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Patterns of Trematode Distribution from Hosts Collected at Lake Winnibigoshish, Minnesota

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at Lake Winnibigoshish, Minnesota

By

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This thesis has been examined and approved by the following members of the thesis committee.

April 5, 2018

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ABSTRACT

A comparative and seasonal study was performed on trematode populations and communities within waterfowl definitive hosts in order to determine important factors in the structuring of these communities. Furthermore, larval trematode populations were also sampled in order to compare larval stages found within snail hosts to adult stages found within collected waterfowl in order to determine where infections could have occurred with the trematode species. 52 birds including 18 blue-winged teal (*Anas discors*), 5 bufflehead (*Bucephala albeola*), 4 common goldeneye (*Bucephala clangula*), 1 greater scaup (*Aythya marila*), 1 green-winged teal (*Anas carolinensis*), 11 lesser scaup (*Aythya affinis*), 1 northern pintail (*Anas acuta*), 1 redhead (*Aythya americana*), and 10 wood duck (*Aix sponsa*) were collected by licensed waterfowl hunters during the fall and spring migration seasons in 2012 and 2013, respectively. Of these species blue-winged teal, bufflehead, lesser scaup, and wood duck were sampled during the fall and spring migration. Overall, trematode populations and communities were highly variable among the hosts sampled and differences during the fall and spring migration season were also present, with the fall migration season harboring a greater number and diversity of trematodes.

In general, a total of 42,118 trematodes were recovered during the fall migration season, representing a total of 13 trematode species, whereas 316 trematodes were recovered during the spring migration, representing a total of 8 trematode species. The role of diet and overall size of the intestinal tract appeared to represent important factors in both the number of trematodes that were found as well as the species richness of trematodes. Overall, mean trematode intensity and species richness was highest in lesser scaup during the fall migration with wood duck harboring fewer trematodes and fewer trematode species than other birds used during community analysis (blue-winged teal and lesser scaup). When adult trematode communities were compared to larval trematode communities that were sampled, 2 trematode species (*Cyathocotyle bushiensis* and *Sphaeridiotrema pseudoglobulus*) appeared to have the ability to represent an autochthonous relationship, meaning that infections could have occurred at the site where both the birds and snails were collected. Although site characteristics could have played a role in the type and prevalence of certain types of trematodes found within the waterfowl hosts, host size (including length of the intestine), diet, and feeding patterns could also be important factors in determining the trematode species present within a given host and the number of trematodes within a given host. Finally, this work represents a comprehensive study on the trematode communities and populations of waterfowl trematodes found within Lake Winnibigoshish, Minnesota and the potential factors responsible for trematode distribution within intermediate and definitive hosts collected from this location.

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INTRODUCTION

Trematodes represent a diverse group of parasites with some estimates suggesting there may be more than 24,000 species (Poulin 2000). While the interactions between trematodes of humans and domestic animals has received considerable attention, many trematode species with influences on wildlife have not received as much attention. The purpose of the studies provided within this thesis was to describe the overall trematode communities present among waterfowl collected from Lake Winnibigoshish, Minnesota, and to compare differences in trematode communities between birds collected during fall and spring migration periods, as well as to compare the adult trematode communities from these birds to those present among intermediate host snails at Lake Winnibigoshish.

The first chapter of this thesis examined the structure of the trematode communities present within waterfowl collected from Lake Winnibigoshish. A variety of factors that had potential influences in the structuring of these communities were analyzed and overall lesser scaup were found to have the highest number of individual trematodes (intensity), as well as the highest trematode species diversity (species richness) of any other bird.

The second study compared the trematode communities present within waterfowl during the fall and spring migration periods. Overall, trematode communities were higher in both infection intensity and species richness during the fall migration season. When adult trematode communities from waterfowl were compared the larval trematode communities that were collected in snails, a total of 5 trematode species were found in common between the two host communities. This suggests that waterfowl which were infected with these trematodes species could have acquired those infections as a result of their exposure at Lake Winnibigoshish if it is assumed that trematode populations are maintained in the snail population from year-to-year at a given site.

CHAPTER ONE: TREMATODE COMMUNITY STRUCTURE OF WATERFOWL COLLECTED FROM LAKE WINNIBIGOSHISH, MINNESOTA

Abstract:

Helminth parasite communities have received considerable attention within many host systems to better understand the factors that structure those communities. Within waterfowl, helminth communities have been studied at a variety of levels to help elucidate important factors that lead to the structuring of these communities. In this study, thirty-eight waterfowl were collected from Lake Winnibigoshish during the fall migration season in 2012, from six different duck species that included 16 blue-winged teal (*Anas discors*), 10 lesser scaup (*Aythya affinis*), 7 wood duck (*Aix sponsa*), 3 bufflehead (*Bucephala albeola*), 1 northern pintail (*Anas acuta*), and 1 redhead (*Aythya americana*). In total, 41,006 trematodes were collected, which were distributed among 12 trematode species and two Family-level identifications. Despite showing some overlap of trematode infections between these three host species, statistically significant differences in mean trematode intensity and mean trematode species richness ($P \leq 0.05$) were detected. By utilizing various methods of community analysis, we are able to highlight important factors to better describe the patterns of trematode distribution within these waterfowl hosts.

INTRODUCTION

Community ecology is typically studied among species living within a specific habitat and is an attempt to assess the effects of biotic interactions, such predation, competition, mutualism, and parasitism, as well as the effect abiotic factors have in determining the structure of the community. The structure of helminth communities has been of interest to community ecologists as they attempt to understand whether these communities are structured in a predictable manner and, if so, what underlying processes may be shaping that structure (Holmes and Price 1986; Gaston et al.1997).

Because parasites represent a group of small, highly specialized organisms, several aspects of their biology are unique relative to larger, more animals that can survive in a variety of environmental conditions or make use of a wide variety of resources for survival. Heteroxenous parasites that require multiple, distinct, host species to complete their life-cycle provide a unique complexity of life stages and recruitment strategies that necessitate accounting for various hierarchical levels in their organization (Esch and Fernandez 1993). Waterfowl species are hosts to a diverse group of both internal and external parasites (Ballweber 2004). Of the parasites that infect waterfowl, helminths have received considerable attention from the standpoint of causing disease and possessing the ability to impair a bird's ability to function normally (McDonald 1981). In a study by Gregory (1990), on parasite community structure within waterfowl, the main factors that determined the diversity of the parasite fauna for a host species were: body size, diet, life-span, migratory movements, and gregarious habits of the host, as well as, whether or not the sample size of the birds collected had an influence on the parasite communities that the researchers were noticing. Other researchers also noticed the importance of helminth communities within waterfowl as excellent systems of study on the trematode communities present within the host, including interactions between various parasite species, and for examining various ecological concepts such as the seasonal transmission of helminths between waterfowl populations, relations of helminth communities with diet of waterfowl species, influences of certain trematodes on the survival of certain waterfowl species, differences in helminth communities between different migration corridors, and spatiotemporal distributions of helminths (Buscher 1965; Drobney et al. 1983; Hoeve and Scott 1988; Garvon et al. 2011; England et al. 2016).

Although studies exist on the helminths found within various waterfowl species throughout much of the United States (Broderson et al. 1977; Shaw and Kocan 1980; Canaris et al. 1981; Thul et al. 1985; Fedynich et al. 1996), no recent studies could be found that examined the characteristics of trematode communities within waterfowl populations in Minnesota. This lack of knowledge is even more troubling given the recent association between mortality-related trematodes and waterfowl in the upper Midwest. In 2002, large numbers of waterbirds experiences mortality events on the Upper Mississippi National Wildlife Refuge during fall and spring migrations (Herrmann and Sorensen 2011). Beginning in 2007, large-scale waterbird losses were noticed at Lake Winnibigoshish, a large lake (22,853 ha) located in northwest Minnesota (Roy 2016). The cause of these mortality events was attributed to three trematodes (*Cyathocotyle bushiensis*, *Leyogonimus polyoon*, and *Sphaeridiotrema pseudoglobulus*), and the waterbird species that have been primarily affected by these mortality events were

American coot (*Fulica americana*) and lesser scaup (*Aythya affinis*). For these mortality-related trematodes, an exotic snail (*Bithynia tentaculata*) was noted to be the first and second intermediate hosts for all three trematode species. While considerable emphasis has been given to the role of mortality-related trematodes and their effects within definitive and intermediate hosts at sites where faucet snails (*B. tentaculata*) occur (Hoeve and Scott 1988; Herrmann and Sorensen 2009; Sandland et al. 2014), little information has been obtained on the overall trematode communities present within the broader waterfowl community in these parasite-host systems.

Trematodes often display the greatest diversity in a given parasite community within waterfowl and are also the most abundant parasite species found in waterfowl (McDonald 1981). The most common trematodes found within waterfowl often rely on freshwater mollusks as their second intermediate hosts (Sorensen and Minchella 2001). Therefore, birds that typically rely on mollusks as a dietary item will routinely encounter infective stages of these parasites from infected mollusks. For example, a study performed by Skirnisson (2015) showed that sea ducks that consume mainly invertebrates in their diet typically have higher helminth diversity than birds that do not feed on invertebrates. Additionally, waterfowl that predominantly feed upon plant matter have been generally found to harbor a lower helminth diversity and infection intensities since this mode of infection relies upon the bird opportunistically feeding upon aquatic invertebrates that serve as intermediate hosts for helminth species (Amundson et al. 2016) or by passively ingesting larval stages (metacercariae) that are attached to the vegetation.

Within this study, trematode communities were compared among 3 species of waterfowl: blue-winged teal (*Anas discors*), lesser scaup (*Aythya affinis*), and wood duck (*Aix sponsa*). Blue-winged teal are a small, dabbling duck that occur throughout much of the north-central United States and Canada. While a majority of their diet is plant matter, molluscs are typically consumed in order to meet energy requirements before periods of migration and egg-laying (Bellrose and Kortright 1976). Blue-winged teal typically inhabit shorelines, as opposed to open water, which typically involve areas with shallow water and emergent vegetation (Baldassare 2015). Wood duck, like blue-winged teal, are also a dabbling duck that inhabit much of the eastern United States year round. The diet of these birds typically involves seeds, fruit, and aquatic arthropods. The areas inhabited by wood duck are typically woodland habitats with freshwater marshes present with vegetation for both cover and foraging (Hepp and Bellrose 1995). Lesser scaup, differ from both blue-winged teal and wood ducks in that they are a large, diving duck that occurs throughout much of the United States with winter distributions into southern areas of Mexico. The diet of these birds varies with the season, but animal matter, especially molluscs and aquatic insects have been known to be a dominant food item in the lesser scaup's diet (Anteau and Afton 2008). Areas typically inhabited by lesser scaup usually involve open water and these birds are typically found feeding in deeper areas of lakes and rivers (Austin et al. 2006). Because these 3 waterfowl species differ in habitat preference, diet, and body size, we were interested in evaluating existing hypotheses that serve as mechanisms for explaining the parasite communities that are found in waterfowl.

The hypotheses that exist for explaining possible causes for variation in the helminth communities present in waterfowl include habitat preference, which plays a role in where these specific waterfowl species are feeding and having contact between each other. By having these various habitat differences between these waterfowl species, this will effect which trematode species will be available since certain dietary items can only be found within certain habitats. Diet of the host species has been explained as a critical factor in the helminth communities present within waterfowl and we were interested in evaluating whether birds that are thought to feed more on invertebrates, namely blue-winged teal and lesser scaup, will be more likely to possess trematode infections since they have a greater chance at receiving an infection by ingesting an intermediate molluscan host. Finally, body size has also been cited as being a factor that can influence the helminth communities within waterfowl; therefore, the hypothesis is proposed that birds with a greater body size will also have more niche space available for parasites to be found (Gregory 1990). In review, we were interested in seeing whether or not molluscivorous birds, such as blue-winged teal and lesser scaup would harbor a greater total number of trematodes and greater species richness than non-molluscivorous birds like wood duck. In addition, we were also interested in examining whether or not lesser scaup, which is typically larger in size than blue-winged teal and wood duck, would harbor a greater number of trematodes and a greater number of species than the other birds being examined in this study.

The primary objective of this study was to compare the trematode infection intensity and richness between blue-winged, lesser scaup, and wood duck. In addition, to evaluate factors such as species evenness, or the relative abundance of different species that make up the richness of a given area, and community diversity, which represents both the number of species present and their evenness in a given habitat, we compared the trematode component communities among the three waterfowl species to determine if host-specific patterns existed. The final objective of this study was to evaluate possible causes for observed intensity, richness among trematodes.

MATERIALS AND METHODS

Waterfowl Species and Collection Site

Thirty-eight waterbirds were collected from Lake Winnibigoshish (Figure 1) in Itasca County, Minnesota (47°21´53.27 N, 95°15´31.13 W) during the fall of 2012 by licensed waterfowl hunters. Altogether, the sample collection, which encompassed a total of six different waterfowl species, included blue-winged teal (*A. discors*), lesser scaup (*A. affinis*), wood duck (*A. sponsa*), bufflehead (*Bucephala albeola*), northern pintail (*Anas acuta*), and redhead (*Aythya americana*). Sampling of such a diverse set of waterfowl species was an initial priority of this study to account for differences in host diet, life-span, migratory movements, social habits of the host, host body size, and the total number of hosts examined, all of which are known to directly influence the structure of the parasite community residing within a given bird host (Gregory 1990).

Parasite Collection and Identification

Prior to dissection, which took place within 24 hours of harvest, the weight and sex of each bird was recorded. The gastrointestinal tract was removed and intestines were stored on ice prior to and during transport to Minnesota State University, Mankato, where they were frozen at -20˚C until examination. At necropsy, the intestinal tract was cut into 15 cm segments with the large intestine and the cecum left intact to subdivide the digestive tract into longitudinal segments that would have possessed different physiological conditions in the intact birds. At the time these segments were examined for parasites, each segment was placed into a separate 15cm petri dish, rinsed with tap water, and examined under a dissecting microscope for the presence of parasites. Any trematodes observed were sorted based on gross morphology and then fixed in formalin for subsequent morphological analysis.

The formalin-fixed trematodes were placed in increasing concentrations (35%, 50%, and 70%) of ethanol, with each step lasting approximately 20 minutes. Specimens were then placed in an acetocarmine stain (Fisher Scientific, Pittsburgh, PA) for 1 hour followed by additional 20 minutes in a series of dehydrating ethanol washes (85% and 95%). The final dehydration step, which was performed in 100% ethanol, was allowed to proceed for an hour. The stained trematodes were then placed in xylene (Fisher Scientific, Pittsburgh, PA) and mounted on microscope slides using Canada balsam (Matheson Coleman and Bell Inc., Norwood, OH). Each specimen was then identified at the Family, Genus or species level using the taxonomic keys provided by McDonald (1981), Schell (1985), Gibson et al. (2002), Jones et al. (2005), and Bray et al. (2008).

Definitions

The different measures of parasitism calculated for the various trematodes recovered from each species of bird include: (1) prevalence, which is defined as the number of hosts infected with 1 or more individuals of a single parasite species divided by the total number of potential hosts examined for that parasite, (2) mean intensity, *i.e.*, the mean number of worms per infected host, (3) mean trematode species richness is the total number of trematode species, found while sampling all individuals of one host species (including non-infected individuals) divided by the total sample size (Bush et al. 1997). Species evenness refers to the relative abundance of different species making up the richness of a given area. Species diversity in turn refers to both the number of species in the community (richness) and their relative abundance (evenness). All values are reported as a mean ± one standard error.

Data Analysis

Prior to considering species-specific influences on parasite diversity or community structure it was necessary to plot species richness curves to determine whether there was evidence that sufficient numbers of birds were collected to provide a reasonable estimate of the parasite diversity within a bird species. This curve was constructed by writing an equation in Microsoft Excel to randomly reorder and resample the original data that was collected from the individual birds. Once the samples had been randomly reassembled 30 times, the relationship between cumulative number of trematode species identified (mean richness) and the number of hosts examined was plotted in a single graph depicting the 30 randomly-assembled replicates. Based on mean values from these replicates, the best-fit curve was calculated. These curves were visually examined to determine whether the relationship between the mean richness and number of worms reached an asymptote, which indicates sufficient sampling effort to assume relatively complete sampling of the component parasite community for each sampled waterfowl species (Figure 2).

Simpson's Diversity Index was used to calculate the diversity of the trematode community with a range of values from 1 (typically representative of monospecific communities) to a maximum of the total number of species richness when all species are represented evenly. For each host species, the diversity of the trematode community was calculated by taking the sum of the richness, or number of trematode species, and multiplied by the frequency of the trematode species in the community. The resulting answer was then divided into 1. Evenness (E) was calculated according to Poulin (1996) in which case $E = D_{obs}/D_{max}$, where $D_{obs} = 1\text{-}1/G$, and G is the diversity of a given community and D_{max} is the maximum obtainable value given the number of species. This measure of evenness was a scaled measure that ranged from 0 (extreme unevenness) to 1 (complete evenness). The Jaccard coefficient of community similarity was used to compare similarity of trematode communities between the different waterfowl species, in a pairwise manner, with indices ranging from 0 (when no species are found in common between the two communities) to 1 (when all species are found in common between communities).

The Kruskal-Wallis H test was used to compare differences in species richness, mean trematode intensity, species diversity, and species evenness values between bird species. The Kolmogorov-Smirnov 2-sample test was then used to compare differences in these calculations among the different host species. Scatterplots were used to visualize relationships between number of trematodes or number of species and bird weight. Pearson's correlation was used as a non-parametric test to determine whether there was statistical support for apparent relationships in the scatterplots. Trendlines between weight and total trematodes or trematode species richness and intestine length and trematode species richness were plotted in order to determine if positive or negative relationships were present. A statistical significance of $P \leq 0.05$ was used for all analyses.

RESULTS

Overall trematode community:

A total of 42,424 trematodes from 12 trematode species were present within the waterfowl collected during this study. These species were *Cyathocotyle bushiensis, Echinoparyphium recurvatum, Echinostoma revolutum, Hypoderaeum conoideum, Maritrema obstipum, Notocotylus attenuatus, Neopsilotrema affine, Neopsilotrema lakotae, Neopsilotrema lisitsynae, Ornithodiplostomum ptychocheilus, Sphaeridiotrema pseudoglobulus*, and *Zygocotyle lunata*. Identification of two trematode species was limited to Family-level identifications (Family Strigeidae and Family Psilostomidae) due to the lack of diagnostic morphological traits that could serve as the basis for species level identifications. Table 1 shows which parasite species were present in each of the bird species. Among all of the birds sampled, the total number of trematode species present in the various waterfowl species ranged from 13 for lesser scaup and 2 for wood duck.

The curves plotted using Microsoft Excel that display the cumulative number of species found as a function of the number of birds samples appeared to asymptote for blue-winged teal, lesser scaup, and wood ducks (Figure 2). This finding suggests that sufficient sampling occurred to warrant further consideration of parasite population and community characteristics for these species.

Comparison of trematode populations among blue-winged teal, lesser scaup, and wood duck:

A total of 41,006 trematodes were collected from 16 blue-winged teal, 10 lesser scaup, and 7 wood ducks. These worms were distributed among the trematode species and Families listed above. All of these birds were infected with at least one trematode, but the majority of these birds possessed many more than 1 worm. Lesser scaup possessed the largest number of trematodes (33,389) and species (12 species and 1 unidentified member of the Family Strigeidae). To the contrary, wood ducks possessed the fewest individual parasites (86) and fewest species (1 species and 1 unidentified member of Family Psilostomidae). Blue-winged teal possessed an intermediate number of trematodes (7531) and species (8), and 1 unidentified member of Family Strigeidae.

Prevalence and mean intensity values for each trematode species collected from blue-winged teal, lesser scaup, and wood duck are reported in Table 2. For lesser scaup, trematode intensity ranged from 20–8,373 worms per bird, providing a mean value of 2513.6 ± 935.5 trematodes per bird. In addition, prevalence ranged from a high of 100% infected with *C. bushiensis* to a low of 20% infected with *N. attenuatus*. Taken together, these intensity and prevalence values yielded mean intensity for each of the trematode species collected from lesser scaup ranging from of 3,688.5 ± 1303.7 worms per birds with *S. pseudoglobulus* to 2.5 ± 1.5 worms per bird with *O. ptychocheilus*. Similarly, within blue-winged teal, the overall mean trematode intensity was 502.1 ± 177.2 worms per host, with individual trematode species values ranging from 8–2,652 worms per bird and prevalence values ranging from 81.3% infected for *N. lisitsynae* to 6.3% infected for *H. conoideum*. Lastly, prevalence values within wood duck ranged from 85.7% infected for *Z. lunata* to 57.1% infected for members of the Family Psilostomidae. Mean intensity ranged from 18.5 ± 9.9 for members of the Family Psilostomidae and 1.67 ± 0.33 for *Z. lunata*. A Kruskal-Wallis one-way analysis of variance revealed significant differences in mean trematode intensity among the three bird species (H = 17.001; P = 0.0002). Kolmogorov-Smirnov two-sample tests among all possible pairwise combinations of bird species also revealed significant differences in all possible host pairings (P = 0.0002) (Figure 4). This finding indicates that lesser scaup possessed significantly more trematodes than both blue-winged teal and wood ducks, while blue-winged teal also possessed significantly more worms than wood ducks.

Comparison of trematode community structure among blue-winged teal, lesser scaup, and wood duck:

Analysis of community structure revealed significant patterns when trematode communities were compared to those from other host species. Species evenness and Simpson's species diversity values for blue-winged teal produced evenness values ranging from 0.31 to 0.99 with a mean of 0.71 ± 0.05 while diversity indices displayed a range of 1 to 3.22 with a mean of 1.58 ± 0.17. For lesser scaup, evenness values ranged from 0.25 to 0.90 with a mean of 0.57 ± 0.08, while diversity indices displayed a range of 1.1 to 3.86 with a mean of 2.11 ± 0.31. Finally, for wood duck, evenness values ranging from 0 to 0.96 with a mean of 0.30 ± 0.15 and diversity indices displayed a range of 1 to 1.77 and a mean of 1.22 ± 0.14. Pairwise analysis using Jaccard's Community Similarity Index showed that blue-winged teal and lesser scaup possessed more similar parasite communities than when either of these waterfowl species are compared with wood duck. Overall, the Similarity Index provided values of 0.11 for blue-winged teal and wood duck, 0.077 for wood duck and lesser scaup, and 0.69 for blue-winged teal and lesser scaup. The Kruskal-Wallis H test showed significant differences in diversity indices between the three bird species ($H = 7.271$; $P = 0.026$). The Kolmogorov-Smirnov 2-sample test showed significant differences with all possible pairings involving wood duck; whereas, the host pairing of blue-winged teal and lesser scaup did not display a significant difference in diversity indices ($Z = 0.868$; $P = 0.438$). A Kruskal-Wallis H test showed that there were significant differences in evenness values between host species ($H = 6.041$; $P = 0.049$). The Kolmogorov-Smirnov 2-sample test revealed significant differences between all pairings involving wood duck ($Z = 1.340$; $P = 0.055$). However, blue-winged teal and lesser scaup showed no significant differences in evenness values ($Z = 0.868$; $P = 0.438036$). Overall, this analysis shows that within measures of community structure, blue-winged teal and lesser scaup displayed similarities, whereas they both differed from wood duck.

The only trematode that was common of all three waterfowl species was *Z. lunata* and it was in found in 31.3%, 50%, and 85.7% of the blue-winged teal, lesser scaup, and wood duck respectively. Similarly, the mean intensity of *Z. lunata* in the 3 bird species ranged from 1.7 ± 0.3 individuals per wood duck to 5 ± 1.7 individuals per lesser scaup, with blue-winged teal having an intermediate value of 2.4 ± 0.68 individuals per bird. Blue-winged teal and lesser scaup shared 9 trematode species, and the mean intensity values for trematodes shared between these 2 species differed significantly ($Z = 1.830$; $P = 0.002$) with lesser scaup possessing more species than blue-winged teal.

The average number of trematode species found among the 3 bird species ranged from less than 2 to greater than 7. More specifically, in blue-winged teal this value ranged from 3-6 species per bird with a mean value of 3.56 ± 0.27 species per bird. In contrast, within lesser scaup, trematode species richness averaged 7.6 ± 0.8 species per bird. Lastly, for wood duck, mean trematode species richness was 1.4 ± 0.2 species per bird. A Kruskal-Wallis H test showed there was a statistically significant difference in trematode species richness between the three bird species (H = 21.928; P = 0.000011). The Kolmogorov-Smirnov 2-sample test revealed significant differences in trematode species richness among all possible host species pairs (blue-winged teal vs. wood duck: Z = 1.931; P = 0.001; blue-winged teal vs lesser scaup: Z = 1.830; P = 0.002; lesser scaup vs. wood duck: Z = 2.029; P = 0.001). This analysis shows that lesser scaup have higher trematode species richness than both blue-winged teal and wood duck, while blue-winged teal also displayed a significantly higher richness than wood duck.

Analysis of portions of the intestine where certain trematode species were found revealed habitat-use patterns for different trematodes species and these patterns were generally consistent among the various trematode species, independent of whether they were found within blue-winged teal, lesser scaup, or wood duck. Within the anterior portion of the intestine, *E. recurvatum* was found within both blue-winged teal and lesser scaup, displaying a mean intensity of 130.5 ± 8.1 and 116 ± 47.3 for blue-winged teal and lesser scaup respectively. *H. conoideum* was only found within 1 blue-winged teal and 3 lesser scaup and displayed infections throughout the anterior portion of the intestine in both birds. A trematode that was typically found in the more posterior portion of the intestine for both blue-winged teal and lesser scaup was *E. revolutum*. The trematodes that were found within the cecum were *C. bushiensis* and *Z. lunata*. Overall, all remaining trematodes were typically found in all habitats within the intestine. The only trematodes that were found within a single waterfowl species were found in lesser scaup and these trematodes were *C. bushiensis*, *N. affine*, *N. lakotae*, and *O. ptychocheilus*. Overall, this analysis showed that a majority of the trematodes shared between blue-winged teal and lesser scaup were found within similar locations in the intestine.

Relationship between bird weight and trematode intensity or richness:

In order to determine whether bird weight could be used to explain trematode intensity and species richness patterns within these bird species, weights of all of the bird species were compared. Within blue-winged teal, lesser scaup, and wood duck, the average weights for all of the birds in the sample were 469.399g ± 20.2g, 734.254g ± 49.0g, and 728.988g ± 18.3g, respectively. A Kruskal-Wallis test was used to determine whether significant differences existed between the weights of the 3 bird species ($H = 20.401$; $P = 0.000037$). The Kolmogorov-Smirnov 2 sample test revealed significant differences when blue-winged teal were compared to lesser scaup ($Z = 1.985$; $P = 0.001$), or wood duck ($Z = 2.069$; $P = 0.000383$). Conversely, no significant difference was detected between lesser scaup and wood duck ($Z = 0.812$; $P = 0.525$). There was no significant correlation between the weight of a bird, within a species and the total number of trematodes or the number of trematode species that they possessed based on regression analysis. However, relationships of weight versus total number of trematodes for blue-winged teal and lesser scaup seemed to show a negative relationship between total number of trematodes in relation to weight (Figure 5A). On the other hand, a different pattern was observed when number of trematode species was plotted relative to weight. In this instance, lesser scaup appeared to show a positive relationship while blue-winged teal appeared to show a negative relationship (Figure 5B). Intestine length was also considered as a possible factor affecting trematode richness, so intestine length was plotted against trematodes species richness, this analysis showed what appeared to be a positive relationship between intestine length and trematode richness for both blue-winged teal and lesser scaup while wood duck appeared to show a negative relationship (Figure 6).

DISCUSSION

The goal of this study was to reveal the trematode community present within different species of waterfowl that were collected during their fall migration period. After removal and identification of all worms, patterns of trematode population distribution, trematode community structure were detected, as were relationships between weight and total number of trematodes or richness. All birds in this study, except one bufflehead, possessed trematodes and there were 14 trematode species, 2 of which were not identified to the species level, among these birds. It was shown that lesser scaup have the most trematodes and wood duck possessed the fewest. In regards to community structure, patterns of species diversity and evenness were comparable between blue-winged teal and lesser scaup whereas both differed significantly in terms of total number of trematodes and species richness. With the relationship between weight and total number of trematodes, data presented herein suggests a negative relationship for both blue-winged teal and lesser scaup. However, a relationship between weight and trematode species richness suggested that there was a positive relationship for lesser scaup, while blue-winged teal appeared to have a negative relationship.

The fact that all but one of the waterfowl in this study possessed trematodes exemplifies the common pattern of trematode presence within waterfowl (McDonald

1981; Gregory 1990; England et al. 2016). None of these 14 trematode species were shared among the 6 waterfowl species examined herein; therefore, trematode-waterfowl interactions show some level of host-specificity. For instance, blue-winged teal and lesser scaup possessed more species of trematodes than the other 4 waterfowl species, and 14 of the trematode species were found only among blue-winged teal and lesser scaup. Furthermore, over one-third of the trematode species were found within lesser scaup. Moreover, 5 of the 7 species found in blue-winged teal and lesser scaup use molluscs as second-intermediate hosts (Schell 1985). This finding is consistent with claims of these two species feeding predominantly on mollusks (Baldassare 2015).

Analysis of trematode populations was restricted to blue-winged teal, lesser scaup, and wood duck because relationships between number of waterfowl individuals sampled and number of trematode species detected suggested that sufficient sampling effort was performed among these species to avoid spurious conclusions. Within these 3 waterfowl species, lesser scaup had significantly more trematodes than both blue-winged teal and wood duck. Additionally, blue-winged teal had significantly more trematodes when compared to wood duck. The finding that blue-winged teal and lesser scaup had significantly more trematodes than wood duck suggest dietary preferences of blue-winged teal and lesser scaup is an important determinant of parasite presence being these birds are described as being molluscivorous. One potential explanation for why lesser scaup harbor a significantly higher number of trematodes than blue-winged teal could be that lesser scaup tend to forage on mollusks to a greater extent than do blue-winged teal. Recent studies have noted that due to declines in amphipod populations, lesser scaup dietary preferences have shifted to more abundant mollusk species (Anteau and Afton

2006; Karatayev et al. 2012; England et al. 2016). Differences in trematode intensity between these two hosts could also be attributed to differences in foraging habitats for these two bird species with lesser scaup tending to forage in open water areas and typically feed by diving below the water whereas blue-winged teal typically feed in shallow waters with emergent vegetation (Hepp and Bellrose 1995). Therefore, lesser scaup could potentially be exposed to a wider range of potential invertebrate hosts, including mollusks that favor deeper water. In addition, other studies have also noted the ability of lesser scaup to be infected with many trematodes (England et al. 2016).

Trematode community structure among the 3 waterfowl species displayed significant differences in species diversity, species evenness, and species richness, but the patterns for species diversity and evenness differed from the patterns for species richness. For species richness and species diversity, significant differences were found in pairings involving wood duck while no differences were found between blue-winged teal and lesser scaup. This finding indicates that although significant differences in species richness were found for all birds, the fact that no differences were found for species diversity and species evenness between blue-winged teal and lesser scaup indicate that although differences occurred in the number of trematodes that were found between these two species, both the evenness and species diversity were not significantly different between these bird species. This finding reinforces the concept that both blue-winged teal and lesser scaup are more likely to be exposed to trematodes more often than non-molluscivorous birds because both feed on mollusks (Skirnisson 2015). In trematode species richness, significant differences were detected in all possible host pairings. These differences could be attributed to physiological or immunological differences between the host species which could in turn result in a difference in the infection intensity by a certain trematode (Herrmann and Sorensen 2011).

Analysis of weight as a potential explanation of higher trematode intensity and species richness within blue-winged teal, lesser scaup, and wood duck revealed that blue-winged teal are significantly smaller than both lesser scaup and wood duck, and that the latter pair of species did not differ in their weight. Negative relationships appeared to exist when total number of trematodes was plotted against weight of the bird for both blue-winged teal and lesser scaup. However, when species richness was plotted against weight, a positive relationship appeared to exist for lesser scaup, while a negative relationship appeared for blue-winged teal. While host body size has been explored as a potential mechanism for determining the diversity of a parasite fauna that a bird will harbor (Gregory 1990), the intestine length of individual bird species could also be an important mechanism in determining the diversity of a trematode fauna in waterfowl. During this study, trematodes were only collected from one organ (intestines). Therefore, length of the intestine plotted against trematode species richness was used to determine if relationships existed between intestine length and the number of worms or the number of trematode species. These plots showed that blue-winged teal and lesser appeared to have a positive slope, while wood duck appeared to have a negative relationship. Overall, this finding shows that greater intestine length results in a greater trematode diversity within blue-winged teal and lesser scaup.

Results from this study suggest that multiple factors are involved in structuring the trematode communities found within waterfowl. Within this study, differences in dietary preferences, habitats encountered by the host, and exposure to potential intermediate hosts appear to be important factors in the structure of these trematode communities. Differences in total number of trematodes and trematode species richness among various waterfowl species remains an important concept as researchers continue to investigate the helminth communities present within waterfowl. Additionally, as wildlife researchers continue to investigate parasites affecting various waterfowl species, the structuring of these communities is an important factor that cannot be overlooked.

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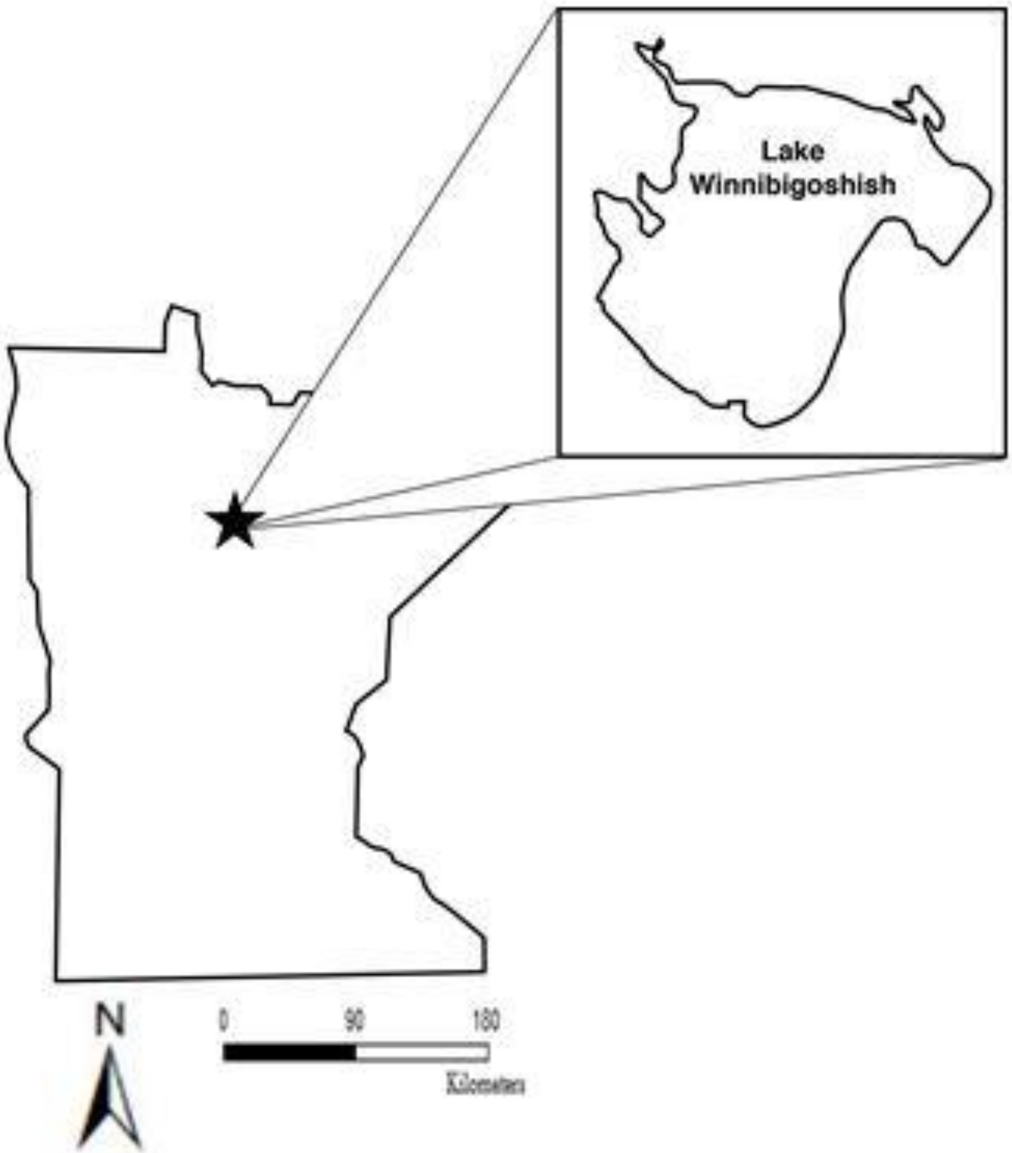

Figure 1. Map of Lake Winnibigoshish location in Minnesota (47°21´53.27 N, 95°15´31.13 W).

Table 1. Trematode species present in waterfowl species collected during fall and spring 2013. Y's represent that the waterfowl species harbored the trematode species*.

	Cb	Er	Ev	S?	Hc	Mo	Na	Nf	Nl	Ns	Op	Ps	Sp	Zl
Blue-Winged Teal N=16	-	Y	Y	Y	Y	Y	Y	-	-	Y	-	-	Y	Y
Lesser Scaup N=10	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	Y	Y
Wood Duck N=7	-	-	-	-	-	-	-	-	-	-	-	Y	-	Y
Bufflehead N=3	-	-	-	-	-	Y	-	-	-	Y	-	Y	Y	-
Northern Pintail N=1	-	-	-	Y	-	-	-	-	-	Y	-	Y	-	-
Redhead N=1	-	-	Y	-	-	-	-	-	-	-	-	-	Y	Y

* Key to trematode species abbreviations: Cb = *Cyathocotyle bushiensis*, Er = *Echinoparyphium recurvatum*, Ev = *Echinostoma revolutum*, S? = Family Strigidae, Hc= *Hypoderaeum conoideum*, Mo = *Maritrema obstipum*, Na = *Notocotylus attenuatus*, Nf = *Neopsilotrema affine*, Nl = *Neopsilotrema lakotae*, Ns = *Neopsilotrema lisitsynae*. Op = *Ornithodiplostomum ptychocheilus*, Ps= Family Psilostomidae, Sp = *Sphaeridiotrema pseudoglobulus*, Zl = *Zygocotyle lunata*

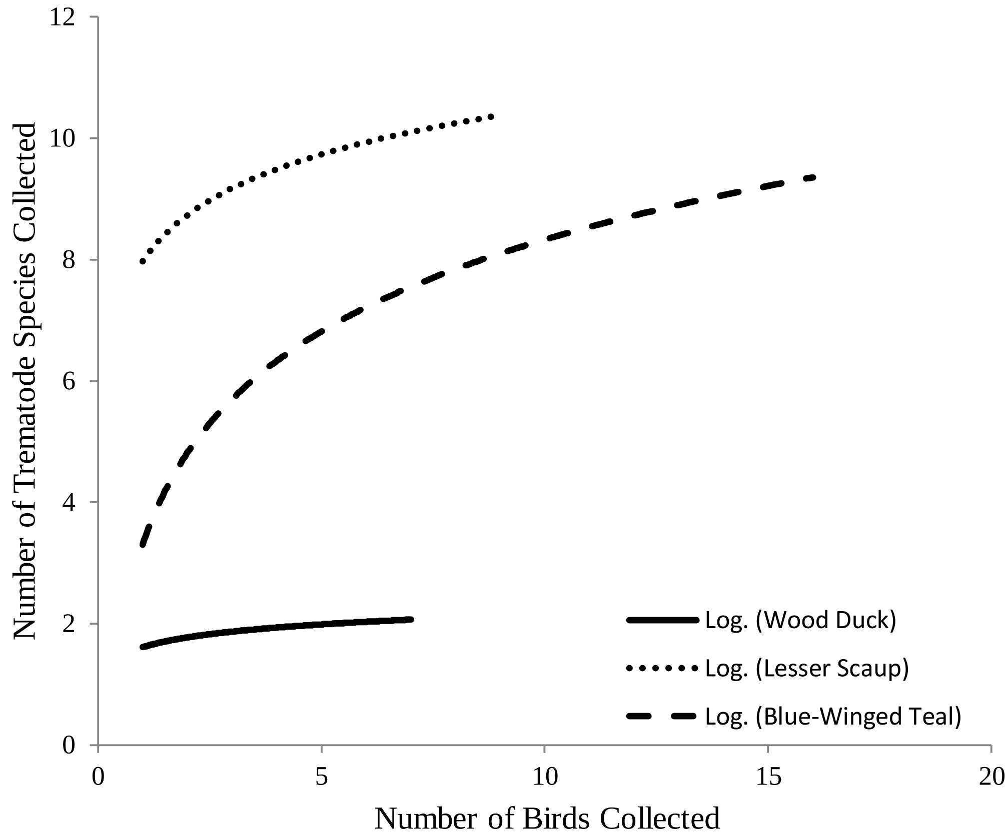

Figure 2. Trendlines representing the logarithmic relationships for the trematode species collected for each bird species as a function of the number of birds sampled. Each trendline is based on the re-sampling of trematode species within each bird species collected 30 times.

Table 2. Prevalence and mean intensity values for trematodes collected from blue-winged teal, lesser scaup, and wood duck. Dashes represent that no trematode species were recovered from that bird.

	Blue-Winged Teal		Lesser Scaup		Wood Duck	
Trematode Species	Prevalence (%)	Mean Intensity	Prevalence (%)	Mean Intensity	Prevalence (%)	Men Intensity
Cyathocotyle bushiensis	-	-	9 (90)	107.7 ± 93.7	-	-
Echinoparyphium recurvatum	2 (12.5)	130.5 ± 8.1	10 (100)	116 ± 47.3	-	-
Echinostoma revolutum	3 (18.8)	20.6 ± 14.9	3 (30)	5 ± 1.7	-	-
Family Strigeidae	12 (75)	9.3 ± 2.4	8 (80)	18.6 ± 4.6	-	-
Hypoderaeum conoideum	1 (6.3)	4	3 (30)	5.7 ± 2.7	-	-
Maritrema obstipum	2 (12.5)	7 ± 6.0	4 (40)	11 ± 3.7	-	-
Neopsilotrema affine	-	-	1 (10)	8 ± 0.0	-	-
Neopsilotrema lakotae	-	-	1 (10)	4 ± 0.0	-	-
Neopsilotrema lisitsynae	13 (81.3)	525.6 ± 25.9	7 (70)	151 ± 73.4	-	-
Notocotylus attenuatus	6 (37.5)	1 ± 0	2 (20)	214 ± 23	-	-
Ornithodiplostomum ptychocheilus	-	-	2 (20)	2.5 ± 1.5	-	-
Family Psilostomidae	-	-	-	-	4 (57.1)	18.5 ± 9.9
Sphaeridiotrema pseudoglobulus	5 (31.3)	45.4 ± 9.0	8 (80)	3688.5 ± 1303.7	-	-
Zygocotyle lunata	5 (31.3)	2.4 ± 0.68	5 (50)	5 ± 1.7	6 (85.7)	1.7 ± 0.3

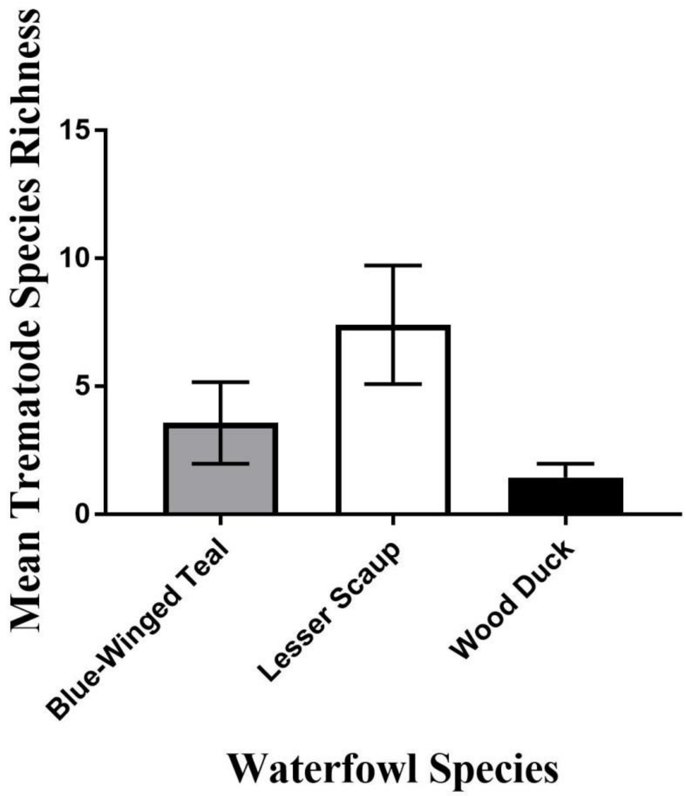

Figure 3. Mean trematode species richness among 3 species of waterfowl. Error bars represent ± 1 SE.

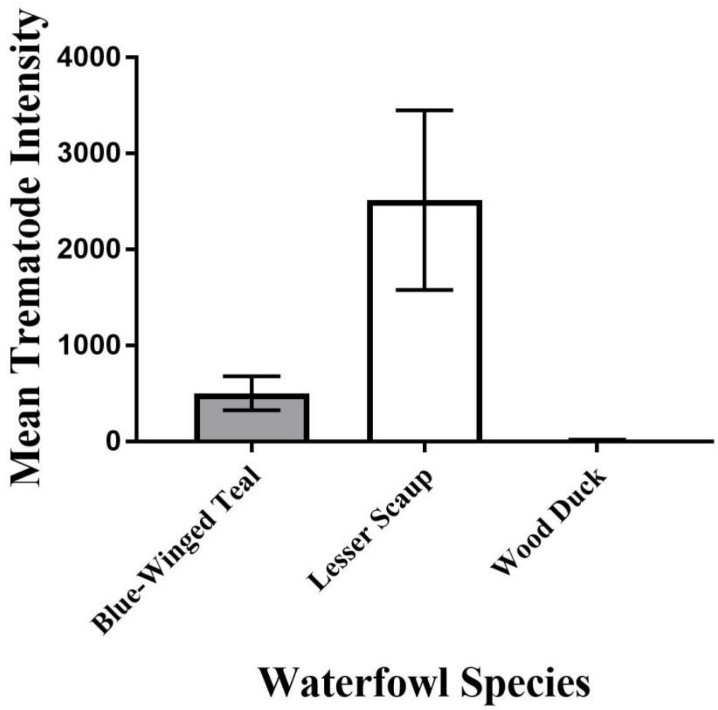

Figure 4. Mean trematode intensity among 3 species of waterfowl. Error bars represent ± 1 SE.

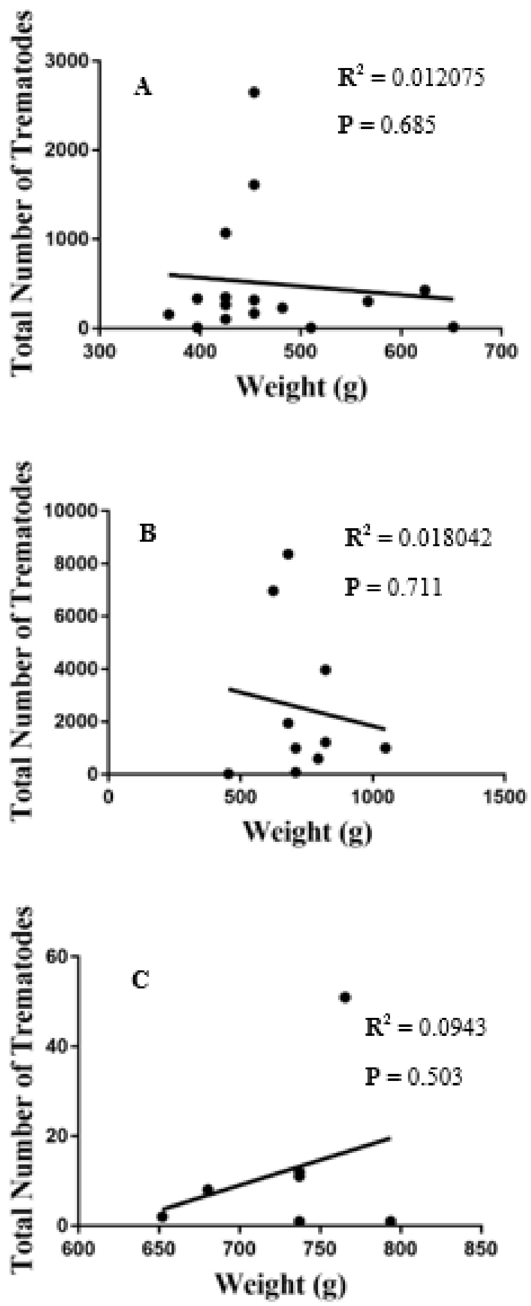

Figure 5. Trendline representing relationships between weight and total trematodes among blue-winged teal (A), lesser scaup (B), and wood duck (C).

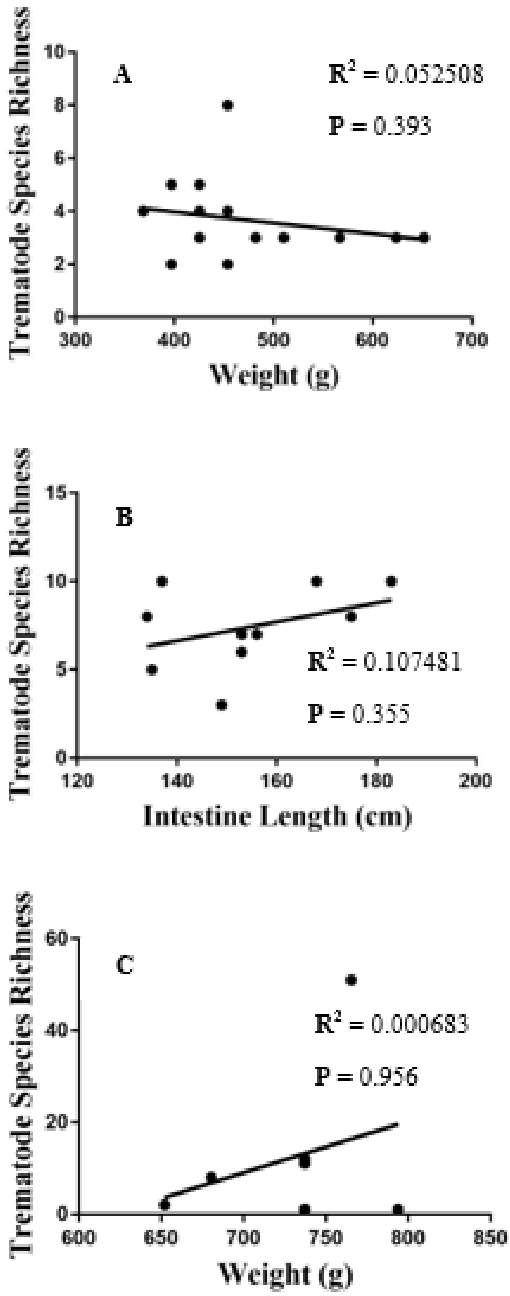

Figure 6. Trendline representing relationship between weight and trematode species richness for blue-winged teal (A), lesser scaup (B), and wood duck (C).

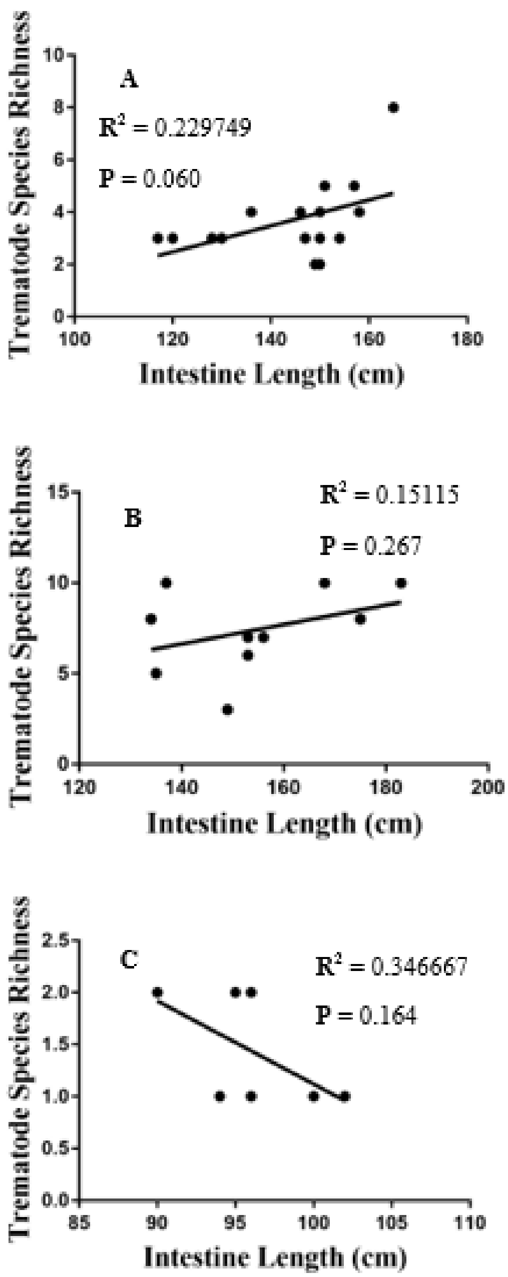

Figure 7. Trendlines representing the relationship between intestine length and trematode species richness among blue-winged teal (A), lesser scaup (B), and wood duck (C).

CHAPTER TWO: A COMPARISON OF ADULT AND LARVAL TREMATODE COMMUNITIES FROM LAKE WINNIBIGOSHISH, MINNESOTA

Abstract: This study compares the trematode communities in waterfowl collected from Lake Winnibigoshish, Minnesota between the fall and spring migration periods. Furthermore, this study also utilized the collection of larval trematode communities from snail hosts and compared those communities to those found within waterfowl. Overall, results from this study found significant differences in the mean trematode intensity and mean species richness in waterfowl hosts collected during the fall and spring migration periods. Additionally, when larval trematode stages were compared to adult stages that were found in waterfowl, 2 trematode species were found reside in both waterfowl and snails at Lake Winnibigoshish.

INTRODUCTION

Fall and spring migrations are critical events for many waterfowl species (Stafford 2014). During these times, acquisition and maintenance of energy reserves is a necessity for completing the migration and successful breeding (Ankeny et al. 1991). However, during this time period, waterfowl can also be exposed to endoparasites acquired during their migration route (Loehle 1995; Waldenstrom et al. 2002; Hoye 2011). When compared to non-migratory birds, birds that migrate are expected to encounter more intermediate hosts that could lead to a diverse assemblage of parasitic infections.

During migration events, patterns of parasite transmission are affected by variations in both temperature and seasonal patterns of infection within the intermediate host population (Esch and Fernandez 1994; Herrmann and Sorensen 2009). Furthermore, migration plays a critical role in the distribution and maintenance of helminths in waterfowl. For example, Buscher (1965) attributed reductions in the intensity of helminth infections to: natural loss of helminths due to physical stresses associated with migration and the loss of species due to short-lived adult forms. Differences in helminth infection intensities and species richness within avian definitive hosts have been attributed to multiple factors including: host feeding ecology, habitat use, and host-parasite coevolution (Brooks 1979; Fedynich et al. 1996; Vickery and Poulin 1998). Further studies also noted differences in helminth community structure due to loss of helminths between fall and spring migrations (Wallace and Pence 1986) and variations in species richness between breeding and wintering grounds (Broderson et al. 1977; Wilkinson et al. 1977; Canaris et al. 1981). Therefore, in this study we were interested in seeing if differences existed between the trematode communities within waterfowl collected during the fall and spring migrations, since differences have been noted to occur within waterfowl species during different periods of migration. Also, we were interested in comparing the larval trematode communities within snail hosts collected from Lake Winnibigoshish, Minnesota and compare those larval stages to adult trematodes found within collected waterfowl to determine if infections of these trematodes could help explain the infection patterns detected in waterfowl at this site.

MATERIALS AND METHODS

Waterbird Species and Collection Site

Fifty-two waterbirds were collected from Lake Winnibigoshish in Itasca County, Minnesota (47°21´53.27 N, 95°15´31.13 W) during the Fall of 2012 or the Spring of 2013. Birds were collected in the fall by licensed waterfowl hunters while in the spring, graduate students from Minnesota State University-Mankato (MNSU-Mankato) and employees of the Minnesota Department of Natural Resources (MN-DNR) harvested the birds under permits from the MN-DNR (Special Permit No.19032) and the United States Fish and Wildlife Service (Scientific Collecting Permit No. MB09296). During the fall migration season, 38 waterfowl were collected, which included 16 blue-winged teal (*Anas discors*), 10 lesser scaup (*Aythya affinis*), 7 wood duck (*Aix sponsa*), 3 bufflehead (*Bucephala albeola*), 1 northern pintail (*Anas acuta*), and 1 redhead (*Aythya americana*). For the spring migration season, 14 birds were collected which included 2 blue-winged teal, 1 lesser scaup, 3 wood duck, 2 bufflehead, 4 common goldeneye, 1 greater scaup, and 1 green-winged teal.

Trematode Collection and Identification

Prior to dissection, which took place within 24 hours of harvest, the weight and sex of each waterbird was recorded. The gastrointestinal tract was then removed and the intestines were stored in a cooler until transport to MNSU-Mankato, where they were frozen at -20˚C until examination. At necropsy, the intestinal tract was cut into 15 cm segments with the large intestine and the cecum left intact. Each segment was placed into a separate petri dish, rinsed with water, and then examined under a dissecting microscope for the presence of parasites. Any trematodes observed were sorted based on gross morphology and then fixed in formalin or frozen for subsequent DNA analysis.

The formalin-fixed trematodes were placed in increasing concentrations (35%, 50%, and 70%) of ethanol, with each step lasting approximately 20 minutes. Specimens were then placed in an acetocarmine stain (Fisher Scientific, Pittsburgh, PA) for 1 hour followed by additional 20 minutes in a series of dehydrating ethanol washes (85% and 95%). The final dehydration step, which was performed in 100% ethanol, was allowed to proceed for an hour. The stained trematodes were then placed in Xylene (Fisher Scientific, Pittsburgh, PA) and mounted on microscope slides using Canada Balsam (Matheson Coleman and Bell Inc., Norwood, OH). Each specimen was then identified to the Family, Genus or species level using the taxonomic keys provided by McDonald (1981), Schell (1985), Gibson et al. (2002), Jones et al. (2005), and Bray et al. (2008).

Once the trematodes were counted and identified from all waterfowl, patterns of distribution within each host species were described using the following indices: prevalence, mean intensity, and species richness. Prevalence is defined as the percentage of infected birds of each species while mean intensity indicates the mean number of worms per infected bird. Species richness is the total number of species found while sampling all individuals of a host species (Bush et al. 1997). All values were expressed as a mean ± 1 SE.

Snail Collection

On August 9, 2016, snails were collected from Lake Winnibigoshish in northern Minnesota (47°21´53.27 N, 95°15´31.13 W). Snails were collected from the western side of the lake in areas where definitive hosts were collected for a previous study on adult trematode communities within waterfowl definitive hosts. Collection was conducted by hand sampling shoreline littoral locations during 30-minute search periods or by a benthic grab sampler (Wildco Petite Ponar, Buffalo, New York, USA) in the limnetic zone of the lake adjacent to the shoreline sites. Snails were transported on ice back to the laboratory at Minnesota State University, Mankato and were placed under incandescent light for 12 hr to observe the presence of cercarial shedding. Individuals that did not shed cercariae were dissected in order to search for metacercarial stages within the snail tissue. Identification of larval stages was based upon the descriptions of Khan (1962), Schell (1985), and references therein. Finally, cercariae types that were shed from collected snails were compared to cercariae types that give rise to adult trematodes from previously studied waterfowl.

Data Analysis:

Data were pooled from all waterfowl species for each collection season to assess patterns in distribution across the two migration seasons. Differences in mean trematode intensity and mean trematode species richness were assessed using a Mann-Whitney U-test. A statistical significance of $P \leq 0.05$ was used for all analyses.

RESULTS

Trematodes from Waterfowl:

A total of 42,118 trematodes were recovered from the intestinal tracts of 38 birds collected during the fall migration season. This included 14 species of trematodes, 12 that were identified as: *Cyathocotyle bushiensis, Echinoparyphium recurvatum, Echinostoma revolutum, Hypoderaeum conoideum, Maritrema obstipum, Notocotylus attenuatus, Neopsilotrema affine, Neopsilotrema lakotae, Neopsilotrema lisitsynae,* *Ornithodiplostomum ptychocheilus, Sphaeridiotrema pseudoglobulus*, and *Zygocotyle lunata*, while 2 species were limited to Family-level identification (Family Psilostomidae and Family Strigeidae) due to a lack of diagnostic morphological traits. During the spring migration, 316 individual trematodes, representing 10 species, 8 of which were identified as: *Diplostomum spathaceum*, *E. recurvatum*, *E. revolutum*, *M. obstipum*, *N. attenuatus*, *N. lisitsynae*, *S. pseudoglobulus*, *Z. lunata*, while the remaining two species were members of the Family Psilostomidae and Family Strigeidae. The prevalence and mean intensity values for each trematode species are reported in Tables 1 and 2 respectively. Relative to parasites collected from birds during the fall and spring migration, 9 trematode species were found in both seasons. Specifically, for birds collected during the fall and spring (namely: blue-winged teal, lesser scaup, wood duck, and bufflehead) all of the trematode species that were collected during the fall migration were also found in the spring migration with the exception of *N. attenuatus* in wood duck.

Among all of the birds sampled during the fall migration, lesser scaup were the most heavily infected and also harbored the greatest trematode species richness of all the birds (Chapter 1). During the spring collection, a single lesser scaup was harvested and it provided the highest total number of trematodes and species richness when compared to the other birds (Table 1). In addition, wood duck also displayed a low mean trematode intensity and low species richness during the spring season when compared to blue-winged teal. These patterns remain similar to those observed in Chapter 1, where wood duck displayed a lower mean trematode intensity and low species richness when compared to blue-winged teal and lesser scaup.

A significant difference (U = 66.500; P = 0.000296) of the mean trematode intensity between the fall and the spring migration was found, with the fall birds possessing more trematodes when all birds independent of species were pooled together. When mean intensity was analyzed on an individual species level, 7 out of the 11 instances where the same trematode was found in the same host species during both migration periods showed higher mean intensities during the fall as compared to the spring. Also, a significant difference in mean trematode species richness was found between the birds collected during fall and the spring (U = 82.000; P = 0.001). This finding indicates that the birds collected during the fall possessed significantly more trematodes and greater species richness than birds collected during the spring.

Trematodes from Snails:

Overall, 1,613 snails comprised of 6 different species were collected from Lake Winnibigoshish (Table 3). The snails collected from the limnetic zone were *Bithynia tentaculata*, *Viviparus georgianus*, and *Cipangopaludina chinensis*, while those from the littoral zone were *Lymnaea stagnalis*, *Helisoma trivolvis*, and *Lymnaea elodes*. The most common snail collected from the limnetic zone was *B. tentaculata*, whereas *L. stagnalis* was the most abundant during the littoral zone collections. In addition, the least abundant snail species collected from the limnetic zone was *C. chinensis* while *L. elodes* was the least common snail during littoral zone collections (Table 3).

Three out of the six snail species were infected as either first or second intermediate hosts by six different types of trematodes. Interestingly, each snail species also had 2 distinct trematode types. Snails from the limnetic zone showed different patterns of infections as compared to those recovered from the littoral zone. Specifically, *B. tentaculata*, which was collected only in the limnetic zone, served as a first and second intermediate host for two trematode species (*Cyathocotyle bushiensis* and *Sphaeridiotrema pseudoglobulus*), with the prevalence of second intermediate hosts being higher than that of first intermediate hosts. Among the infected snails from the littoral zone, *L. stagnalis* was a first intermediate host for 2 trematode types and was both a first and second intermediate host for 1 of these trematodes (Table 4). Contrary to the pattern in *B. tentaculata*, prevalence of first intermediate hosts from the littoral zone was higher than that of second intermediate hosts for trematodes that were present as first and second intermediate host stages.

DISCUSSION

The aims of the current investigation were two-fold. The first objective was to determine if the trematode communities found within waterfowl collected from Lake Winnibigoshish were different between the fall and spring migratory seasons. Secondly, the study focused on examining trematode life cycles within snail hosts from the same lake to determine whether or not the previously surveyed waterfowl could have been infected during their time at Lake Winnibigoshish. Our findings indicate that differences in mean trematode intensity and trematode species richness occur in waterfowl during their fall and spring migrations. With regard to the second objective, *C. bushiensis* and *S. pseudoglobulus*, two pathogenic trematode species, were maintained likely to be maintained at Lake Winnibigoshish given that waterfowl were infected with the adult forms of the abovementioned species and the snails examined, specifically *B. tentaculata*, were found to serve as both the first and second intermediate hosts.

In the 52 birds examined in the present study, variation in the number of trematodes as well as the trematode species present was observed among the 9 different species of waterfowl. These differences were also noted among the waterfowl collected during each migration season. For instance, when the trematodes from the birds collected during the fall were compared to those harvested during the spring, the greatest mean intensity (Table 2) and species richness (14 vs. 8 for the fall and spring migratory seasons, respectively) was found in the collection of fall-harvested birds. This finding is not surprising given that Buscher (1965) also noted differences in the helminth communities of waterfowl based on the timing of their migration. It is interesting to note among the 38 fall-harvested birds examined in this study, 5 trematodes species, namely *C. bushiensis, H. conoideum, N. affine, N. lakotae,* and *O. ptychocheilus,* were unique to this group whereas only 1 species, *D. spathaceum*, was exclusive to the group of waterfowl collected during the spring.

According to Buscher (1965), there is a reduction in helminth intensity in waterfowl depending on when the birds are collected during the migratory season. In the current investigation, the spring-harvested birds displayed a much lower rate of infection that those collected during the fall. The decreased trematode intensities and species richness of the 14 spring-harvested birds may be attributed to the fact that during the spring migration, the birds travel north (as opposed to the south in the fall), thus there is a greater distance before reaching Lake Winnibigoshish. Consequently, there may be a greater possibility of the birds shedding the trematodes in route and/or less availability of an intermediate host to obtain an infection.

Of the 1,613 snails examined in this work, ~ 20 percent were infected by one or more trematode species. Interestingly, *B. tentaculata,* one of the 6 snail species observed, exhibited the highest rate of infection, a finding that is consistent with those presented in other published reports (Herrmann and Sorensen 2009). It should also be noted that during sample collection, this species of snail was found to exhibit a clustered distribution in the limnetic zone, meaning that many individuals were isolated in a small sample location. In contrast, *H. trivolvis* and *L. stagnalis,* the other two commonly infected snail species, were typically more dispersed throughout the littoral zone. This clustering pattern suggests that infected *B. tentaculata* snails will likely infect other snails as a second intermediate host at a higher rate than would infected *H. trivolvis* and *L. stagnalis*.

Subsequent comparisons of the adult and larval trematode species suggested that in our sample the proper larval stage and intermediate host was not present at Lake Winnibigoshish, with the exception of two trematode species, *C. bushiensis* and *S. pseudoglobulus*, which were present in both the snails (larval) and the birds (adult) examined. This suggests that these two trematode species permanently reside in the snail population at Lake Winnibigoshish and that these snails provide a source for subsequent infections of migrating waterfowl.

Altogether, the results of this study indicate that multiple factors could be involved in the patterns observed in the trematode communities found within Lake Winnibigoshish. The differences noted between the fall- and spring-harvested birds is likely attributed to the timing of collection (which would allow for potential exposure to intermediate hosts), potential loss of trematodes, and/or differences in host feeding ecology. This variation in trematode diversity between the fall and spring migratory seasons may provide important clues to other scientists examining trematode communities within waterfowl. Finally, this study illustrates that larval stages can successfully be used to determine if infections could have originated in a particular ecosystem, such as the one studied in this report.

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Table 1. Prevalence values for collected trematode species from waterfowl harvested during the fall (F) or spring (S) migration.

Species	BWT (F)	BWT (S)	LS (F)	LS (S)	WD (F)	WD (S)	B (F)	B (S)	CG (S)	GS (S)	GWT (S)	NP (F)	R (F)
C. bushiensis	-	-	90 (9)	-	-	-	-	-	-	-	-	-	-
D. spathaceum	-	-	-	-	-	-	-	-	25 (1)	-	-	-	-
E. recurvatum	12.5 (2)	-	100 (10)	100 (1)	-	-	-	-	-	100 (1)	-	-	-
E. revolutum	18.8 (3)	50 (1)	30 (3)	100 (1)	-	-	-	-	-	-	-	-	100 (1)
Family Psilostomidae	-	-	-	-	57.1 (4)	-	-	50 (1)	25 (1)	-	-	100 (1)	-
Family Strigeidae	75 (12)	100 (2)	80 (8)	-	-	-	-	-	-	-	-	100 (1)	-
H. conoideum	6.3 (1)	-	30 (3)	-	-	-	-	-	-	-	-	-	-
M. obstipum	12.5 (2)	-	40 (4)	100 (1)	-	-	66.7 (2)	50 (1)	25 (1)	-	-	-	-
N. affine	-	-	10 (1)	-	-	-	-	-	-	-	-	-	-
N. lakotae	-	-	10 (1)	-	-	-	-	-	-	-	-	-	-
N. lisitsynae	81.3 (13)	-	70 (7)	100 (1)	-	-	-	-	-	-	-	-	-
N. attenuatus	37.5 (6)	-	20 (2)	-	-	33.3 (1)	-	-	25 (1)	-	-	-	-
O. ptychocheilus	-	-	20 (2)	-	-	-	-	-	-	-	-	-	-
S. pseudoglobulus	31.3 (5)	-	80 (8)	100 (1)	-	-	33.3 (1)	-	-	-	-	-	100 (1)
Z. lunata	31.3 (5)	50 (1)	50 (5)	100 (1)	85.7 (6)	33.3 (1)	-	-	-	-	-	-	100 (1)

Table 2. Mean intensity of trematodes collected from waterfowl harvested during the fall (F) or spring (S) migration season.

Species	BWT (F)	BWT (S)	LS (F)	LS (S)	WD (F)	WD (S)	B (F)	B (S)	CG (S)	GS (S)	GWT (S)	NP (F)	R (F)
C. bushiensis	-	-	107.7±93.7	-	-	-	-	-	-	-	-	-	-
D. spathaceum	-	-	-	-	-	-	-	-	1	-	-	-	-
E. recurvatum	130.5±8.1	-	116±47.3	32	-	-	-	-	-	21	-	-	-
E. revolutum	20.6±14.9	1	5±1.7	35	-	-	-	-	-	-	-	-	26
Family Psilostomidae	9.3±2.4	2.5±2.1	-	-	18.5±9.9	-	-	37	2	-	-	3	-
Family Strigeidae	4	-	18.6±4.6	-	-	-	-	-	-	-	-	15	-
H. conoideum	7±6.0	-	5.7±2.7	200	-	-	-	-	1	-	-	-	-
M. obstipum	-	-	11±3.7	-	-	-	144±2.8	100	-	-	-	-	-
N. affine	-	-	8±0.0	-	-	-	-	-	-	-	-	-	-
N. lakotae	525.6± 25.9	-	4±0.0	7	-	-	-	-	-	-	-	-	-
N. lisitsynae	1±0	-	151±73.4	-	-	3	-	-	1	-	-	-	-
N. attenuatus	-	-	214±23	-	-	-	-	-	-	-	-	-	-
O. ptychocheilus	-	-	2.5±1.5	-	-	-	-	-	-	-	-	-	-
S. pseudoglobulus	45.4±9.0	-	3688.5±1303.7	1	-	-	52	-	-	-	-	-	1033
Z. lunata	2.4±0.68	4	5±1.7	1	1.7±0.3	1	-	-	-	-	-	-	1

Table 3. Total number of snails collected from Lake Winnibigoshish, Minnesota.

Snail Species	Zone	Total
Bithynia tentaculata	Limnetic	760
Viviparus georgianus	Limnetic	284
Cipangopaludina chinensis	Limnetic	13
Lymnaea stagnalis	Littoral	401
Helisoma trivolvis	Littoral	145
Lymnaea elodes	Littoral	10

Table 4. Prevalence of snail species infected as either a 1st or 2nd intermediate host.

	Snail Species					
	B. tentaculata		*H. trivolvis*		*L. stagnalis*	
Larval Trematodes	1st	2nd	1st	2nd	1st	2nd
C. bushiensis	0.13%	23.4%	-	-	-	-
E. trivolvis	-	-	12%	-	-	-
Family Echinostomatidae	-	-	-	2.1%	-	-
Family Schistosomatidae	-	-	-	-	4.7%	-
Family Strigeidae	-	-	-	-	4.2%	2.2%
Magnacauda Cercariae	-	-	1.4%	-	-	-
S. pseudoglobulus	0.66%	12.1%	-	-	-	-

CONCLUSION

Trematode communities of waterfowl examined during this study exhibited a total of 14 trematode species. Seasonally, trematode populations and communities were highly variable during the fall and spring migration and were also variable within host species that were sampled during the fall and spring migration. Within the 3 waterfowl species used for community analysis, statistically significant differences in mean trematode intensity and mean trematode species richness were detected. Overall, lesser scaup were found to have significantly higher number of trematodes and species richness when compared to blue-winged teal and wood duck. These results could be attributed to lesser scaup feeding on more abundant invertebrate species, which would increase the likelihood for a trematode infection.

Furthermore, results from the comparison of fall migration to spring migration found significant differences in the mean trematode intensity and mean species richness between waterfowl hosts collected during those periods with the fall migration period having significantly higher mean trematode intensity and mean trematode species richness. Additionally, when larval trematode stages were compared to adult stages that were found in waterfowl, 2 trematode species were found to have potential infections occur at Lake Winnibigoshish. Overall, this thesis presents a comprehensive study on the trematode communities found within waterfowl definitive hosts as well as larval trematode communities that are essential for wildlife researchers as work is being performed on both this system and other large lake systems. Due to the large amount of trematode diversity found within this study in both intermediate and definitive hosts, wildlife researchers will be able to use this information to aid in the identifications of trematodes at this location including the patterns of trematode community structure which could change over time at this location. Future experiments will need to look at the various life cycles of these trematodes to see which hosts (both intermediate and definitive) are compatible with one another.