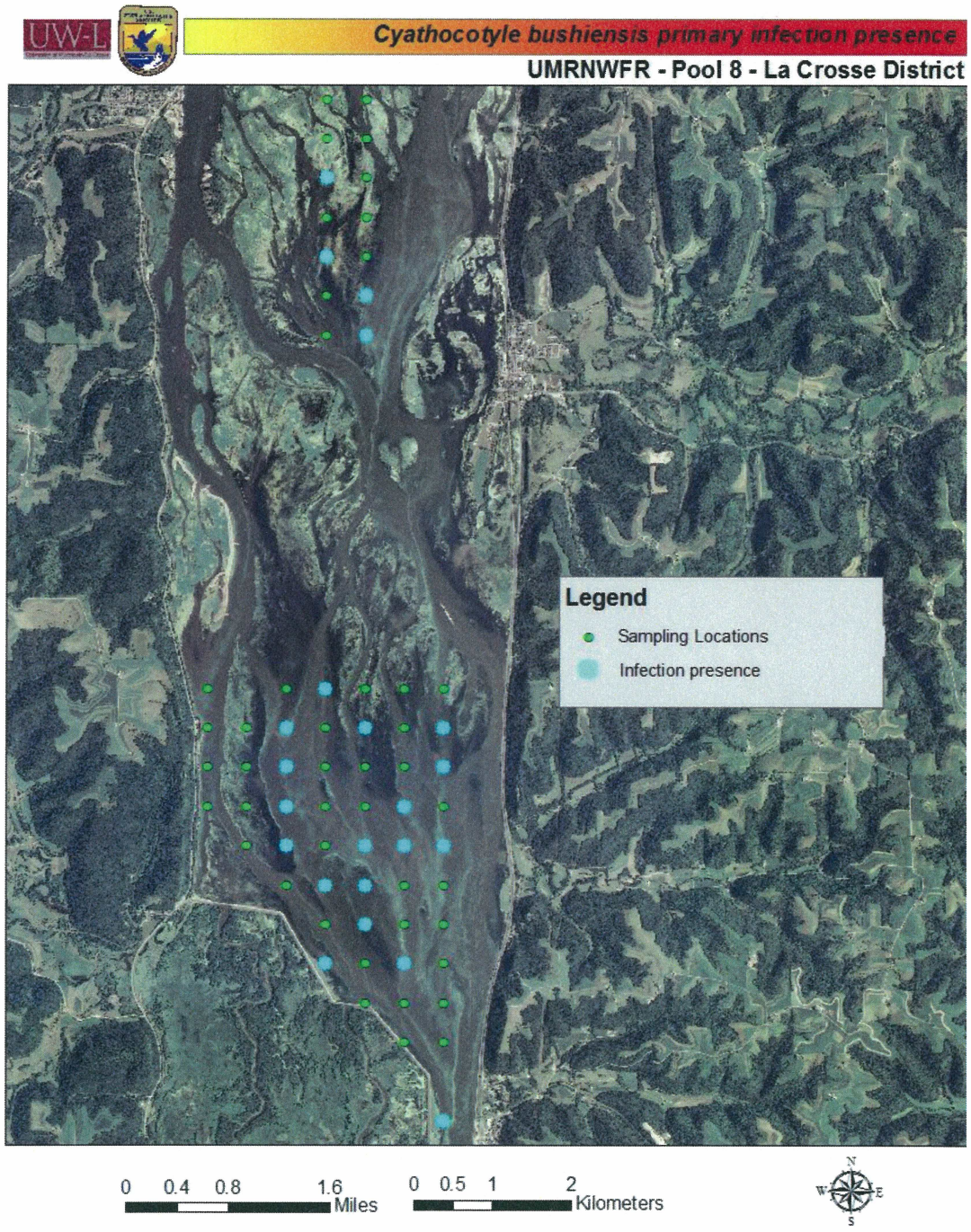


Figure 15. The sampling locations in the Pool 8 closed area of the Upper Mississippi River. The small circles (green) indicate sampling points and the larger highlighted circles (blue) indicate the presence of snails infected with the primary stage of *Cyathocotyle bushiensis.*

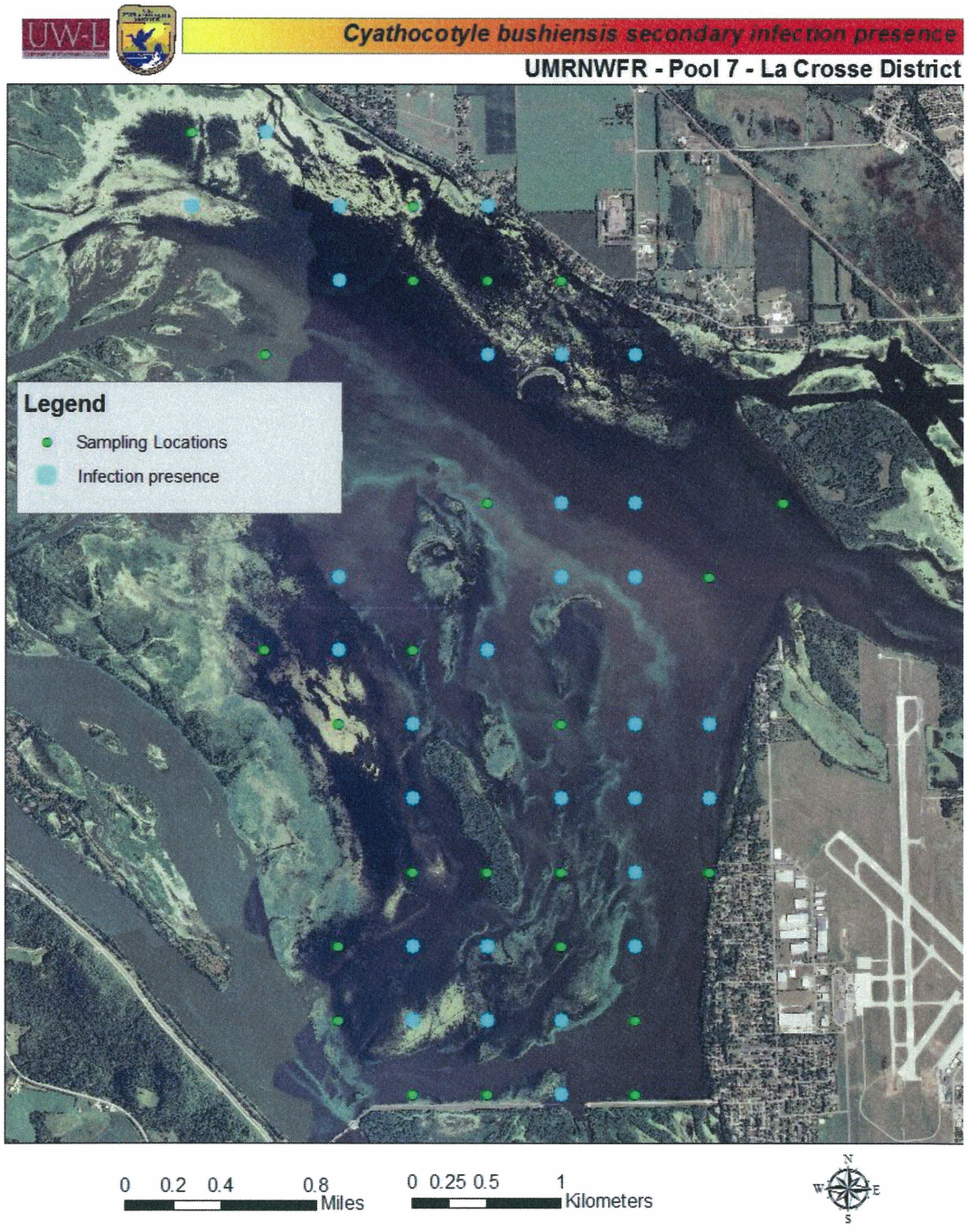


Figure 16. The sampling locations in the Pool 7 closed area of the Upper Mississippi River. The small circles (green) indicate sampling points and the larger highlighted circles (blue) indicate the presence of snails infected with the secondary stage (metacercariae) of *Cyathocotyle bushiensis.*

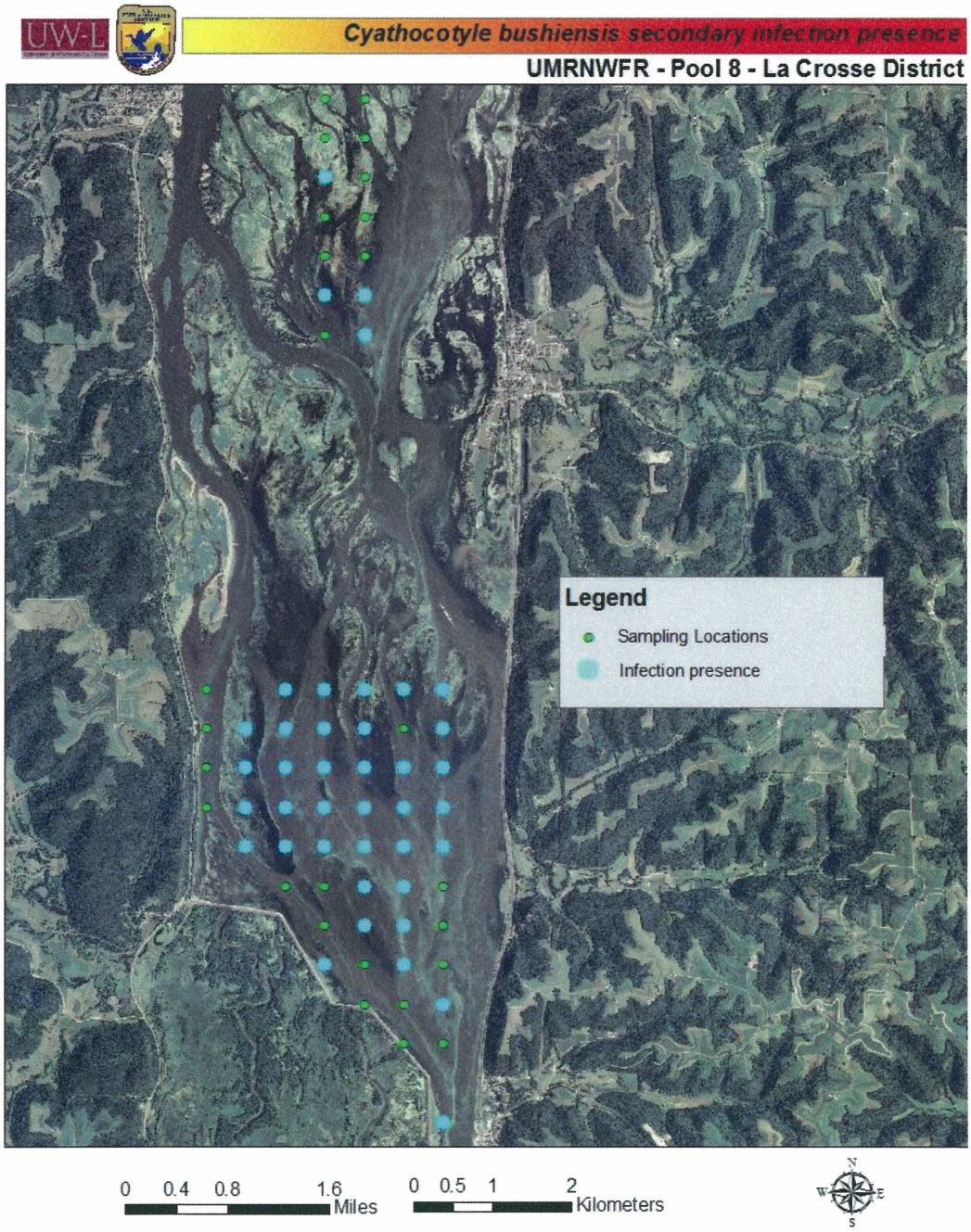


Figure 17. The sampling locations in the Pool 8 closed area of the Upper Mississippi River. The small circles (green) indicate sampling points and the larger highlighted circles (blue) indicate the presence of snails infected with the secondary stage (metacercariae) of *Cyathocotyle bushiensis.*

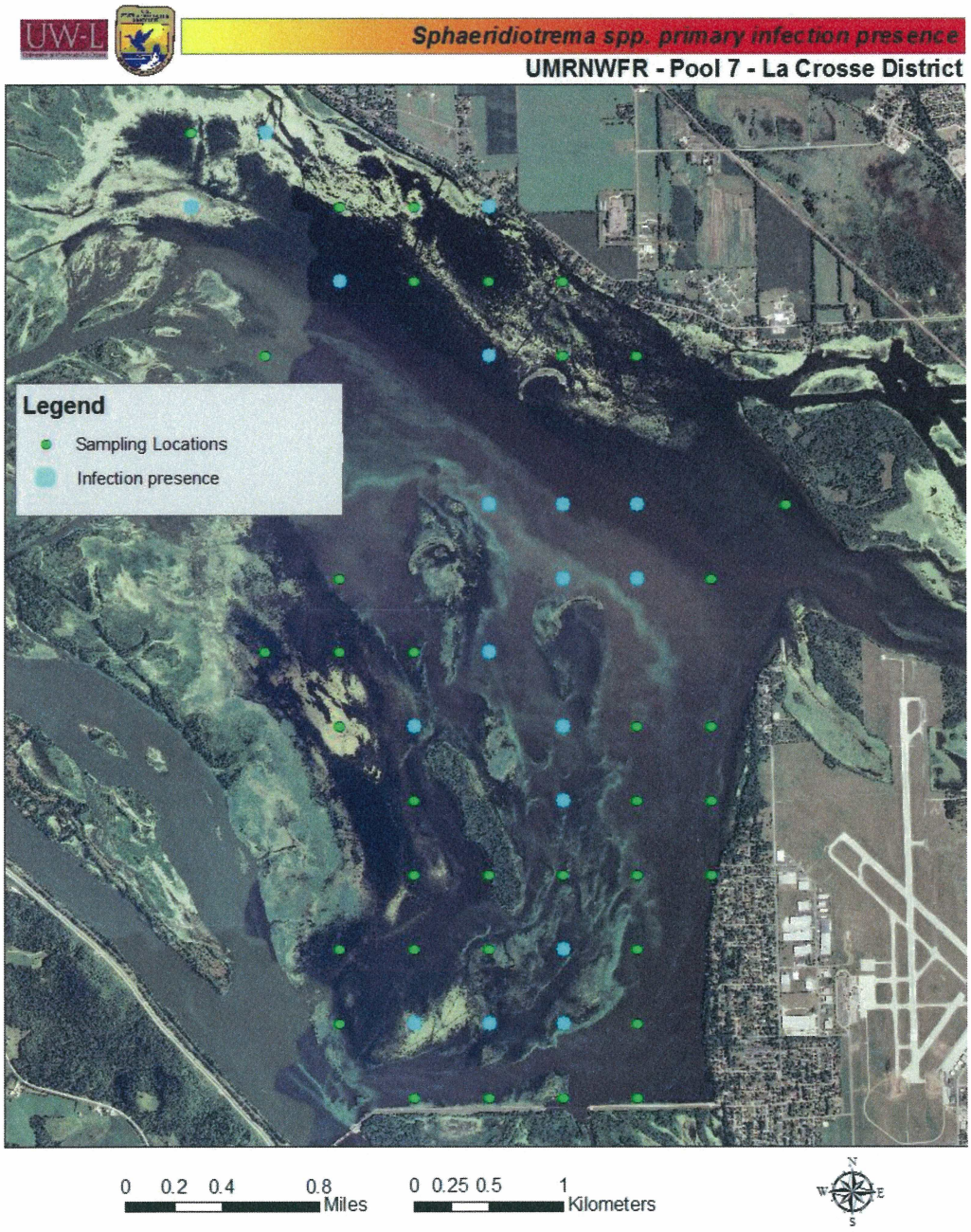


Figure 18. The sampling locations in the Pool 7 closed area of the Upper Mississippi River. The small circles (green) indicate sampling points and the larger highlighted circles (blue) indicate the presence of snails infected with the primary stage of *Sphaeridiotrema* spp.

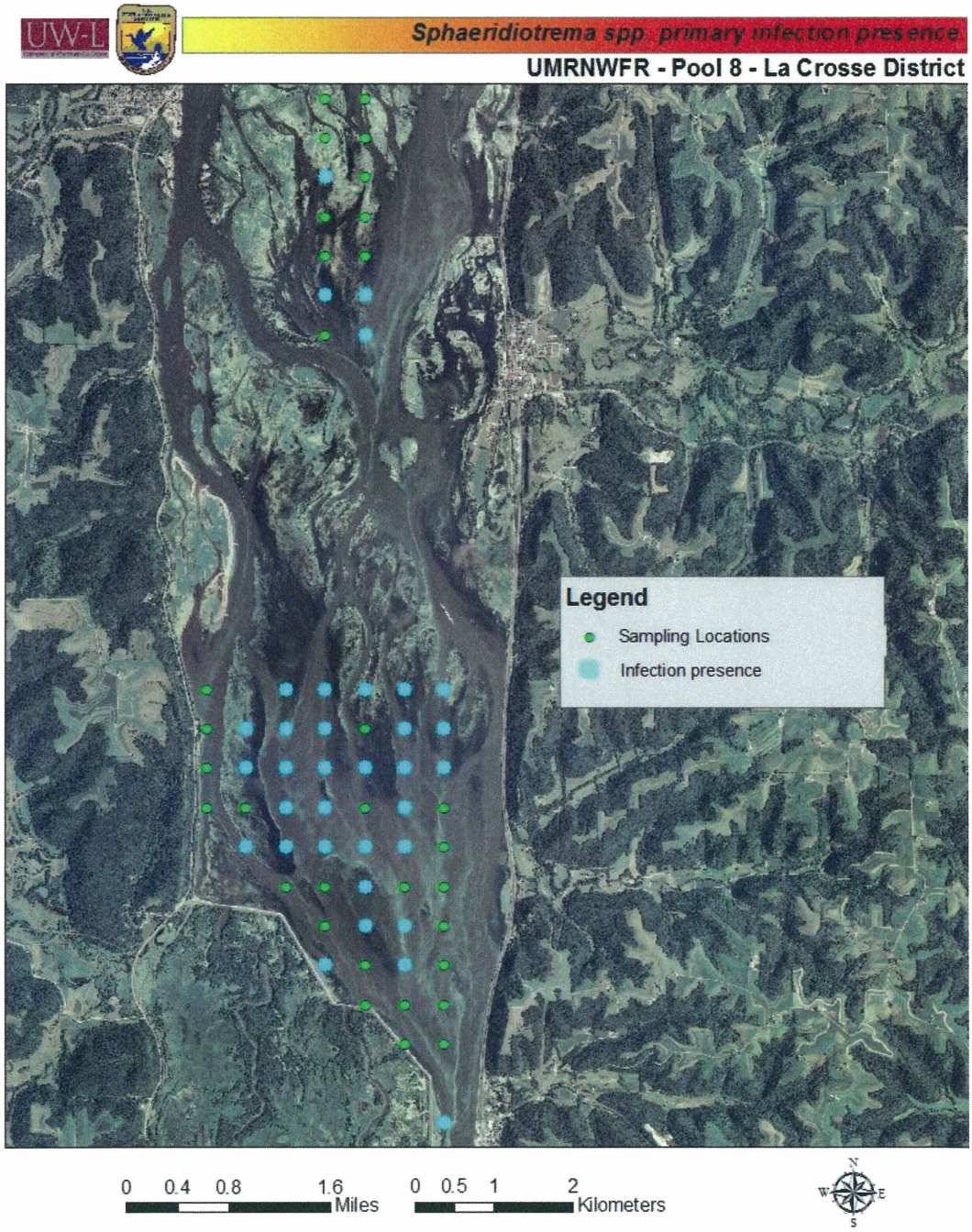


Figure 19. The sampling locations in the Pool 8 closed area of the Upper Mississippi River. The small circles (green) indicate sampling points and the larger highlighted circles (blue) indicate the presence of snails infected with the primary stage of *Sphaeridiotrema* spp.

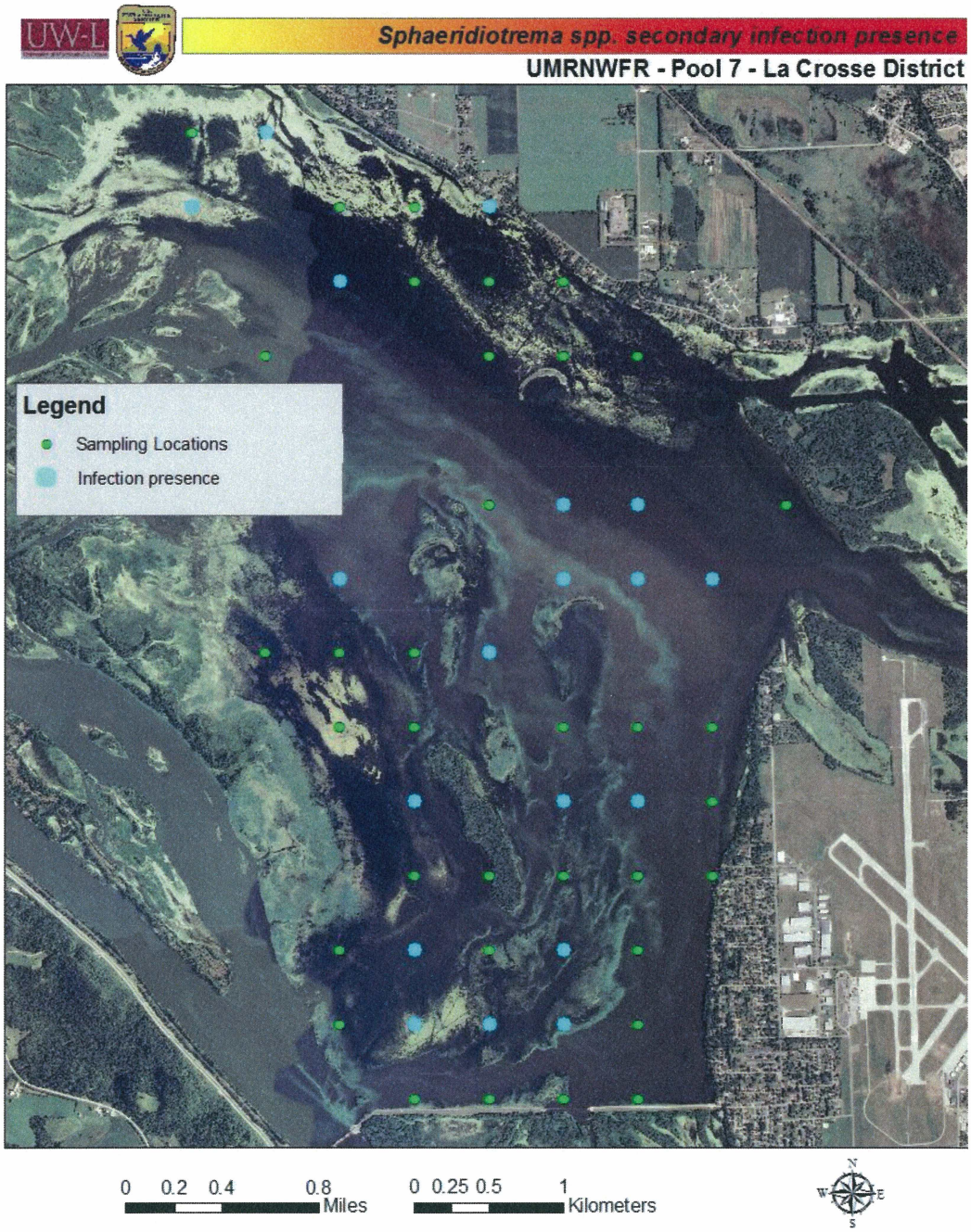


Figure 20. The sampling locations in the Pool 7 closed area of the Upper Mississippi River. The small circles (green) indicate sampling points and the larger highlighted circles (blue) indicate the presence of snails infected with the secondary stage of *Sphaeridiotrema* spp.

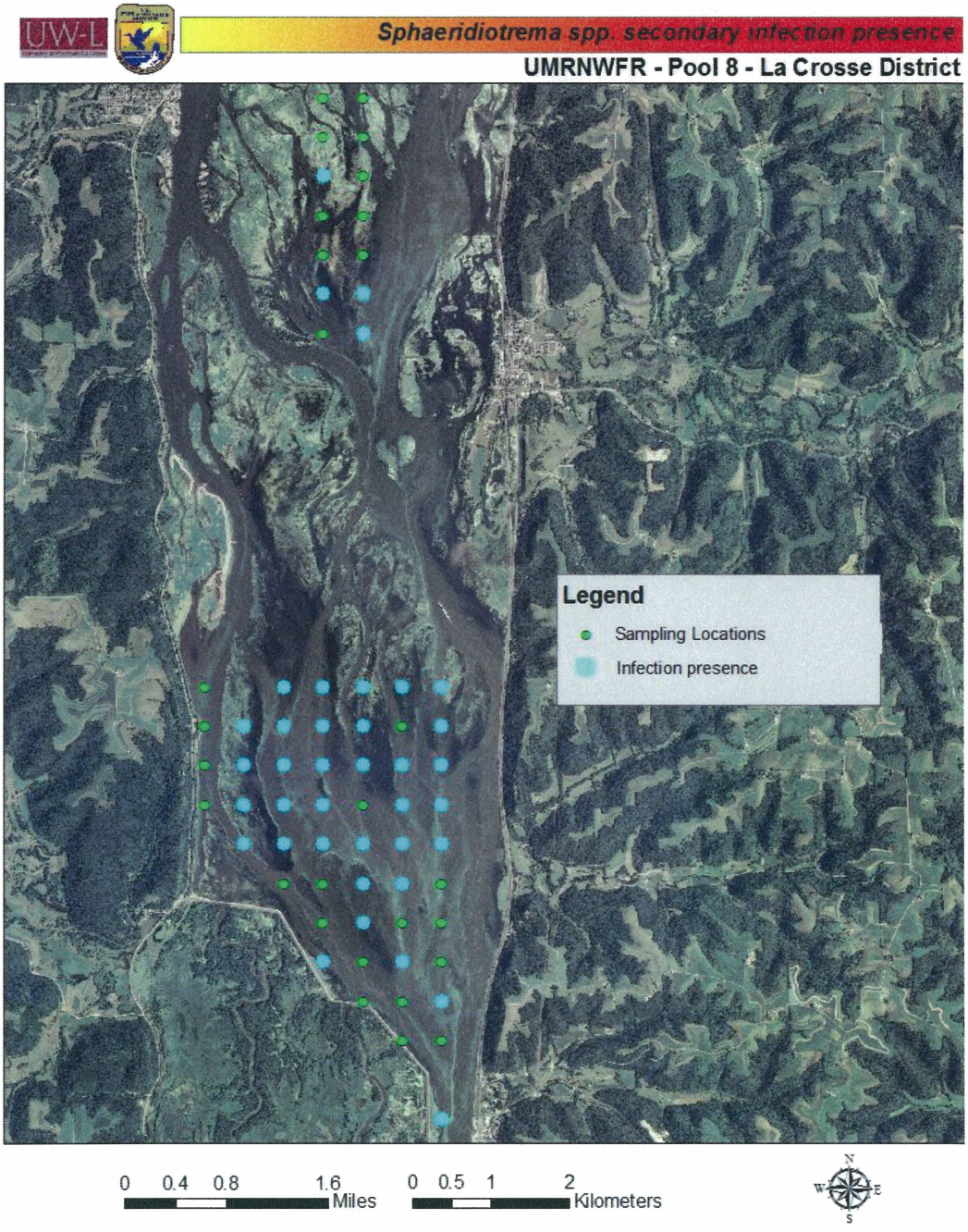


Figure 21. The sampling locations in the Pool 8 closed area of the Upper Mississippi River. The small circles (green) indicate sampling points and the larger highlighted circles (blue) indicate the presence of snails infected with the secondary stage of *Sphaeridiotrema* spp.

DISCUSSION

Bithynia tentaculata's presence throughout the majority of the sampling region illustrates the success of this snail in navigation pools of the UMR. Based on previous work, the highest densities of *B. tentaculata* are often found in association with vegetation and areas of low water velocity. For example, Vincent *et al.* (1981) found the highest *B. tentaculata*'s densities on aquatic vegetation in Quebec, Canada. Richter (2001) also found *B. tentaculata* to be most abundant in areas with aquatic vegetation and in regions of low water velocity in Europe, and found that river banks free of vegetation, were also free of *B. tentaculata*. A number of factors could generate this pattern including enhanced feeding capacity (Richter, 2001) and/or enhanced reproductive output due to the stability of macrophytes (Vincent *et al.* 1981). Regardless of the mechanism, *B. tentaculata* densities might be expected to continue to increase in these pools due to ongoing habitat rehabilitation projects aimed at increasing the densities of aquatic macrophytes.

Water velocity has also been shown to be important for *B. tentaculata* establishment. For example, Lilly (1953) found that *B. tentaculata* avoided areas where aerators increased the water velocity in commercial pipes, suggesting that this factor is an important determinant of snail distributions. Furthermore work by Gabel *et al.* (2008) found that increases in hydrological shear stress reduced *B. tentaculata*'s ability to adhere to substrates including stones. Together these reports suggest that areas of higher water velocity may limit the distribution of *B. tentaculata*. This may help to explain why these snails were absent from areas in the UMR that exhibit relatively high water flow, such as areas towards the periphery of each pool and in the main channel in Pool 8. Interestingly, a combination

of increased water velocity and specific morphological attributes of *B. tentaculata* could facilitate dissemination of these snails to areas of low flow. It is well established that *B. tentaculata* will close its operculum in times of environmental stress or the presence of predators (Kelly and Cory, 1987; Mitchell and Cole, 2008). If *B. tentaculata* also uses this strategy during times of high water velocity to avoid injury, it could seal itself inside its shell allowing passive dispersal to other sites. The independent effects of vegetation and flow cannot be disentangled based on this work, but would be an interesting research avenue for future study.

Bithynia tentaculata distribution could also be influenced by water chemistry. Mitchell and Cole (2008) found that *B. tentaculata* can withstand extreme abiotic conditions when mature, through the use of the operculum. However, evidence is accumulating to suggest that *B. tentaculata* are relatively sensitive to anoxic conditions. For example, low levels of oxygen can harm the eggs and immature *B. tentaculata* which would not allow populations to persist (Richter, 2001; Wood *et al.*, 2011). This may restrict *B. tentaculata* to habitats of shallow, open water where oxygen is readily available. For example, Legendre *et al.* (1984) found that snails were absent in high productivity areas where oxygen levels are depleted for long periods of time. Areas with low levels of dissolved oxygen may hinder the reproductive success of *B. tentaculata*, therefore restricting its distribution in the UMR.

Bithynia tentaculata was able to occupy a variety of depths under 3 m in the Pools 7 and 8 closed areas although deeper water (> 3 m) high-flow sites exhibited lower *B. tentaculata* densities. Past research has documented that *B. tentaculata* can be found in a variety of depths depending on the water system. For example, Hermann and Sorensen (2009) reported snails up to their maximum sampling depth of 2 m. Furthermore, Baker

(1918) found snails up to 5 meters in depth. Combined these studies suggest that depth is not likely a key variable dictating *B. tentaculata* distribution patterns in the UMRNW&FR and that the lower densities observed at some of the deeper sites in this work may have more to do with water velocity or substrate conditions.

The presence of primary infections is a good indicator that ecological overlap of the definitive host exists in that area. All three parasite species were present at a number of sites and prevalence values were consistent with previous studies (Hermann and Sorensen, 2009). The presence/absence patterns of primary infections in each of the pools may be explained by the avian definitive host acting as a dispersal mechanism. For example, Smith (2001) found that the mangrove snail, *Cerithidea scalariformis,* had higher infection levels where wading birds would frequently perch. Similarly, Jokela and Lively (1995) found that the freshwater snail *Potamopyrgus antipodarum* had higher prevalence levels of the trematode parasite *Microphallus* sp. in shallow waters where large numbers of waterfowl where most commonly found. Although the presence of primary infections was found to be widespread in both navigation pools, this pattern may not adequately reflect transmission hotspots as the actual numbers of primary infections were not taken into account. This will be further addressed using GIS techniques in Chapter 3.

Cyathocotyle bushiensis and *Sphaeridiotrema* spp. secondary infections were variable throughout the closed areas of Pools 7 and 8, suggesting that secondary transmission can be site specific. This could be attributed to the poor swimming ability of the cercarial stages and/or the presence/absence of snails containing primary infections at the site level. The observed variability can also be explained by the lack of snail movement during active months. For example, using a mark-recapture technique, Lepitzki (1993) found that *B. tentaculata,* on average only traveled 25 cm from where it was previous found

3 weeks earlier. This suggests that the metacercarial distribution within *B. tentaculata* is dependent on the primary infected host's proximity to other susceptible snails, the longevity of the cercariae, and/or patterns of water flow in the area.

The distribution of parasites was highly aggregated for secondary forms (metacercariae). Aggregated parasite distributions, like the one observed for metacercariae, can be explained by a number of factors including the degree of host exposure to infective parasite stage and the degree of susceptibility expressed by hosts themselves (Wilson *et al.*, 2002). For example, Anderson and May (1978) found that small changes in the amount of exposure a snail receives or the level of snail susceptibility can substantially shift parasite distributions. Oftentimes these factors can be influenced by variability in the surrounding microhabitats (Sandland *et al.*, 2001). In Pools 7 and 8, factors such as substrate, vegetation, depth, and water velocity were observed to vary dramatically among sites and even among replicates within a site. Given that these environmental variables can have important impacts on transmission features, particularly the probability of exposure (Anderson and May, 1978; May and Anderson, 1978; Sandland *et al.*, 2001; Wilson *et al.*, 2002), the observation was not overly surprising. However, little work has been done investigating secondary parasite patterns in snail hosts, so this result provides novel insight into a relatively understudied area of host-parasite interactions.

Another understudied area is the effects of parasitism on host life history, which can drastically influence parasite transmission. Gigantism is a condition that occurs during the primary infection stage mollusk hosts. The parasite will sterilize the host in order to increase the growth rate of the host. This is believed to benefit the parasite as a larger host habitat equates to a large volume for parasite exploitation and development (Sandland and Minchella, 2003). In native populations, *B. tentaculata* will reach a shell size of 12 mm

(Jokinen, 1992; Lepitzki *et al.*, 1994; Richter, 2001; Hermann and Sorensen, 2009). In this study, the average size of uninfected snails was 7.9 mm whereas infected snails were significantly larger (9.7 mm). This suggests that gigantism may be occurring in this system, a result which has recently been supported by laboratory infections (Sandland *et al.*, 2011). This condition benefits the parasites as more cercariae can be produced which can change the secondary transmission dynamics within a snail population. Increased transmission could ultimately equate to higher levels of waterbird mortality, as susceptible hosts would have an increased probability of becoming infected. Secondary infection intensities did not correlate with snail size, thus it appears that secondary infections do not alter growth response in *B. tentaculata* from the UMR.

Habitat alteration can facilitate the establishment of a number of important snail species that serve as intermediate hosts for trematodes. For example, the creation of Lake Nasser from Aswan dam generated new habitat in which snails and other invertebrates thrived (Heyneman, 1979). This was extremely problematic as the higher snail populations resulted in an increase of human schistosomiasis in the region. Similarly, recent work by Richardson *et al.* (2005) has found that marsh restoration projects in Iraq have enhanced the preferred habitat for snails transmitting *Schistosoma hematobium*. Recent habitat rehabilitation work in Pool 8 has increased food abundance for waterbirds but has also increased the available habitat for *Bithynia tentaculata* colonization (Sandland, pers.obs). Due to habitat restoration in these areas, extremely high numbers (over 16,000) of scaup congregate seasonally in the Pool 8 closed area which also contains large numbers of infected snails. Increasing the densities of the definitive host through environmental modifications could ultimately lead to higher parasite transmission and greater waterfowl mortality.

This work sheds light on the spatial patterns of primary and secondary infections of waterfowl-killing parasites in *B. tentaculata*. Although these data provide a snapshot of the infection dynamics occurring between *Sphaeridiotrema* spp./*C. bushiensis* and *B. tentaculata*, it provides important insight into the distribution of parasites and hosts in areas of heavy waterfowl use, and the factors that may be driving them. Site specific habitat rehabilitation likely increased the available snail habitat, thereby affecting the disease transmission of a large migrating waterbird population. This work stresses the need for snail intermediate host monitoring in other areas that receive high levels of waterbird use, as a means of mitigating future disease outbreaks. The work also serves as a research foundation for the temporal data presented in Chapter 2 and the risk-mapping described in Chapter 3.

REFERENCES

Afton, A.D. and Anderson, M.G. (2001). Declining scaup populations: a retrospective analysis of long-term population and harvest survey data. *The Journal of Wildlife Management*, 65, 781-796.

Afton, A.D. and Ankney, C.D. (1991). Nutrient-reserve dynamics of breeding lesser scaup: a test of competing hypotheses. *The Condor*, 93, 89-97.

Al-Kandari, W.Y., Abdul-Salam, J. and Meakins, R. (2000). Temporal variations in the infection of a population of *Cerithidea cingulata* by larval trematodes in Kuwait Bay. *Journal of Helminthology*, 74, 17-22.

Anderson, R.M. and May, R.M. (1978). Regulation and stability of host-parasite population interactions: I. regulatory processes. *Journal of Animal Ecology*, 47, 219 – 247.

Anteau, M.J. and Afton, A.D. (2004) Nutrient reserves of lesser scaup (*Aythya affinia*) during spring migration in the Mississippi Flyway: a test of the spring condition hypothesis. *The Auk*, 121, 917-929.

Austin, J.E., Afton, A.D., Anderson, M.G., Clark, R.G., Custer, C.M., Lawrence, J.S., Pollard, J.B. and Ringelman, J.K. (1999). Declines of greater and lesser scaup populations: issues, hypotheses, and research directions. U.S. Department of the Interior, U.S. Geological Survey Report, Northern Prairie Wildlife Research Center, Jamestown, North Dakota, USA.

Baker, F.C. (1918). The productivity of invertebrate fish food on the bottom of Oneida Lake, with special reference to the mollusks. *New York State College of Forestry at Syracuse University Technical Publication*, 9, 1-264.

Baker, F.C. (1928). The fresh water Mollusca of Wisconsin part 1. Gastropoda. *Wisconsin Geological and Natural History Survey Bulletin*, 70, 1-494.

Berry, E.G. (1943). The Amnicolidae of Michigan: distribution, ecology, and taxonomy. Miscellaneous Publications of the Museum of Zoology (University of Michigan), 57, 1-68.

Bundy, D.P. (1981). Survival characteristics of the free-living miracidial population of *Transversotrema patialense* (Soparkar) (Digenea: Transversotrematidae). *Journal of Parasitology*, 67, 531-534.

Bush, A.O., Lafferty, K.D., Lotz, J.M. and Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology*, 83, 575-583.

Chen, M. (1999). Progress in schistosomiasis control in China. *Chinese Medical Journal*, 112, 930-933.

Clarke, A.H. (1981). *The freshwater molluscs of Canada*. National Museum of Natural Sciences, National Museum of Canada, Ottawa. 446 p.

Dussart G.B.J. (1979). Life cycles and distribution of the aquatic gastropod molluscs *Bithynia tentaculata* (L.), *Gyraulus albus* (Muller), *Planorbisp lanorbis* (L.) and *Lymnaeap eregra* (Muller) in relation to water chemistry. *Hydrobiologia,* 67, 223-239.

Elliot, J.M. (1977). *Statistical analysis of samples of benthic invertebrates*. Ambleside, Freshwater Biological Association. 160 p.

Fretter, V. and Graham, A. (1962). *British prosobranch molluscs; their functional anatomy and ecology*. The Ray Society, London.

Gabel, F., Garcia, X.F., Brauns, M., Sukhodolov, A., Leszinski, M. and Pusch, M.T. (2008). Resistance to ship-induced waves of benthic invertebrates in various littoral habitats. *Freshwater Biology*, 53, 1567-1578.

Gibson, G.G., Broughton, E. and Choquette, L.E. (1972). Waterfowl mortality caused by *Cyathocotyle bushiensis* Khan, 1962 (Trematoda: Cyathocotylidae), St. Lawrence River, Quebec. *Canadian Journal of Zoology*, 50, 1351-1356.

Green, W. (1970). Ecological changes on the Upper Mississippi River Wildlife and Fish Refuge since inception of the 9-foot channel. USDI, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife Report. 132 p.

Herrmann, K.K. and Sorensen, R.E. (2009). Seasonal dynamics of two mortality-related trematodes using an introduced snail. *Journal of Parasitology*, 95, 823-828.

Herrmann, K.K. and Sorensen, R.E. (2011). Differences in natural infections of two mortality-related trematodes in lesser scaup and American coot. *Journal of Parasitology*, 95, 823-828.

Heyneman, D. (1979). Dams and disease. *Human Nature*, 2, 50-57.

Hoeve, J. and Scott, M.E. (1988). Ecological studies on *Cyathocotyle bushiensis* (Digenea) and *Sphaeridiotrema globulus* (Digenea), possible pathogens of dabbling ducks in southern Quebec. *Journal of Wildlife Diseases*, 24, 407–421.

Hudson, P.J., Rizzoli, A., Grenfell, B.T., Heesterbeek, H. and Dobson, A.P. (2002). *The Ecology of Wildlife Diseases*, Oxford, UK, Oxford Press. 197 p.

Jokinen, E. (1992). *The Freshwater Snails* (Mollusca: Gastropoda*) of New York State*. The University of the State of New York, The State Education Department, The New York State Museum, Albany, New York 12230. 112 p.

Joklea, J. and Lively, C.M. (1995). Spatial variation in infection by digenetic trematodes in a population of fresh water snails (*Potamopyrgus antipodarum*). *Oecologia*, 103, 509-517

Kazibwe, F., Makanga, B., Rubaire-Akiiki, C., Ouma, J., Kariuki, C., Kabatereine, N.B., Booth, M., Vennervald, B.J., Sturrock, R.F. and Stothard, J.R. (2006). Ecology of *Biomphalaria* (Gastropoda: Planorbidae) in Lake Albert, Western Uganda: snail distributions, infection with schistosomes and temporal associations with environmental dynamics. *Hydrobiologia*, 568, 433-444.

Kelly, P.M. and Cory, J.S. (1987). Operculum closing as a defense against predatory leeches in four British freshwater prosobranch snails. *Hydrobiologia*, 144, 121-124.

Khan, D. (1962). Studies on larval trematodes infecting freshwater snails in London (U.K.) and some adjoining areas. Part VI. The cercariae of the ''Vivax'' group and the life history of *Cercaria bushiensis* n. sp. (*Cyathocotyle bushiensis* n. sp.). *Journal of Helminthology*, 36, 67–94.

Korschgen, C., Kirsch, E. and Kenow, K. (1999). *Birds. In Ecological status and trends of the Upper Mississippi River System 1998: A report of the Long Term Resource Monitoring Program.* U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse Wisconsin. LTRMP. 236 p.

Kube, S., Kube, J. and Bick, A. (2002). Component community of larval trematodes in the mudsnail *Hydrobia ventrosa*: temporal variation in prevalence in relation to host life history. *Journal of Parasitology*, 88, 730-737.

Legendre, P., Planas, D., et Auclair, M.J. (1984). Succession des communautes de gasteropodes dan deux milieu different par leur degree d'eutrophisation. *Canadian Journal of Zoology*, 62, 2317-2327.

Lepitzki, D.W. (1993). *Epizootiology and transmission of snail-inhabiting metacercariae of the duck digeneans Cyathocotyle bushiensis and Sphaeridiotrema globulus*. Unpublished Ph.D. dissertation. McGill University, Montreal, Canada, 354 p.

Lepitzki D.W., Scott, M.E. and Mclaughlin, J.D. (1994). Influence of storage and examination methods on the recovery and size of metacercariae of *Cyathocotyle bushiensis* and *Sphaeridiotrema pseudoglobulus* (Digenea). *Journal of Parasitology*, 80, 454–460.

Lilly, M.M. (1953). The mode of life and the structure and functioning of the reproductive ducts of *Bithynia tentaculata* (L.). *Journal of Molluscan Studies*, 30, 87-110.

Mattice, J.S. (1972). Production of a natural population of *Bithynia tentaculata* L. (Gastropoda, Mollusca). *Ekologia Polska*, 20, 525-539.

Mckindsey, C.W. and Mclaughlin, J.D. (1994). Transmission of *Cyclocoelum mutabile* (Digenea) to snails: the influence of temperature on the egg and miracidium. *Canadian Journal of Zoology*, 71, 1745-1751.

McLaughlin, J.D., Scott, M.E. and Huffman, J.E. (1993). *Sphaeridiotrema globulus* (Rudolphi, 1814) (Digenea): evidence for two species known under a single name and a description of *Sphaeridiotrema pseudoglobulus* n.sp. *Canadian Journal of Zoology*, 71, 700-707.

Meier-Brook, C. and Kim, C.H. (1977). Notes on ciliary feeding in two Korean *Bithynia* species. *Malacologia*, 16, 159-163.

Menard, L. and Scott, M.E. (1987). Seasonal occurrence of *Cyathocotyle bushiensis* Khan, 1962 (Digenea: Cyathocotylidae) metacercariae in the intermediate host *Bithynia tentaculata* L. (Gastropoda: Prosobranchia). *Canadian Journal of Zoology*, 65, 2980-2992.

Mitchell, A.J. and Cole, R.A. (2008). Survival of the faucet snail after chemical disinfection, *p*H extremes and heated water bath treatments. *North American Journal of Fisheries Management*, 28, 1597-1600.

Peirce, J.P., Sandland, G.J., Sutter, C. and Haro, R.J. (2011). Predicting the ecological outcomes of species invasions and parasite transmission in the upper Mississippi river. *Journal of Biological Dynamics*, 0, 1-18.

Pinel-Alloul, B. and Magnin, E. (1971). Cycle vital et croissance de *Bithynia tentaculata* L. (Mollusca, Gastropoda, Prosobranchia) du Lac St. Louis pres de Montreal. *Candian Journal of Zoology*, 49, 759-766.

Price, E.W. (1934). Losses among wild ducks due to infestation with *Sphaeridiotrema globulus* (Rudolphi) (Trematoda; Psilostomidae). *Helminthological Society*, 1, 31-34.

Reid, F., Kelley, J., Taylor, T. and Frederickson, L. (1989). *Upper Mississippi Valley Wetlands – refuges and moist soil impoundments*. 202 p.

Richardson, C.J., Reiss, P., Hussain, N.A., Alwash, A.J. and Pool, D.J. (2005). The restoration potential of the Mesopotamian marshes of Iraq. *Science*, 307, 1307-1311.

Richter, T. (2001). *Reproductive biology and life history strategy of Bithynia tentaculata (Linnaeus, 1758) and Bithynia leachii (Sheppard, 1823)*. (Unpublsihed Ph.D. Dissertation), Universitat Hannover, Saxony, Germany.

Sandland, G.J. and Minchella, D.J. (2003). Effects of diet and *Echinostoma revolutum* infection on energy allocation patterns in juvenile *Lymnaea elodes* snails. *Oecologia*, 134, 479-486.

Sauer, J. S., Cole, R.A. and Nissen, J.M. (2007). Finding the exotic faucet snail (*Bithynia tentaculata*): Investigation of waterbird die-offs on the Upper Mississippi River National Wildlife and Fish Refuge. *U.S. Geological Survey Open-File Report 2007-1065*, 1–3.

Schell, S.C. (1985). *Trematodes of North America, north of Mexico*. University Press of Idaho, Moscow, Idaho, 263 p.

Smith, N.F. (2001). Spatial heterogeneity in recruitment of larval trematodes to snail intermediate hosts. *Oecologia*, 127, 115-122

Southwood, T.R.E. (1966). *Ecological Methods*. London, Chapman and Hall.

Strayer, D.L. (1999). Effects of alien species on freshwater mollusks in North America. *Journal of North American Benthological Society*, 18, 74-98.

Szidat, L. (1937). Uber die Entwicklungsgeschichte von *Sphaeridiotrema globulus* Rud. 1814 und die Stellung der Psilostomidae Odhner im naturlichen System. I. Die Entwicklungsge-chichte von Sphaeridiotrema globulus Rud. Ztschr. *Paraisitenk.* 9, 529-542.

U.S. Fish and Wildlife Service. (2006). *Upper Mississippi River National Wildlife and Fish Refuge comprehensive conservation plan*. 242 p.

Vincent B. and Vaillancourt, G. (1981). Methode de determination de l'age, longevite et croissance annuelle de *Bithynia tentaculata* L. (Gastropoda: Prosobranchia) dans le Saint-Laurent (Quebec). *Canadian Journal of Zoology*, 59, 982-985.

Vincent, B., Vaillancourt, G. and Harvey, M. (1981). Cycle de développement, croissance, effectifs, biomasse et production de *Bithynia tentaculata* L. (Gastropoda : Prosobranchia) dans le Saint-Laurent (Québec). *Canadian Journal of Zoology*, 59, 1237-1250.

Vincent B, Gaucher M (1983) Variations de la fecondite et de la structure des populations chez *Bithynia tentaculata* L. (Gastropoda: Prosobranchia). *Canadian Journal of Zoology*, 61, 2417-2423

Vincent, B. and Letourneau, C. (1985). Inter-population variations in the age structure and growth of the prosobranch *Bithynia tentaculata* L. *Canadian Journal of Zoology*, 63, 1345-1353.

White, D.H. and James, D. (1978). Differential use of fresh water environments by wintering waterfowl of coastal Texas. *The Wilson Bulletin*, 90, 99-111.

Wilson, K., Bjornstad, O.N., Dobson, A.P., Merler, S., Poglayen, G., Randolph, S.E., Read, A.F. and Skorping, A. (2002). *Heterogeneities in macroparasite infections: patterns and processes.* – In: Hudson, P. J. et al. (eds). The Ecology of Wildlife Diseases. Oxford Univ. Press, 6 – 44.

Wood, A.M., Haro, C.R., Haro, R.J. and Sandland, G.J. (2011). Effects of dessication on two life stages of an invasive snail and its native cohabitant. *Hydrobiologia*, 675, 167-174.

Yamaguti, S. (1958). Systema Helminthum. Vol. I. *The digenetic trematodes of vertebrates-part I.* Interscience Publishers, Inc., New York. 979 p.

Yurlova, N.I., Vodyanitskaya, S.N., Serbina E.A., Biserkov, V.Y, Georgiev, B.B. and Chipev, N.H. (2006). Temporal variation in prevalence and abundance of metacercariae in the pulmonate snail *Lymnaea stagnalis* in Chany Lake, West Siberia, Russia: long-term patterns and environmental covariates. *Journal of Parasitology*, 92, 249-259.

CHAPTER II

THE SEASONAL DYNAMICS OF BITHYNIA TENTACULATA AND THREE TREMATODES IN THE UPPER MISSISSIPPI RIVER NATIONAL WILDLIFE AND FISH REFUGE (UMRNW&FR)

ABSTRACT

Temporal dynamics of an intermediate host can affect the transmission dynamics of a parasite to the definitive host. The prosobranch snail, *Bithynia tentaculata* invaded North America in the late nineteenth century. Since 2002, over 60,000 waterbirds have died in the Upper Mississippi River National Wildlife and Fish Refuge (UMRNW&FR). Three trematode species, *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. are responsible for the mortality. Little is known about the seasonal dynamics of the parasite and the intermediate hosts in the Upper Mississippi River system. The objective of this study was to investigate snail and parasite populations temporally, using a quantitative sampling procedure at select sites. A total of ten sites were sampled seasonally in the Pool 7 Lake Onalaska closed area and parasite infection status was assessed in each snail host. *Bithynia tentaculata* was the most abundant mollusk found, making up 86.6% of the snails collected. In terms of parasitism, primary (rediae/cercariae) infections decreased in the fall resulting in a stable metacercarial population during the ice-free period. The decrease of primary infections could be attributed to mortality of older, infected snails and/or predation of larger snails by migrating waterfowl. Snails with concurrent secondary (metacercarial) infections had higher intensities suggesting that cercarial exposure is unequal throughout the closed area.

This can be attributed to the spatial distribution of the definitive hosts that may select feeding areas based on macroinvertebrate availability.

INTRODUCTION

Seasonal life-history responses of parasites are known to impact infection patterns in intermediate hosts, which in turn, can alter the transmission dynamics of these parasites to definitive hosts (Esch and Fernandez, 1994). Allogenic parasite species (whose definitive host is a temporary visitor) such as trematodes, typically follow a predictable temporal pattern due to the presence of hosts at similar times each year (Sandland *et al.*, 2001). For example, Esch and Fernandez (1994) found that many trematode species display two peaks in transmission. The temporal patterns can fluctuate predictably both between years and across seasons based on relatively consistent environmental cycles in cold temperate habitats which influence distribution patterns of definitive and intermediate hosts (Al-Kandan *et al.*, 2000; Sandland *et al.*, 2001; Kube *et al.*, 2002; Kazibwe *et al.*, 2006; Yurlova *et al.* 2006).

One of the key features dictating seasonal patterns in trematode transmission dynamics is the response of invertebrate intermediate hosts to changing environmental factors. In temperate regions, water temperature plays a critical role in the seasonal fluctuations and migrations of invertebrates (Clampitt, 1974), including snails (Luzon-Pena *et al.,* 1994) which serve as key hosts in the life cycle of trematodes. Snails typically have a narrow range of preferred temperatures wherein optimal growth and reproduction can occur (Von der Schalie and Berry, 1973). During unfavorable periods, snail intermediate hosts will often retreat to warmer, deeper waters until the water temperature begins to rise (Mcmahon, 1983; Fernandez and Esch, 1994; Takada, 2003). During colder periods, snails can experience mortality, which can have important influences on snail densities.

Furthermore, parasites infecting snails can also be influenced through host mortality and a reduction in reproductive output due to temperature-dependent reductions in metabolic capacity. Together these processes can significantly alter transmission dynamics between hosts and parasites across seasons (Richter, 2001).

The seasonal dynamics of the prosobranch snail, *Bithynia tentaculata* has gained research interest over the past decade due to the fact that it is an invasive species in North America, and because it serves as host to three parasite species (*Sphaeridiotrema* spp. and *Cyathocotyle bushiensis*) which kill thousands of birds annually in the Upper Mississippi River (UMR) region. Range expansion of *B. tentaculata* and its parasites are now believed to be responsible for waterbird deaths exceeding 60,000 individuals (Sandland *et al.*, 2011). The majority of the waterfowl succumbing to infection are lesser scaup, which is of conservation concern because population sizes of this species continue to remain under the North American Waterfowl Management's population goal since 1985 (Afton and Anderson, 2001; U. S. Fish and Wildlife Service, 2006). For reasons that are poorly understood, waterfowl mortality fluctuates temporally, across years and between migration events within a year.

The seasonal dynamics of *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. have been studied throughout the summer months and into the fall migration season in Pool 7 of the UMR and in other areas of North America (Menard and Scott, 1987; Herman and Sorenson, 2009; Chapter 1). For instance, Hermann and Sorenson (2009) investigated infection dynamics during the summer months in shallow and deep-water sites around constructed islands in Pool 7 of the UMR. They found that *Sphaeridiotrema* spp. and *C. bushiensis* infections fluctuated monthly and attributed these changes to seasonal migration patterns of both definitive and intermediate hosts. They reported that metacercarial

prevalence peaked in late summer and early fall, and attributed this pattern to primary infections established during the spring waterfowl migration. A similar temporal pattern of metacercarial intensities was also reported for *C. bushiensis* by Menard and Scott (1987). Although these studies have been important for understanding temporal changes in the parasites during the ice-free period, no information exists for how host densities and parasite infection vary during the winter (ice-on) period. This is a significant shortcoming as waterfowl return to areas of the UMR shortly after ice-off during their spring migrations northward. Understanding interactions between snails and the waterfowl-killing parasites during this critical period may allow us to better understand patterns of waterfowl mortality during spring migrations.

The goal of this study was to investigate the overwintering dynamics of *Bithynia tentaculata* and both *Sphaeridiotrema* spp. and *C. bushiensis*. Work from this study provides insight into mollusk populations and their parasites in the closed areas of Pool 7 during ice-on and ice-free periods from Summer 2010-April 2011.

METHODS

Lake Onalaska (Pool 7) of the Upper Mississippi River was selected for this work based on waterbird use, ease of access during the winter months, and site locations outside of the waterfowl avoidance areas (Figure 22). Although these sampling sites were located outside of the closed area, the sites remain important feeding areas for lesser scaup and therefore experience high seasonal waterbird use. Ten sites were established and sampled for *B. tenatculata* and its parasites from the summer 2010 to spring 2011. During this period, collections were made during the months of July, October, February, and April. Collection times during the fall and spring coincided with waterbird migrational events to better understand snail and parasite patterns during periods of overlap with definitive hosts.

During the ice-free period, quantitative benthic sampling was conducted as described in Chapter 1. Three replicate samples were collected at each sampling location and each replicate was taken from a different side of the boat (starboard, bow, and port sides) to ensure representative sampling. During the ice-on period (February), sites were accessed by foot. After arriving at each site, a gas-powered ice auger was used to remove a sufficient ice volume to accommodate the ponar. Due to the extreme weather conditions and safety concerns, only a single replicate was obtained at each of the ten sites during this period.

Snails collected as part of each replicate were placed into designated 2-gallon plastic bags and transported back to the University of Wisconsin – La Crosse (UWL) for further processing. This was consistent across sampling dates. In addition, the presence or absence of aquatic-vegetation species was noted for each sample and the number of *Vallisneria*

americana stems were quantified. Any vegetation outside of the ponar was discarded to ensure consistency among samples.

After transportation to the University of Wisconsin – La Crosse, samples were either immediately processed or were placed in the refrigerator and processed later (within 24 hours of initial collection). The length of each snail was recorded using digital calipers. Glass plates were used to crack the shell of each snail and tissues were teased apart using fine forceps. Parasite stages and species were identified and recorded using trematode keys (Yamaguti, 1972; Schell, 1985) and parasite descriptions from primary sources (Khan, 1962, Mclaughlin *et al.*, 1993). Primary-infections (sporocyst/rediae) were reported as presence/absence data. The presence/absence of metacercariae was also recorded along with intensities.

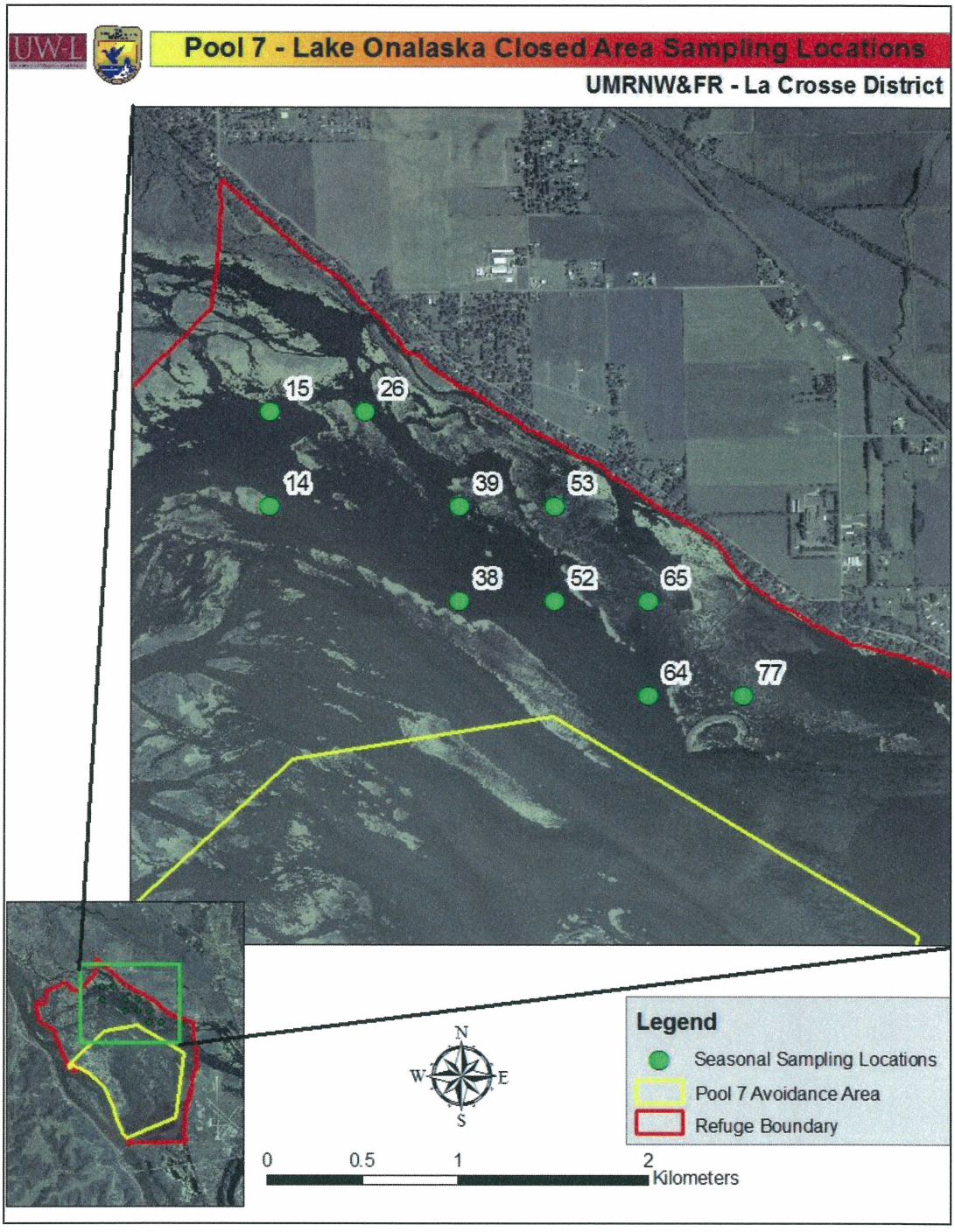


Figure 22. Ten established sampling sites in Pool 7 of the UMR. Circles represent sampling sites, the light line represents the waterfowl avoidance area, and the darker line outlines the refuge boundary. Sites were sampled from summer 2010-Spring 2011.

Supplemental data acquisition

To gain an idea of how seasonal sampling of snails and parasites relate to other variables in the UMR, I supplemented this research with waterfowl and temperature data obtained by U.S. Fish and Wildlife and the US Army Corps of Engineers respectively. Waterfowl locations and numbers were determined via aerial flight surveys. These surveys were conducted throughout the refuge using a fixed-wing aircraft to estimate the numbers of each waterbird species present in an area. Fall aerial flight surveys were conducted weekly from September 27, 2010 until November 29, 2010. The only exception was October 25th due to aircraft maintenance. Aerial flight surveys were not conducted in the spring of 2011 due to their occurrence every other year.

Waterbird mortality data was obtained through the La Crosse district of the Upper Mississippi River National Wildlife and Fish Refuge. Surveys were conducted from the beginning of waterbird migration in early September until the river froze in early December (C. Gehri. Pers. comm.). Mortality assessments focused on the constructed islands in Lake Onalaska where waterbird carcasses have been observed in the past. Each week, the islands were surveyed and the numbers of sick and dead waterbirds were recorded.

Water temperature data were obtained through the U.S. Army Corps of Engineers River Gages website. Daily temperature measurements were obtained at Lock and Dam 7 near La Crescent, MN. Each Lock and Dam records hourly weather and river parameters including temperature and flow. The temperature readings for the Pool 7 closed area were obtained from July 1, 2010 until June 30, 2011. Although, the temperature information was not site specific, it did provide an overall indication of the general temperature fluctuations in Pool 7 during the collection period of this study.

Statistical Analysis

Data were assessed for parametric assumptions such as normality and homogeneity of variance. When assumptions were not met, non-parametric tests were utilized. This included most of the variables of interest. In addition, prevalence values were arcsin-square root transformed prior to analysis (Zar, 1974). Kruskal-Wallis tests were used to compare seasonal difference in snail densities and most parasite metrics. The Mann-Whitney U-test was used to assess the relationship between *Bithynia tentaculata* size and the presence/absence of primary infections. Data were analyzed using SPSS statistical software (IBM)

RESULTS

A total of 561 snails were collected throughout the seasons for this study (July 2010 – April 2011). Species composition of the snails varied throughout the seasons, however native snails were found at lower densities (Figure 23). *Bithynia tenacualata* was the most abundant species, making up 86.6% from the pooled seasonal data (Figure 24). Due to high site variation, a difference in *B. tentaculata* density was not detected among sampling dates (Kruskall Wallis test, $P > 0.05$) (Figure 25) and the mean size of *B. tentaculata* did not differ among seasons (Kruskall Wallis test, $P > 0.05$) (Figure 26).

Throughout the study, 3.7% of the snails collected harbored primary infections and of these, 85.7% were collected during the summer sampling period. Primary infection prevalences for both *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. were significantly higher in the summer when compared to other seasons (Mann-Whitney U-test, $P < 0.05$) (Figure 27). Mean prevalence values for primary infections of *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. were 0.07 and 0.05 respectively, in the summer but then fell close to zero across the remaining sampling dates. Pooling snails across sampling dates revealed that snails containing primary infections were significantly larger than uninfected individuals with infected snails exhibiting a means size of 9.18 mm compared to 7.36 mm in uninfected hosts (Mann-Whitney U-test, $P < 0.001$) (Figure 28).

Metacercarial prevalence did not differ for either parasite species across seasons (Kruskal-Wallis, $P > 0.05$). However, *Cyathocotyle bushiensis* had a higher seasonal prevalence mean than *Sphaeridiotrema* spp. when seasonal data were pooled (Mann-Whitney U-test, $P < 0.001$) (Figure 29). Metacercarial intensity did not differ for either

parasite among the seasons (Kruskal-Wallis, $P > 0.05$), and intensity did not differ between parasite species (Mann-Whitney U-test, $P > 0.05$) (Figure 30). Interestingly, when looking at singly versus co-infected snails, the mean metacercarial intensity of both parasite species was higher when compared to their singly infected counterparts (Mann-Whitney U-tests, $P < 0.05$) (Figure 31).

Based on the USFW aerial surveys, lesser scaup began resting and feeding in the Pool 7 closed area during the week of October 12, 2010 (Figure 32), and peaked during the week of November 1, 2010 in the Pool 7. The following week, lesser scaup population numbers began to decrease and were absent from the Pool 7 after the week of November 29, 2010. Large numbers of American coot arrived earlier than lesser scaup (September 27, 2010), peaked during the week of October 12, 2010 and decreased thereafter (Figure 32).

Waterbird mortality was not detected in the Pool 7 closed area until the week of October 17, 2010 where 16 American coots had succumbed to trematodiasis (Figure 33). Waterbird mortality peaked during the week of October 24, 2010 in the Pool 7 closed area where 48 dead American coots were collected while only 2 lesser scaup were collected. The weeks following the peak mortality event, only a few birds of each species were collected.

The water temperature reached a maximum of 27.7° C on August 14, 2010 and began decreasing after this date (Figure 34). The water temperature reached 0° C on November 25, 2010 where the water temperature remained until March 13, 2011 as it began to increase. The water temperature fluctuated below 10° C until May 2, 2011 as it began to rise with warming air temperature. Ice cover lasted for about 100 days in Pool 7.

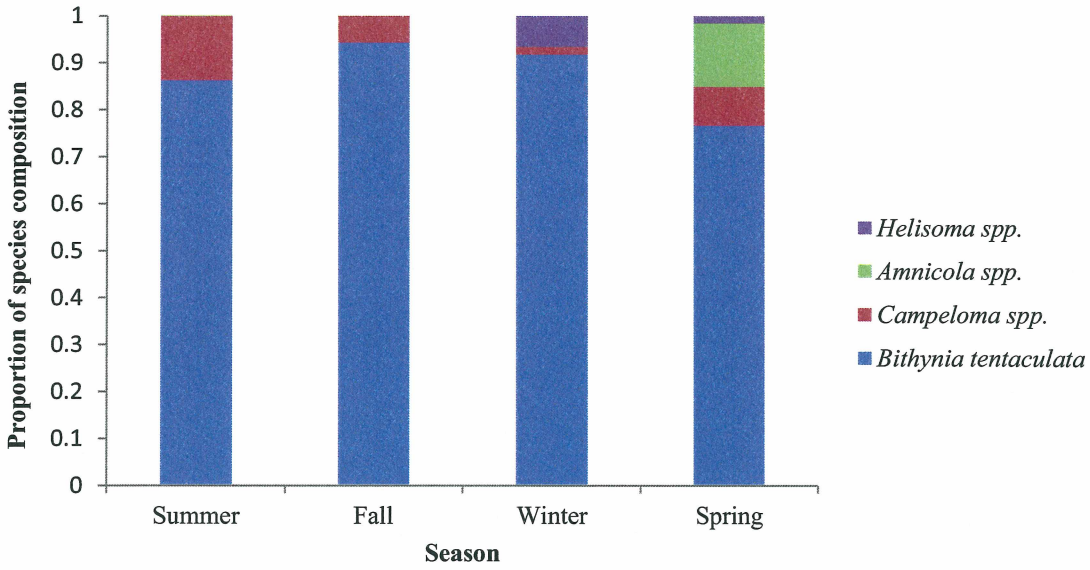


Figure 23. Seasonal species composition of snails collected at four sampling periods (summer, fall, winter and spring) in the Pool 7 closed area from July 2010 to April 2011.

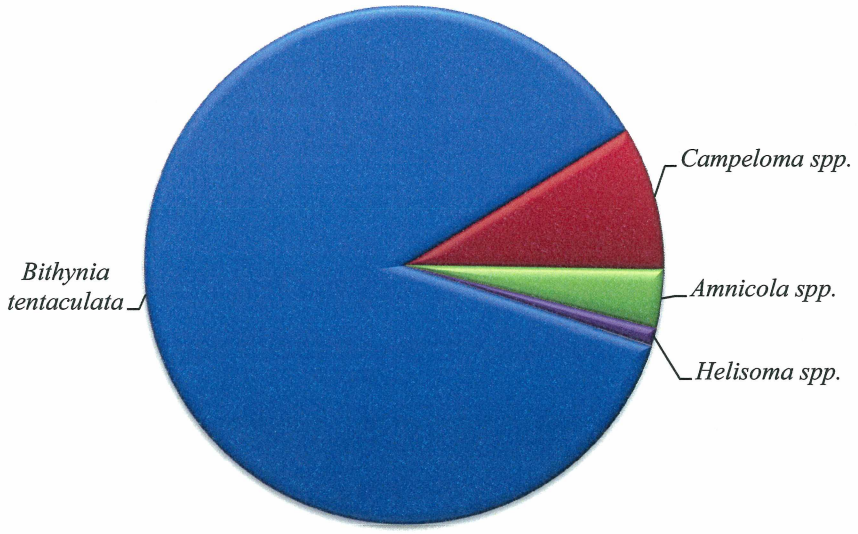


Figure 24. Overall species composition of the pooled snail data obtained over the four sampling periods in the Pool 7 closed area from July 2010 to April 2011.

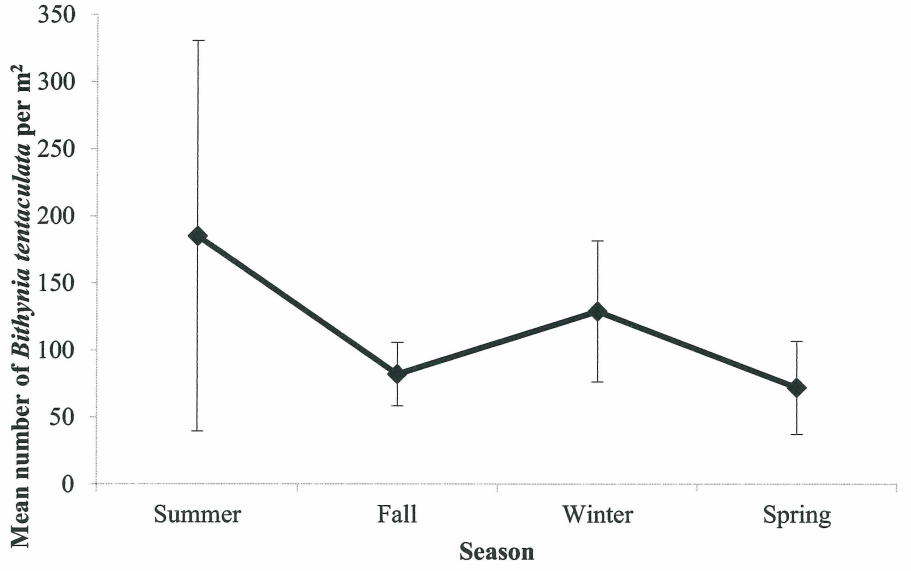


Figure 25. Mean number of *Bithynia tentaculata* (± SE) collected from 10 sites in Pool 7 across 4 sampling periods from July 2010-April 2011.

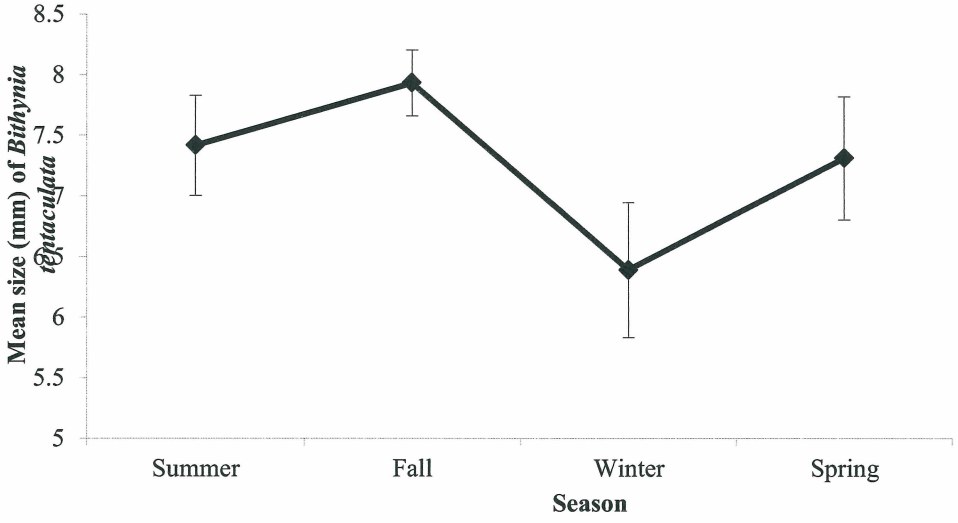


Figure 26. The mean size (±SE) of *Bithynia tentaculata* across four points in time, collected from July 2010 to April 2011.

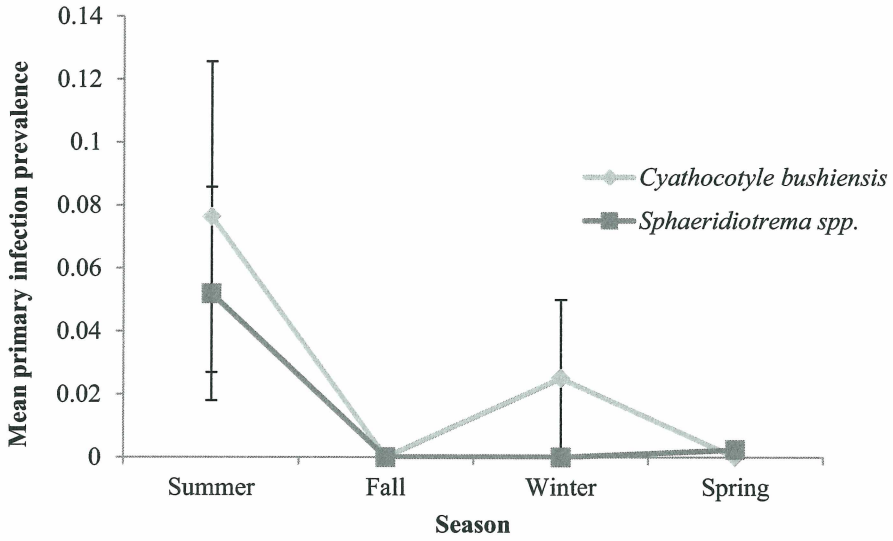


Figure 27. The mean primary infection prevalence (±SE) of two trematode species using the intermediate host *Bithynia tentaculata* across four sampling points.

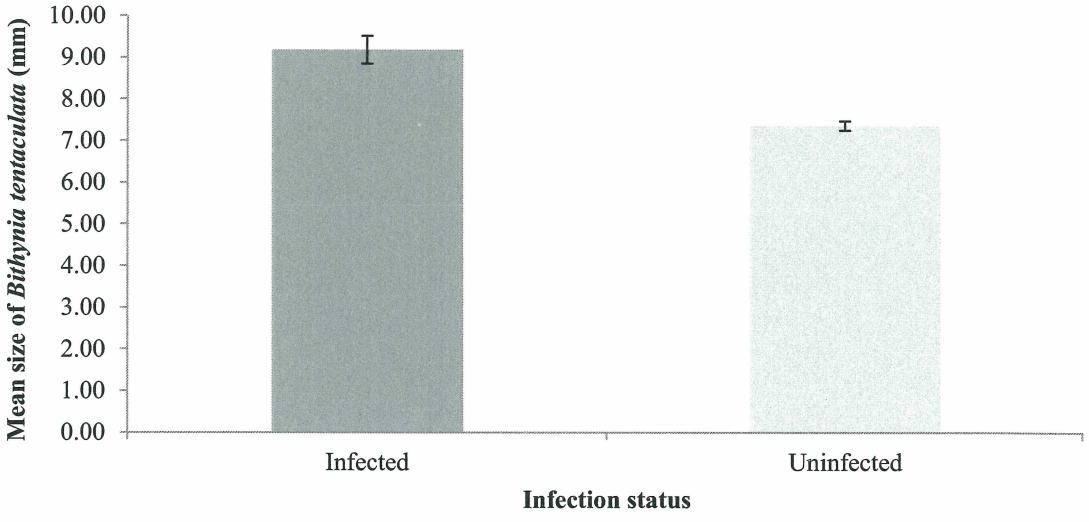


Figure 28. The mean size (±SE) of *B. tentaculata* infected with primary infections compared to snails uninfected with primary infections.

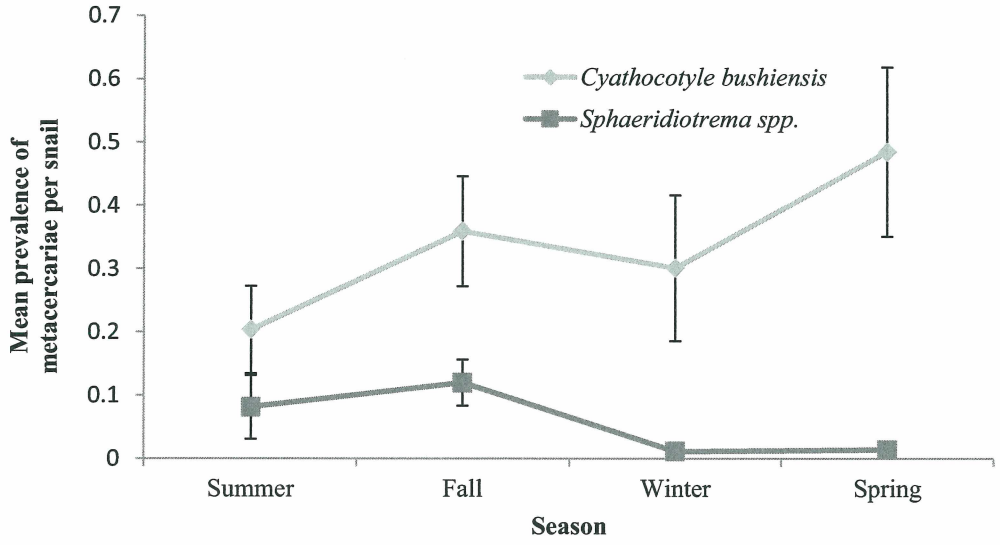


Figure 29. The mean prevalence (±SE) of secondary infections (metacercariae) in each season of *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. in *Bithynia tentaculata*.

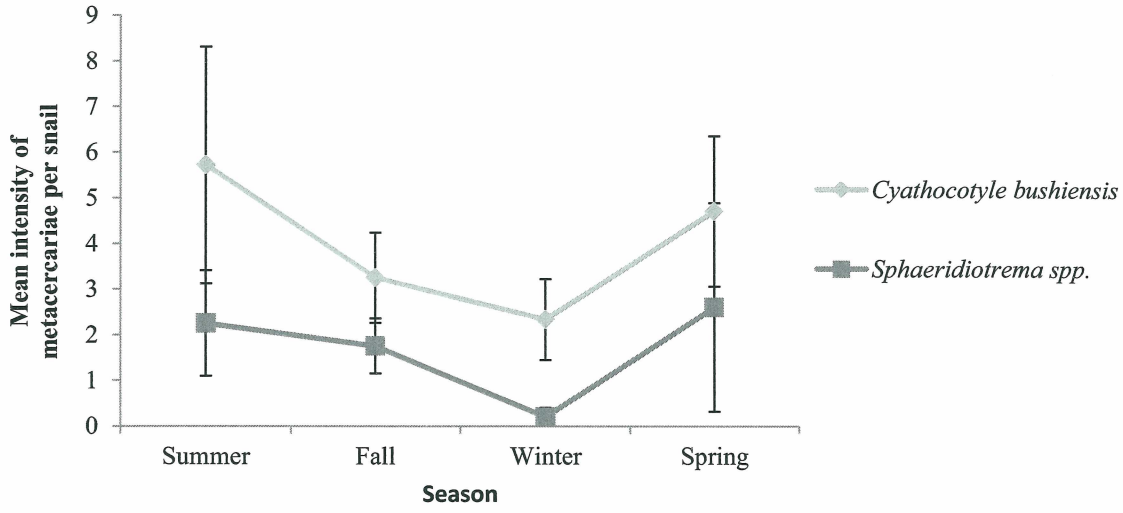


Figure 30. The mean intensity (±SE) of secondary infections (metacercariae) in each season of *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. in *Bithynia tentaculata*.

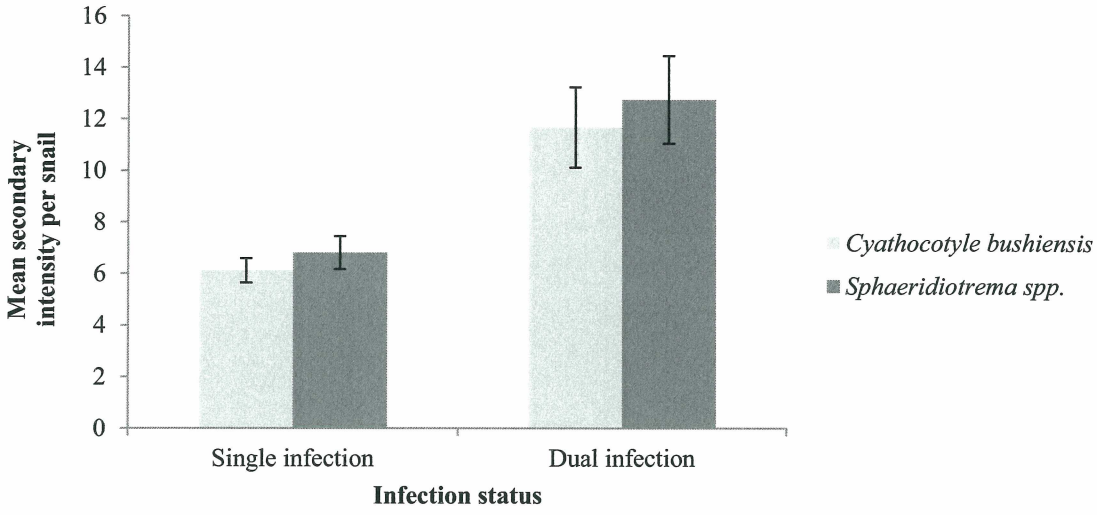


Figure 31. The mean metacercarial intensity (±SE) of three trematode species infecting *Bithynia tentaculata* with a single species infection and with concurrent infections.

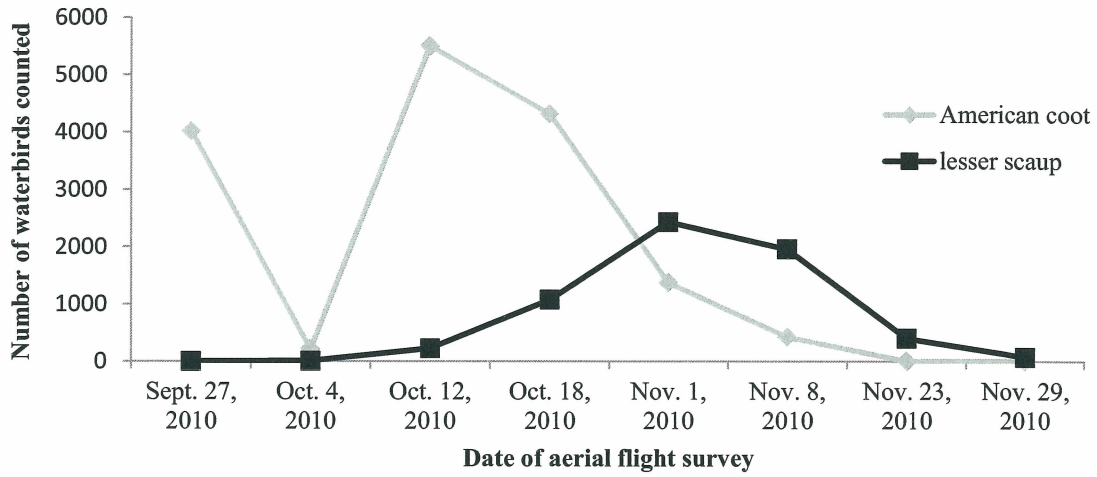


Figure 32. The number of waterbirds resting and feeding in the Pool 7 Lake Onalaska Closed Area during the fall of 2010. The data was obtained from the aerial flight surveys flown by the U.S. Fish and Wildlife Service.

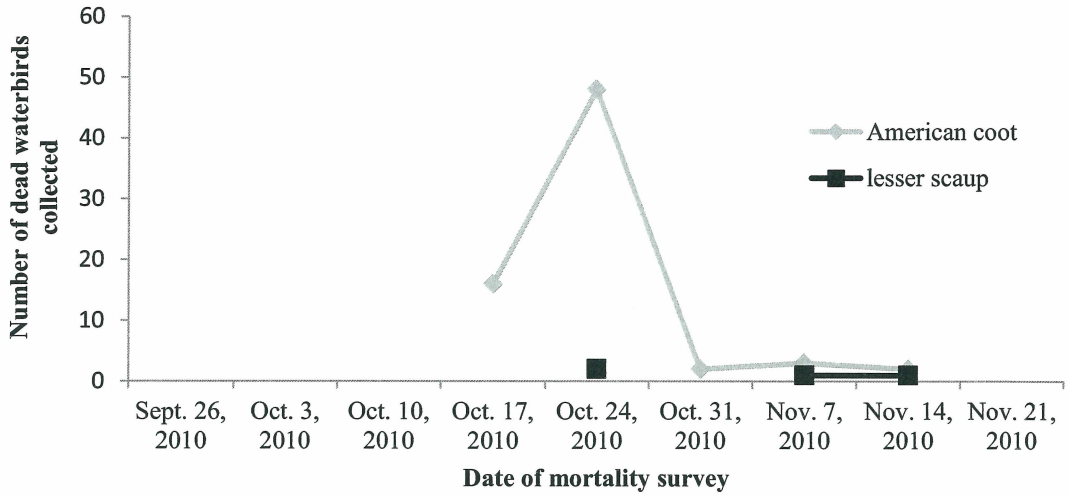


Figure 33. The number of dead American coot and lesser scaup collected from the Pool 7 closed area during the fall of 2010.

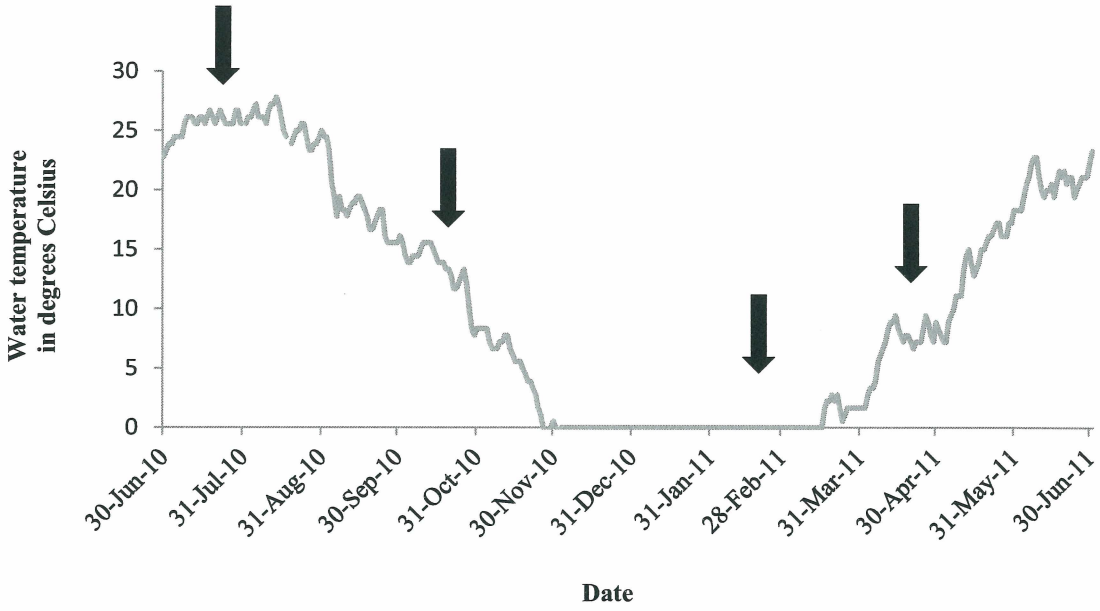


Figure 34. The water temperature collected at Lock and Dam 7 of the Upper Mississippi River. Site sampling times are designated by the black arrows. Temperature was collected for this study from July 1, 2010 till June 30, 2011.

DISCUSSION

The seasonal variability in populations of hosts and parasites can have important consequences for transmission dynamics and disease occurrence within a system. Although recent work has been done to investigate spatial (Chapter 1) and temporal patterns of *C. bushiensis* and *Sphaeridiotrema* spp. infections in *B. tentaculata*, little is known about snail and parasite dynamics during periods prior to, during, and immediately after ice-on. Work conducted as part of this chapter focused on snail and parasite distribution patterns during this understudied period.

One of the key patterns observed in this work was the general reduction in both *C. bushiensis* and *Sphaeridiotrema* spp. primary infections during the ice-on period. It has been established that snails can experience pathology at lower temperatures. For example, Jensen *et al.* (1996) found that the mud snail, *Hydrobia ventrosa* can lose up to 78% of its population during freezing temperatures. This, in combination with the fact that trematodes can cause increases in mortality of their snail hosts (Sandland and Minchella, 2003) suggests that the pattern may be the result of environmentally-mediated parasite-induced mortality where lower temperatures actually exacerbate the negative effects of the primary infections (Richter, 2001). Alternatively, colder temperatures may actually have an asymmetrical impact on parasites relative to their hosts, leading to a loss of infections (but not the hosts themselves) overwinter (Esch and Fernandez, 1994). For instance, work by Goater (1989) found that snails lose their trematode infections overwinter and actually reverse the castration process even though extensive tissue damage may have been done to the hosts. Another possibility is that the loss of infections simply corresponds with the loss of older,

mature snails through general senescence patterns of the host. If parasites tend to become more prevalent in more mature snails (due to their increased time of exposure), the prevalence of these infections would be expected to decline as the oldest year-class dies off. However, Richter (2001) found that high mortality of mature *B. tentaculata* typically took place after reproduction in the spring, which makes this explanation less likely than one that considers parasitism as an additional factor. A final possibility involves the behaviors of infected versus uninfected individuals. It has been shown that trematodes can modify the behaviors (and therefore distributional patterns) of their infected snail hosts (Miller and Poulin, 2001). If infected *B. tentaculata* migrate to different areas of Pool 7 relative to uninfected individuals during the onset of ice-on (such as shallower, or deeper areas), they may not have been acquired during the latter stages of this study.

The lack of new primary infections during the overwintering period likely ties in with the interaction between temperature and the development of both *C. bushiensis* and *Sphaeridiotrema* spp. in the external environment. Menard and Scott (1987) found that decreased temperatures slow the development of the miracidium (within the deposited eggs) and reduce the viability of these larvae if hatching occurs. Similarly, research by McKindsey and McLaughlin (1992) showed experimentally that *S. pseudoglobulus* failed to hatch during the winter in a Quebec lake. Thus, trematode eggs deposited by birds migrating through the UMR in the fall of 2010 were unlikely to have been at an infective stage to snails during my sampling periods prior to, during, and immediately after ice-on preventing any new primary infections in *B. tentaculata* during this period. However, McKindsey and McLaughlin (1992) also showed that although eggs fail to hatch over winter, they can exhibit high hatching success when temperatures increase in the spring. This would predict a wave of new primary infections in the summer (Mernard and Scott,

1987; McKindsey and McLaughlin, 1994). Although my data cannot confirm this temporal pattern directly, the relatively high prevalence values detected during the initial August sample suggest that this temperature-based process may be occurring in the UMR as well.

In terms of secondary infections, *Cyathocotyle bushiensis* had higher mean metacercarial prevalences relative to *Sphaeridiotrema* spp. across sampling dates. In addition, mean cercarial intensities of *C. bushiensis* tended to be higher in snails relative to *Sphaeridiotrema* spp. across the same time points (although these differences were not significant). One reason for the difference between parasite species could relate to the level of specificity exhibited by the cercariae of each trematode. Although a large body of work has demonstrated that trematode cercariae tend to be relatively generalist in nature (Lepitzki, 1993; Esch and Fernandez, 1994), specificity for particular species of second intermediate-host still exists. This has been shown for a number of systems involving secondary infections in snails (Evans and Gordon, 1983; McCarthy and Kanev, 1990). In this system, the less invasive establishment of *Sphaeridiotrema* spp. cercariae may allow this species to infect a greater breadth of snails, including native species (Lepitzki, 1993). Indeed, recent evidence on *S. pseudoglobulus* cercariae suggests that this species can infect not only *B. tentaculata* but a number of native snail species as well (Sandland *et al.*, submitted). This may actually lead to a dilution effect where *Sphaeridiotrema* spp. cercariae could infect a greater number of species in the area (compared to *C. bushiensis*) if present, which could results in fewer metacercariae/snail for this species. Alternatively, differences in cercariae production/cercariae dispersal between the two species may help to explain the observed pattern. Future comparative work investigating differences in cercarial traits between these parasites could help to better resolve the mechanisms underlying prevalence patterns in *B. tentaculata* collected from the UMR.

Mean metacercarial prevalences and intensities did not differ seasonally for either *C. bushiensis* or *Sphaeridiotrema* spp. This was not surprising given the reductions in primary infections observed at the sampling sites. A loss of primary infections in snails would be predicted to lead to relatively stable metacercariae numbers as no new cercariae would be accrued by hosts. Changes in metacercarial prevalence/intensities would be more likely to occur in the summer after primary infections reach patency in first-intermediate hosts.

Interestingly, comparing pooled metacercariae revealed that co-infected snails tended to have greater larval intensities that snails infected with just a single species. One possibility is that this pattern simply reflects aggregation patterns of bird definitive hosts (Lepitzki, 1993). Areas where both coot and lesser scaup forage together at high densities would be expected to generate more primary infections by each parasite species, which in turn could enhance overall metacercarial numbers and the occurrence of co-infection. These infection hotspots could be enhanced by the reduced movement of sick waterbirds further enhancing egg release in these areas. Site level observations from this study provide some support for this, as in areas where co-infection was observed, higher levels of metacercariae were found in snails infected with one trematode species. A second intriguing explanation for this pattern is that cercarial infection by one of the trematode species actually facilitates the establishment of the other. Past work provides support for this idea (Christensen *et al.*, 1987). For example, work by Noland *et al.* (2005) reported that mice infected with the trematode, *Echinostoma caproni* had increased malaria parasitemias. Although the specific mechanism underlying this pattern was not known, the authors suggested that it was likely immunologically mediated. A similar mechanism may also be responsible for the pattern observed in my study. For example, if cercarial infection by one species alters the responses

of snail immuno-components such as hemocytes, it may facilitate the invasion and establishment of other species.

In 2010, waterbirds began to die two weeks after the arrival of large waterbird populations. Others have also reported delays in mortality when investigating this system. For example, in Quebec Canada, Menard and Scott (1987) found that dead waterbirds could be found months after initial arrival at sites containing both *B. tentaculata* and their parasites. The observed delay could be attributed to trematode development in the intestines and the time required for the waterbird to ingest a lethal amount of metacercariae. Waterbirds with low fat reserves tend to forage aggressively in the UMRNW&FR in order to refill their fat stores during migration, which may increase their chance of ingesting a lethal dose of metacercariae.

The transmission dynamics in this system are complex as evidenced by the host-parasite patterns observed from summer to spring in this study. This work suggests that both parasite species are able to successfully overwinter in *Bithynia tentaculata* and the metacercariae numbers are high enough to generate waterfowl mortality in the spring. Future work should attempt to better resolve the interaction between host densities, parasite intensities and waterfowl occurrence during both migrational events. In addition it will be important to continue to follow host-parasite dynamics during the ice-free period (after April) to determine whether parasite transmission peaks occur and what the consequences of these peaks may be to waterfowl during fall migration.

REFERENCES

Afton, A.D. and Anderson, M.G. (2001). Declining scaup populations: a retrospective analysis of long-term population and harvest survey data. *The Journal of Wildlife Management*, 65, 781-796.

Anteau, M. J., and A. D. Afton. (2004). Nutrient reserves of lesser scaup (*Aythya affinis*) during spring migration in the Mississippi Flyway: a test of the spring condition hypothesis. *Auk*, 121:917-929

Burch, J. B. (1989). *North American freshwater snails*. Malacological Publications, Hamburg, Michigan.

Bush, A.O., Lafferty, K.D., Lotz, J.M. and Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology*, 83, 575–583.

Clampitt, P.T. (1974). Seasonal migratory cycle and related movements of the fresh-water pulmonate snail, *Physa integra*. *The American Midlands Naturalist*, 92, 275-300.

Cheng, T.C. and Snyder, R.W. (1962). Studies on host-parasite relationships between larval trematodes and their hosts. I. A review. II. The utilization of host's glycogen by the intramollscan larvae of *Glypthelmins pennsylvaniensis* Cheng, and associated phenomena. *Transactions of the American Microscopical Society*, 81, 209-225.

Emmel, M.W. (1942). Field experiments in the use of sulfur to control lice, fleas, and mites of chickens. *Florida Agriculture Experiment Station Bulletin*, 374, 1-8

Esch, G.W. and Fernandez, J.C. (1994). Snail-trematode interactions and parasite community dynamics in aquatic systems: A review. *American Midland Naturalist,* 131: 209–237.

Evans, N.A. and Gordon, D.M. (1983). Experimental observations on the specificity of *Echinoparyphium recurvatum* toward second intermediate hosts. *Parasitology Research*, 69, 214-222.

Gibson, G.G., Broughton, E. and Choquette, L.E. (1972). Waterfowl mortality cause by *Cyathocotyle bushiensis* Khan, 1962 (Trematoda: Cyathocotylidae), St. Lawrence River, Quebec. *Canadian Journal of Zoology*, 50, 1351-1356.

Goater, T.M., Shistak, A.W. and Williams, J.A. (1989). A mark recapture study of trematode parasitism in overwintered *Helisoma anceps* (Pulmonata), with special reference to *Halipegus occidualis* (Hemiuridae). *Journal of Parasitology*, 75, 553-560.

Havera, S.P., Boens, L.R., Georgi, M.M. and Shealy, R.T. (1992). Human disturbance of waterfowl on Keokuk pool, Mississippi River. *Wildlife Society Bulletin*, 20, 290-298.

Herrmann, K.K. and Sorensen, R.E. (2009). Seasonal dynamics of two mortality-related trematodes using an introduced snail. *Journal of Parasitology*, 95, 823-828.

Herrmann, K.K. and Sorensen, R.E. (2011). Differences in natural infections of two mortality-related trematodes in lesser scaup and American coot. *Journal of Parasitology*, 95, 823-828.

Jensen, K.T., Latama, G. and Mouritsen, K.N. (1996). The effect of larval trematodes on the survival rates of two species of mud snails (Hydrobiidae) experimentally exposed to desiccation, freezing, and anoxia. Helgolander Meeresunters, 50, 327-335.

Jokinen, E. (1992). *The Freshwater Snails (Mollusca: Gastropoda) of New York State*. The University of the State of New York, The State Education Department, The New York State Museum, Albany, New York 12230. 112 pp.

Khan, D. (1962). Studies on larval trematodes infecting freshwater snails in London (U.K.) and some adjoining areas. Part VI. The cercariae of the ''Vivax'' group and the life history of *Cercaria bushiensis* n. sp. (*Cyathocotyle bushiensis* n. sp.). *Journal of Helminthology*, 36, 67–94.

Korschgen, C.E. and Dahlgren, R.B. (1992). Human disturbance of waterfowl: causes, effects, and management. *Fish and Wildlife Leaflet,* 13.2.15, 1-8.

Kube, J., Kube, S. and Dierschke, V. (2002). Spatial and temporal variations in the trematode component community of the mudsnail *Hydobia ventrosa* in relation to the occurrence of waterfowl as definitive hosts. *Journal of Parasitology*, 88, 1075-1086.

Lepitzki, D.W. (1993). Epizootiology and transmission of snail-inhabiting metacercariae of the duck digeneans *Cyathocotyle bushiensis* and *Sphaeridiotrema globulus*. (Unpublished Ph.D. dissertation). McGill University, Montreal, Canada, 354 p.

Lepitzki D.W., Scott, M.E. and Mclaughlin, J.D. (1994). Influence of storage and examination methods on the recovery and size of metacercariae of *Cyathocotyle bushiensis* and *Sphaeridiotrema pseudoglobulus* (Digenea). *Journal of Parasitology*, 80, 454–460.

Luzon-Pena, M., Rojo-Vazquez, F.A. and Gomez-Bautista, M. (1994). The overwintering of eggs, intramolluscal stages and metacercariae of *Fasciola hepatica* under the temperatures of a Mediterranean area (Madrid, Spain). *Veterinary Parasitology*, 55, 143-148.

Mattice, J.S. (1972). Production of a natural population of *Bithynia tentaculata* L. (Gastropoda, Mollusca). *Ekologia Polska*, 20, 525-539.

McCarthy, A.M. and Kanev, I. (1990). *Pseudechinoparyphium echinatum* (Digenea: Echinostomatidae): experimental observations on cercarial specificity toward second intermediate hosts. *Parasitology*, 100, 423-428.

Mckindsey, C.W. and Mclaughlin, J.D. (1993). The viability of *Sphaeridiotrema pseudoglobulus* (Digenea) eggs following cold water storage as a possible overwintering strategy. *Parasitology*, 107, 441-447.

McLaughlin, J.D., Scott, M.E. and Huffman, J.E. (1993). *Sphaeridiotrema globulus* (Rudolphi, 1814) (Digenea): evidence for two species known under a single name and a description of *Sphaeridiotrema pseudoglobulus* n.sp. *Canadian Journal of Zoology*, 71, 700-707.

McMahon R.F. (1983). *Physiological ecology of freshwater pulmonates. In: Russell-Hunter WD (ed) The Mollusca 6 Ecology*. Academic Press, London, pp 359-430

Meier-Brook, C. and Kim, C.H. (1977). Notes on ciliary feeding in two Korean *Bithynia* species. *Malacologia*, 16, 159-163.

Menard, L. and Scott, M.E. (1987). Seasonal occurrence of *Cyathocotyle bushiensis* Khan, 1962 (Digenea: Cyathocotylidae) metacercariae in the intermediate host *Bithynia tentaculata* L. (Gastropoda: Prosobranchia). *Canadian Journal of Zoology*, 65, 2980–2992.

Miller, A.A. and Poulin, R. (2001). Parasitism, movement, and distribution of the snail *Diloma subrostrata* (Trochidae) in a soft-sediment intertidal zone. *Canadian Journal of Zoology*, 79, 2029-2035.

Mitchell, A.J. and Cole, R.A. (2008). Survival of the faucet snail after chemical disinfection, pH extremes and heated water bath treatments. *North American Journal of Fisheries Management*, 28, 1597-1600.

Moore, M.V. and Lee, R.E. (1991). Surviving the big chill: overwintering strategies of aquatic and terrestrial insects. *American Entomologist*, 111-118

Murkin, H.R. and Kadlec, J.A. (1986). Relationship between waterfowl and macroinvertebrate densities in a norther prairie marsh. *Journal of Wildlife Management*, 50, 212-217.

Noland, G.S., Graczyk, T.K., Fried, B., Fitzgerald, E.J. and Kumar, N. (2005). Exacerbation of *Plasmodium yoelii marlaria* in *Echinostoma caproni* infected mice and abatement through anthelmintic treatment. *Journal of Parasitology*, 91, 944-948.

Olsson, T.I. (1983). Seasonal variation in the lateral distribution of mayfly nymphs in a boreal river. *Holarctic Ecology*, 6, 333-339.

Olsson, T.I. (1984). Winter sites and cold-hardiness of two gastropod species in a boreal river. *Polar Biology*, 3, 227–230.

Peirce, J.P., Sandland, G.J., Sutter, C. and Haro, R.J. (2011). Predicting the ecological outcomes of species invasions and parasite transmission in the upper Mississippi river. *Journal of Biological Dynamics*, 0, 1-18.

Pimentel, D. (1957). Life history of *Australorbis glabaratus*, the snail intermediate host of *Schistosoma mansoni* in Puerto Rico. *Ecology*, 38: 576-580.

Richter, T., (2001). *Reproductive biology and life history strategy of Bithynia tentaculata (Linnaeus, 1758) and Bithynia leachii (Sheppard, 1823)*. Unpublished Ph.D. dissertation). Universitat Hannover. Saxony, Germany.

Sandland, G.J., Houk, S., Walker, B.A. and Gillis, R. (2012). Differential life-history expression in invasive and native hosts exposed to a parasite of conservation concern. Submitted.

Sauer, J. S., Cole, R.A. and Nissen, J.M. (2007). Finding the exotic faucet snail (*Bithynia tentaculata*): investigation of waterbird die-offs on the Upper Mississippi River National Wildlife and Fish Refuge. *U.S. Geological Survey Open-File Report 2007-1065*, 1-3.

Strayer, D.L. (1999). Effects of alien species on freshwater mollusks in North America. *Journal of North American Benthological Society*, 18, 74-98.

Takada, Y. (2003). Dimorphic migration, growth, and fecundity in a seasonally split population of *Littorina brevicula* (Mollusca: Gastropoda) on a boulder shore. *Population Ecology*, 45, 141-148.

Tashiro J.S. and Colman, S.D. (1982). Filter feeding in the freshwater prosobranch snail *Bithynia tentaculata*: bioenergetic partitioning of ingested carbon and nitrogen. *American Midland Naturalist*, 197, 114-132.

Von der Schalie, H. and Berry, E.G. (1973). The effects of temperature on growth and reproduction of aquatic snails. S*terkiana*, 50, 1-92.

Vincent, B., Vaillancourt, G. and Harvey, M. (1981) Cycle de développement, croissance, effectifs, biomasse et production de *Bithynia tentaculata* L. (Gastropoda : Prosobranchia) dans le Saint-Laurent (Québec). *Canadian Journal of Zoology*, 59, 1237-1250.

Vincent, B. and Letourneau, C. (1985). Interpopulation variations in the age structure and growth of the prosobranch *Bithynia tentaculata* L. *Canadian Journal of Zoology*, 63, 1345-1353.

Vincent, B., Vaillancourt, G. and Harvey, M. (1981) Cycle de développement, croissance, effectifs, biomasse et production de *Bithynia tentaculata* L. (Gastropoda: Prosobranchia) dans le Saint-Laurent (Québec). *Canadian Journal of Zoology*, 59, 1237-1250.

White, D.H. and James, D. (1978). Differential use of fresh water environments by wintering waterfowl of coastal Texas. *The Wilson Bulletin*, 90, 99-111.

Wood, A.M., Haro, C.R., Haro, R.J. and Sandland, G.J. (2011). Effects of dessication on two life stages of an invasive snail and its native cohabitant. *Hydrobiologia*, 675, 167-174.

Yamaguti, S. (1958). *Systema Helminthum. Vol. I. The digenetic trematodes of vertebrates-part I.* Interscience Publishers, Inc., New York. 979 p.

Zar, J. H. (1974). *Biostatistical analysis*. Prentice-Hall Inc., Englewood Cliffs, NJ.

CHAPTER III

ASSESSING THE ECOLOGICAL RISK OF THREE TREMATODE SPECIES IMPLICATED IN WATERFOWL MORTALITY BY THE USE OF GEOGRAPHIC INFORMATION SYSTEMS (GIS)

ABSTRACT

Trematodiasis, a disease caused by exotic trematodes has caused large mortality events in the Upper Mississippi River National Wildlife and Fish Refuge (UMRNW&FR) since 2002. The spatial distribution of the intermediate host, *Bithynia tentaculata* and the trematodes, *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. are relatively unknown in the Upper Mississippi River (UMR). The objective of this study was to use a quantitative sampling method to assess the disease risk and environmental factors contributing to disease transmission in the closed areas. Recent habitat rehabilitation programs implemented by the U.S. Army Corps of Engineers, U.S. Fish and Wildlife Service, and the U.S. Geological Survey have created new habitats that allow the snails to flourish. These habitats are also located in an area that is closed to all migratory waterbird hunting giving large numbers of migrating waterbirds a place to feed without human disturbance. Ecological overlap among infected snails and waterfowl is responsible for the die-offs that occur seasonally. The static risk maps will help managers focus resources on an area that could help to lessen the severity of this disease through mitigation strategies.

INTRODUCTION

Spatial epidemiology was developed to understand the impacts of the spatial variation of infectious diseases (Ostfeld *et al.*, 2005). This involves spatially linking variables associated with infection risk and the occurrences of disease, which is often accomplished by modeling (Ostfeld *et al.*, 2005; Hudson *et al.*, 2002). This approach allows users to determine the spatial distribution of both hosts and parasites, and correlate these data with the abiotic and biotic factors that potentially modulate the interaction (Husdson *et al.*, 2002). By combining these data layers, users can create maps that identify foci of infection, where the probability of transmission and disease occurrence is highest. The most famous use of this idea was John Snow's maps created by hand in 1854, which found that cholera cases clustered around water pumps (Howe, 1972). With advancements in computer technology, risk maps can be digitally created to show the distribution and risk of infection of a particular disease. These maps focus on the disease variation which can provide insight into the actions most important for controlling the disease and/or aiding infected individuals (Ostfeld *et al.*, 2005). For example, Omumbo and Snow (2004) used risk maps to identify high-risk areas for *Plasmodium falciparum* occurrence so that they could prioritize allocation of limited aid resources. The use of GIS and risk maps has become common with human disease in recent years (Beyers *et al.*, 1996; Kitron *et al.*, 1996; Hong Hu *et al.*, 1998; Brooker *et al.*, 2003; Danson *et al.*, 2004); however widespread application has been limited in other disease fields (Eastman *et al.*, 1995).

Using GIS to understand livestock and wildlife diseases has gained popularity in recent years (Mortarino *et al.*, 2008; Musella *et al.*, 2011; Murphy *et al.*, 2011). Wildlife researchers have used GIS to track disease spread in large mammals such as bighorn sheep, mountain goats, deer and moose (Kitron *et al.*, 1992; Kitron and Kazmierczak, 1996; Douglas, 2001; Gross, 2001; Johnson and Swift, 2001; Cobb *et al.*, 2004; Conner and Miller, 2004; Daniel *et al.*, 2004; Lemke, 2004; Wolfe *et al.*, 2004; Lenarz, 2006; Richomme *et al.*, 2006; Rinaldi *et al.*, 2007; Singer *et al.*, 2008; Kilpatrick *et al.*, 2009; Larter, 2009; Rinaldi *et al.*, 2009). Few avian studies existed until the outbreak of the avian influenza (H5N1) virus starting in 2003. After that point, a number of models were developed to assess migrational routes of waterfowl and the level of exposure the waterfowl might receive (Ward *et al.*, 2007; East *et al.*, 2008; Ward *et al.*, 2008; Prosser *et al.*, 2009; Gilbert *et al.*, 2010). However, the use of GIS in monitoring other waterfowl diseases of conservation concern has been underutilized.

Since 2002, large waterbird mortality events have occurred in the Upper Mississippi River National Wildlife and Fish Refuge (UMRNW&FR). It is now estimated that over 60,000 waterbirds have succumbed to a disease caused by a number of trematodes including *Cyathocotyle bushiensis* and *Sphaeridiotrema pseudoglobulus* (U.S. Fish and Wildlife Service, 2006; Sauer *et al.*, 2007; Calvin Gehri pers. comm.). The parasites have complex lifecycles that use the exotic snail *Bithynia tentaculata* as an intermediate host for both the primary (cercarial) and secondary (metacercarial) stages of infection (Sauer *et al.*, 2007; Sandland *et al.,* 2011). Waterbirds become infected with the trematodes while feeding on the snails and can perish in as little as 24 hours, however most die within 3-8 days (Sauer *et al.*, 2007). Trematodiasis on the Upper Mississippi River (UMR) primarily affects lesser scaup (*Aythya affinis*) and American coot (*Fulica americana*); however 15 other species

have been affected by this disease (Sauer *et al.*, 2007). Management strategies, which include risk mapping, will have to be established in order to better understand transmission dynamics in the UMR and develop mitigation strategies to reduce the impacts of this disease migrating waterbirds.

GIS-based infection risk maps have not been generated for trematode-based waterfowl disease in the UMR. This study combined data for *Bithynia tentaculata* and its parasites with additional abiotic and biotic variables in order to generate novel epidemiological risk maps for Pool 8 of the UMR. Results of this work will assist with mitigating the spread of *B. tentaculata* and its parasites.

METHODS

Pool 8 data were used to develop invasion/transmission maps in this study due to the completeness of snail and parasite information (Chapter 1) along with the fact that this pool is included within the USGS Long Term Resource Monitoring Program (LTRMP) which has collected extensive data on vegetation, flow, bathymetry, and water quality over a number of decades. Pool 8 is 39 kilometers long and stretches from its northern point at La Crescent, MN (N 43.826566 W 91.305091) to its southernmost point at Genoa, WI (N 43.574155 W 91.226494). To facilitate successful waterfowl migrations, habitat rehabilitation (island construction) was initiated in 1989 within this pool. The first islands were finished in 2002 and the project was completed in the fall of 2011.

To further enhance the success of waterfowl migrations, a closed area (26.3 km^2) has been established in the southern half of Pool 8. This area provides a resting and feeding stop for migrating waterbirds during the spring and fall (Figure 28). For this study, waterbird use and distribution in Pool 8 was verified by aerial flight surveys conducted by the U.S. Fish and Wildlife Service (U.S. Fish and Wildlife Service, 2006) during fall migration. The aerial flight surveys were performed weekly during both migrational periods using a fixed wing aircraft (U.S. Fish and Wildlife Service, 2006; Bill Thrune pers. comm.). Based on waterbird feeding distribution and locations where carcasses were collected, a standard square grid with a cell area of 0.24 km^2 was created to sample Pool 8. Each cell intersection represented a single sampling point ensuring coverage over a large area while minimizing costs (Figure 35). Coordinates of each sampling location were loaded onto a handheld global position system (GPS) to aid in navigation to each site and to ensure spatial

location. Sampling in the northern portion of the Pool 8 closed area could not be conducted due to island construction. Sample and collection processes were carried out in Pool 8 as outlined in Chapter 1.

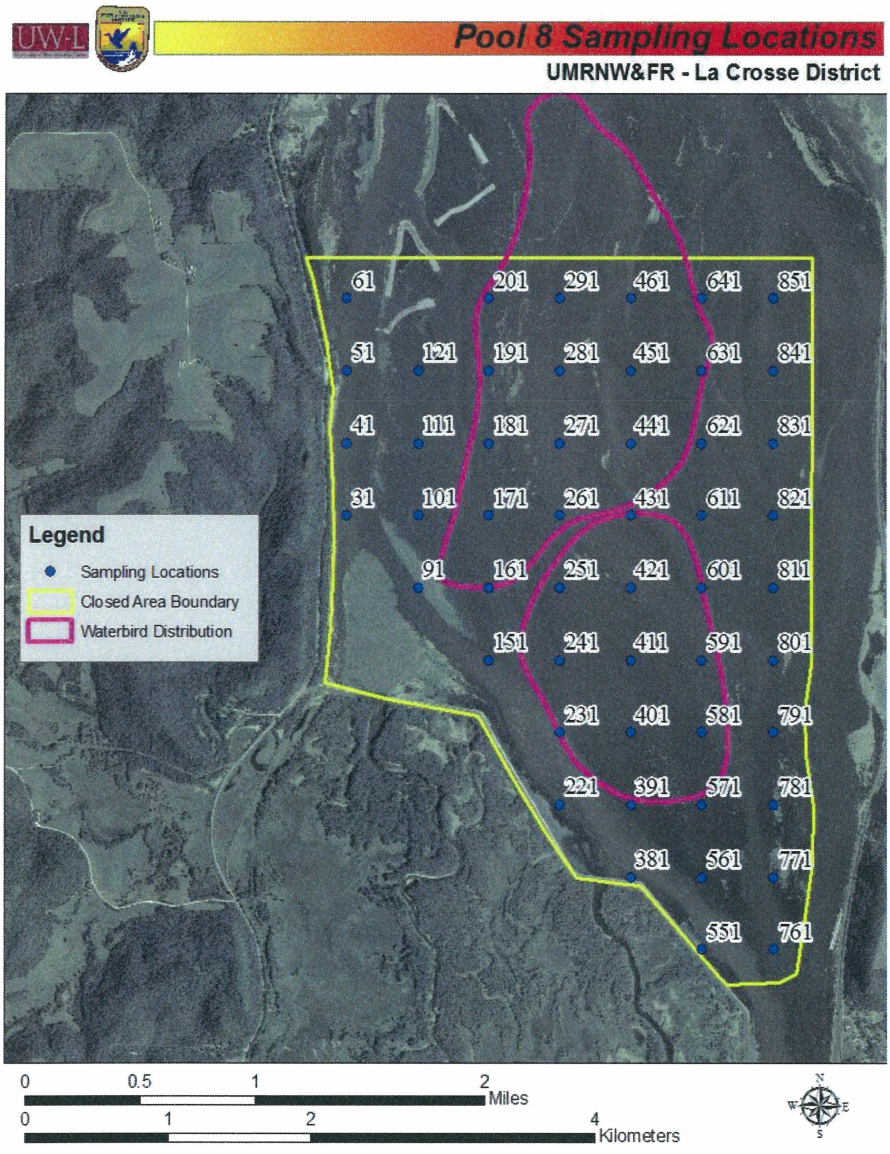


Figure 35. The Wisconsin Islands Closed area showing the benthic sampling sites and lesser scaup distribution. The yellow perimeter designates the boundary of the southern portion of the Wisconsin Islands closed area, the polygons show the feeding and resting distribution of lesser scaup, and the smaller blue circles represent the sampling locations.

Additional Data Acquisition

Bathymetry, water velocity and vegetation data from summer 2010 were obtained from the U.S. Geological Survey's Long Term Resource Monitoring Program (LTMRP). This study is implemented by the U.S. Geological Survey's Upper Midwest Environmental Sciences Center (UMESC) and state agencies products (Soballe and Fischer, 2004). Depth data were collected using an automated survey boat, which records depth soundings while navigating the Pool (Soballe and Fischer, 2004). Water velocity was measured with an electromagnetic velocity meter (Marsh McBimey model 201D); six readings were collected per site and the mean of those observations was recorded at fixed sites and stratified random sites outlined in the LTMRP technical report (Soballe and Fischer, 2004). The submerged aquatic vegetation species cover was created by combining aerial photography with vegetation surveys (Rogers and Owens, 1995). The vegetation was surveyed by following the methods outlined by Soballe and Fischer (2004). Predetermined transects were sampled using modified vegetation rakes (3 m long) and identifying each plant to species and estimating percent density. In addition, vegetation cover was estimated in a 2 m circle around the sampling site. Data were compiled and incorporated into land cover use files that UMESC creates and publically distributes.

Aerial flight survey data were obtained through the La Crosse District of the Upper Mississippi River National Wildlife and Fish Refuge. Surveys are conducted throughout the refuge to monitor the numbers of migrating waterfowl and their areas of use. Survey observers follow predetermined transects using a fixed-wing aircraft and estimate the number of each waterbird species that are present in an area. Fall aerial flight surveys were conducted weekly from September 27, 2010 until November 29, 2010. During the week of

October 25, 2010, the aerial flight survey was not conducted due to maintenance requirements for the aircraft.

Data Processing

A GIS (ArcGIS 9.3, ESRI, Redlands, CA, USA) was created of the sampling area, utilizing 2010 aerial photography, bathymetry, flow, and land-use cover. In addition, a series of maps were generated based on land use cover from 1989, 1994, 2000, 2002, and 2010. A waterbird distribution file was created from the fall 2010 aerial flight surveys and information provided from surveyors. To assess differences between sites, site replicates for host and parasite variables were collapsed into site-level means before incorporation into GIS software. Using linear kriging, the sample point data were interpolated into raster files to show overall distributions. Areas where high or low values clustered spatially or features (*Bithynia tentaculata* density, primary infections, or secondary infections) that differed significantly from the other features were identified using the cluster and outlier analyses (Anselin Local Moran's I value) (Anselin, 1995). Waterbird distribution was digitized into a shapefile, based on aerial flight survey locations from 2010. A Kruskal-Wallis test was used to compare the number of hectares of submerged aquatic vegetation over a 21 year period. Mann-Whitney U-tests were used to compare *B. tentaculata* densities and infection metrics (of all parasites) to the presence/absence of vegetation.

RESULTS

Submerged aquatic vegetation was widespread in 1989 and decreased until 1994 (Figure 36 and Figure 37). Low levels of submerged aquatic vegetation persisted throughout the late 1990s until 2000 when a habitat rehabilitation program was implemented to reconstruct islands that had disappeared from erosion (Figure 38). Additionally, water level reduction occurred during the summers of 2001 and 2002 in Pool 8 to aid in the establishment of aquatic vegetation (Figure 39). The submerged aquatic vegetation was very abundant in 2010, which provided a large habitat area that was relatively non-existent prior to 2000 (Figure 40). The amount of submerged aquatic vegetation covered the fewest hectares in 1994 and began increasing in subsequent years until it reached its peak in 2010 (Figure 41). The submerged aquatic vegetation patches differed in area over the years (Figure 42) (Kruskal-Wallis, $P < 0.001$). In 2002 the average patch was only 2.53 hectares, whereas in 2010 the average patch was 16.33 hectares.

In 2010, bathymetry was variable throughout the Pool 8 closed area in the UMRNW&FR (Figure 43). Higher depths were found on the western and eastern sides of the closed area where two channels were present. The main navigation channel on the eastern side had a range from 3 m to greater than 10 m in depth whereas depth in the other channel ranged from 2.5 m to 8 m. The interior of the study area had a narrower range of depths, from 0.2 m to 6 m. Water velocity was also highly variable throughout the study area (Figure 44). The greatest velocity was present in the channels on the western and eastern portion of the study area, where the water velocity was greater than 1.26 m/s. The

interior portion of the study area had reduced levels of water velocity that ranged from 0.07 m/s – 1.26 m/s.

Bithynia tentaculata was widespread throughout the closed area. Two areas, one to the northeast corner (sites 841, and 851) and one in the center (Site 251) of Pool 8, exhibited significantly higher snail densities (1,635-3,708 snails per m^2) relative to other sites (Anselin Local Moran's analysis, $P < 0.05$) (Figure 45). Relatively low snail numbers (< 254 snails per m^2) were found around the perimeter of the closed area (Figure 45) and in deeper areas (over 3 m) where higher water flow was present (> 0.63 m/sec). The mean densities of *B. tentaculata* at sites with submerged aquatic vegetation was significantly greater at sites with vegetation (mean of 630.4 snails per m^2) compared to sites without macrophytes (mean of 125.8 snails per m^2) (Figure 46) (Mann-Whitney U-test, $P < 0.05$). Waterbird distribution overlapped in areas with intermediate (254.4-1,017.6 snails per m^2) snail densities. Two of the sites with the highest densities (sites 841 and 851) were located outside of the waterbird aggregation area; however the central site (site 251) with high *B. tentaculata* density was located in the feeding/resting area (Figure 45).

Cyathocotyle bushiensis infections were low or absent in areas of increased water velocity (> 0.63 m/sec) and depth (> 3 m) for both life stages (Figure 47 and Figure 48). *Cyathocotyle bushiensis* primary infections were found at low levels (6.4-12.8 infections per m^2) throughout most of Pool 8. The exceptions were snails from sites 161, 251, 241, and 411 in the west-central portion of the closed. Infections here were significantly higher than other sites in the pool (Anselin Local Moran's analysis, $P < 0.05$). High levels (> 954 metacercariae per m^2) of secondary infections were found throughout the interior and northeast portion of the closed area, where low levels (< 636 metacercariae per m^2) were found throughout the perimeter of the closed area. Sites which differed from others,

clustered in the middle of the closed area (sites 181, 251, 261, 271, 281, 421 and 601) (Anselin Local Moran's analysis, $P < 0.05$). Migrating waterbirds tended to forage and rest in the middle of the closed area where the range of metacercariae varied (range 0 – 4,910 metacercariae per m^2). A difference in metacercariae density was not detected between resting/feeding areas and those outside the resting/feeding areas in the closed area (Mann-Whitney U-test, $P > 0.05$).

Infections of both life stages of *Sphaeridiotrema* spp. were low or absent in areas of increased water velocity (> 0.63 m/sec) and depth (> 3 m) (Figure 49 and Figure 50). *Sphaeridiotrema* spp. primary infections were located in the north-central and north-eastern portion of the closed area. The perimeter of the closed area was absent of primary infections of *Sphaeridiotrema* spp. Sites that differed when compared to other sites clustered in the center (site 271) and the northeast portion of the closed area (sites 841, and 851) (Anselin Local Moran's analysis, $P < 0.05$). Metacercariae levels were high in the northeast corner (>1,144.8 metacercariae per m^2), intermediate (80 -150 metacercariae per site) throughout the interior portion, and low (< 381.6 metacercariae per m^2) around the perimeter of the closed area except the northeast corner. Sites that differed from others, clustered in the eastern and central portion of the closed area (sites 271, 831, 841, 851) (Anselin Local Moran's analysis, $P < 0.05$). *Sphaeridiotrema* spp. metacercariae levels varied (range 0 – 1,749 metacercariae per m^2) in the waterbird's resting and feeding areas. A difference in metacercariae density was not detected between resting/feeding areas and those outside the resting/feeding areas in the closed area (Mann-Whitney U-test, $P > 0.05$).

Higher numbers of *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. primary infections were found at sites with submerged aquatic vegetation compared to sites without macrophytes (Both analyses - Mann-Whitney U-test, $P < 0.05$) (Figure 51). Similar patterns

were also observed for metacercariae of both parasite species (Both analyses - Mann-Whitney U-test, $P < 0.05$) (Figures 52).

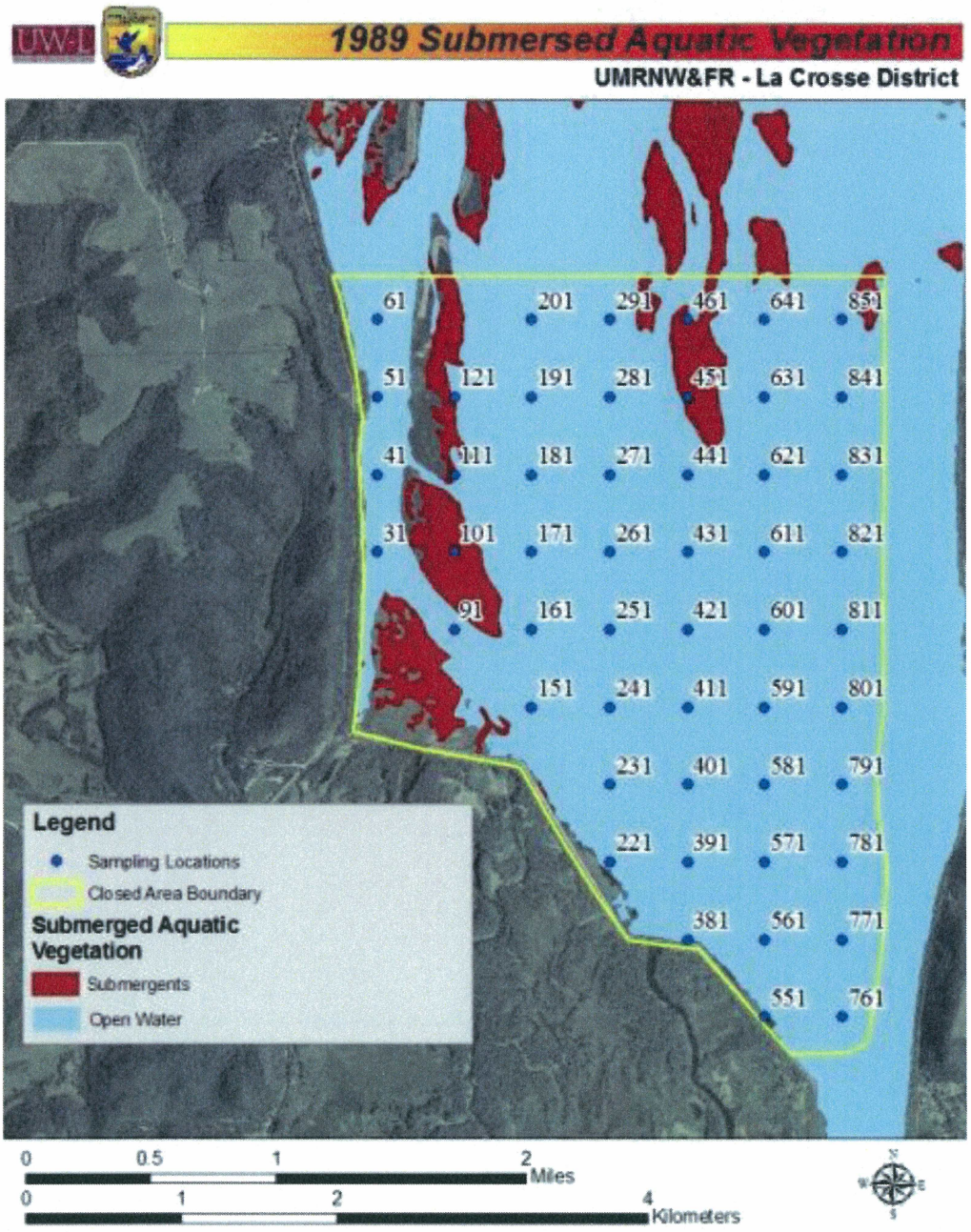


Figure 36. The benthic sampling sites relative to the submersed aquatic vegetation present in Pool 8 of the Upper Mississippi River in 1989. The yellow perimeter signifies the southern portion of the Pool 8 closed area and the red polygons represent the spatial distribution of submerged aquatic vegetation.

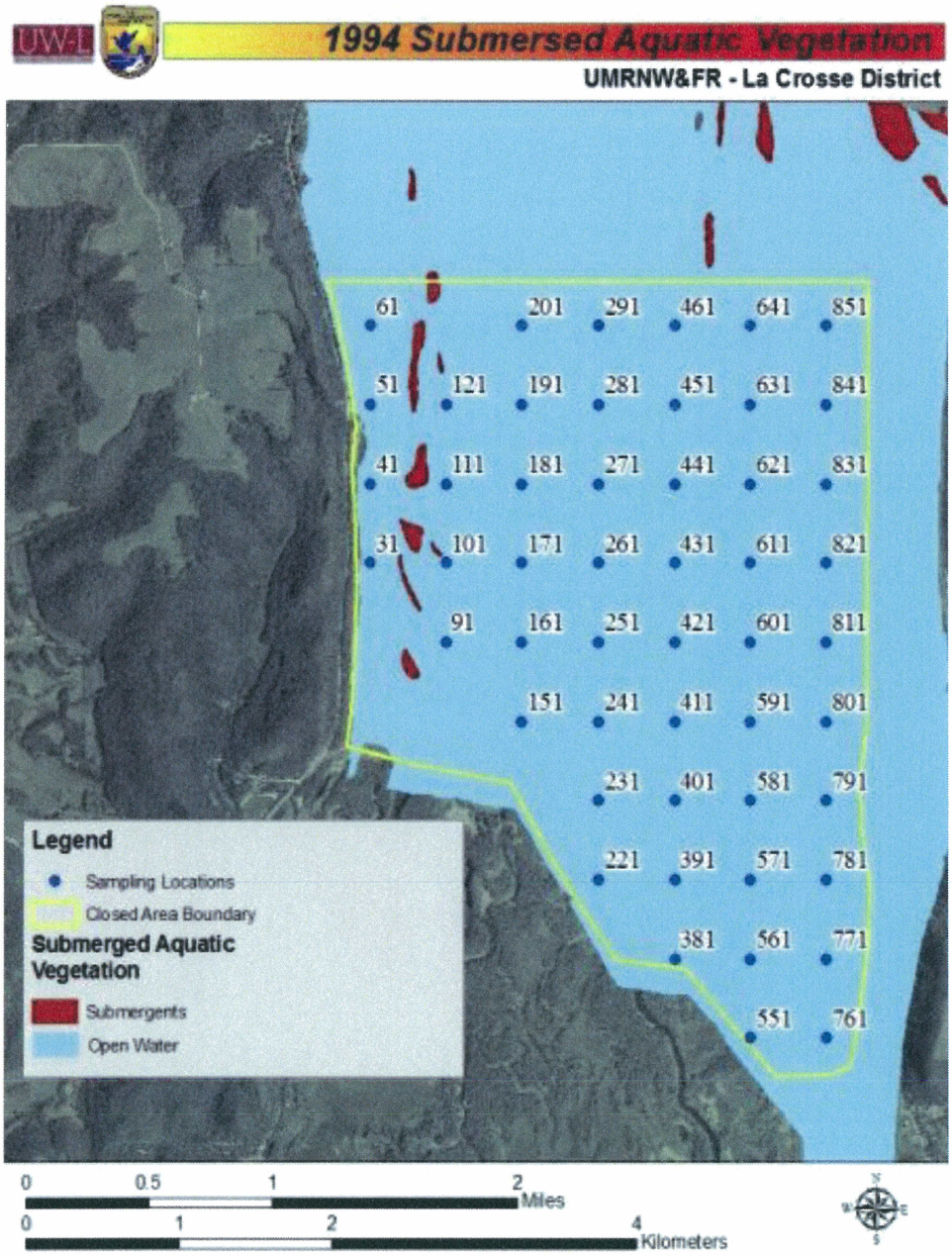


Figure 37. The benthic sampling sites relative to the submersed aquatic vegetation present in Pool 8 of the Upper Mississippi River in 1994. The yellow perimeter signifies the southern portion of the Pool 8 closed area and the red polygons represent the spatial distribution of submerged aquatic vegetation.

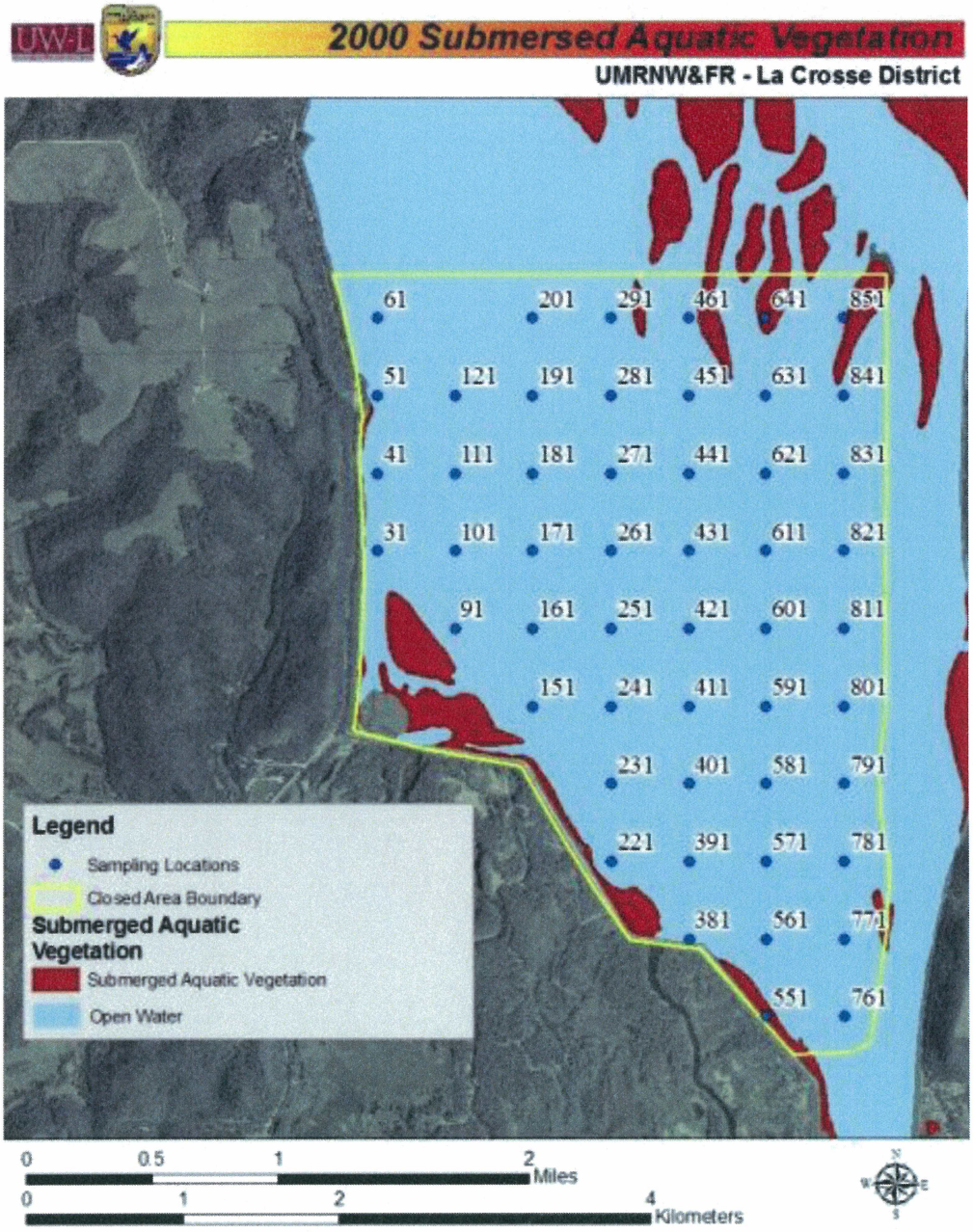


Figure 38. The benthic sampling sites relative to the submersed aquatic vegetation present in Pool 8 of the Upper Mississippi River in 2000. The yellow perimeter signifies the southern portion of the Pool 8 closed area and the red polygons represent the spatial distribution of submerged aquatic vegetation.

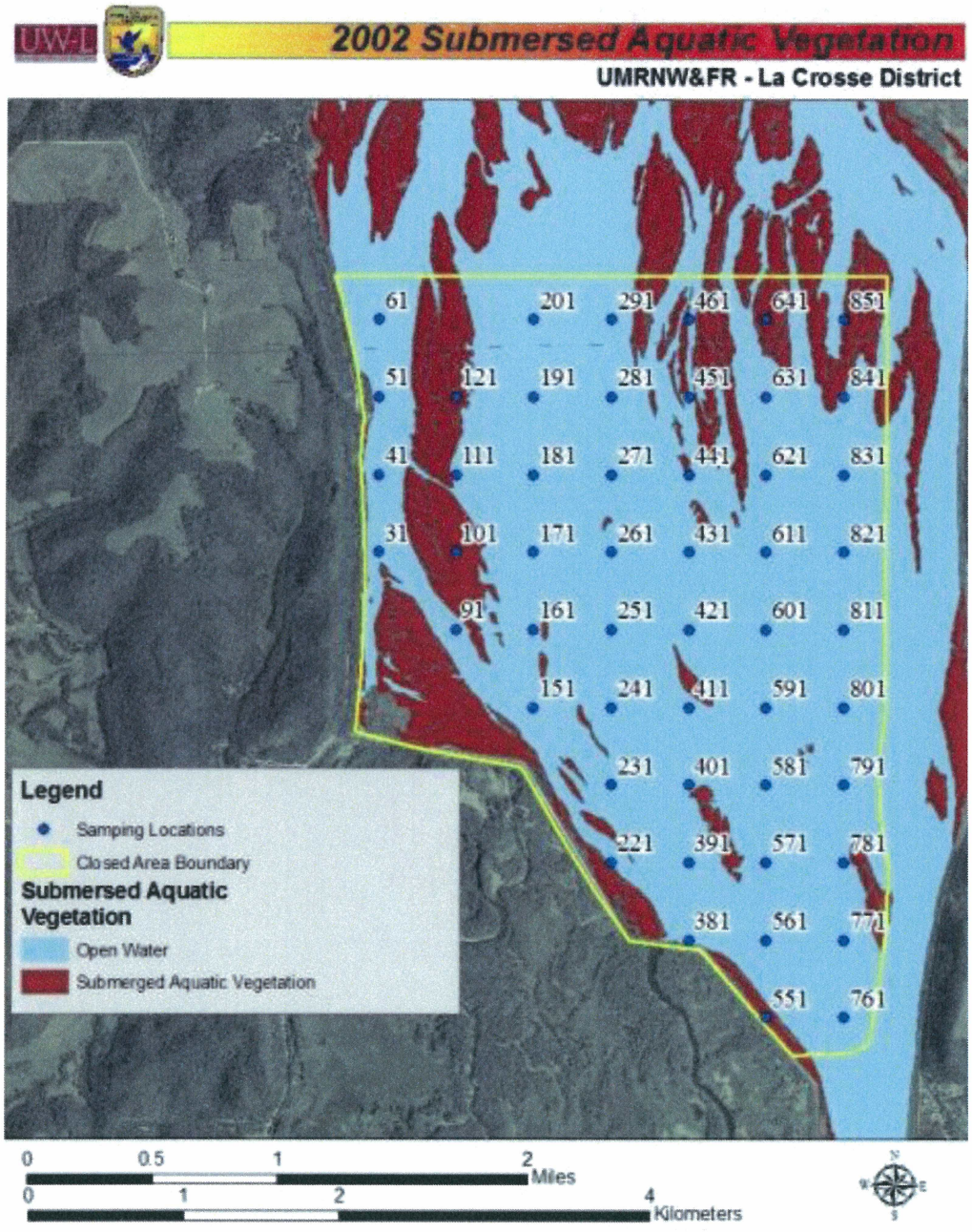


Figure 39. The benthic sampling sites relative to the submersed aquatic vegetation present in Pool 8 of the Upper Mississippi River in 2002. The yellow perimeter signifies the southern portion of the Pool 8 closed area and the red polygons represent the spatial distribution of submerged aquatic vegetation.

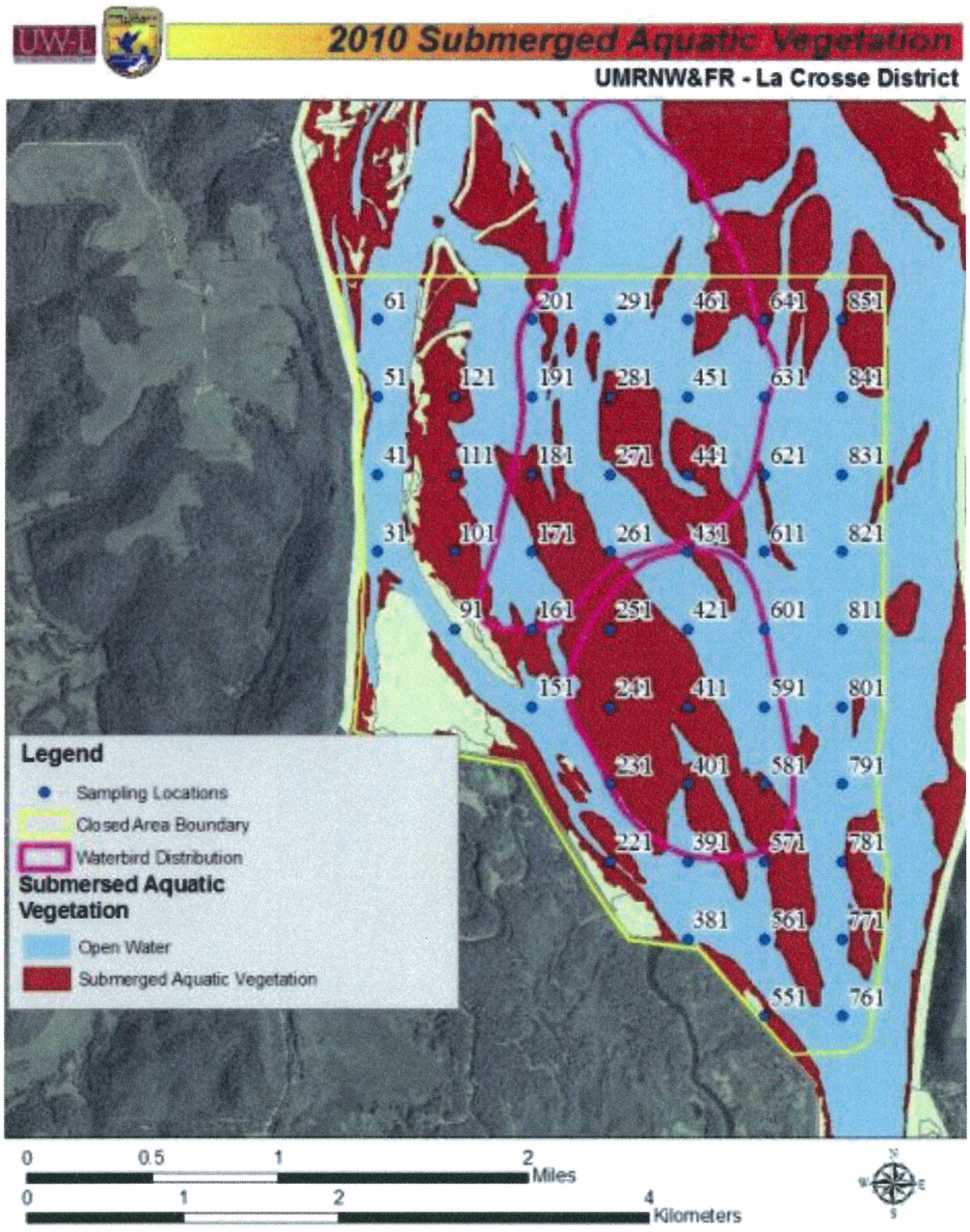


Figure 40. The benthic sampling sites relative to the submersed aquatic vegetation present in Pool 8 of the Upper Mississippi River in 2010. The yellow perimeter signifies the southern portion of the Pool 8 closed area and the red polygons represent the spatial distribution of submerged aquatic vegetation.

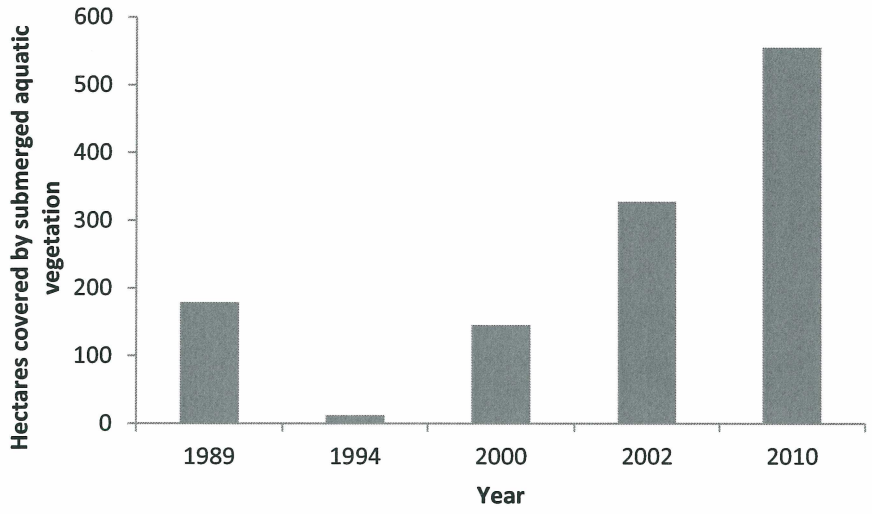


Figure 41. Total hectares of submerged aquatic vegetation present in the Pool 8 closed area during a 21-year period. Submerged aquatic vegetation data was obtained from the Land Cover Use dataset created by the U.S. Geological Survey's UMESC.

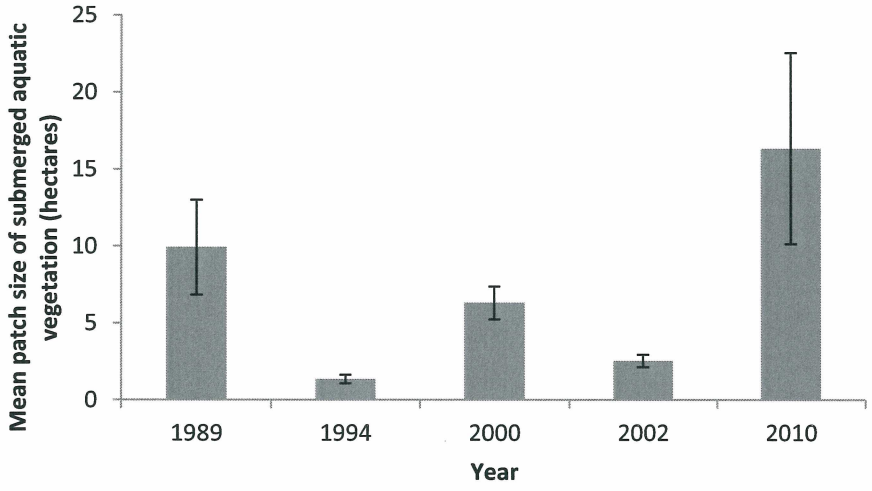


Figure 42. Mean hectares (±SE) of submerged aquatic vegetation patches in the closed area of Pool 8 during a 21-year period.

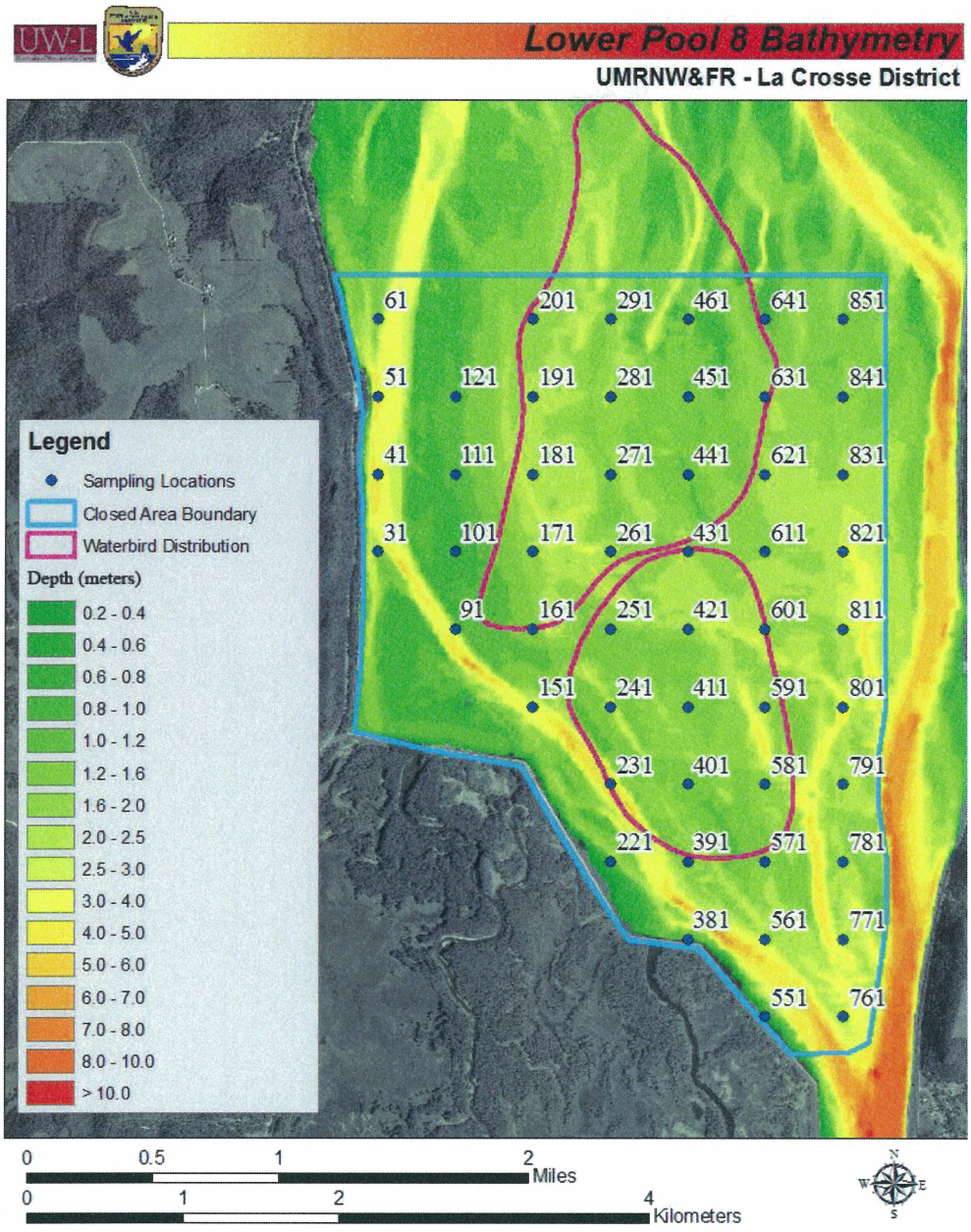


Figure 43. Bathymetry of the lower portion of the Wisconsin Islands Closed Area in Pool 8 of the Upper Mississippi River. The green areas represent shallower waters while the red areas represent deeper waters.

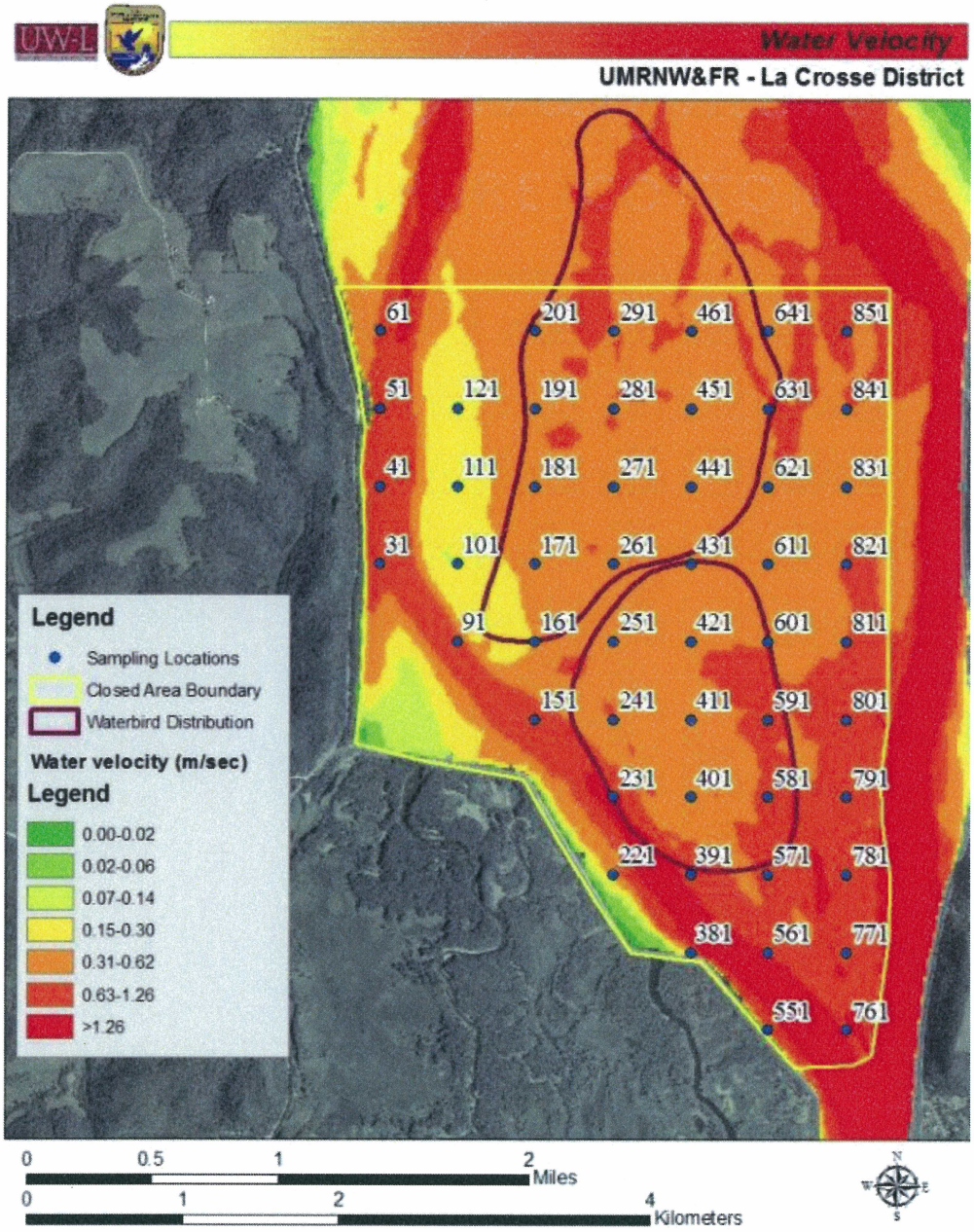


Figure 44. Water velocity of the lower portion of the Wisconsin Islands Closed Area in Pool 8 of the Upper Mississippi River. The green areas represent less velocity while the red areas represent higher velocity.

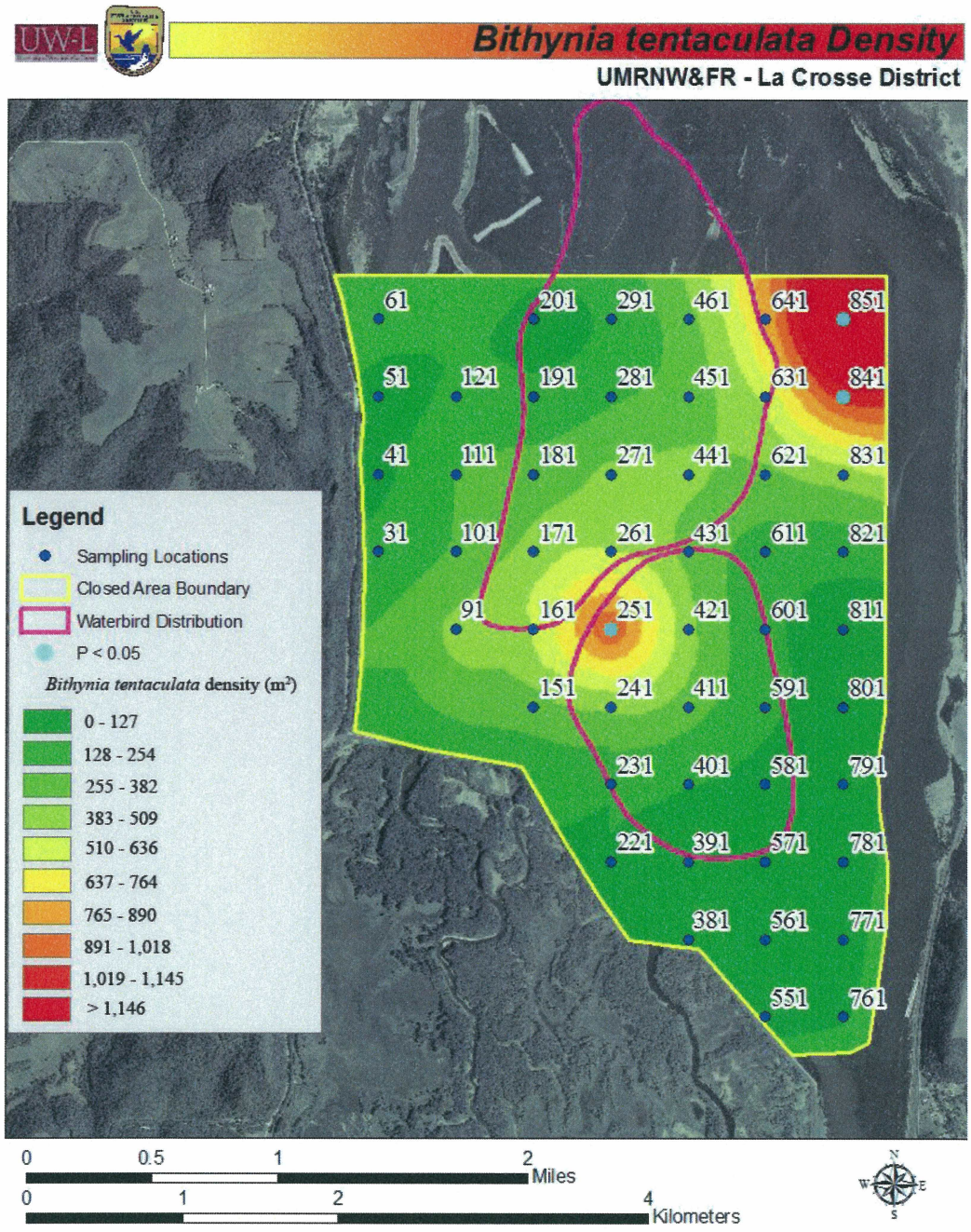


Figure 45. Raster map created from the *Bithynia tentaculata* density at each sampling location throughout the lower portion of the Pool 8 Wisconsin Island's Closed Area. Sites highlighted in red had the highest density while those in green had the lowest levels.

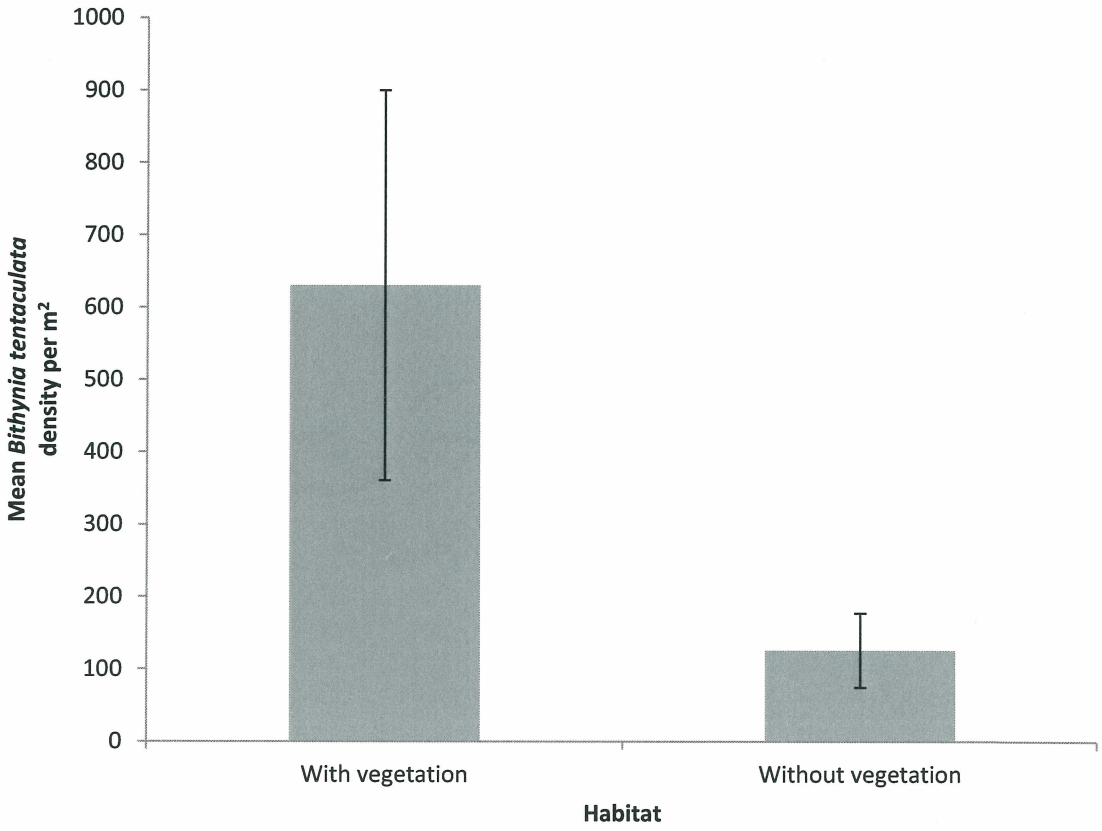


Figure 46. The mean density (±SE) of *Bithynia tentaculata* at sites with and without submerged aquatic vegetation in the Pool 8 closed area.

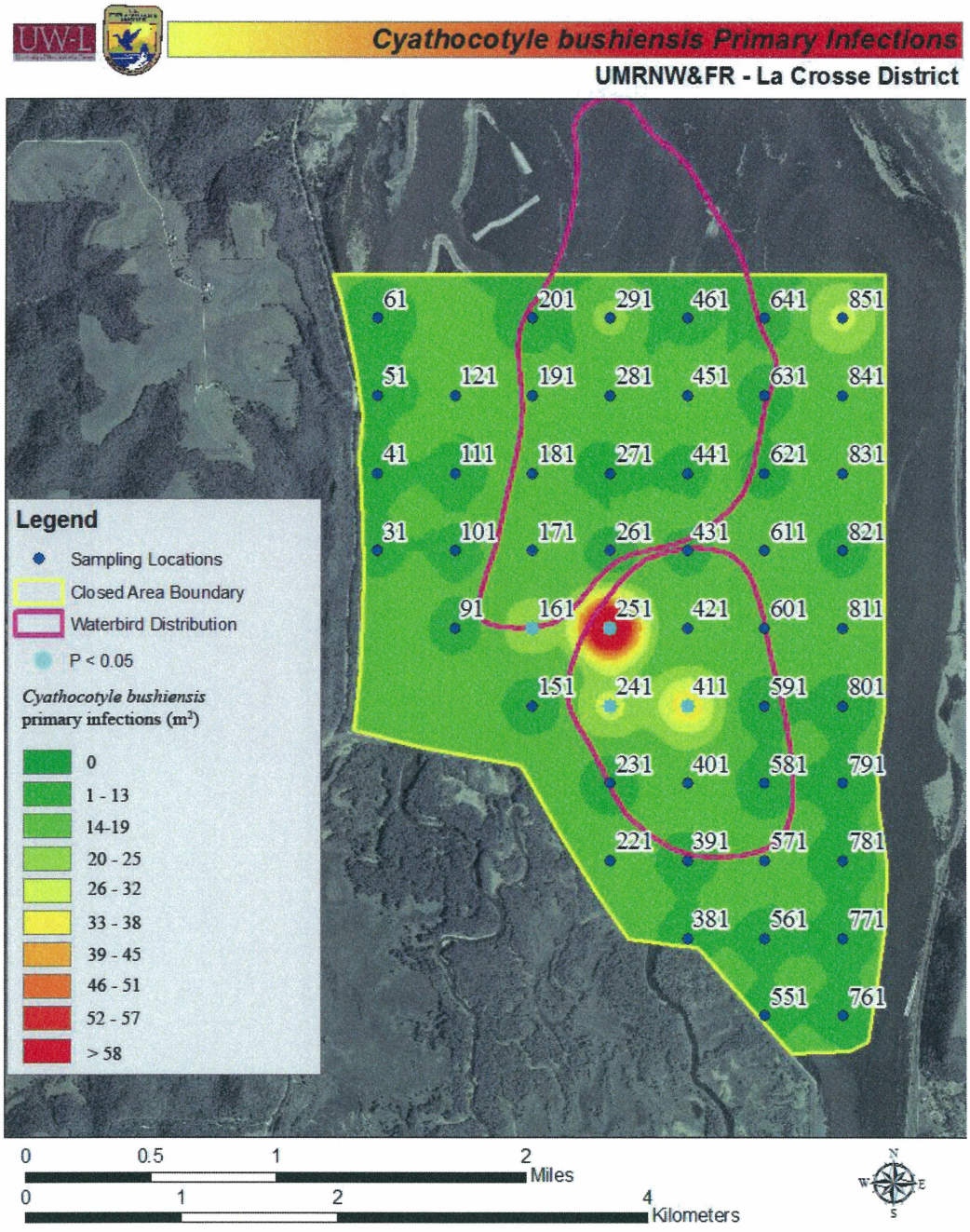


Figure 47. A) Static risk map showing the distribution of *Cyathocotyle bushiensis* primary infections throughout the lower portion of the Wisconsin Islands Closed Area. Sites highlighted in red had the highest infection levels, those in green the lowest infection levels.

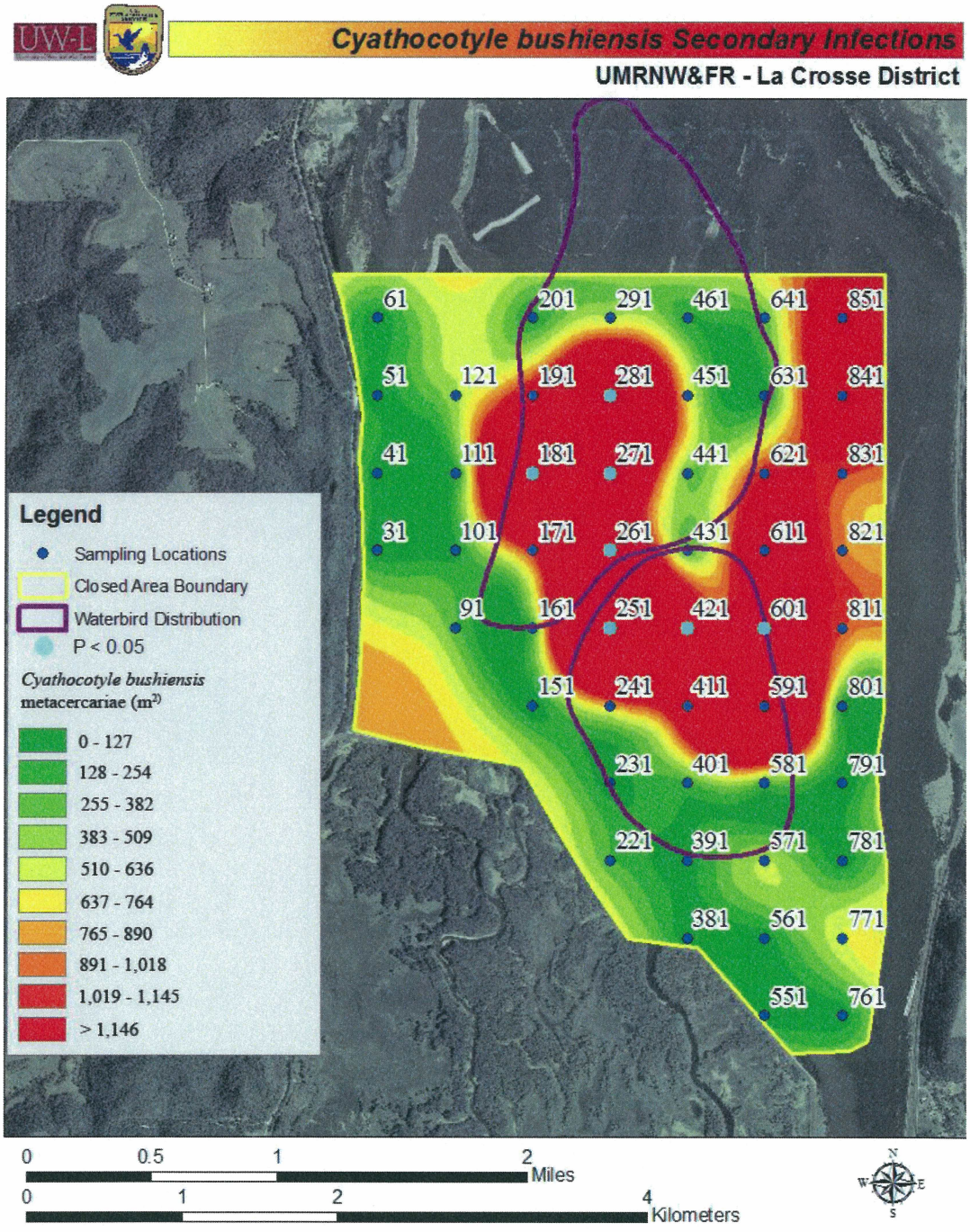


Figure 48. A) Static risk map showing the distribution of *Cyathocotyle bushiensis* secondary infections throughout the lower portion of the Wisconsin Islands Closed Area. Sites highlighted in red had the highest infection levels, those in green the lowest infection levels.

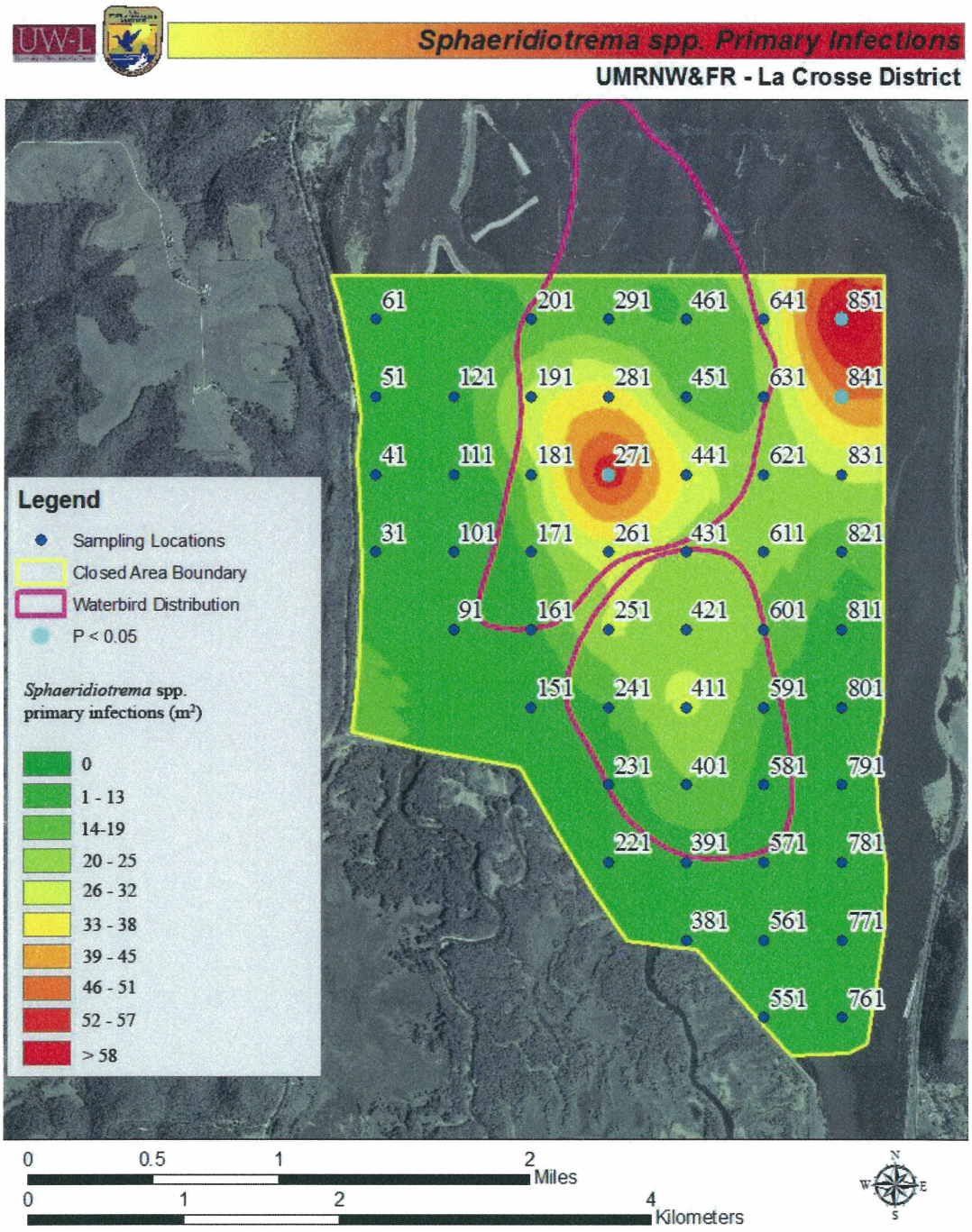


Figure 49. A) Static risk map showing the distribution of *Sphaeridiotrema* spp. primary infections throughout the lower portion of the Pool 8 Wisconsin Islands Closed Area. Sites highlighted in red had the highest infection levels, those in green the lowest infection levels.

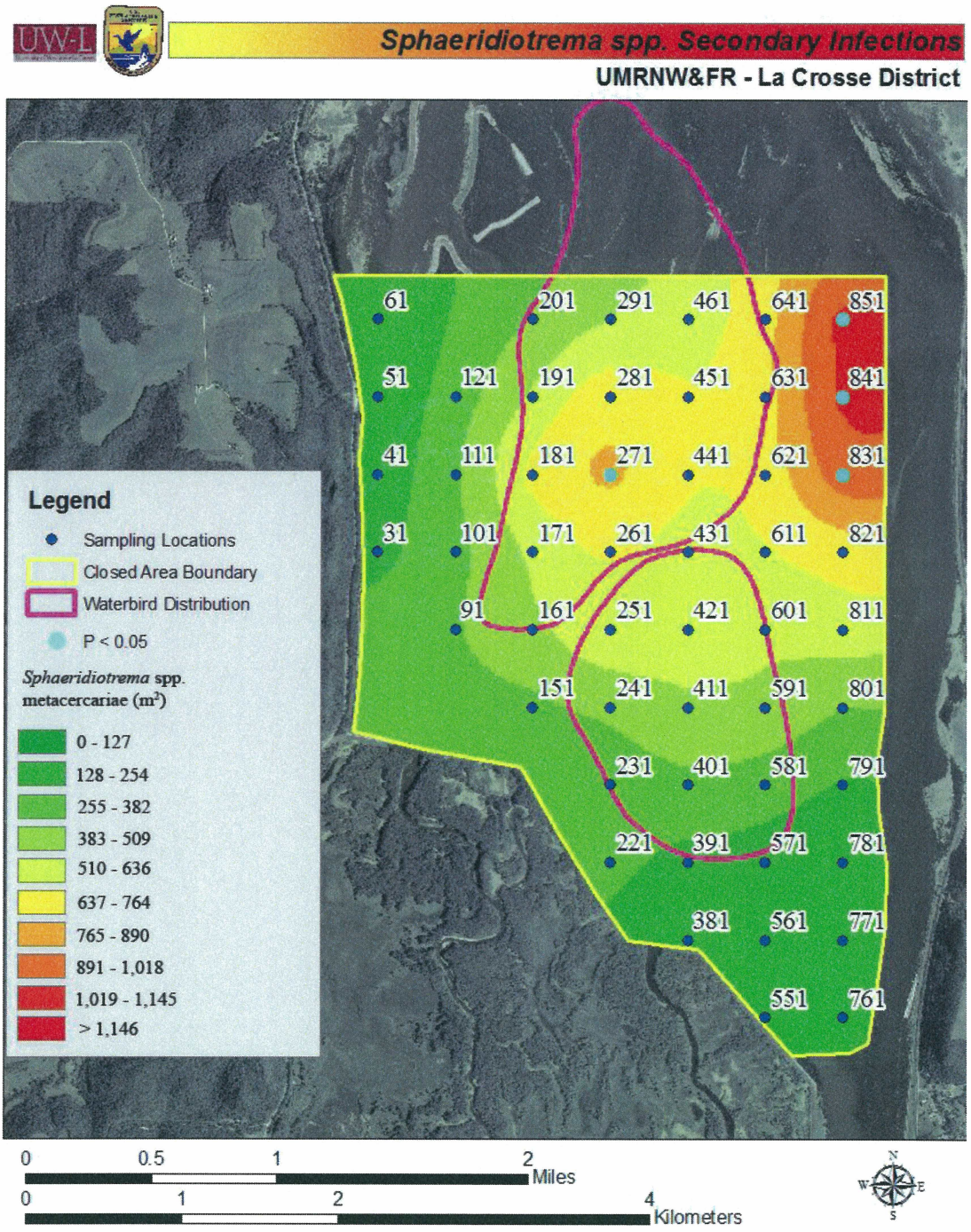


Figure 50. Static risk map showing the distribution of *Sphaeridiotrema* spp. secondary infections throughout the lower portion of the Pool 8 Wisconsin Islands Closed Area. Sites highlighted in red had the highest infection levels, those in green the lowest infection levels.

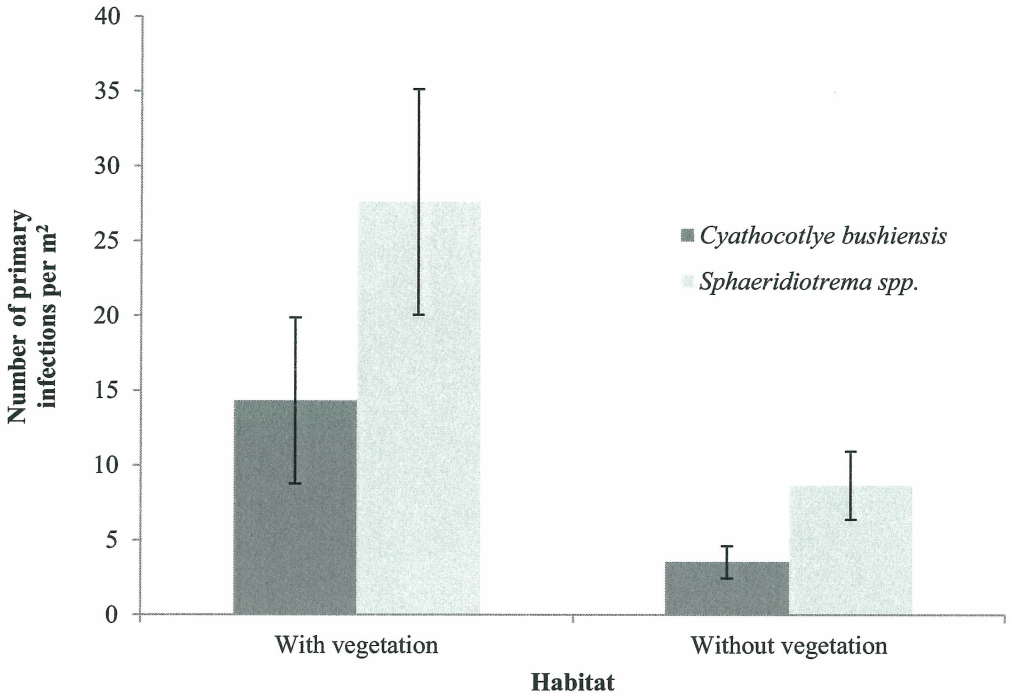


Figure 51. The mean number of primary infections per m^2 (±SE) of *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. per m^2 with submerged aquatic vegetation and at sites without vegetation in Pool 8 of the UMR.

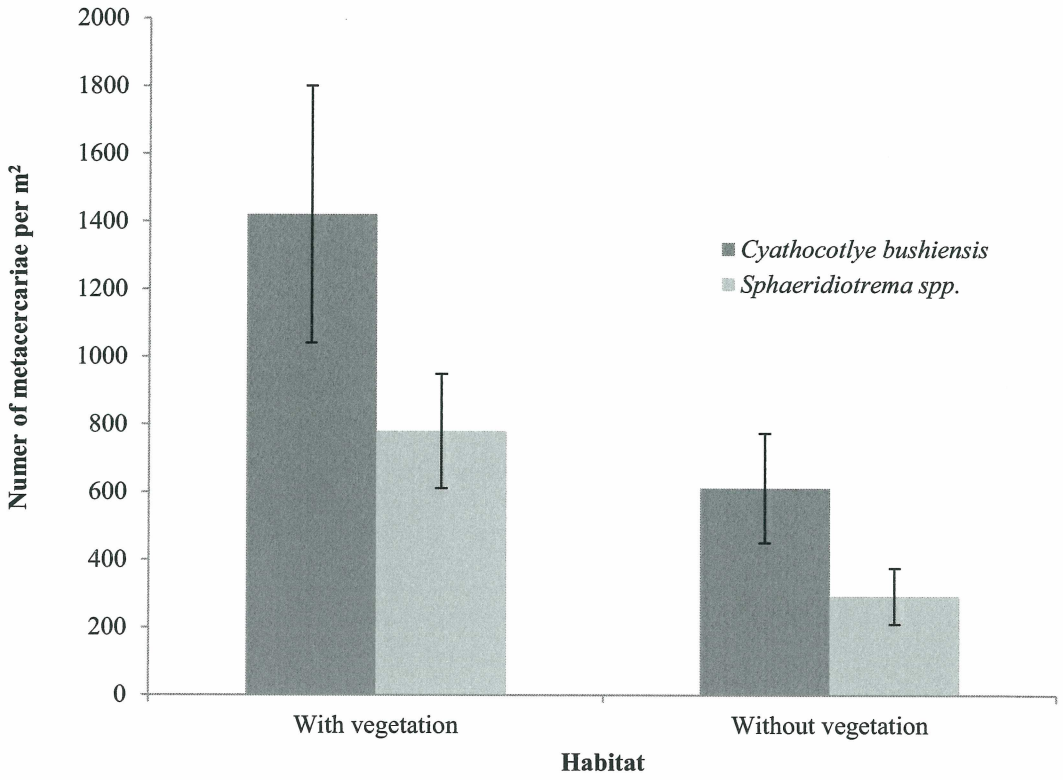


Figure 52. The mean number of metacercariae per m^2 (±SE) of *Cyathocotyle bushiensis* and *Sphaeridiotrema* spp. per m^2 with submerged aquatic vegetation and at sites without vegetation in Pool 8 of the UMR.

DISCUSSION

Understanding the mechanisms underlying infectious disease has been a goal of ecologists and epidemiologists for decades. More recently, GIS has been used as an important tool for exploring the risk associated with host and parasite distributions. To date however, much of this work has focused exclusively on diseases of humans and livestock, which has left the application of these techniques to wildlife and especially waterfowl, relatively unexplored. This study aimed to use GIS mapping to investigate the variables most strongly associated with distributions of *Bithynia tentaculata* and two trematode species that are responsible for waterbird mortality.

Numerous environmental variables such as water flow, temperature, water body size and the type and density of aquatic vegetation can influence the distribution of aquatic mollusks (Anderson, 1972; Dussart, 1979; Murphy, 1985; Laamrani *et al.,* 1997; Arruda and Amaral, 2003; Skirnisson *et al.*, 2004; Kwong *et al.*, 2008). More specifically, previous work has demonstrated the importance of factors such as substrate sizes, vegetation, water velocity, and water chemistry on the distribution of *B. tentaculata* (Pinel-Allouol and Magnin, 1971; Vincent *et al.,* 1981; Vincent and Gaucher, 1983; Hoeve and Scott, 1988; Lepitzki, 1993; Richter, 2001; Herrmann and Sorensen, 2009). For example, *B. tentaculata* prefers rocky substrates, however when hard substrates are not available they can aggregate in areas where submersed aquatic vegetation is present (Tashiro and Colman, 1982; Vincent and Letourneau, 1985; Lepitzki, 1993; Richter, 2001). The addition of a third dimension of habitat, such as aquatic vegetation, can contribute to high *Bithynia tentaculata* densities. For example, Richter (2001) found that large numbers of snails congregated on vegetation to

enhance their filter feeding capacity and to reproduce. In fact, female snails were found to preferentially lay eggs on vegetation when hard substrates were not present (Richter, 2001). The importance of vegetation for *B. tentaculata* nutrient acquisition and reproduction likely explains why such high snail numbers were found in areas with relatively low flow and high macrophytes abundance. This association also generates concern given the current trend towards greater macrophyte cover in areas of Pool 8. Further manipulation of habitat to generate greater macrophyte coverage in Pool 8 may inadvertently lead to the spread of *B. tentaculata* to additional sites in Pool 8 of the UMR. It may also lead to dispersal of snails to additional sites of the Mississippi River during flood events which could transport large volumes of macrophytes (and hitchhiking snails) southwards.

A single primary-infection hotspot was seen for *Cyathocotyle bushiensis* in the middle region of Pool 8. This area corresponded to the region of highest *B. tentaculata* densities, suggesting that snail numbers may be an important driver of primary infections of this parasite. This result mirrors work by others showing a similar association. For instance Mouritsen *et al.* (1997) found that the snail *Hydrobia ulvae* exhibited higher trematode prevalence in regions with higher host densities. However, snail densities alone are not the only predictors of primary infections in this system as evidenced by the distribution of *Sphaeridiotrema* spp. For this species, primary-infection hotspots correlated with relatively low *B. tentaculata* densities. In this case, other factors such as microhabitat attributes (such as low water velocity) may facilitate host finding by parasite larvae (Upatham, 1973). Differences in the primary infection hotspots between each species may also reflect habitat preferences of foraging/loafing definitive hosts. Huffman and Roscoe (1989) found that infected waterbirds exhibit muscular weakness, reducing their ability to fly and swim which confines the host to the area of infection. Although the lifespan of an infected bird is short

(5-8 days), it is a sufficient amount of time for the developed parasite to introduce eggs back into the environment, starting the cycle over again (Hoeve and Scott, 1988; Sandland *et al.*, 2011). Therefore, primary infections of a parasite species distribution may be influenced by the definitive host's spatial distribution as found by Kube *et al.* (2002). Recent work by Herrmann and Sorensen (2011) suggests that American coot commonly harbor more *C. bushiensis* while lesser scaup commonly harbor more *Sphaeridiotrema* spp. Differences in the preferred areas of the two avian species and the host specificity exhibited by the parasite, may explain the differences of the observed distribution of primary infections.

Cyathocotyle bushiensis metacercariae infections were much more widespread than primary infections. In fact, snails from approximately half of the area in Pool 8 contained relatively high numbers of metacercariae. This was somewhat unexpected, as primary infections often dictate the infection patterns observed in subsequent hosts in the life cycle (Lysne *et al.*, 1995; Fingernut *et al.*, 2003; Kazibwe *et al.*, 2010). A number of factors may help to explain this discrepancy. First, the sampling design may have been coarse enough to have missed areas where *C. bushiensis* primary infections were occurring in *B. tentaculata* and contributing to the broad distribution of secondary infections. Second, it is possible that snails containing primary infections occurred throughout this region earlier in the year, but had since died due to natural senescence or parasite induced mortality. Third, *C. bushiensis* cercariae may have been introduced to snails, particularly along the eastern portion of the pool, from areas upstream. The eastern area of Pool 8 does have higher flow, which may facilitate the transport of parasites from non-sampled northern areas, inflating the metacercariae numbers observed in the study region. A final possibility which ties in with the aforementioned idea is that *C. bushiensis* cercariae have a greater capacity for dispersal than *Sphaeridiotrema* spp. larvae. This may have allowed *C. bushiensis* to spread to snails

throughout areas of Pool 8 from this single infection hotspot (particularly during periods of low water velocity).

Unlike *Cyathocotyle bushiensis*, high *Sphaeridiotrema* spp. metacercariae infections were localized in the same area as primary infections following the more typical patterns observed for other snail-trematode systems (Peterson, 2007). One factor contributing to this pattern could be deterioration of the cercariae shortly after release from primarily infected snails. Indeed, Toledo *et al.* (1999) found that the transmission efficiency of *Hypoderaeum conoideum* and *Euparyphium albuferensis* cercaraie decreased rapidly after they were shed from infected hosts. This would hinder the spatial distribution of *Sphaeridiotrema* spp. metacercariae resulting in infection of snails that are within close proximity of the primary infected intermediate host.

The closed areas in the Upper Mississippi River were created in nutrient rich areas were waterbirds could feed and rest, free of human disturbance. Recent waterbird use has increased in previous years and consequently, *Bithynia tentaculata* populations have increased as well. This study aimed to map and understand the restricting factors of *B. tentaculata* and its parasites. This work suggests that reduced flow and high vegetation coverage positively influenced *B. tentaculata* populations and increased trematode transmission. This information and the predictive risk maps can be used to better understand the infection dynamics of these two parasite species in the UMRNW&FR.

REFERENCES

Anderson, S.S. (1972). The ecology of Morecambe Bay. II. Intertidal invertebrates and factors affecting their distribution. *Journal of Applied Ecology*, 9, 161-178.

Anselin, L. (1995). Local indicator of spatial association – LISA. *Geographical Analysis*, 27, 93-115.

Arruda, E.P. Amaral, A.C. (2003). Spatial distribution of mollusks in the intertidal zone of sheltered beaches in southeastern Brazil. *Revista Brasileira de Zoologia*, 20, 291-300.

Cobb, M.A., Gogan, P.J., Kozie, K.D., Olexa, E.M., Lawrence, R.L. and Route, W.T. (2004). Relative spatial distributions and habitat use patterns of sympatric moose and white-tailed deer in Voyageurs National Park, Minnesota. *Alces*, 40, 169-191.

Conner, M.M. and Miller, M.W. (2004). Movement patterns and spatial epidemiology of a prion disease in mule deer population units. *Ecological Applications*, 14, 1870-1881.

Douglas, C.L. (2001). Weather, disease, and bighorn lamb survival during 23 years in Canyonlands National Park. *Wildlife Society Bulletin*, 29, 297-305.

Dussart G.B.J. (1979). Life cycles and distribution of the aquatic gastropod molluscs *Bithynia tentaculata* (L.), *Gyraulus albus* (Muller), *Planorbis planorbis*(L.) and *Lymnaea peregra* (Muller) in relation to water chemistry. *Hydrobiologia*, 67, 223-239.

Daniel, M., Kolar, J. and Zeman, P. (2004). GIS tools for tick and tick borne disease occurrence. *Parasitology*, 129, Supplement S1.

East, I.J., Hamilton, S.A., Sharp, L.A. and Garner, M.G. (2008). Identifying areas of Australia at risk for H5N1 avian influenza infection from exposure to nomadic waterfowl moving throughout the Australo-Papuan region. *Geospatial Health*, 3, 17-27.

Fernandez, J. and Esch, G.W. (1991). Guild structure of larval trematodes in the snail *Helisoma anceps*: patterns and processes at the individual host level. *Journal of Parasitology*, 77, 528-539.

Fingernut, J.T., Zimmer, C.A. and Zimmer, R.K. (2003). Larval swimming overpowers turbulent mixing and facilitates transmission of a marine parasite. *Ecology*, 84, 2502-2515.

Gilbert, M., Newman, S.H., Takekawa, J.Y., Loth, L., Biradar, C., Prosser, D.J., Balachandran, S., Subba Rao, M.V., Mundkur, T., Yan, B., Xing, Z., Hou, Y., Batbayar, N., Natsagdorj, T., Hogerwerf, L., Slingenbergh, J. and Xiao, X. (2010). Flying over an infected landscape: distribution of highly pathogenic avian influenza H5N1 risk in South Asia and satellite tracking of wild waterfowl. *EcoHealth*, 7, 448-458.

Gross, J.E. (2001). Evaluating effects of an expanding mountain goat population on native bighorn sheep: a simulation model of competition and disease. *Biological Conservation*, 101, 171-185.

Hoeve, J. and Scott, M.E. (1988). Ecological studies on *Cyathocotyle bushiensis* (Digenea) and *Sphaeridiotrema globulus* (Digenea), possible pathogens of dabbling ducks in southern Quebec. *Journal of Wildlife Diseases*, 24, 407–421.

Hohman, W. L. (1985). Feeding ecology of ring-necked ducks in northwestern Minnesota. *Journal of Wildlife Management*, 49: 546-557.

Howe, G.M. (1972). *Man, environment, and disease in Britain*. New York. Barnes and Nobles Books. 285 p.

Huffman, J.E. and Roscoe, D.E. (1989). Experimental infections of waterfowl with *Sphaeridiotrema globulus* (Digenea). *Journal of Wildlife Diseases*, 25, 143-146.

Hudson, P.J., Rizzoli, A., Grenfell, B.T., Heesterbeek, H. and Dobson, A.P. (2002). *The Ecology of Wildlife Diseases*. Oxford, UK, Oxford Press.

Johnson, T.L. and Swift, D.M. (2000). A test of a habitat evaluation procedure for rocky mountain bighorn sheep. *Restoration Ecology*, 8, 47-56.

Kazibwe, F., Makanga, B., Rubaire-Akiiki, C., Ouma, J., Kariuki, C., Kabatereine, N.B., Vennervald, B.J., Rollinson, D. and Stothard, J.R. (2010). Transmission studies of intestinal schistosomiasis in Lake Albert, Uganda and experimental compatibility of local *Biomphalaria* spp. *Parasitology International*, 59, 49-53.

Kitron, U., Jones, C.J., Bouseman, J.K., Nelson, J.A. and Baumgartner, D.L. (1992). Spatial analysis of the distribution of *Ixodes dammini* (Acari: Ixodidae) on white-tailed deer in Ogle County, Illinois. *Journal of Medical Entomology*, 29, 259-266.

Kitron, U. and Kazmierczak, J.J. (1997). Spatial analysis of the distribution of lyme disease in Wisconsin. *American Journal of Epidemiology*, 145, 558-566.

Kitron, U. (1998). Landscape ecology and epidemiology of vector-borne diseases: Tools for spatial analysis. *Journal of Medical Entomology*, 35, 435-445.

Kwong, K.L., Wong, P.K., Lau, S.S. and Qiu, J.W. (2008). Determinants of the distribution of apple snails in Hong Kong two decades after their initial invasion. *Malacologia*, 50, 293-302.

Laamrani, H., Khallayoune, K., Delay, B. and Pointier, J.P. (1997). Factors affecting the distribution and abundance of two prosobranch snails in a thermal spring. *Journal of Freshwater Ecology*, 12, 75-79.

Larter, N.C. (2009). A program to monitor moose populations in the Dehcho region, Northwest Territories, Canada. *Alces*, 45, 89-99.

Lemke, T.O. (2004). Origin, expansion, and status of mountain goats in Yellowstone National Park. *Wildlife Society Bulletin*, 32, 532-541.

Lepitzki, D.A.W. (1993). Epizootiology and transmission of snail-inhabiting metacercariae of the duck digeneans *Cyathocotyle bushiensis* and *Sphaeridiotrema globulus*. (Unpublished Ph.D. dissertation). McGill University, Montreal, Canada, 354 p.

Lepitzki D.W., Scott, M.E. and Mclaughlin, J.D. (1994). Influence of storage and examination methods on the recovery and size of metacercariae of *Cyathocotyle bushiensis* and *Sphaeridiotrema pseudoglobulus* (Digenea). *Journal of Parasitology*, 80, 454–460.

Lysne, D.A., Skorping, A. and Hemmingsen, W. (1995). Trasmission of *Cryptocotyle lingua* cercariae in natural environments: a field experiment. *Journal of Fish Biology*, 53, 879-885.

Menard, L. and Scott, M.E. (1987). Seasonal occurrence of *Cyathocotyle bushiensis* Khan, 1962 (Digenea: Cyathocotylidae) metacercariae in the intermediate host *Bithynia tentaculata* L. (Gastropoda: Prosobranchia). *Canadian Journal of Zoology*, 65, 2980–2992.

Mortarino, M., Vincenzo, M. and Valeria, C. (2008). GIS modeling for canine dirofilariosis risk assessment in central Italy. *Geospatial Health*, 2, 253-261.

Murphy, R.C. (1985). Factors affecting the distribution of the introduced bivalve, *Mercenaria mercenaria*, in a California lagoon – the importance of bioturbation. *Journal of Marine Research*, 43, 673-692.

Musacchio, L.R. and Coulson, R.N. (2001). Landscape ecological planning process for wetland, waterfowl, and farmland conservation. *Landscape and Urban Planning*, 56, 125-147.

Ostfeld R.S., Hazler, K.R. and Cepeda, O.M. (1996). Temporal and spatial dynamics of *Ixodes scapularis* (Acari: Ixodidae) in a rural landscape. *Journal of Medical Entomology*, 33, 90-95.

Peterson, N.A. (2007). Seasonal prevalence of *Ribeiroia ondatrae* in one population of *Planorbella trivolvis* (*Helisoma trivolvis*), including notes on the larval trematode component community. *Comparative Parasitology*, 74, 312-318.

Prosser, D.J., Takekawa, J.Y., Newman, S.H., Yan, B., Douglas, D.C., Hou, Y., Xing, Z., Zhang, D., Li, T., Li, Y., Zhao, D., Perry, W.M. and Palm, E.C. (2009). Satellite-marked waterfowl reveal migratory connection between H5N1 outbreak areas in China and Mongolia. *Ibis*, 151, 568-576.

Richomme, C., Gauthier, D. and Fromont, E. (2006). Contact rates and exposure to inter-species disease transmission in mountain ungulates. *Epidemiology and Infection*, 134, 21-30.

Richter, T., 2001. *Reproductive biology and life history strategy of Bithynia tentaculata (Linnaeus, 1758) and Bithynia leachii (Sheppard, 1823)*. (Unpublished Ph.D. dissertation). Universitat Hannover. Saxony, Germany.

Rogers, S. J., and T. W. Owens. (1995). Long Term Resource Monitoring Program Procedures: Vegetation monitoring. National Biological Service, Environmental Management Technical Center, Onalaska, Wisconsin, July 1995. LTRMP 95-P002-3. 10 pp. + Appendixes A-E.

Runstadler, J.A., Happ, G.M., Slemons, R.D., Sheng, Z.M., Gundlach, N., Petrula, M., Senne, D., Nolting, J., Evers, D.L. and Modrell, A. (2007). Using RRT-PCR analysis and virus isolation to determine the prevalence of avian influenza virus infections in ducks at Minto Flats State Game Refuge, Alaska, during August 2005. *Archives of Virology*, 152, 1901-1910.

Sauer, J. S., Cole, R.A. and Nissen, J.M. (2007). Finding the exotic faucet snail (*Bithynia tentaculata*): Investigation of waterbird die-offs on the Upper Mississippi River National Wildlife and Fish Refuge. *U.S. Geological Survey Open-File Report 2007-1065*, 1–3.

Sandland, G.J., Houk, S., Walker, B.A., Gillis, R. (2012). Differential life-history expression in invasive and native hosts exposed to a parasite of conservation concern. Submitted.

Skirnisson, K., Galaktionov K.V. and Kozminsky, E.V. (2004). Factors influencing the distribution of digenetic trematode infections in a mudsnail (*Hydrobia ventrosa*) population inhabiting salt march ponds in Iceland. *Journal of Parasitology*, 90, 50-59.

Singer, F.J., Zeigenfuss, L.C. and Spicer, L. (2001). Role of patch size, disease, and movement in rapid extinction of bighorn sheep. *Conservation Biology*, 15, 1347-1354.

Tashiro J.S., Colman, S.D. (1982). Filter feeding in the freshwater prosobranch snail *Bithynia tentaculata*: bioenergetic partitioning of ingested carbon and nitrogen. *American Midland Naturalist*, 197, 114-132.

Upatham, E.S. (1973). Location of biomphalaria glabrata (say) by miracidia of *Schistosoma mansoni* sambon in natural standing and running waters on the West Indian Island of St. Lucia. *International Journal for Parasitology*, 3, 289-297.

Toledo, R., Munoz-Antoli, C., Perez, M. and Esteban, J.G. (1999). Survival and infectivity of *Hypoderaeum conoideum* and *euparyphium albuferensis* cercariae under laboratory conditions. *Journal of Helminthology*, 73, 177-182.

U.S. Fish and Wildlife Service. (2006). *Upper Mississippi River National Wildlife and Fish Refuge comprehensive conservation plan.* 242 p.

Vincent, B., Vaillancourt, G. and Harvey, M. (1981). Cycle de développement, croissance, effectifs, biomasse et production de *Bithynia tentaculata* L. (Gastropoda: Prosobranchia) dans le Saint-Laurent (Québec). *Canadian Journal of Zoology*, 59, 1237-1250.

Vincent, B. and Letourneau, C. (1985). Inter-population variations in the age structure and growth of the prosobranch *Bithynia tentaculata* L. *Canadian Journal of Zoology*, 63, 1345-1353.

Ward, M.P., Maftei, D.N., Apostu, C.L. and Suru, A.R. (2009). Association between outbreaks of highly pathogenic avian influenza subtype H5N1 and migratory waterfowl (family Anatidae) populations. *Microbiology and Virology*, 56, 1-9.

Wolfe, L.L., Miller, M.W. and Williams, E.S. (2004). Feasibility of "test-and-cull" for managing chronic wasting disease in urban mule deer. *Wildlife Society Bulletin*, 32, 500-505.