CORNERSTONE
MINNESOTA STATE UNIVERSITY MANKATO

Journal of Undergraduate Research at Minnesota State University, Mankato

Volume 13 | Article 6

2013

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Recommended Citation
Ogunnowo, Omolayo (2013) "The Abundance and Diversity of Intestinal Trematodes Collected from Blue Winged Teal and Ring-Neck Ducks Inhabiting Lake Winnibigoshish, Minnesota," *Journal of Undergraduate Research at Minnesota State University, Mankato*: Vol. 13, Article 6.
DOI: https://doi.org/10.56816/2378-6949.1005
Available at: https://cornerstone.lib.mnsu.edu/jur/vol13/iss1/6

The Abundance and Diversity of Intestinal Trematodes Collected From Blue Winged Teal

and

Ring-Neck Ducks Inhabiting Lake Winnibigoshish, Minnesota

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and

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Abstract

This study investigated the abundance and diversity of parasites residing in the digestive tracts of blue winged teal and ring-necked ducks collected at Lake Winnibigoshish, MN. The trematode parasites we focused on in this study were removed from the anterior-most, 15cm segment of the small intestine of 10 individuals of each bird species. The parasites were initially stored in 10% formalin and were subsequently stained with Schneider's aceto carmine, then mounted on microscope slides for diagnostic purposes. A total of 1605 trematodes were recovered from the birds. We found that blue wing teal and ring-necked ducks tended to contain different species of parasites. Six of the 8 trematode "Types" we collected were identified to the species-level. Based on the identification of these parasites, we were able to determine that all of them are transmitted to their vertebrate hosts as metacercariae. This finding is consistent with our hypothesis that these birds are encountering different parasites based on differences in their feeding behaviors and diet preference.

DOI: 10.56816/2378-6949.1005

Introduction

Interactions between parasites and hosts are common in nature. The community of parasites that interacts with a particular host species is limited by the extent to which the behavior of individual hosts puts them in the proximity of the parasite. Many intestinal parasites rely on the feeding behavior and diet preferences of their hosts as a means of gaining access to the host's digestive system. In other words, intestinal parasites rely upon having their hosts ingest them. In this study, we assess the diversity of parasites residing in the gastrointestinal (GI) tracts of two waterfowl species that are often found in sympatry, that is, they exist in the same geographical location, especially during the migratory season,and have the potential to interact with one another.

Blue winged teal, *Anas discors*, and ring-necked duck, *Aythya collaris*, are both common in North America, with the teal primarily being found in freshwater lakes, streams, and ponds (American Museum of Natural History, AMNH, 2009, p. 64) while the ring-necked duck is found mainly in freshwater ponds or lakes (AMNH, 2009, p. 71). Characteristics of the diet and habitat of these two duck species provide for an interesting comparison. Ring-necked ducks are diving feeders. That is, they dive in shallow freshwater lakes or ponds to feed on submerged plant and invertebrate animal matter. Blue winged teal, on the other hand, are surface feeders and obtain food near the surface of waters they inhabit. Similar to the ring-neck duck, blue winged teal feed on invertebrate animal matter. More specifically, blue winged teal feed primarily on insect larvae and snails when breeding and on plant seeds during the winter season ((Rohwer, Johnson & Loos, 2002). Ring-necked ducks also feed on snails, in addition to small clams and plant tubers (Hohman & Eberhardt, 1998).

We hypothesize that there will be differences in the gastrointestinal (GI) parasite communities for these two bird species that relates to differences in the invertebrates they ingest. More specifically, if different invertebrate species are found at different depths in the water column and these different invertebrate species are infected by different parasites, then the two duck species will be collecting different parasites based on their differing feeding behaviors.

Methods

Collection of Birds

The birds utilized in this study were obtained in the fall of 2012, at Lake Winnibigoshish, in northern Minnesota, as part of a study being conducted by Ms. Holly Bloom, a graduate student being advised by Dr. Robert Sorensen, Ph.D. Ms. Bloom collected the GI tracts of these birds from waterfowl hunters at this site. These GI tracts were placed in zip-lock bags and labeled with identifying information about the bird and the approximate location where the bird was harvested. These bags were kept on ice and transported to Minnesota State University, Mankato, where they were stored in a freezer at -20° C. until they were thawed for parasite removal.

Removal of the Parasites

Frozen GI tracts were thawed using room temperature water to float sample bags until the contents were easily manipulated. GI tracts were dissected into 15-cm portions beginning post-gizzard and working down the length of the tract (Herrmann 2007). Each GI segment was placed into a Petri dish, where it was cut longitudinally and its contents rinsed into the Petri dish using tap water. Intestinal material and rinsate were examined using a dissecting microscope for the presence of parasites. All parasites within the GI tract were given preliminary “Type”

DOI: 10.56816/2378-6949.1005

identifications, counted, recorded, and stored in 10% formalin until more complete identifications were performed.

Identification of Parasite Species

Identification of the parasite species relied upon comparing morphological features and morphometric measurements of our specimens to the same features of previously recorded species. Several published dichotomous keys (McDonald, 1981; Yamaguti, 1958) guided this process. Identification of the morphological features and measurement of the morphometric features required that some of worms from each parasite “Type” be stained, cleared, and mounted on microscope slides to assess those features.

Attempts were made to identify all collected parasites to the species level, if possible, and to the most specific level, if species level distinction was not possible. Identification involved removing the specimens from the formalin they were stored in by moving the worms through a series of ethanol solutions, staining them Schneider’s aceto-carmine stain to visual the diagnostic features, and clearing them with xylene to remove the ethanol (Pritchard, 1982). The cleared worms were placed in a Kleermount medium on standard glass microscope slides and covered standard glass cover slips. The slides were examined by light microscopy at magnifications up to 400X. Digital photomicrographs were captured of the stained and unstained worms using Motic Images Plus 2ML software and a Motic digital camera. Measurements of the morphometric features were made using the Motic Images Plus software.

RESULTS

Parasite Identification

Identification of GI parasites from both bird species was restricted to the trematode worms (Class Trematoda) inhabiting the anterior-most 15-cm segment of the birds’ intestine for

10 birds from each species. Analysis was restricted to the first segment of the small intestine due to the abundance and diversity of parasites that were encountered throughout the entire intestine. A total of 1605 trematode worms were recovered from the anterior-most segment of the 20 birds. These worms were initially grouped into 8 types based on morphological characteristics at the time the worms were removed.

Further analysis of these worms allowed us to identify 6 trematode species among the 8 “Types.” Types C and F (Figure 1) from blue winged teal are yet to be assigned to a species. We are certain that “Type C” is a member of Family Echinostomatidae and “Type F” is believed to be a member of Family Psilostomatidae. The six identified species are *Apatemon gracilis* (Family Strigeidae), *Cotylurus flabelliformis* (Family Strigeidae), *Diplostomum mergi* (Family Diplostomatidae), *Echinoparyphium aconiatum* (Family Echinostomatidae), *Psilotrema mediopora* (Family Psilostomatidae), and *Sphaeridiotrema pseudoglobulus* (Family Psilostomatidae) (Figure 1).

All of these species are transmitted to their vertebrate hosts as metacercariae, which are a larval resting stage that resides in or on other hosts. The metacercariae of members of Family Strigeidae inhabit fish, amphibians, and leeches (McDonald, 1981). Metacercariae of members of Families Echinostomatidae and Psilostomatidae encyst within amphibian or molluscan intermediate hosts (McDonald, 1981). Metacercariae of Family Diplostomatidae encyst on a variety surfaces including amphibian skin, mollusk shells, and vegetation.

Apatemon gracilis

Cotylurus flabelliformis

Diplostomum mergi

DOI: 10.56816/2378-6949.1005

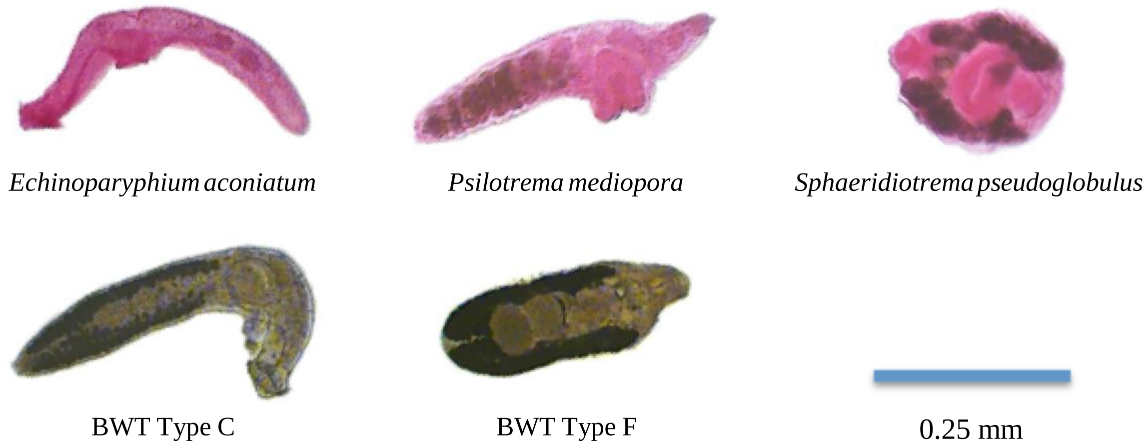

Figure 1. Representative stained and unstained trematodes. Images were captured using a digital camera at 40X magnification through the ocular lens of a compound dissecting microscope. All worm images are at the same scale. Scale bar shows the length of a 0.25mm line. Stained worms were treated with Schneider's aceto-carmine prior to mounting on microscope slides.

Parasite Diversity and Abundance

There were 1041 trematodes recovered from blue winged teal 564 recovered from ring-necked ducks (Tables 1 and 2). These worms were categorized into 6 trematode "Types" recovered from ring-neck ducks and 4 from blue winged teal.

Table 1. Abundance and diversity of worms found in ring-necked ducks. The AP–CF, columns represent the number of worms of different trematode species found within 10 ring-necked ducks (RN1-RN10). The "Total" column gives the number of worms of all species found within that duck. The "Total" row describes the number of worms found in the first 15-cm segment of the small intestine of all the birds examined. The "AVG row describes the average number of worms of a given species found among all 10 ducks. The SE row displays the standard error of that mean. The abbreviations for the parasite species are as follows: AP=*Apatemon gracilis*, SP=*Sphaeridiotrema pseudoglobulus*, EA=*Echinoparyphium aconiatum*, DM=*Diplostomum mergi*, PM=*Psilotrema mediopora*, and CF=*Cotylurus flabelliformis*.

Bird #	Parasite species						
	AP	SP	EA	DM	PM	CF	Total
RN1						4	4
RN2	5	8				8	21
RN3	8			17			25
RN4	1	1					2
RN5	21		1				22
RN6			1	207		3	211
RN7	57		39		20	2	118
RN8	23	3		16	57		99
RN9	3						3
RN10	52		2	5			59

Total	170	12	43	245	77	17	564
AVG	21.25	4.00	10.75	61.25	39.00	4.25	56.4
SE	15.27	0.81	4.61	23.84	8.36	0.68	20.37

Table 2. Abundance and diversity of worms found in blue winged teal. The PM–F columns represents the number of worms of a trematode species found within 10 blue winged teal (BWT1–BWT10) The "Total" column gives the number of worms of all species found within that duck. The "Total" row describes the number of worms found in the first 15-cm segment of the small intestine of all the birds examined. The "AVG row describes the average number of worms of a given species found among all 10 ducks. The SE row displays the standard error of that mean. The abbreviations for the parasite species are as follows: PM=*Psilotrema mediopora*, CF=*Cotylurus flabelliformis*, C=unassigned species from Family Echninostomatidae, and F=unassigned species from Family Psilostomatidae.

Bird #			Parasite species		
	PM	CF	C	F	Total
BWT1	80	17			97
BWT2					0
BWT3	2	1			3
BWT4	34	2	11		47
BWT5	64	4		30	98
BWT6	22			1	23
BWT7		3	3	1	7
BWT8	459		1		460
BWT9	47		6		53
BWT10	189		64		253
Total	897	27	85	32	1041
AVG	118.38	5.40	17.00	10.67	104.1
SE	87.82	2.86	9.84	4.30	43.80

The largest number of worms was found in a blue winged teal that possessed 459 *Psilotrema mediopora* worms and 1 "Type C" worm. A large number of *P. mediopora* (N=189) were also found in another blue winged teal (Table 2). In fact, *P. mediopora* was the most common parasite in blue winged teal and was found in 8 of those 10 birds, with a range of 2–459 worms among infected birds (±SE=112.13±50.03). *Cotylurus flabelliformis* and "Type C" worms were present in 5 of the 10 birds and had ranges of 1–17 and 1–64 among infected birds, respectively.

Apatemon gracilis was the most common trematode in the ring-necked ducks and was found in 8 of the 10 birds (Table 1). The average number of *A. gracilis* in these birds was 21.25±7.30 and the range was 1–52. *Diplostomum mergi* was the most abundant trematode in the ring-necked ducks, primarily due to one bird that possessed 207 individual worms. *D. mergi* was one of 4 trematodes that were found in 4 of the ring-necked ducks.

DOI: 10.56816/2378-6949.1005

C. flabelliformis and *P. mediopora* (Figure 2) were the only trematodes that were found in the anterior-most segment of the small intestine of both bird species; however both were more likely to be found in blue wing teal based on their abundance within and among individuals of the two bird species (Tables 1 and 2).

Discussion

This research demonstrates the abundance and diversity of trematode parasites in the intestinal tract of the two duck species studied. Although trematode parasites were found to be very common in these birds, there was a great deal of variation among individual birds in the number of worms they possessed and the specific identity of those worms. It was interesting that all, but one, of the birds examined possessed trematode parasites in the anterior-most part of their small intestines. Likewise, all but 3 birds, possessed at least 2 species of trematodes in that segment of their intestine..

With respect to the diversity of intestinal parasites, we found that blue wing teal appear to harbor more trematodes than ring-necked ducks; however, the teal also appear to harbor fewer species of trematodes in the anterior-most part of their intestine. The 2 bird species tended to contain different species of parasites, based on the information presented in Tables 1 and 2, but they did share 2 of the species. This finding is consistent with our hypothesis that these birds are encountering different parasites based on differences in their feeding behaviors. The fact that all these parasite species possess metacercariae, which could be found in prey items consumed by these birds also supports our hypothesis about diet preferences affecting the diversity of parasites that these birds encounter.

These findings suggest that it could be worthwhile to sample invertebrates at various depths in the water column where these birds were collected. Metacercariae of the parasites found in ring-necked ducks should be found in animals residing deeper in the water column than the metacercariae of the species primarily infecting blue winged teal. Likewise, since *C. flabelliformis* and *P. mediopora* were found in both bird species and these birds primarily feed a different depths, the metacercariae of these two species may be in a more diverse group of prey items than the other trematodes or the hosts that harbor metacercariae of *C. flabelliformis* and *P. mediopora* must make fuller use of the water column.

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Professional Biographies

Omolayo Ogunnowo

I am originally from Lagos, Nigeria in West-Africa and now a Nigerian-American, St. Paul, Minnesota is where I call Home. I am currently an undergraduate student at Minnesota State University, Mankato double majoring in Biomedical Sciences with concentration in Pre-Medicine and Gender & Women's Studies. I anticipate graduating in May 2015 with a BS in Biomedical Sciences and a BS in Gender & Women's Studies. I used this research as a means of increasing my knowledge and understanding in Biological Sciences. I have presented this research at the 2013 Minnesota Conferences of Undergraduate Scholarly and Creative Activity and the 2013 Undergraduate Research Symposium. I anticipate presenting this research at the 21st Annual California McNair Scholars Symposium hosted by University of California at Berkeley. I plan to begin medical school in 2015 working towards an MD/MPH dual degree.

Dr. Robert Sorensen

Dr. Sorensen is an Associate Professor at the Department of Biological Sciences. Dr. Sorensen received his Ph.D. for Purdue University in 1997. Dr. Sorensen teaches a variety of Biological Sciences courses including Biology 105: General Biology I, Biology 316: Animal Diversity and Biology 420: Diagnostic Parasitology. Dr. Sorensen's research focuses on the population biology, evolution and genetics of host-parasite interactions by utilizing molecular and organismal approaches in the laboratory and the field.

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DOI: 10.56816/2378-6949.1005