

The parasite communities of two populations of walleye (*Sander vitreus*) from Black Bay and Circle Lake, Ontario, Canada

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Abstract

The parasite communities of fishes can provide important information on certain aspects of host biology such as where they have been, what they are eating, and what's eating them. Unfortunately, fish parasite studies in the Laurentian Great Lakes have been considerably neglected in recent years—with this being especially true for Lake Superior. Black Bay once harboured the most economically important walleye (*Sander vitreus*) population on Lake Superior; however, historically it has been subject to a wide array of anthropogenic influences that ultimately contributed to a population collapse. Recently, this population has shown signs of recovery. This in turn presents a unique opportunity to investigate its parasite community and gain insight on the biology of the host. In the absence of historical parasite data within Black Bay, walleye parasite communities were compared to that of Circle Lake instead. Circle Lake is considerably different in both abiotic and biotic composition; however it is close in geographic proximity and resides in a watershed that drains into Black Bay. Thus, available parasite species were predicted to be similar. Quantitative parasite data were gathered from 43 walleye from Black Bay, and 46 walleye from Circle Lake. In total, 7 parasite species were found infecting Black Bay walleye (*Ergasilus caeruleus*, *Urocleidus aculeatus*, *Bothriocephalus cuspidatus*, *Azygia angusticauda*, *Proisorhynchoides pusilla*, *Diplostomum* sp., *Echinorhynchus* sp.) and 14 were found infecting Circle Lake walleye (*Ergasilus caeruleus*, *Urocleidus aculeatus*, *Clinostomum complanatum*, *Glochidia*, *Bothriocephalus cuspidatus*, *Proisorhynchoides* sp., *Triaenophorus nodulosus*, *Azygia angusticauda*, *Sanguinicola occidentalis*, *Diplostomum* sp., *Proisorhynchoides pusilla*, *Neascus* sp., *Camallanus oxycephalus*, *Raphidascaris* sp.). The relationship between host attributes and parasite abundance and intensity was variable across both sample locations. There was no relationship between host attributes and species richness or diversity. In 2 of 6 co-occurring species, prevalence was significantly higher in Circle Lake walleye. There was no statistically significant difference in the intensity of shared parasite species. Circle Lake walleye had significantly higher mean infracommunity richness and diversity. Nestedness analysis was used to determine if walleye parasite communities from either sample location were structured. All groupings exhibited a significant nested structure for both sample locations. Despite differences in richness, the order in which species are added as communities assemble showed a similar pattern across sample locations—that is, directly transmitted parasites are acquired first, followed by those using copepods/invertebrates as an intermediate host, and lastly those using forage fish as intermediate hosts. The information gathered in this study reveals that the parasite communities of Black Bay and Circle Lake, although markedly different, perhaps have similar processes contributing to their assembly.

Lay Summary

Mission Statement:

“Faculty and students in the Department of Biology are bound together by a common interest in explaining the diversity of life, the fit between form and function, and the distribution and abundance of organisms.”

Parasites can provide important ecological information on host dietary habits, behaviour, and population health. Black Bay once harboured the most economically important walleye (*Sander vitreus*) on Lake Superior. However, anthropogenic pressures lead to a population collapse. Prior to the present study, baseline information on the parasite community of Black Bay walleye has never existed. As these fish enter a state of recovery, parasitological information can be used to supplement management geared towards rehabilitation. Furthermore, the current status of the stock presents a unique situation to compare the walleye parasite community of a historically exploited population to that of one which has been unaltered. Cross-sample comparisons can provide insight into species regionally available as well as the local factors contributing to the assembly of parasite communities.

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My obsession with fish starting at a young age has somehow guided me to where I am now: studying the little critters that live inside of them.

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Table of Contents

ABSTRACT	II
LAY SUMMARY	I
ACKNOWLEDGEMENTS	II
TABLE OF CONTENTS	III
LIST OF TABLES	IV
LIST OF FIGURES	V
INTRODUCTION	1
WALLEYE PARASITES IN LAKE SUPERIOR.....	2
PARASITE COMMUNITY ECOLOGY	3
HYPOTHESES	6
MATERIALS AND METHODS	8
STUDY AREA - BLACK BAY	8
STUDY AREA - CIRCLE LAKE	10
HOST AND PARASITE SPECIMEN COLLECTION	13
<i>Black Bay</i>	13
<i>Circle Lake</i>	13
<i>Host Measurements and Necropsies</i>	14
<i>Parasite Specimen Processing</i>	14
ANALYSIS.....	15
<i>Diversity</i>	16
<i>Cross-Sample Comparison</i>	17
<i>Nestedness Analysis</i>	17
RESULTS	21
BLACK BAY & CIRCLE LAKE.....	21
ECTOPARASITES	21
ENDOPARASITES.....	32
COMPARISON OF CO-OCCURRING PARASITES.....	42
COMPARISON OF PARASITE DIVERSITY.....	42
NESTEDNESS ANALYSIS	47
DISCUSSION	50
LITERATURE CITED	66
APPENDIX A – CODE USED IN R PROGRAMMING LANGUAGE	73
APPENDIX B – OUTPUT OF NED (NESTEDNESS) SOFTWARE	76

List of Tables

TABLE 1 BLACK BAY FISH COMMUNITY.....	9
TABLE 2. RESULTS OF KRUSKAL-WALLIS TEST COMPARING HOST ATTRIBUTES BETWEEN BLACK BAY AND CIRCLE LAKE.....	22
TABLE 3. STOMACH CONTENTS OF BLACK BAY AND CIRCLE LAKE WALLEYE.	23
TABLE 4. PREVALENCE, MEAN ABUNDANCE, MEAN INTENSITY, AND INTENSITY RANGE OF ECTOPARASITES	24
TABLE 5. VARIANCE TO MEAN RATIOS FOR BLACK BAY AND CIRCLE LAKE ECTOPARASITE	26
TABLE 6. RESULTS OF KENDALL’S RANK CORRELATION COEFFICIENT COMPARING BLACK BAY AND CIRCLE LAKE WALLEYE ATTRIBUTES TO ECTOPARASITE ABUNDANCE.	29
TABLE 7. RESULTS OF KENDALL’S RANK CORRELATION COEFFICIENT COMPARING BLACK BAY AND CIRCLE LAKE WALLEYE ATTRIBUTES TO ECTOPARASITE INTENSITY.....	30
TABLE 8. RESULTS OF MANN WHITNEY U TEST COMPARING BLACK BAY AND CIRCLE LAKE WALLEYE GENDER TO PARASITE ABUNDANCE. .	31
TABLE 9. PREVALENCE, MEAN ABUNDANCE, MEAN INTENSITY, AND INTENSITY RANGE OF ENDOPARASITES I	33
TABLE 10. VARIANCE TO MEAN RATIOS FOR BLACK BAY ENDOPARASITE SPECIES.....	35
TABLE 11. RESULTS OF KENDALL’S RANK CORRELATION COEFFICIENT COMPARING BLACK BAY AND CIRCLE LAKE WALLEYE ATTRIBUTES TO ENDOPARASITE ABUNDANCE.	39
TABLE 12. RESULTS OF KENDALL’S RANK CORRELATION COEFFICIENT COMPARING BLACK BAY AND CIRCLE LAKE WALLEYE ATTRIBUTES TO ENDOPARASITE INTENSITY.	40
TABLE 13. RESULTS OF MANN WHITNEY U TEST COMPARING BLACK BAY AND CIRCLE LAKE HOST GENDER AGAINST ENDOPARASITE ABUNDANCE.....	41
TABLE 14. RESULTS OF FISHER’S EXACT TEST OF INDEPENDENCE AND BOOTSTRAP TEST COMPARING THE PREVALENCE AND MEAN INTENSITIES OF PARASITES IN COMMON TO BLACK BAY AND CIRCLE LAKE WALLEYE.	43
TABLE 15. RESULTS OF KENDALL’S RANK CORRELATION COEFFICIENT TESTING THE EFFECTS OF HOST ATTRIBUTES ON INFRACOMMUNITY RICHNESS AND INFRACOMMUNITY DIVERSITY.	44
TABLE 16. RESULTS OF NESTEDNESS ANALYSIS.	48

List of Figures

FIGURE 1. BLACK BAY, LAKE SUPERIOR.	10
FIGURE 2. CIRCLE LAKE.....	12
FIGURE 3. AN EXAMPLE OF A PERFECTLY NESTED COMMUNITY (L) AGAINST ONE THAT IS RANDOMLY ASSEMBLED (R).	18
FIGURE 4. NUMERICAL DISTRIBUTION OF THE ECTOPARASITES INFECTING BLACK BAY WALLEYE.....	27
FIGURE 5. NUMERICAL DISTRIBUTION OF THE ECTOPARASITES INFECTING CIRCLE LAKE WALLEYE.	28
FIGURE 6. NUMERICAL DISTRIBUTION OF ENDOPARASITES INFECTING BLACK BAY WALLEYE.....	36
FIGURE 7. NUMERICAL DISTRIBUTION OF ENDOPARASITES INFECTING CIRCLE LAKE WALLEYE.....	37
FIGURE 8. NUMERICAL DISTRIBUTION OF PARASITE INFRACOMMUNITY RICHNESS ACROSS EACH SAMPLE LOCATION.	45
FIGURE 9. ORDERED PLOT SHOWING SIMPSON’S DIVERSITY INDEX FOR PARASITE INFRACOMMUNITIES IN BOTH BLACK BAY AND CIRCLE LAKE. EACH CIRCLE REPRESENTS AN INFRACOMMUNITY.....	46
FIGURE 10. IMPORTANCE OF PARASITE SPECIES AND THEIR CONTRIBUTION TO A NESTED PATTERN FOR A GIVEN CATEGORY.	49
FIGURE 11 NESTEDNESS ANALYSIS FOR ALL PARASITE SPECIES FOUND IN BLACK BAY WALLEYE.	78
FIGURE 12 NESTEDNESS ANALYSIS FOR ALL ADULT PARASITE SPECIES FOUND IN BLACK BAY WALLEYE.....	79
FIGURE 13 NESTEDNESS ANALYSIS FOR ALL ECTOPARASITE SPECIES FOUND IN BLACK BAY WALLEYE	80
FIGURE 14 NESTEDNESS ANALYSIS FOR ALL ENDOPARASITE SPECIES FOUND IN BLACK BAY WALLEYE.	81
FIGURE 15 NESTEDNESS ANALYSIS FOR ALL PARASITE SPECIES FOUND IN CIRCLE LAKE WALLEYE.	82
FIGURE 16 NESTEDNESS ANALYSIS FOR ALL ADULT PARASITE SPECIES FOUND IN CIRCLE LAKE WALLEYE.....	83
FIGURE 17 NESTEDNESS ANALYSIS FOR ALL ECTOPARASITE SPECIES FOUND IN CIRCLE LAKE WALLEYE	84
FIGURE 18 NESTEDNESS ANALYSIS FOR ALL ENDOPARASITE SPECIES FOUND IN CIRCLE LAKE WALLEYE.....	85

Introduction

Walleye (*Sander vitreus*; Mitchill 1818) is one of the most important freshwater fish species in North America from a sport and commercial standpoint (Scott and Crossman 1973). In Northwestern Ontario, the Ministry of Natural Resources recognizes 1368 walleye lakes readily accessible to anglers within a 300 km radius of Thunder Bay (Ministry of Natural Resources and Forestry 2012). Historically, one of the most significant walleye populations in the Thunder Bay region existed in Black Bay, on Lake Superior. During the mid to late 1960's, Black Bay harboured the largest commercial walleye fishery on Lake Superior (Berglund 2016). A combination of overfishing, dam construction, and other anthropogenic pressures led to a rapid decline in walleye abundance in the late 1960's and eventual collapse of the fishery in the early 1970's (Bobrowicz 2010). Since the collapse of the fishery, various efforts have been employed in an attempt to rehabilitate the population, including closure of the fishery, stocking, and the addition of management boundaries (Furlong et al. 2006). A series of recent surveys, starting in the early 2000's, suggest that the Black Bay walleye population is beginning to recover (Berglund 2016).

Parasites often have a negative connotation due to their capacity to cause harm to host populations; however their application to ecological studies should not be overlooked. Identifying and quantifying the parasite community of a host species can provide valuable information on host diet, behaviour, and life history. For example, examination of the stomach contents of a fish will tell you what that fish was feeding on prior to capture. However, because parasites often rely on trophic transmission to infect their host, they provide clues as to what that host has consumed over a longer period of time. Thus, information on complex parasitic lifecycles can in turn identify important prey items that are not represented in the stomach contents. Considering host behaviour, if a fish is infected with digenetic parasites in which the definitive host is a bird then it can be recognized that the fish frequents shallow water where gastropod intermediate hosts occur (Faltýnková et al. 2008). Lastly,

parasites have also been applied to differentiate between fish stocks exhibiting different life history characteristics, but have an overlapping geographic range (Margolis 1965, Mackenzie and Abauza 1998, Marcogliese and Jacobson 2015).

The comparison of parasite communities across sample locations allows for insights into the mechanisms contributing to similarities or differences in parasite abundance, species composition, and community structure. Circle Lake represents a waterbody with considerably different abiotic and biotic features in comparison to Black Bay. However, Circle Lake resides in a watershed that drains into Black Bay and their geographic proximity to one another suggests that their parasites communities may exhibit some degree of similarity. Poulin (2003) hypothesized that similarity in the parasite community composition decays with increasing distance between samples. Therefore, considering the proximity of Circle Lake and Black Bay, we might predict similar parasite communities following Poulin (2003).

Walleye Parasites in Lake Superior

Walleye parasites have been well documented throughout North America due to their popularity as a recreational species and importance as a commercial fish species (Hoffman 1999). There has been much less attention directed towards parasites infecting walleye populations in the Upper Great Lakes (Muzzall and Haas 1998). Lake Superior has only one published survey that characterizes walleye parasites, after examining 15 fish from Eastern Lake Superior (Dechtiar and Lawrie 1988). Furthermore, Lake Superior has the least number of fish parasitological surveys of any of the Laurentian Great Lakes (see Muzzall and Whelan 2011 for review). This lack of information on walleye parasites specifically and Lake Superior fish parasites in general may be due to the limited number of strong walleye populations currently existing in Lake Superior and the smaller human population around Lake Superior. In the northwest quadrant of Lake Superior, Thunder Bay, Black Bay, and Nipigon Bay all harboured significant walleye fisheries historically. However, each have been subjected to extensive fishing pressures that contributed to stock decline (Goodier 1982). The same pattern of fishing pressure

leading to stock decline appears to be apparent on the east end of Lake Superior—with strong historic populations existing in Batchawana and Goulais Bay, as well as sporadically in the rivers draining down the east shore (Goodier 1982). Noteworthy is that at times in the early 20th century, walleye were thought of as nuisance species locally because of their predatory interaction with desirable trout species (Goodier 1982). Furthermore, Scholz and Choudhury (2014) published a review highlighting the decreasing number of studies conducted on the parasites of freshwater fish species in North America over the course of the 20th century. Scholz and Choudhury (2014) attributed this decline to a lack of funding and a decline in the number of new parasitologists. This suggests that perhaps a combination of rapid stock decline, early local disinterest in the host, and a reduction in funding and personnel are important factors that have caused the low number of studies on walleye parasites and fish parasites in general in Lake Superior.

Parasite Community Ecology

Studying parasites offers a unique opportunity for ecological studies in that each host essentially serves as a replicate community once the effect of size, sex, and age on parasite numbers has been controlled (Bush et al. 2001). The study of the complex processes involved in parasite community assembly (i.e. how a parasite community is put together) and structure (i.e. the parasite composition) began decades ago (Dogiel, 1964; Crofton 1971; Holmes 1961, 1962) and has continued to be a significant area of research in modern day parasitology (Poulin 2011). It is the curiosity surrounding the sporadic and dynamic nature of parasites that has sustained this research over the course of the last century (Scholz and Choudhury 2014), with efforts attempting to answer questions such as: “are there general patterns in parasite communities” (Poulin 2007), “is the structure of a parasite community random or predictable” (Poulin 1996), and “what are the ramifications of environmental change on parasite communities” (Lafferty 2003, Palm 2011)?

Due to the complexity of a field conceived by the merging of two parent disciplines (i.e. ecology and parasitology), parasite ecology has developed its own terminology (Margolis et al. 1982, Bush et al. 1997). The infrapopulation, infracommunity, and component community are specific to parasite ecology. The infrapopulation represents the number of individuals of a particular parasite species infecting a single host. The infracommunity is the sum of all infrapopulations existing within an individual host. The component community refers to the set of parasites species present in all of the infracommunities within a host population. This infers that the infracommunity exists as a subset of the component community. While these two terms are fundamental in understanding the importance that species richness plays in parasite community ecology (Poulin 2011), they are restrictive in the sense that they are merely the product of presence-absence data, and thus studies involving parasite diversity must also be accompanied by information on abundance. In the context of parasite communities, mean abundance is the number of individuals of a parasite species present in a host sample, regardless of whether or not the host is infected. Concomitant with mean abundance is mean intensity—which refers to the number of individuals of a distinct parasite species infecting members of a host population. Because intensity omits instances where a parasite is not present, intensity generally plays a more central role with regards to the study of parasite communities simply because if there are no parasites, there is no community. With that said abundance data can assist in identifying transitions in situations where there is a difference between uninfected and infected hosts.

The terms autogenic and allogenic are used to describe whether parasite life cycles require hosts existing across ecosystem boundaries. In autogenic species, a parasite can complete its life cycle within an aquatic ecosystem. In allogenic species, fish or other aquatic vertebrates serve as intermediate hosts and the definitive host is a bird or a mammal (Bush et al. 2001).

Since what we see in a given infracommunity or component community is always some subset of a theoretical maximum (Poulin 1998), we look to species richness patterns to provide insight on

factors contributing to the structuring of a community. If the composition of a community does not exist as a random subset drawn from the available species pool, then perhaps there are rules governing how the community is assembled. Assembly rules can be considered as factors that dictate the persistence of a species in a given host at a given time. Examples of assembly rules guiding the assembly of a parasite community are environmental conditions, biogeography, and host diversity (Krasnov, et al. 2015). Prior to making any assumptions on these rules, a structured pattern must first be recognized. Nestedness has been a popular concept in ecology since its re-emergence in 1986, with its application to insular mammalian fauna and island biogeography (Patterson and Atmar, 1986). Nestedness has since been applied to parasite ecology, however results are inconsistent (Poulin 2007, Carney and Dick 2000, Guégan and Hugueny 1994, Zelmer and Arai 2004). The function of a nestedness analysis is to identify non-random patterns of community structure under the approach that species poor communities exist as subsets of progressively richer ones. This provides insight on the order by which species are added as communities are assembled—with common species forming the base of the community structure and then increasingly rare species are successively added (Poulin 1997). If nestedness is demonstrated then hypotheses can be developed on the processes involved in generating the pattern. In the context of parasite communities, the parasite component community would represent a local pool that all infracommunities draw species from and a nested pattern would hence infer that species-poor infracommunities are recognizable subsets of progressively richer infracommunities.

Hypotheses

Because of the differences in both biotic and abiotic characteristics among sample locations, it can be assumed that the parasite communities of Black Bay and Circle Lake walleye will be different. The purpose of my study is to identify similarities and differences among parasite communities, as well as identify reasons why those patterns exist. The following are a set of hypotheses tested using the data collected by this study.

Hypothesis 1: Black Bay walleye have fewer parasite species than Circle Lake walleye

This prediction is made under the assumption that a collapse in the walleye population would be concomitant with a decline in parasites. The exploitation of a host has been shown to affect parasite richness, abundance, prevalence, and community structure in studies in marine environments (Lafferty et al. 2008, Wood et al. 2010, Wood et al. 2014, Wood and Lafferty 2015). Black Bay walleye had not shown signs of recovery until recently and overall abundance is still considered to be low (Berglund 2016). With Lake Superior representing a salmonid dominated community at large, a collapse in the walleye population should have a pronounced effect on its parasite community as reservoirs retaining walleye parasite species lakewide are low (i.e. walleye and salmonids carry different parasite species). Further, while yellow perch (*Perca flavescens*) likely serve as a reservoir for certain species infecting walleye (Poulin 2010), they were also exposed to a high degree of exploitation over the history of Black Bay (Goodier 1982).

Hypothesis 2: Species shared among sample locations will be less prevalent and intense in Black Bay.

For the same reasons I predict Black Bay walleye will have fewer parasite species, I also predict that prevalence and intensity will be reduced within the sample.

Hypothesis 3: The parasite community of Black Bay walleye will not exhibit a nested structure because the host population has been altered, whereas the parasite community of Circle Lake walleye will have a nested structure because the hosts have not been subject to anthropogenic influence.

This prediction operates under the assumption that the severe depletion of a host's abundance (ex. collapse of a fishery) creates a significant barrier to parasite life cycles. There have been a number of processes thought to generate nested patterns in parasite communities (Geugan and Hugueny 1994; Poulin and Valtonen 2001; Zelmer and Arai 2004), and relevant to the present study is the ability of parasites to colonize. The Black Bay walleye population is currently in a state of rehabilitation, and I predict that the lagging parasite community is also undergoing changes as parasite species attempt to recolonize their host. If a parasite community is reduced in both diversity and abundance due to the removal of their host, then in order for the parasite community to reestablish there must first be a recovery of the host population. If the Black Bay walleye parasites are currently in a tumultuous state, a structured parasite community should not be observed. Noteworthy is that Morley 2007 suggests that parasites with direct life cycles can establish themselves in 2-3 years, whereas parasites employing trophic transmission can take anywhere from 4-10 years depending on their invertebrate intermediate host.

Because of the nature of the questions stated above, this study requires the identification of parasite species, the collection of presence/absence data, and the collection of quantitative data. This study will not only provide baseline parasitological information for an important fish species and stock in Northwestern Ontario, but will also identify important parasite species that are regionally available.

Materials and Methods

Study Area - Black Bay

Black Bay is on the north shore of Lake Superior (48.54723° N, -88.58408° W) and is situated between Thunder Bay and Nipigon Bay (Figure 1). Lake Superior is a deep, cold, oligotrophic lake. It has mean and maximum depths of 147 m and 405 m, respectively, which is the deepest of the Laurentian Great Lakes (United States Environmental Protection Agency 2006). Black Bay by contrast, is an area of relatively shallow and warm water on Lake Superior's northern shore. The surface area of Black Bay is roughly 60,000 hectares with approximately 30% of its surface area consisting of water less than 5 meters in depth and 50% consisting of water between 5 and 15 meters in depth (Furlong 2006). The most northerly section of the bay holds major river inflows and sheltered wetlands, likely serving as important nursery grounds for fish. The largest river flowing into Black Bay is the Black Sturgeon River. The Black Sturgeon is the seventh largest tributary on Lake Superior and is an area of prominent interest due to its importance with regards to potamodromous fish species (Bobrowicz 2010). Presently, there is a dam that acts as an absolute barrier to fish located approximately 17 km upstream of the estuary. This dam was originally constructed to facilitate log driving efforts in the mid-20th century. The south end of the bay is exposed to the colder water of the main basin and is variable in physical characteristics due to the constant mixing of physical zones.

The fish community in Black Bay is fairly diverse in comparison to other major embayments on Lake Superior (pers. comm. Eric Berglund, Ontario Ministry of Natural Resources). Because of its productive waters (i.e. more optimal conditions for the growth of organisms within the ecological community; Wetzel 2001), Black Bay harbours a wide variety of percids, salmonids, coregonids, and cyprinids, as well as a number of other fish species from miscellaneous families (Table 1).

Table 1 Black Bay fish community. Information on the Black Bay Fish Community was gathered from recent data collected from the Upper Great Lake Management Unit (OMNRF) Fall Walleye Index Netting program and the United States Geological Survey trawling programs.

Order	Common Name	Scientific Name
Perciformes	Walleye	<i>Sander vitreus</i>
	Yellow Perch	<i>Perca flavescens</i>
	Smallmouth Bass	<i>Micropterus dolomieu</i>
	Eurasian Ruffe	<i>Gymnocephalus cernua</i>
Esociformes	Northern Pike	<i>Esox lucius</i>
Salmoniformes	Lake Trout	<i>Salvelinus namaycush</i>
	Brook Trout	<i>Salvelinus fontinalis</i>
	Rainbow Trout	<i>Onchorhynchus mykiss</i>
	Chinook Salmon	<i>Onchorhynchus tshawytscha</i>
	Coho Salmon	<i>Onchorhynchus kisutch</i>
	Pink Salmon	<i>Onchorhynchus gorbuscha</i>
	Lake Whitefish	<i>Coregonus clupeiformis</i>
	Lake Cisco	<i>Coregonus artedii</i>
	Round Whitefish	<i>Prosopium cylindraceum</i>
Deepwater Cisco	<i>Coregonus sp.</i>	
Cypriniformes	Common White Sucker	<i>Catostomus commersonii</i>
	Longnose Sucker	<i>Catostomus catostomus</i>
	Common Carp	<i>Cyprinus carpio</i>
	Shorthead Redhorse	<i>Moxostoma macrolepidotum</i>
	Spottail Shiner	<i>Notropis hudsonius</i>
Acipenseriformes	Lake Sturgeon	<i>Acipenser fulvescens</i>
Percopsiformes	Trout-Perch	<i>Percopsis omiscomaycus</i>
Gadiformes	Burbot	<i>Lota lota</i>
Scorpaeniformes	Slimy Sculpin	<i>Cottus cognatus</i>
	Spoonhead Sculpin	<i>Cottus ricei</i>
	Deepwater Sculpin	<i>Myoxocephalus thompsonii</i>
Clupeiformes	Alewife	<i>Alosa pseudoharengus</i>
Gasterosteiformes	Ninespine Stickleback	<i>Pungitius pungitius</i>
Osmeriformes	Rainbow Smelt	<i>Osmerus mordax</i>

Figure 1. Black Bay, Lake Superior.



Study Area - Circle Lake

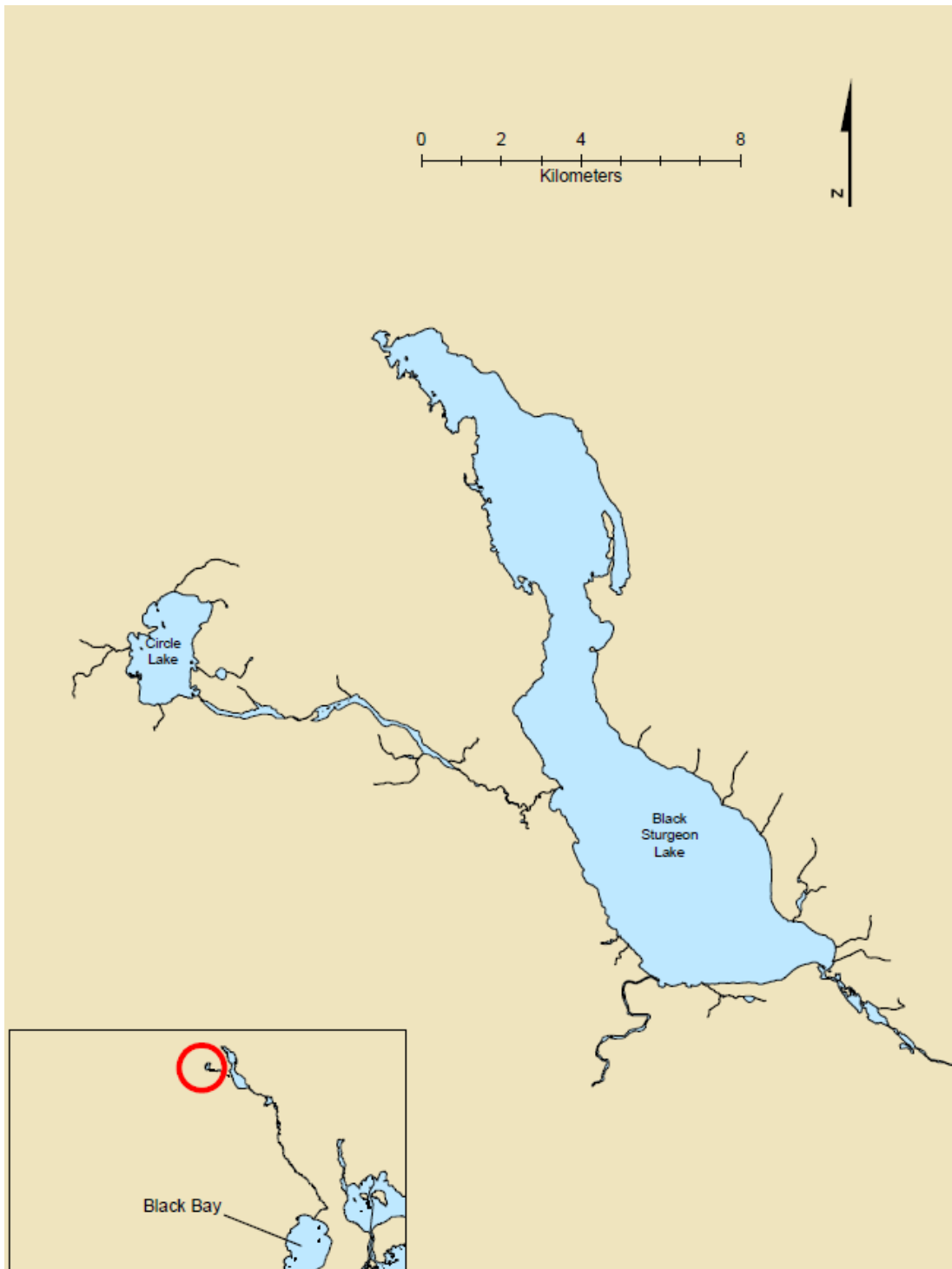
Circle Lake is a small (~390 ha) inland lake in Northwestern Ontario and is part of the Black Sturgeon watershed (Figure 2). It is connected to Pucker Lake, Mikinak Lake and Black Sturgeon Lake all via Circle Creek. Black Sturgeon Lake is a reservoir draining into the Black Sturgeon River and is the closest lake to Circle Lake that has been subject to monitoring (Broad-scale Fisheries Monitoring Bulletin: Black Sturgeon Lake 2008-2012).

There is little information available on Circle Lake, and thus, the following description is based on the work completed for this study. Lake area was determined using ArcMap geographic information system. As a whole, Circle Lake appeared to be relatively shallow (< 10m) aside from a deep area surrounding a small island in the northwest quadrant of the lake. This speculation is supported by the presence of aquatic vegetation extending through the majority of lake's surface. The shoreline surrounding the northwest island appeared to be the only area with significant rock and boulder substrate.

There is no published information pertaining to the fish community in Circle Lake, however, broadscale monitoring data (Broad-scale Fisheries Monitoring Bulletin: Black Sturgeon Lake 2008-2012) revealed that in addition to walleye, Black Sturgeon Lake harbours Lake Whitefish (*Coregonus clupeaformis*, Mitchill 1818), Lake Cisco (*Coregonus artedii*, Le Sueur 1818), Burbot (*Lota lota*, Linnaeus 1758), Smallmouth Bass (*Micropterus dolomieu*, La Cépède 1802) and Lake Trout (*Salvelinus namaycush*, Walbaum 1792). This information can perhaps be used to speculate on the structure of the Circle Lake fish community, however, it is important to take note that Circle Lake is much smaller than Black Sturgeon Lake (≈5000 ha).

Circle Lake is known to be a sport fishery for walleye, however, angling pressure has never been documented. It is likely that angling pressure is fairly limited as it is relatively remote, and access is laborious.

Figure 2. Circle Lake



Host and Parasite Specimen Collection

Black Bay

Walleye specimens from Black Bay were collected with the help of the Ministry of Natural Resources and Forests - Upper Great Lake Management Unit (UGLMU). Fish were caught using gillnets between September 15th, 2014 and September 30th, 2014 during the Fall Walleye Index Netting (FWIN) program. A standard gang length was composed of eight 25' panels (mesh sizes in mm: 25, 38, 51, 64, 76, 102, 127, 152). Walleye were selected at random by the UGLMU staff with the condition that there was to be an even representation of small, medium, and large fish relative to the minimum and maximum size of fish caught (122 mm - 706 mm). Fish were individually bagged and placed on ice in order to preserve the host and parasite specimens. Fish were then frozen at the UGLMU laboratory no later than 5 hours after capture and once completely frozen were transferred to freezers at Lakehead University and stored at -4° C.

Circle Lake

Circle Lake walleye were collected between September 30th and October 1st of 2014 using trap nets. Nets were set with a 100' lead line running off shore, which connected to the housing that was set at approximately 8' - 10' deep. Nets were left over night and were lifted the following morning. Fish were selected based on the criteria that there would be an even representation of small, medium, and large fish relative to the minimum and maximum size of fish caught (199 mm - 509 mm). The size criteria for Circle Lake walleye had to be reduced as the average size of these fish is much less than those captured in Black Bay. All bycatch was released. Selected fish were immediately euthanized using a tricaine methanesulfonate immersion bath (400 mg/L in 50L of water; SOP Fish and Amphibian Euthanasia). Fish were then immediately placed on ice for transport to Lakehead University. Each fish was individually bagged and stored at -4° C on the day of capture.

Host Measurements and Necropsies

Total length and round weight were measured the day fish were to be necropsied. Otoliths, and dorsal spines were removed for ageing purposes. Walleye were aged with the help of the UGLMU facilities and staff. Necropsies followed standard parasitological procedure (Hanson Pritchard and Kruse 1982). Fish were thawed and the exterior was examined for ectoparasites. Fish were cut along the ventral surface in order to access the internal organs. Sex was determined for each fish. Structures examined for parasites are as follows: swim bladder, gills, stomach, pyloric caecae, intestine, urinary bladder, eyes, muscle tissue, liver, and heart. All organs and their contents were examined under a dissecting microscope

Parasite Specimen Processing

Parasites were collected and enumerated. Platyhelminths were immediately fixed in alcohol-formalin-acetic acid (AFA) solution (Hanson-Pritchard and Kruse 1982) for a minimum of 48 hours and then preserved in 70% alcohol (EtOH). Specimens were stained with acetocarmine (Hanson-Pritchard and Kruse 1982), dehydrated using a graded series of alcohol, cleared using a graded series of xylene, and mounted on slides in Canada balsam. Nematode parasites were preserved in glycerin alcohol (10:90; 70% EtOH) and were identified once cleared enough that the internal organs became apparent. Copepods were immediately fixed in AFA for 48 hours and preserved in 70% EtOH, or, placed in potassium hydroxide (KOH) to digest the organic tissue and stored in 70% EtOH. Parasites were initially identified using Gibson (1996), Kabata (1988), Margolis et al. (1989), Schell (1985), Freze (1969), and Hoffman (1999). Final identifications were confirmed by reference to species descriptions in the primary literature.

Analysis

All statistics were computed using the R statistical programming language unless stated otherwise (R Core Team 2016). Results of statistical tests were considered to be significant at $p \leq 0.05$. Definitions of prevalence, mean abundance, and mean intensity follow Margolis et al. (1982) and Bush et al. (1997). Prevalence of a parasite species refers to the number of hosts within the sample infected by at least one individual of that parasite species, and is expressed as a percentage. The mean abundance of a parasite species refers to the average number of individuals infecting the host sample, including those hosts that are not infected. The mean intensity of a parasite species refers to the average number of parasite individuals infecting the host sample in instances where the hosts are infected by at least one individual parasite. Parasites were grouped into ecto- (parasitizes the external features of the host) and endo- (parasitizes the internal anatomy of the host) so that different compartments of the parasite community could be analyzed. Prevalence, mean abundance, and mean intensity were determined for all parasite species (Refer to Appendix A for the code outlining the user defined functions for these calculations).

An issue arises when operating under the assumption that each host represents a replicate parasite community, that is, there is a potential correlation between host attributes and parasite quantities (Bush et al. 2001). In order to control for this, host attributes were compared to parasite number. Parasites were grouped into ecto- and endoparasites. Kendall rank correlation coefficient (tau-b) was calculated to test if parasite abundance is associated with host age (year), round weight (g), and total length (mm). The tau-b statistic was used as it is more suitable for data containing ties (i.e. abundance data often has multiple 0's). Kendall rank correlation coefficient was also used to test whether parasite intensity is associated with host attributes (tau-a). The Mann-Whitney U test was used to determine if individual parasite abundance, or total parasite abundance differed between sexes of hosts.

In order to provide visual representation of parasite numerical distribution throughout a sample, histograms were constructed using the 'ggplot2' package for the R programming language. Parasite distributions have been historically described as random, overdispersed (clumped or aggregated), and underdispersed (regular), however, this contradicts the terminology familiar to 'free-living' ecologists, who know overdispersion as being uniform, and underdispersion as being clumped (Bush et al. 2001). Bush et al. (2001) recommend the use of the terms random, aggregated (clumped), and uniform (regular) to avoid future confusion and this recommendation will be followed in this thesis. The variance (s^2)-to-mean (\bar{x}) ratio was calculated in order to mathematically determine the distribution of the parasite populations in this study. If $s^2 \approx \bar{x}$, then the population is determined to be 'random', if $s^2 < \bar{x}$, then the population is determined to be uniform, and if $s^2 > \bar{x}$, then the population is determined to be aggregated.

Diversity

Species richness was determined for each individual host. Mean, maximum, and minimum infracommunity richness was determined for both Circle Lake and Black Bay walleye. A two sample t-test was used to test if there was a significant difference between mean infracommunity richness between sample locations. Component community richness was also determined for each study location. Kendall tau rank correlation coefficient was used to test if infracommunity richness was associated with host weight, length, and age. Diversity was measured using Simpson's index of diversity and calculated using the Community Ecology Package 'vegan', following the equation:

$$D_1 = 1 - \sum_{i=1}^S p_i^2$$

where p_i is the proportion of species i , and S is the number of species.

Cross-Sample Comparison

Host populations were compared by weight, length, and age using the Kruskal-Wallis test. Prevalence between parasite fauna common to both sample locations was compared using Fisher's exact test. Intensities between common parasite taxa was compared using a distribution-free bootstrap test (Ròsza et al. 2000), as comparisons that rely on normal distributions are generally not applicable to parasites as a result of their aggregated nature (Bush et al. 2001). Because there was no statistical package available to perform this comparison of mean intensity, a code was developed to compute the results (Appendix A).

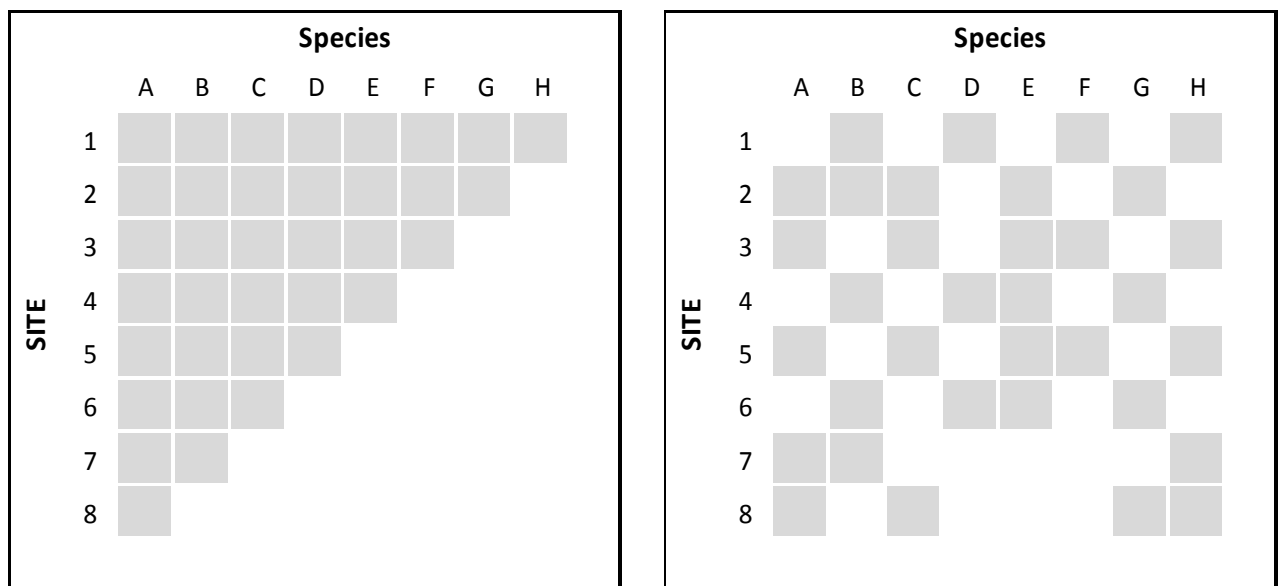
Nestedness Analysis

Nestedness analysis was conducted in order to assess if the parasite communities exhibited a structured assemblage. A nested structure refers to species poor communities existing as distinct subsets of progressively richer ones (Figure 3). If nestedness analysis does not reveal a distinct ordered pattern, then it is said to be randomly structured. The NeD software (Nestedness for Dummies; Strona and Fattorini 2014) was used to carry out the nestedness analysis. The metric chosen for the present study is NODF (Nestedness measure based on overlap and decreasing fills; Almeida-Neto et al. 2008). This metric is based on two matrix properties; decreasing fill (DF) and paired overlap (PO). The computation of the metric follows a series of rules. As described from Almeida-Neto et al. (2008), for a matrix with rows i descending downward to j , and columns k moving rightwards to l , DF_{ij} (i.e. DF of rows) is determined by the following set of rules:

$$\begin{aligned} \text{if } MT_j < MT_i, & \quad \text{then } DF_{ij} = 100 \\ \text{if } MT_j \geq MT_i, & \quad \text{then } DF_{ij} = 0 \end{aligned}$$

where MT is the row sum, or marginal total. These same rules apply for determining DF of columns, i.e. (DF_{kl}). The second property, PO, is calculated as, for PO_{kl} , the percentage of presences in column l that

Figure 3. An example of a perfectly nested community (L) against one that is randomly assembled (R). In the context of parasite communities the y axis, or “Site”, represents host individuals.



have coinciding presences in column k —and vice versa for PO_{ij} . With measures of DF_{paired} and PO , the value of N_{paired} is given by the set of rules:

$$\begin{aligned} \text{if } DF_{paired} = 0, & \quad \text{then } N_{paired} = 0 \\ \text{if } DF_{paired} = 100, & \quad \text{then } N_{paired} = PO \end{aligned}$$

Lastly, the $NODF$ measure is calculated as:

$$NODF = \frac{\sum N_{paired}}{\left[\frac{n(n-1)}{2} \right] + \left[\frac{m(m-1)}{2} \right]}$$

where n is the number of columns in a matrix, m is the number of rows in a matrix, and N_{paired} is the degree of paired nestedness. With increasing nestedness, the $NODF$ measure will increase, and a perfectly nested matrix is imposed at $NODF = 100$. The process of computing $NODF$ for a given matrix allows for the independent assessment of nestedness among rows or among columns ($NODF_{row}$ and $NODF_{col}$). Stated differently, this allows for the evaluation of nestedness solely among sites (i.e. rows; species composition) or among species (columns; species occupancy) (Almeida-Neto, et al. 2008).

In order to assess the significance of a nestedness index for an observed community, results must be compared to a set of nestedness measures generated from a null model. I computed my results to 100 randomly generated matrices (Ulrich and Gotelli 2007). The null model used in the present study is CE, which gives each cell in the null matrix a probability to be occupied based on the proportion of row and column totals (Strona et al. 2014). The CE null model is computed as:

$$P_{ij} = \frac{\frac{TotR_i}{C} + \frac{TotC_j}{R}}{2}$$

Where i represents the matrix row, j represents the matrix column, C represents the number of columns, R represents the number of rows, and P_{ij} is the probability of a cell being occupied. The variables $TotR_i$ and $TotC_j$ are the number of row and column presences, respectively.

Assessing the significance of an observed nestedness measure is achieved by computation of the *Z-value*, calculated as:

$$Z = \frac{[Nlr - \overline{NIs}]}{\sigma(NIs)}$$

where *Nlr* is the nestedness index (NODF measure) for the observed community, \overline{NIs} is the mean of nestedness indices computed for the generated null matrices and $\sigma(NIs)$ is the standard deviation of the null matrices. A *Z-value* > 1.64 indicates statistical significance at $p = 0.05$ for the NODF metric.

While the *Z-value* provides insight on statistical significance, it does not help make inference on the magnitude of nestedness. NODF is sensitive to matrix fill (Strona and Fattorini 2014); meaning that two empirical matrices can have different NODF values based on species occurrences, even though they both display the same degree of nestedness. This prevents the direct comparison of NODF values across empirical matrices. Bascompte et al. 2003 proposed an alternative metric, RN (relative nestedness), which replaces $\sigma(NIs)$ with (\overline{NIs}) as the divisor, and in turn calculates the degree or magnitude of nestedness. The RN value is given as:

$$RN = \frac{(Nlr - \overline{NIs})}{(\overline{NIs})}$$

and increases with increasing degree of nestedness.

Nestedness analysis was conducted on four different facets of the parasite community: (1) including all parasite species, (2) including all adult parasite species, (3) including only ectoparasites, and (4) including only endoparasites. Compartmentalizing the parasite community allows nestedness to be assessed for the parasite community as a whole, for parasites in which walleye is the definitive host (adult), and furthermore, it allows for a comparison of nested structure between direct and indirect life cycles (ecto-, endo-).

Results

Black Bay & Circle Lake

In total, 2943 parasites representing 7 different species was recovered from 43 Black Bay walleye and 11987 parasites representing 14 species was recovered from 46 Circle Lake walleye. The ageing of Black Bay walleye revealed five distinct age classes: 1, 2, 3, 4, and 8. Mean weight, total length, and age for Black Bay walleye was 840 grams, 403 millimeters, and 3.25 years, respectively. There were 8 different year classes in the Circle Lake walleye sample: 1, 2, 3, 4, 5, 6, 8 and 9. Mean weight, total length, and age for Circle Lake walleye was 347 g, 318 mm, and 3.6 years, respectively. The Black Bay walleye hosts were on average heavier and longer than Circle Lake walleye, however, the median age of the hosts was not significantly different (Table 2).

Stomach contents revealed that Black Bay walleye were primarily feeding on rainbow smelt prior to capture (Table 3). Invertebrates were found in the stomach of only two Black Bay walleye. Most fish in the stomachs of Circle Lake walleye were unidentifiable. However, those that could be identified showed Circle Lake walleye were feeding on yellow perch, white sucker, and logperch. This information provides insight into the fish community of Circle Lake, as it has not been subject to a fish community study. Circle Lake walleye were also feeding on a diversity of invertebrates, including amphipods, copepods, cladocerans, ephemeropterans, and terrestrial insects.

Ectoparasites

Two species of parasites were found infecting the gills of Black Bay walleye, the copepod *Ergasilus caeruleus* (Wilson, 1911) and the monogenean *Urocleidus aculeatus* (Van Cleave and Mueller

Table 2. Results of Kruskal-Wallis test comparing host attributes between Black Bay and Circle Lake.

	Black Bay (n = 43)		Circle Lake (n = 46)		Kruskal-Wallis	
	\bar{x}	sd	\bar{x}	sd	Statistic	<i>p</i> -value
Weight	840.33	781.37	346.96	249.27	12.49	0.0004
Total Length	403.81	128.86	318.72	75.79	11.95	0.0005
Age	3.26	2.60	3.63	1.76	3.75	0.0528

Table 3. Stomach contents of Black Bay and Circle Lake walleye. Numerals refer to the number of walleye found with a given prey type in their stomach. Stomachs may contain more than one type of prey item.

Stomach contents	Black Bay (n = 43)	Circle Lake (n = 46)
Empty	6	5
Fish		
Rainbow Smelt	24	---
Lake Cisco	1	---
Yellow Perch	---	8
Common White Sucker	---	5
Logperch	---	1
Unidentified fish	6	27
Invertebrate		
Amphipod	---	2
Copepod	---	1
Cladoceran	---	2
Ephemeropteran larvae	---	2
Trichopteran larvae	---	---
Unidentified terrestrial insects	---	2
Unidentified insect parts	1	9
Snails	1	---

Table 4. Prevalence, mean abundance, mean intensity, and intensity range of ectoparasites infecting Black Bay and Circle Lake walleye. Prevalence is reported as a percentage. Mean abundance and Mean intensity are reported as mean \pm standard deviation. † = larval. Site: g = gills, ga = gillarch

Location	Ectoparasite	Site	Prevalence	Mean Abundance	Mean Intensity	Range
Black Bay n = 43	<i>Ergasilus caeruleus</i>	g	88.37	26.98 \pm 36.81	30.53 \pm 37.77	1 - 173
	<i>Urocleidus aculeatus</i>	g	4.65	0.07 \pm 0.34	1.5 \pm 0.71	1 - 2
Circle Lake n = 46	<i>Ergasilus caeruleus</i>	g	100.00	28.35 \pm 21.1	28.35 \pm 21.1	5 - 87
	<i>Urocleidus aculeatus</i>	g	97.83	82.28 \pm 109.43	84.11 \pm 109.95	2 - 568
	<i>Clinostomum complanatum</i> †	ga	4.35	0.04 \pm 0.21	1 \pm 0	1 - 1
	Glochidia †	g	63.04	48.39 \pm 74.17	76.76 \pm 81.16	1 - 338

1932). *Urocleidus aculeatus* was only prevalent in 4.7% of the sample and existed at low intensities (1 - 2). The gill parasite community was dominated almost entirely by *E. caeruleus*— which had a prevalence of 88.37% and a mean intensity of 30.53 (Table 4). No *E. caeruleus* had egg sacs. The variance to mean ratio indicated that both *E. caeruleus* and *U. aculeatus* exhibited an aggregated distribution (Table 5, Figure 4).

Four parasite species infected the gills of Circle Lake walleye: *E. caeruleus*, *U. aculeatus*, the glochidia of a species of unionid freshwater mussel, and larval encysted *Clinostomum complanatum* (Rudolphi 1814) (Table 4). While characteristically not an ectoparasite, *C. complanatum* was grouped into this category because it was only found encysted on the gill arch of two walleye. The gills of Circle Lake walleye were always infested by at least two of the four parasites identified, and harboured three species 63% of the time. The copepod *E. caeruleus* and monogenean *U. aculeatus* in Circle Lake were the most prevalent of all parasite species, occurring in 100% and 98% of the walleye, respectively. *Ergasilus caeruleus* was never found with egg sacs. *Urocleidus aculeatus* and glochidia infections in Circle Lake exhibited the highest and third highest mean intensities of all parasite species at 84.11 and 76.76 (Table 4). *Ergasilus caeruleus*, *U. aculeatus*, and glochidia all exhibited an aggregated distribution (Table 5; Figure 5).

E. caeruleus abundance was negatively correlated to and host age (Kendall tau $z = -2.5$, $\tau = -0.29$, $p = 0.012$), weight (Kendall tau $z = -2.1$, $\tau = -0.23$, $p = 0.031$), and length (Kendall tau $z = -2.2$, $\tau = -0.24$, $p = 0.026$) in Black Bay walleye (Table 4). There was no significant relationship between *U. aculeatus* abundance and Black Bay walleye host attributes. In Circle Lake, ectoparasites *U. aculeatus* and glochidia abundance was strongly correlated to walleye attributes (Table 6). Glochidia abundance significantly decreased with increasing host age (Kendall tau $z = -3$, $\tau = -0.35$, $p = 0.002$), weight (Kendall tau $z = -2.9$,

Table 5. Variance to mean ratios for Black Bay and Circle Lake ectoparasite species.

	Ectoparasite	s²	\bar{x}	s²:\bar{x}
Black Bay	<i>Ergasilus caeruleus</i>	1354.98	26.98	50.23
	<i>Urocleidus aculeatus</i>	0.11	0.07	1.63
Circle Lake	<i>Ergasilus caeruleus</i>	445.03	28.35	15.70
	<i>Urocleidus aculeatus</i>	11973.94	82.28	145.52
	Glochidia	0.04	0.04	0.98
	<i>Clinostomum complanatum</i>	5501.76	48.39	113.69

Figure 4. Numerical distribution of the ectoparasites infecting Black Bay walleye. The y-axis refers to the number of hosts within the allotted bin.

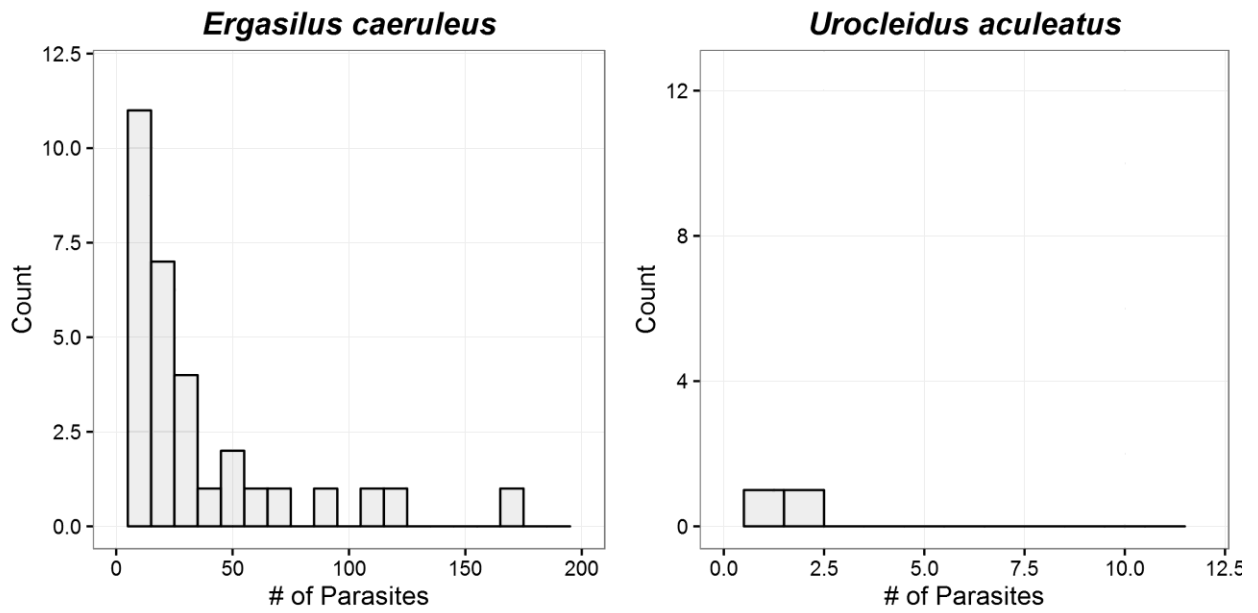


Figure 5. Numerical distribution of the ectoparasites infecting Circle Lake walleye. The y-axis refers to the number of hosts within the allotted bin.

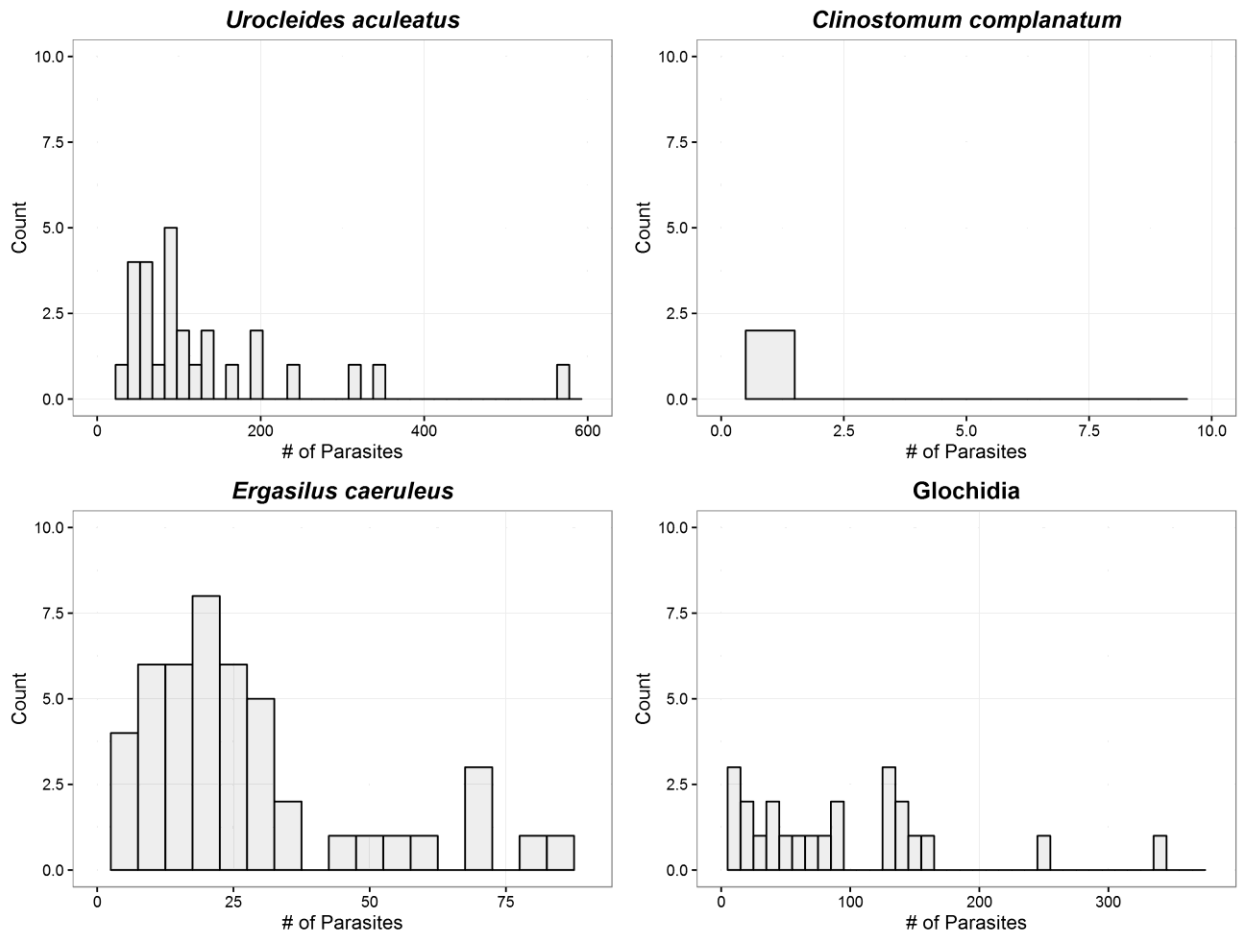


Table 6. Kendall's rank correlation coefficients comparing Black Bay and Circle Lake walleye attributes to ectoparasite abundance. Grey blocks indicate instances of significant correlation.

Parasite	Age			Weight			Length		
	<i>p</i> -value	<i>z</i>	<i>tau</i>	<i>p</i> -value	<i>z</i>	<i>tau</i>	<i>p</i> -value	<i>z</i>	<i>tau</i>
Black Bay <i>Ergasilus caeruleus</i>	0.012	-2.513	-0.290	0.031	-2.16	-0.232	0.026	-2.223	-0.238
<i>Urocleidus aculeatus</i>	0.287	-1.065	-0.146	0.226	-1.211	-0.154	0.237	-1.182	-0.150
Total Ectoparasite	0.012	-2.523	-0.291	0.028	-2.192	-0.235	0.024	-2.254	-0.241
Circle Lake <i>Ergasilus caeruleus</i>	0.146	-1.452	-0.161	0.221	-1.223	-0.126	0.178	-1.346	-0.139
<i>Urocleidus aculeatus</i>	0.000	4.988	0.551	0.000	5.201	0.533	0.000	5.154	0.529
Glochidia	0.002	-3.041	-0.351	0.003	-2.92	-0.313	0.004	-2.911	-0.312
<i>Clinostomum complanatum</i>	0.294	1.050	0.140	0.467	0.727	0.090	0.572	0.566	0.070
Total Ectoparasite	0.345	0.945	0.104	0.208	1.259	0.129	0.264	1.118	0.114

Table 7. Kendall's rank correlation coefficient comparing Black Bay and Circle Lake walleye attributes to ectoparasite intensity. Kendall's rho could not be computed for *U. aculeatus* or *C. complanatum* due to insufficient number of presences. Grey blocks indicate instances of significant correlation.

Parasite	Age			Weight			Length			
	<i>p</i> -value	<i>z</i>	<i>tau</i>	<i>p</i> -value	<i>z</i>	<i>tau</i>	<i>p</i> -value	<i>z</i>	<i>tau</i>	
Black Bay	<i>Ergasilus caeruleus</i>	0.003	-2.939	-0.362	0.015	-2.443	-0.279	0.011	-2.530	-0.289
	<i>Urocleidus aculeatus</i>	---	---	---	---	---	---	---	---	---
Circle Lake	<i>Ergasilus caeruleus</i>	0.146	-1.452	-0.161	0.221	-1.223	-0.126	0.178	-1.346	-0.139
	<i>Urocleidus aculeatus</i>	0.000	4.077	0.597	0.000	4.529	0.610	0.000	4.392	0.592
	Glochidia	0.086	-1.718	-0.248	0.081	-1.747	-0.231	0.091	-1.691	-0.224
	<i>Clinostomum complanatum</i>	---	---	---	---	---	---	---	---	---

Table 8. Results of Mann Whitney U test comparing Black Bay and Circle Lake walleye gender to parasite abundance.

	Ectoparasite	<i>p</i>-value	<i>U</i>
Black Bay n. male = 10 n. female = 26	<i>Ergasilus caeruleus</i>	0.46	108.5
	<i>Urocleidus aculeatus</i>	0.40	120
	Total ectoparasite	0.43	107
Circle Lake n. male = 16 n. female = 25	<i>Ergasilus caeruleus</i>	0.70	185
	<i>Urocleidus aculeatus</i>	0.76	212
	Glochidia	0.88	206
	<i>Clinostomum complanatum</i>	0.08	225
	Total ectoparasite	0.38	234

$\tau = -0.31, p = 0.003$) and length (Kendall tau $z = -2.9, \tau = -0.31, p = 0.004$). By contrast, *U. aculeatus* abundance significantly increased with host age (Kendall tau $z = 4.9, \tau = 0.55, p < 0.001$), weight (Kendall tau $z = 5.2, \tau = 0.53, p < 0.001$), and length (Kendall tau $z = 5.2, \tau = 0.53, p < 0.001$). There was no statistically significant relationship between *E. caeruleus* and *C. complanatum* abundance and host attributes. Furthermore, no statistically significant relationship between total ectoparasite abundance and host attributes was revealed from either sample location.

The intensity of *E. caeruleus* infections showed a weak but statistically significant negative relationship to Black Bay walleye age (Kendall tau $z = -2.9, \tau = -0.362, p = 0.003$), weight (Kendall tau $z = -2.443, \tau = -0.28, p = 0.015$), and length (Kendall tau $z = -2.53, \tau = -0.289, p = 0.011$) (Table 7). By contrast, the only parasite that exhibited a statistically significant relationship between intensity and host attributes in Circle Lake walleye was *U. aculeatus*, with infections showing a strong increase with host age (Kendall tau $z = 4.1, \tau = 0.59, p < 0.001$), weight (Kendall tau $z = 4.5, \tau = 0.61, p < 0.001$), and length (Kendall tau $z = 4.4, \tau = 0.59, p < 0.001$). *Urocleides aculeatus* intensity relationships in Black Bay could not be tested due to the insufficient number of associations. The results of the Mann-Whitney U test revealed no significant relationship between ectoparasite abundance and host sex in either sample location (Table 8).

Endoparasites

The endoparasite component community of Black Bay walleye was composed of one cestode (*Bothriocephalus cuspidatus*), three digeneans (*Proisorhynchoides pusilla*, *Azygia angusticauda*, and *Diplostomum* sp.), and one acanthocephalan (*Echinorhynchus* sp.). The cestode *Bothriocephalus cuspidatus* (Cooper 1917) was consistently found infecting the stomach and caeca of the hosts and the majority of the specimens were found as gravid adults. The digenean *Proisorhynchoides pusilla* (Dollfus 1929) was found infecting the stomach, caeca and intestinal tract of Black Bay walleye, and had the

Table 9. Prevalence, mean abundance, mean intensity, and intensity range of endoparasites infecting Black Bay and Circle Lake walleye. Prevalence is reported as a percentage. Mean abundance and Mean intensity are reported as mean \pm standard deviation. † = larval. Site: I = intestine and caeca, s = stomach, e = eye, m = mesenteries, h = heart, l = liver, sk = skin.

Location	Endoparasite	Site	Prevalence	Mean Abundance	Mean Intensity	Range
Black Bay n = 43	<i>Bothriocephalus cuspidatus</i>	i	81.40	30.86 \pm 84.51	37.91 \pm 92.45	1 - 433
	<i>Azygia angusticauda</i>	s	2.33	0.05 \pm 0.30	2 \pm 0	2 - 2
	<i>Prosorhynchoides pusilla</i>	i	55.81	8.47 \pm 17.39	15.17 \pm 21.12	1 - 90
	<i>Diplostomum</i> sp. †	e	32.56	1.65 \pm 3.39	5.07 \pm 4.3	1 - 15
	<i>Echinorhynchus</i> sp.	i	18.60	0.37 \pm 1.16	2 \pm 2.07	1 - 7
Circle Lake n = 46	<i>Bothriocephalus cuspidatus</i>	i	93.48	78.70 \pm 75.16	84.19 \pm 74.69	1 - 297
	<i>Proteocephalus</i> sp.	i	82.61	9.04 \pm 10.8	10.95 \pm 10.97	1 - 51
	<i>Triaenophorus nodulosus</i>	s	10.87	0.13 \pm 0.40	1.2 \pm 0.45	1 - 2
	<i>Azygia angusticauda</i>	s	15.22	0.22 \pm 0.55	1.43 \pm 0.53	1 - 2
	<i>Sanguinicola occidentalis</i>	h	32.61	0.72 \pm 1.42	2.2 \pm 1.74	1 - 7
	<i>Diplostomum</i> sp. †	e	45.65	0.96 \pm 1.60	2.1 \pm 1.81	1 - 7
	<i>Prosorhynchoides pusilla</i>	i	56.52	7.67 \pm 15.44	13.58 \pm 18.59	1 - 81
	<i>Neascus</i> sp. †	sk	73.91	---	---	---
	<i>Camallanus oxycephalus</i>	i	21.74	0.39 \pm 1.04	1.8 \pm 1.62	1 - 6
<i>Raphidascaris</i> sp. †	l	60.87	2.93 \pm 3.34	4.82 \pm 3.03	1 - 11	

second highest prevalence within the sample at 55.81%. *Azygia angusticauda* (Manter 1926) infected the stomach of Black Bay walleye. Larval *Diplostomum* sp. (Poirier 1886) infected at least one eye in 32% of the sample at low intensities (1 – 5). The acanthocephalan *Echinorhynchus* sp. (Cobbold 1876) was found infecting the posterior section of the intestinal tract of 18% of the Black Bay sample. *Echinorhynchus* sp. could not be identified to species with certainty due to the lack of specimens with an extended proboscis (Margolis et al. 1989). This is likely a consequence of working with frozen host specimens.

The endoparasite community of Circle Lake walleye was composed of three cestodes (*Bothriocephalus cuspidatus*, *Proteocephalus* sp., and *Triaenophorus nodulosus*), five digeneans (*Azygia angusticauda*, *Diplostomum* sp., *Neascus* sp., *Prosorhynchoides pusilla*, and *Sanguinicola occidentalis*), and two nematodes (*Camallanus oxycephalus*, and *Raphidascaaris* sp.). *Bothriocephalus cuspidatus* and *Proteocephalus* sp. (La Rue 1911) were found infecting the stomach, caeca, and intestinal tract of Circle Lake walleye. *Bothriocephalus cuspidatus* and *Proteocephalus* sp. were also the most prevalent endoparasites throughout either sample location, at 93% and 85% respectively (Table 9). *Proteocephalus* sp. could not be identified to species due to the lack of specimens with mature proglottids. The third cestode, *T. nodulosus* (Rudolphi 1919) infected the stomach. The digenean *A. angusticauda* was found at low intensities (1 -2) infecting the stomach. *Sanguinicola occidentalis* (Van Cleave and Mueller 1932) infected the heart. Because *S. occidentalis* is an extremely small blood fluke (<1.4 mm; Muzzall 2000), they were likely present at higher prevalence or intensity than what was observed due to their capacity to infect various host organs connected by the vascular system. Furthermore, because of its size *S. occidentalis* specimens were extremely delicate and did not take well to the procedures of staining and mounting, and thus, specimens had to be identified immediately after collection via wetmount. *Diplostomum* sp. and *Neascus* sp. (Poirier 1886) metacercaria could not be identified to species. *Neascus* sp. was not

Table 10. Variance to mean ratios for Black Bay endoparasite species.

	Endoparasite	s²	\bar{x}	s²: \bar{x}
Black Bay	<i>Bothriocephalus cuspidatus</i>	7141.12	30.86	231.40
	<i>Azygia angusticauda</i>	0.09	0.05	2.00
	<i>Diplostomum</i> sp.	11.52	1.65	6.98
	<i>Prosorhynchoides pusilla</i>	302.35	8.47	35.72
	<i>Echinorhynchus</i> sp.	1.33	0.37	3.59
Circle Lake	<i>Bothriocephalus cuspidatus</i>	5648.35	78.70	71.77
	<i>Proteocephalus</i> sp.	116.58	9.04	12.89
	<i>Triaenophorus nodulosus</i>	0.16	0.13	1.23
	<i>Azygia angusticauda</i>	0.31	0.22	1.41
	<i>Sanguinicola occidentalis</i>	2.03	0.72	2.83
	<i>Diplostomum</i> sp.	2.58	0.96	2.69
	<i>Prosorhynchoides pusilla</i>	238.31	7.67	31.06
	<i>Camallanus oxycephalus</i>	1.09	0.39	2.78
<i>Raphidascaaris</i> sp.	11.17	2.93	3.81	

Figure 6. Histograms of endoparasite infections in Black Bay walleye.

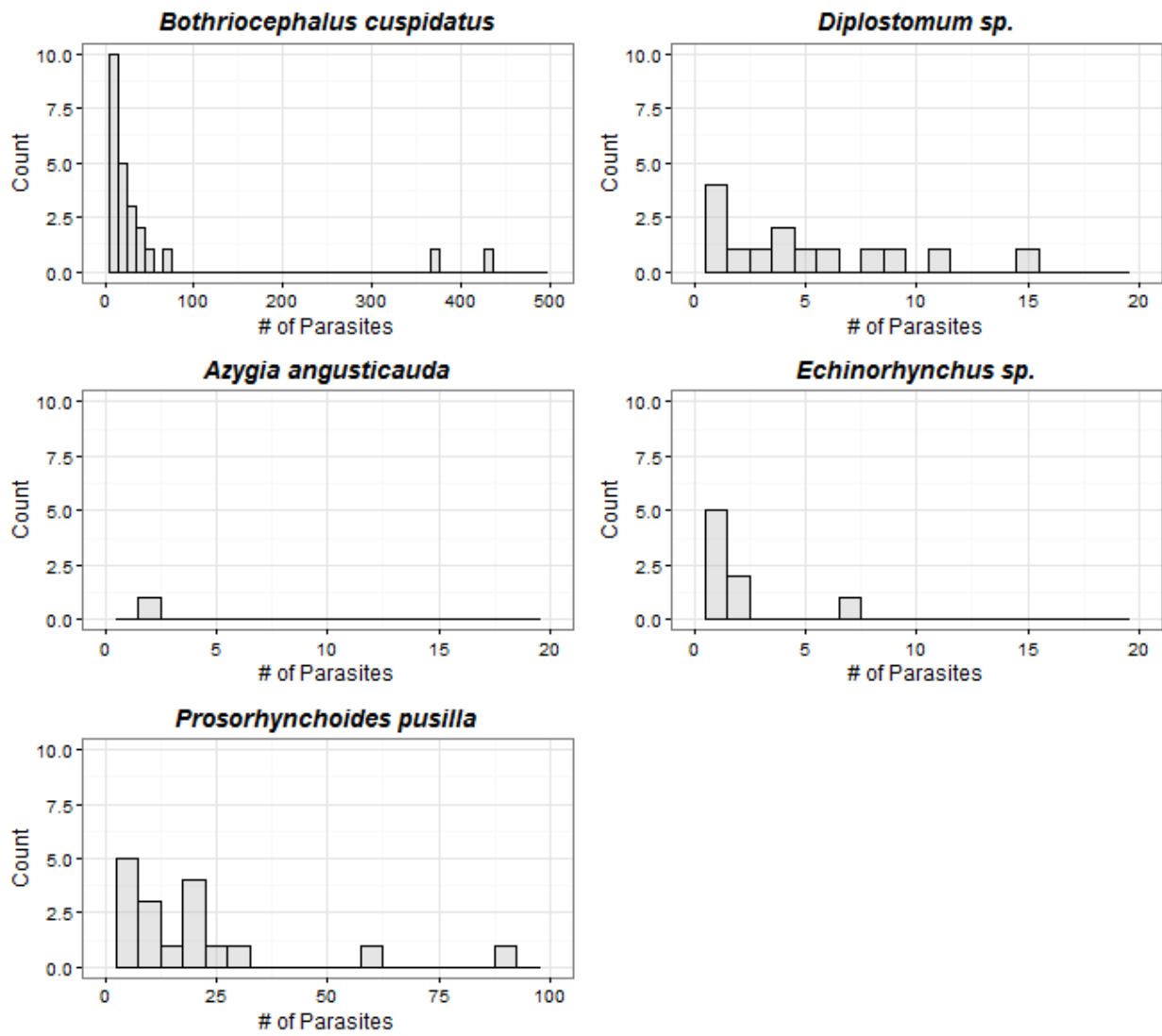
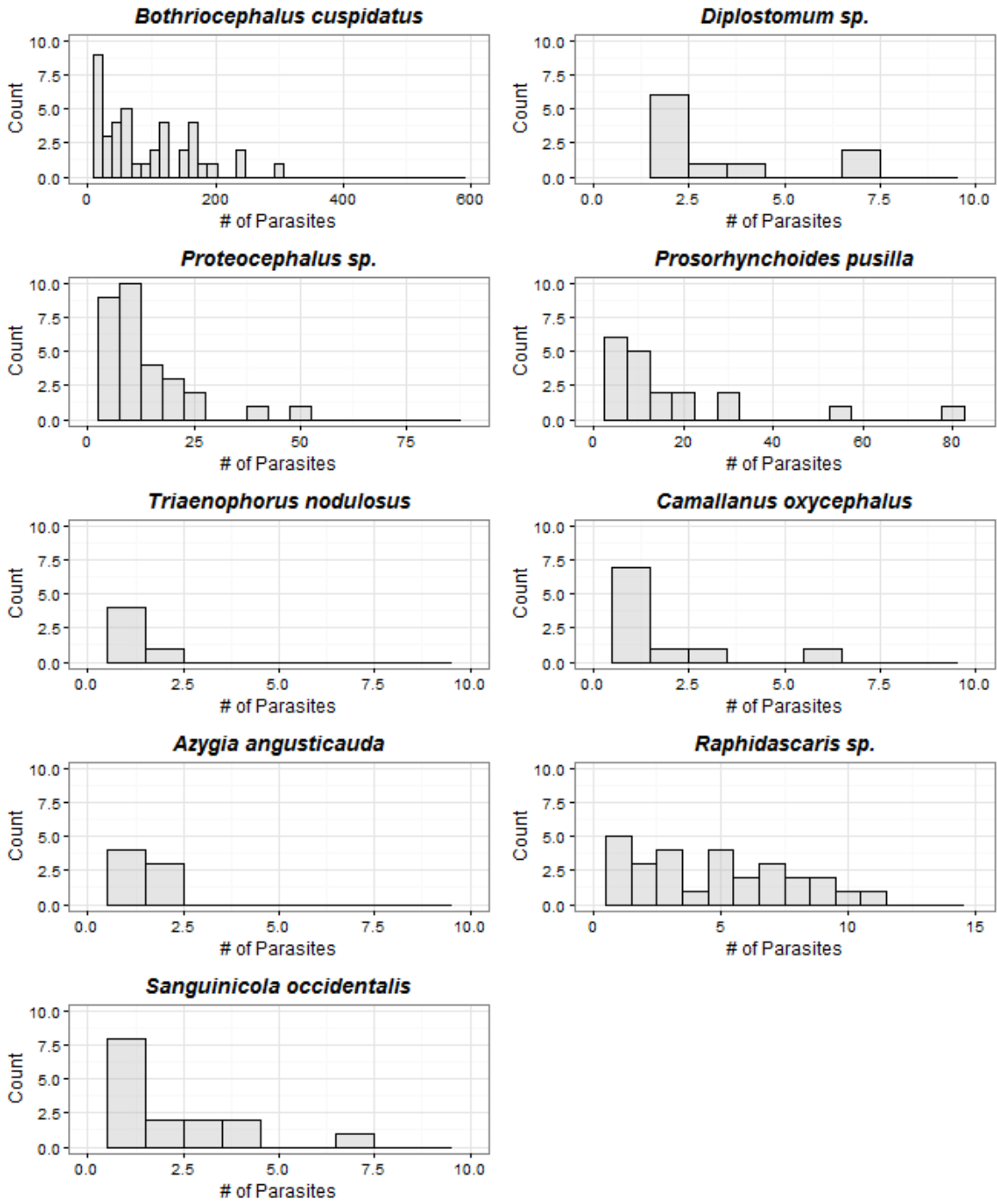


Figure 7. Numerical distribution of endoparasites infecting Circle Lake walleye.



enumerated due to the difficulty associated with removing specimens from their thick host capsule, and based on the fact that multiple individuals can reside in a single cyst (Duru et al. 1981). Six endoparasites were prevalent in at least 30% of the Circle Lake sample and five were prevalent in at least 50% (Table 9).

The variance to mean ratio revealed an aggregated distribution for all endoparasite species present in Black Bay walleye (Table 10, Figure 6). This pattern was also true for endoparasites infecting Circle Lake walleye (Table 10, Figure 7).

There were no relationships between Black Bay walleye endoparasite abundance and host weight, length and age (Table 11). By contrast, *B. cuspidatus* abundance in Circle Lake walleye had a strong negative correlation to weight (Kendall tau $z = -2.6$, $\tau = -0.27$, $p = 0.008$) and length (Kendall tau $z = -2.7$, $\tau = -0.28$, $p = 0.007$), with statistical significance, however there was no statistically significant relationship to host age (Table 11). *Proisorhynchoides pusilla* abundance also had a strong correlation with Circle Lake walleye attributes although abundance tended to increase with host size and age (Kendall tau $z = 2.1$, $\tau = 0.25$, $p = 0.032$; Table 11). Additionally, total endoparasite abundance had a negative relationship to both weight (Kendall tau $z = -2.1$, $\tau = -0.22$, $p = 0.032$) and length (Kendall tau $z = -2.4$, $\tau = -0.25$, $p = 0.017$) but not age (Table 11).

The only endoparasite that exhibited a statistically significant relationship between intensity and host attributes in either study location was *B. cuspidatus* in Circle Lake. The relationship was weak and negative for all host attributes (Table 12). There were no statistically significant relationships between individual or total endoparasite intensity and host gender (Table 13).

Table 11. Results of Kendall's rank correlation coefficient comparing Black Bay and Circle Lake walleye attributes to endoparasite abundance. Grey blocks indicate instances of statistical significance.

	Parasite	Age			Weight			Length		
		p-value	z	tau	p-value	z	tau	p-value	z	tau
Black Bay	<i>Bothriocephalus cuspidatus</i>	0.144	1.461	0.170	0.288	1.063	0.116	0.333	0.968	0.105
	<i>Azygia angusticauda</i>	0.741	-0.331	-0.046	0.904	-0.121	-0.015	0.872	-0.161	-0.021
	<i>Prosorhynchoides</i> sp.	0.982	0.023	0.003	0.869	0.165	0.019	0.895	0.132	0.015
	<i>Diplostomum</i> sp.	0.816	-0.233	-0.030	1.000	0.000	0.000	0.930	-0.088	-0.011
	<i>Echinorhynchus</i> sp.	0.077	1.768	0.237	0.069	1.816	0.227	0.069	1.815	0.226
	Total Endoparasite	0.145	1.456	0.168	0.228	1.206	0.130	0.266	1.112	0.119
Circle Lake	<i>Bothriocephalus cuspidatus</i>	0.056	-1.910	-0.211	0.008	-2.634	-0.270	0.007	-2.701	-0.278
	<i>Proteocephalus</i> sp.	0.922	-0.098	-0.011	0.575	-0.561	-0.059	0.458	-0.742	-0.078
	<i>Trienophorus nodulosus</i>	0.416	-0.814	-0.107	0.259	-1.128	-0.138	0.332	-0.969	-0.119
	<i>Azygia angusticauda</i>	0.356	0.923	0.121	0.772	0.289	0.035	0.807	0.244	0.030
	<i>Sanguinicola occidentalis</i>	0.249	1.152	0.146	0.354	0.928	0.109	0.372	0.893	0.105
	<i>Diplostomum</i> sp.	0.983	-0.021	-0.003	0.933	-0.084	-0.010	0.950	-0.063	-0.007
	<i>Prosorhynchoides pusilla</i>	0.032	2.139	0.252	0.009	2.626	0.287	0.012	2.508	0.274
	<i>Camallanus oxycephalus</i>	0.786	0.271	0.035	0.653	0.450	0.054	0.570	0.569	0.068
	<i>Raphidascaris</i> sp.	0.062	-1.863	-0.219	0.079	-1.754	-0.191	0.100	-1.647	-0.179
	Total Endoparasite	0.141	-1.471	-0.162	0.032	-2.140	-0.219	0.017	-2.397	-0.246

Table 12. Results of Kendall's rank correlation coefficient comparing Black Bay and Circle Lake walleye attributes to endoparasite intensity. Kendall's rho could not be computed for *A. angusticauda* in Black Bay due to insufficient number of presences. Grey blocks indicate instances of statistical significance.

Parasite	Age			Weight			Length		
	<i>p</i> -value	<i>z</i>	<i>tau</i>	<i>p</i> -value	<i>z</i>	<i>tau</i>	<i>p</i> -value	<i>z</i>	<i>tau</i>
<i>Bothriocephalus cuspidatus</i>	0.183	1.332	0.172	0.400	0.841	0.101	0.476	0.712	0.086
<i>Azygia angusticauda</i>	---	---	---	---	---	---	---	---	---
Black Bay <i>Prosorhynchoides</i> sp.	0.531	0.626	0.101	0.841	0.200	0.030	0.881	0.150	0.022
<i>Diplostomum</i> sp.	0.524	0.637	0.140	0.656	0.446	0.093	0.657	0.444	0.092
<i>Echinorhynchus</i> sp.	0.654	0.449	0.149	0.664	0.435	0.138	0.664	0.435	0.138
<i>Bothriocephalus cuspidatus</i>	0.038	-2.079	-0.238	0.005	-2.796	-0.297	0.005	-2.838	-0.302
<i>Proteocephalus</i> sp.	0.678	-0.415	-0.052	0.405	-0.832	-0.096	0.278	-1.085	-0.125
<i>Triaenophorus nodulosus</i>	0.264	-1.118	-0.530	0.480	-0.707	-0.316	0.480	-0.707	-0.316
<i>Azygia angusticauda</i>	0.589	-0.540	-0.199	1.000	0.000	0.000	1.000	0.000	0.000
Circle Lake <i>Sanguinicola occidentalis</i>	0.223	1.217	0.268	0.254	1.140	0.239	0.277	1.088	0.230
<i>Diplostomum</i> sp.	0.891	0.136	0.026	0.571	-0.566	-0.100	0.594	-0.533	-0.094
<i>Prosorhynchoides pusilla</i>	0.683	0.408	0.062	0.465	0.731	0.104	0.492	0.687	0.098
<i>Camallanus oxycephalus</i>	0.565	0.576	0.168	0.824	0.223	0.061	0.738	-0.335	-0.092
<i>Raphidascaris</i> sp.	0.660	-0.440	-0.066	0.349	-0.937	-0.130	0.402	-0.837	-0.116

Table 13. Results of Mann Whitney U test comparing Black Bay and Circle Lake host gender against endoparasite abundance.

	Endoparasite	p-value	U
Black Bay n. male = 10 n. female = 26	<i>Bothriocephalus cuspidatus</i>	0.90	126
	<i>Azygia angusticauda</i>	0.58	125
	<i>Prosorhynchoides pusilla</i>	0.11	86.5
	<i>Diplostomum</i> sp.	0.98	131
	<i>Echinorhynchus</i> sp.	0.44	114.5
	Total endoparasite	0.58	114
Circle Lake n. male = 16 n. female = 25	<i>Bothriocephalus cuspidatus</i>	0.70	215
	<i>Proteocephalus</i> sp.	1.00	200.5
	<i>Triaenophorus nodulosus</i>	0.16	176
	<i>Azygia angusticauda</i>	0.48	182
	<i>Sanguinicola occidentalis</i>	0.90	204.5
	<i>Diplostomum</i> sp.	0.92	196
	<i>Prosorhynchoides pusilla</i>	0.97	198
	<i>Camallanus oxycephalus</i>	0.84	206
	<i>Raphidascaris</i> sp.	0.80	209.5
	Total endoparasite	0.82	209

Comparison of co-occurring parasites

Based on sample of hosts collected in this study, the component community was 7 for Black Bay walleye, and 14 for Circle Lake walleye. Six species occurred in both study locations: *E. caeruleus*, *U. aculeatus*, *B. cuspidatus*, *P. pusilla*, *A. angusticauda* and *Diplostomum* sp. The results of the Fisher's exact test comparing prevalence among species shared by both study locations showed that gill parasites *E. caeruleus* and *U. aculeatus* were more prevalent in Circle Lake walleye (Table 14). The distribution free bootstrap test did not reveal any statistically significant differences in mean intensities between any of the co-occurring parasite species (Table 14).

Comparison of parasite diversity

Mean infracommunity richness was significantly higher in Circle Lake (mean = 7.59) as compared to Black Bay (mean = 2.84) (two sample t-test: $t = 15.2$, $df = 80.44$, p -value = <0.001 ; Figure 8). The maximum infracommunity richness exhibited by individual walleye from Circle Lake was 11—which is more than twice that of Black Bay's richest infracommunity (max = 5; Figure 8). There was no significant correlation between infracommunity richness and host age, length, and weight for either sample location (Table 15).

Mean Simpson's diversity index was significantly different between both samples (two sample t-test: $t = -4.9$, $df = 73.64$, p -value = <0.001), indicating that on average Circle Lake infracommunities had higher diversity than Black Bay walleye (Figure 9), with mean diversity indices of 0.637 and 0.483, respectively. There were no significant relationships between diversity and host attributes for either sample location (Table 15).

Table 14. Results of Fisher’s exact test of independence and bootstrap test comparing the prevalence and mean intensities of parasites in common to Black Bay and Circle Lake walleye. The bootstrap test could not be performed on *U. aculeatus* or *A. angusticauda* due to insufficient number of infections in Black Bay.

	Black Bay	Circle Lake	Prevalence		Intensity	
	n	n	p-value	estimate	mean p-value	mean t
<i>E. caeruleus</i>	38	46	0.023	0.000	0.245	0.120
<i>U. aculeatus</i>	2	45	< 0.001	0.000	---	---
<i>B. cuspidatus</i>	35	43	0.110	0.310	0.970	-0.290
<i>P. pusilla</i>	24	26	1.000	0.970	0.218	0.030
<i>A. angusticauda</i>	1	7	0.060	0.140	---	---
<i>Diplostomum</i> sp.	14	21	0.280	0.580	0.949	0.000

Table 15. Kendall's rank correlation coefficient testing the relationship of host attributes on infracommunity richness and infracommunity diversity.

		Age			Weight			Length		
		<i>p-value</i>	<i>z</i>	<i>tau</i>	<i>p-value</i>	<i>z</i>	<i>tau</i>	<i>p-value</i>	<i>z</i>	<i>tau</i>
Black Bay n = 43	richness	0.833	0.211	0.026	0.619	0.497	0.057	0.682	0.410	0.047
	diversity	0.518	0.647	0.074	0.564	0.577	0.062	0.564	0.577	0.062
Circle Lake n = 46	richness	0.395	-0.851	-0.100	0.519	-0.645	-0.070	0.500	-0.674	-0.073
	diversity	0.961	-0.049	-0.005	0.962	-0.047	-0.005	0.925	-0.095	-0.010

Figure 8. Numerical distribution of parasite infracommunity richness across each sample location.

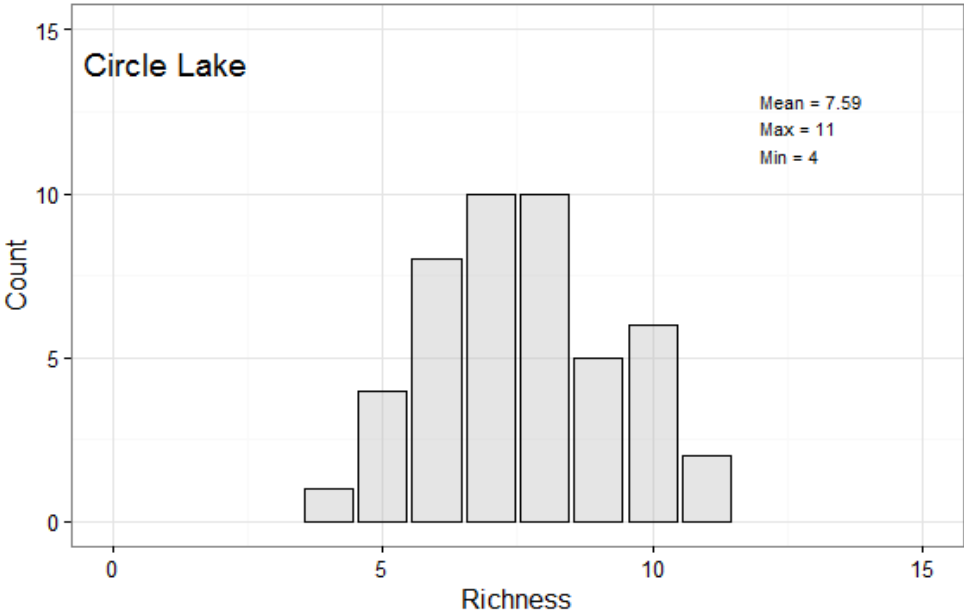
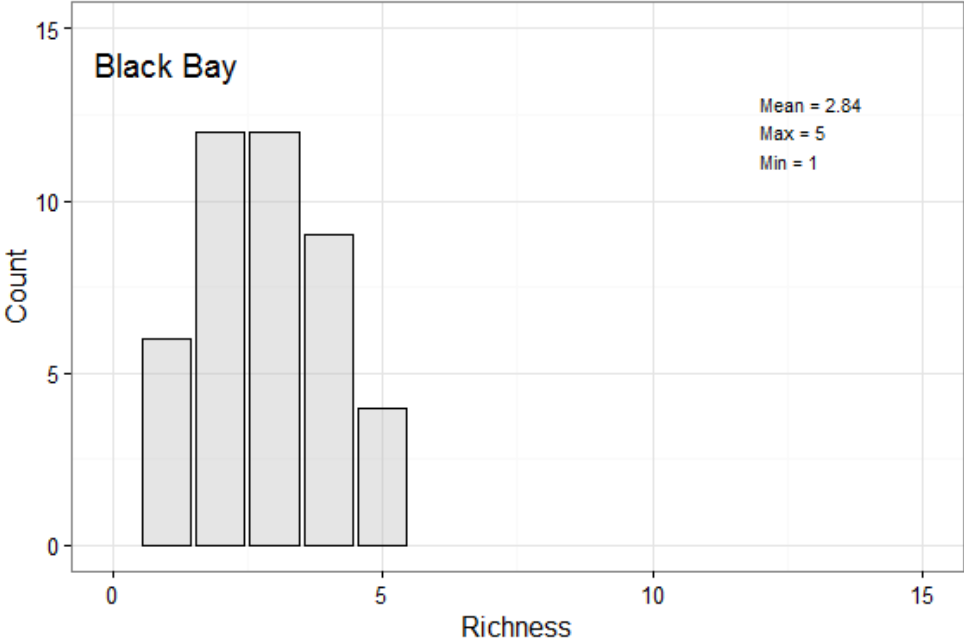
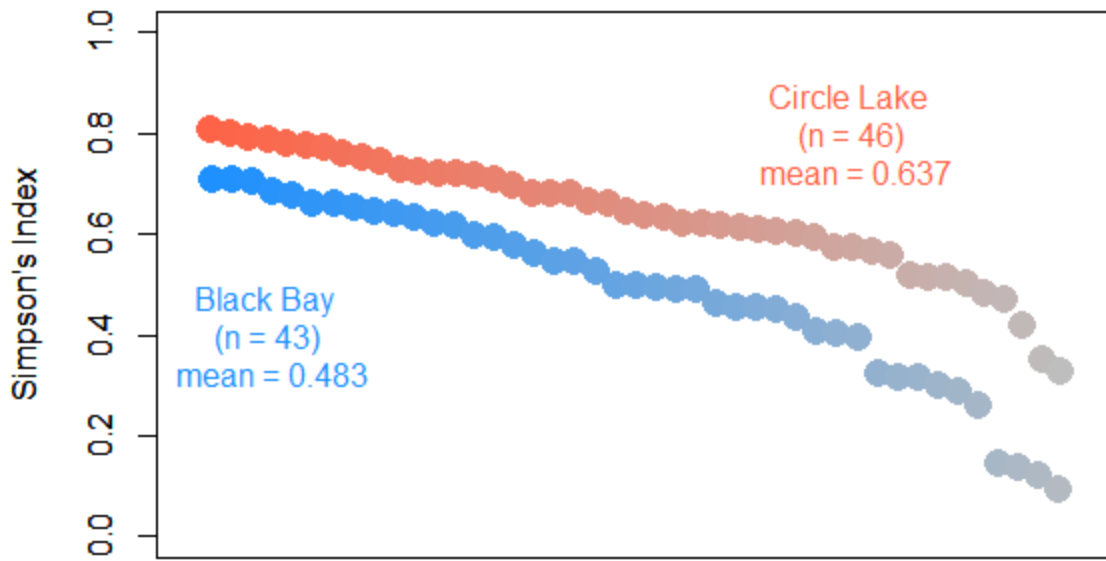


Figure 9. Ordered plot showing Simpson's diversity index for parasite infracommunities in both Black Bay and Circle Lake. Each circle represents an infracommunity.



Nestedness Analysis

The results of the nestedness analysis revealed that all facets of the parasite community tested (all parasites, all adult parasites, ectoparasites, and endoparasites) exhibited some evidence of a structured assemblage for both Black Bay and Circle Lake walleye (Table 16). Nestedness for the whole matrix ranged from $10.369 < NODF < 71.146$ ($\overline{NODF} = 54.9$) for Black Bay and $53.983 < NODF < 72.752$ ($\overline{NODF} = 66.5$) for Circle Lake. The results also suggest that nestedness in the Black Bay walleye parasite community is more dependent on differences among sites (i.e. walleye hosts), as in 3 of 4 of the facets analyzed the measured $NODF_{row}$ was higher than $NODF_{col}$. The only facet in which $NODF_{col}$ was higher than $NODF_{row}$ was for Black Bay ectoparasites, which can be explained by the presence of only two parasites in the ectoparasite community, and because *U. aculeatus* was always found coexisting with *E. caeruleus*. By contrast, in Circle Lake, $NODF_{col}$ was found to be higher than $NODF_{row}$ in all four circumstances—indicating that nestedness is more dependent on parasite species occupancy.

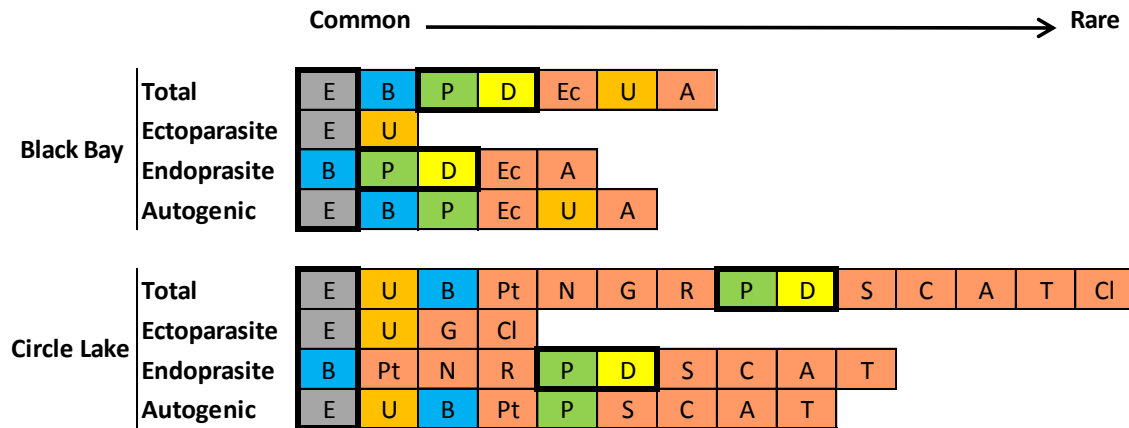
Although not statistically significant, the RN value of Black Bay total parasites, adult parasites, ectoparasites, and endoparasites was always higher than that of Circle Lake (two sample t-test; $t = 1.3$, $df = 5.69$, p -value = 0.1), implying a greater degree of nestedness. $NODF$ values for both total and row measures in Circle Lake ectoparasites were the only components that were not statistically significant. Aside from the $NODF_{col}$ for Black Bay endoparasites ($p < 0.05$), all nestedness measures that were significant at the $p < 0.001$ level (Table 16).

The packing of matrices revealed that both *E. caeruleus* and *B. cuspidatus* serve as the most important baseline parasite in their respective categories for both sample locations (Figure 10). Furthermore, *P. pusilla* and *Diplostomum* sp. exhibited similar patterns in their nesting order across both sample locations. See Appendix A for visualization on how matrices were re-ordered for nestedness analysis.

Table 16. Results of nestedness analysis. NODF values are given for empirical and null matrices pertaining to each facet analyzed (All, Adult, Ectoparasite, and Endoparasite). Grey blocks indicate the location where higher relative nestedness values (RN) were observed.

	METRIC	Black Bay				Circle Lake				
		INDEX	Z-SCORE	RN	NESTED?	INDEX	Z-SCORE	RN	NESTED?	
All	Observed	NODF	71.146	7.269	0.516	Yes (p<0.001)	72.621	7.604	0.283	Yes (p<0.001)
		NODF_row	71.355	7.315	0.522	Yes (p<0.001)	72.229	7.302	0.283	Yes (p<0.001)
		NODF_col	62.16	3.911	0.286	Yes (p<0.001)	77.077	8.699	0.28	Yes (p<0.001)
	Null		MEAN	ST.DEV.	MIN	MAX	MEAN	ST.DEV.	MIN	MAX
		NODF	46.232	2.894	37.665	53.431	56.62	2.104	51.505	61.883
		NODF_row	46.191	2.898	37.514	53.415	56.304	2.181	50.95	61.678
	NODF_col	47.968	3.732	37.209	60.679	60.216	1.938	54.813	64.208	
Adult	Observed	NODF	70.06	7.263	0.494	Yes (p<0.001)	72.752	5.389	0.249	Yes (p<0.001)
		NODF_row	70.118	7.298	0.496	Yes (p<0.001)	72.435	5.259	0.247	Yes (p<0.001)
		NODF_col	66.548	4.446	0.367	Yes (p<0.001)	81.891	6.914	0.3	Yes (p<0.001)
	Null		MEAN	ST.DEV.	MIN	MAX	MEAN	ST.DEV.	MIN	MAX
		NODF	46.9	3.189	40.529	55.089	58.251	2.691	49.199	64.339
		NODF_row	46.87	3.186	40.495	55.076	58.086	2.728	48.88	64.379
	NODF_col	48.677	4.019	38.44	57.16	62.997	2.733	56.034	69.114	
Ectoparasite	Observed	NODF	10.369	32.674	0.012	Yes (p<0.001)	60.935	2.207	0.047	Yes (p<0.05)
		NODF_row	10.242	5.04E+10	0.0	Yes (p<0.001)	60.725	2.131	0.045	Yes (p<0.05)
		NODF_col	100.0	32.759	6.158	Yes (p<0.001)	97.211	1.697	0.25	Yes (p<0.05)
	Null		MEAN	ST.DEV.	MIN	MAX	MEAN	ST.DEV.	MIN	MAX
		NODF	10.247	0.004	10.242	10.259	58.2	1.239	53.459	59.787
		NODF_row	10.242	0.0	10.242	10.242	58.086	1.238	53.333	59.614
	NODF_col	13.97	2.626	10.526	22.222	77.777	11.45	43.243	91.42	
Endoparasite	Observed	NODF	68.168	6.581	0.472	Yes (p<0.001)	59.832	5.247	0.266	Yes (p<0.001)
		NODF_row	68.279	6.645	0.474	Yes (p<0.001)	59.647	5.181	0.264	Yes (p<0.001)
		NODF_col	60.357	2.292	0.271	Yes (p<0.05)	64.09	5.103	0.299	Yes (p<0.001)
	Null		MEAN	ST.DEV.	MIN	MAX	MEAN	ST.DEV.	MIN	MAX
		NODF	45.784	3.194	37.537	53.044	47.27	2.394	41.925	53.86
		NODF_row	45.769	3.185	37.482	53.011	47.181	2.406	41.647	53.704
	NODF_col	46.897	5.286	28.077	64.748	49.33	2.892	41.482	57.438	

Figure 10. Importance of parasite species and their contribution to a nested pattern for a given category. Grey, blue, green, yellow, and orange indicate parasites that exist in both sample locations and red indicates parasites that are sample exclusive. Boxes highlighting similarities in community structure are emboldened. E = *E. caeruleus*, B = *B. cuspidatus*, P = *P. pusilla*, D = *Diplostomum* sp., Ec = *Echinorhynchus* sp., U = *U. aculeatus*, Pt = *Proteocephalus* sp., N = *Neascus* sp., G = Glochidia, R = *Raphidascaris* sp., S = *S. occidentalis*, C = *C. oxycephalus*, A = *A. angusticauda*, T = *T. nodulosus*, Cl = *C. complanatum*.



Discussion

Premise

The premise of my study was to compare the parasite community of Black Bay walleye to a population deemed more representative of Northwestern Ontario as a whole. The Black Bay walleye population collapsed in the early 1970's as a result of anthropogenic pressures (S. Bobrowicz 2010). Currently, the population appears to be trending towards a state of rehabilitation (Berglund 2016). A priority for the Ontario Ministry of Natural Resources is the recovery of the Black Bay walleye population. Baseline parasite information can be used to supplement the information guiding management decisions directed towards walleye recovery. The information gathered from this study will hopefully provide some valuable insight into the biology of walleye in population of economic significance.

Black Bay is a unique waterbody in that it is tied to a large open system that is predominantly oligotrophic. Its boundaries contain the shallow, turbid water preferred by walleye (Scott and Crossman 1973) that is uncharacteristic of Lake Superior at large. However, overall size and constant circulation of cold water from the main basin keep water temperatures relatively low in comparison to most inland lakes. By contrast, Circle Lake is a small, warm, and more comparable to the vast amount of lakes that flood Northwestern Ontario. It was chosen as a surrogate because of its geographic proximity to Black Bay, the fact that it resides on a watershed draining into Black Bay, and under the assumption that parasites present in Circle Lake should represent species that are regionally available. While trivial, it can be assumed that the parasite communities of walleye in both Black Bay and Circle Lake are different. The aim of my study was to address the reasons why they are different, while at the same time also highlighting any apparent similarities.

Hosts & Parasites

Black Bay walleye were both longer and heavier than Circle Lake walleye. However, mean host age between samples did not significantly differ. This indicates that Black Bay walleye have a higher growth rate compared to Circle Lake walleye as they are achieving a greater size at a younger age. These results should not be surprising as the Black Bay fish community is much more diverse, and thus forage fish as suitable prey options are likely more abundant and available. The stomach content analysis supports this as forage fish were more common in the diet of Black Bay walleye. Higher growth rates in a fish population are often indicative of ontogenetic shifts towards piscivory occurring at an earlier age (Olson 1996). Therefore, the difference in size apparent in Black Bay walleye should be attributed to their diet.

Black Bay walleye were infested with two ectoparasites, both of which were adults and employ a direct life cycle: *U. aculeatus* and *E. caeruleus*. Both these parasites were also found infesting Circle Lake walleye. The prevalence of both *E. caeruleus* and *U. aculeatus* was significantly higher in Circle Lake walleye; however, Black Bay infestations still occurred at a high rate (88%). By contrast, *U. aculeatus* was rare in Black Bay walleye. Both of these parasites transmit directly to their host. Further study is required to provide more definitive answers as to why these differences in prevalence were observed; although it is likely that host abundance or density are an important factor in determining rates of infestation for parasites transmitting directly (Arneberg 2002). Black Bay has just recently entered a state of recovery and walleye biomass is still considered to be low (Berglund 2016). Considering a large open system, if transmission and colonization potential for ectoparasites is dependent on, or influenced by host density (Arneberg, Skorping, et al. 1998), then consistent contact events between infested and uninfested hosts may be significantly reduced outside of annual spawning aggregations. As a walleye population increases it may be predicted that host numbers become sufficient to establish higher rates of ectoparasite infestation. Comparatively, Circle Lake is a small and

confined waterbody and therefore chances of contact between infested and uninfested hosts should be higher. Furthermore, with recent research suggesting that Black Bay walleye exhibit migratory behavioural polymorphism (UGLMU, unpublished), the actual density of walleye within the boundaries of Black Bay at given time is likely variable. If host density and abundance are important factors contributing to rates of ectoparasite infestation in Black Bay, then as walleye populations increase so should ectoparasite numbers.

Host Size

A unique aspect of using parasites as models in community ecology is that each host represents a replicate location after the effects of host biology on parasite numbers has been controlled for. The effect of host biology on parasites is variable (Lo et al. 1998, Zelmer and Arai 1998, Carney and Dick 2000, Poulin 2011). The general hypothesis is that larger hosts should harbour more parasites—they eat more (exposure), they provide more habitat options, they are larger targets for direct transmission, and they have been alive for a longer period of time (accumulate) (Bush et al. 2001). Host attributes (i.e. size, weight, age) have been shown to influence parasite numbers in fish (Bush, et al. 2001), and more specifically in percids (Zelmer and Arai 1998, Carney and Dick 2000), with the latter studies being contradictory.

I evaluated the effect of host size, weight, age and sex on both ectoparasite and endoparasite numbers separately. In Black Bay, *E. caeruleus* abundance showed negative correlation to all three host attributes. Additionally, *E. caeruleus* intensity in Black Bay was correlated to all three host attributes tested. By contrast, in Circle Lake walleye there were no statistically significant correlations with *E. caeruleus* in host attributes. Lo et al. (1998) investigated host age and size relationships with parasites in reef fishes from French Polynesia and consistently found positive correlations between individual ectoparasite abundance and host length, and total ectoparasites abundance and host age. Furthermore, Carney and Dick (2000) reported that ectoparasites abundance and intensity were rarely correlated to

yellow perch attributes. Yellow perch represent a comparable host species to walleye as they are phylogenetically similar and can be infected by similar or identical parasites (Carney and Dick 2000, Poulin 2010, Muzzall and Whelan 2011). Contrasting patterns between Black Bay and Circle Lake indicate that the results of my study are more in line with those of Carney and Dick (2000), and suggest that ectoparasites infrapopulations are inconsistently associated with host attributes.

The bathymetry of Black Bay changes considerably across its breadth. Different lake characteristics such as depth and size have been shown to influence parasite community structure (Marcogliese and Cone 1991). It is possible that habitat preference may be a factor dictating *E. caeruleus* abundance as smaller walleye tend to be more infested. This may be explained by the tendency for smaller walleye to inhabit shallower water (Scott and Crossman 1973). Recent research tracking walleye movement in Lake Superior revealed that a substantial proportion of large Black Bay walleye migrate beyond the productive boundaries of the bay and into the more oligotrophic waters of the open lake (UGLMU, unpublished). If smaller walleye refrain from migrating, then this evidence would support the prediction that smaller walleye are more prone to *E. caeruleus* infestations because of their tendency to reside in the shallow zones within the Bay (Scott and Crossman 1973). This would also assume that walleye exist at a higher density in the shallow zones in Black Bay and thus contact events between infested and uninfested walleye occur at a higher rate. Alternatively, decreasing copepod numbers in larger walleye may be a result of host immunological responses (Lo et al. 1998) being more effective with age. Undoubtedly, reasons for negative correlations between *E. caeruleus* and host attributes require further investigation. It may be possible to address this question through experimental infestations in a laboratory environment or by conducting a specialized field study that is parasite species specific. Further, if the walleye migrating out of Black Bay are in fact less infested with *E. caeruleus*, then this would present an opportunity to use parasites as a biological tag (Mackenzie and Abaunza 1998).

Considering positive correlations in *U. aculeatus* infections and host attributes, perhaps this pattern can be explained by the increase in surface area in the gills of larger fish and thus greater available niche space. Additionally, as the surface area of the gills increases with fish size the amount of water that regularly passes over the gills also increases, potentially assisting *U. aculeatus* transmission potential (Lo et al. 1998). By contrast, the negative correlation in glochida abundance by host attributes may be indicative of life-history stage. Some mussels of the Family Unionidae employ a unique strategy to transmit their larvae to their host; that is, they bait a host with a modified extension of their mantle disguised as a prey item and then quickly collapse their shell to eject the larval glochidia onto the fish's gills (Burch 1975). While there has been a diversity of specialized "lures" identified in unionids (Haag and Warren 1999), if those present in Circle Lake mimic a prey item more readily targeted by younger and smaller fish then a negative correlation with size should be observed.

Bothriocephalus cuspidatus and *P. pusilla* abundance in Circle Lake was correlated to host attributes. Zelmer and Arai (1998) found that all the yellow perch parasites examined in their study increased in abundance as fish grew older and larger. Further, Carney and Dick (2000) found that both host length and age contributed to higher total enteric and allogenic parasite abundance in some, but not all yellow perch communities. The contrasting results between studies suggest that there is no general pattern for endoparasites and host attributes in yellow perch. The results of my study are more in line with Carney and Dick (2000) in that there is no apparent pattern of endoparasites increasing with host attributes. Lack of correlations between endoparasite numbers and host attributes may be the result of a number of sample specific, non-mutually exclusive factors, including available forage, host biology, and lake characteristics

Contrary to both Zelmer and Arai (1998) and Carney and Dick (2000) is that *B. cuspidatus* abundance relationships in Circle Lake were negative for both weight and length, but not age. Negative correlations with larger host size are far more infrequent within the literature. This result may be

related to dietary preferences among size classes, or perhaps immunological history. As fish grow diet preferences change as they are capable of consuming larger, more energy rewarding prey. This shift can be used to describe the transition from invertebrates as a primary food source, to forage fish as a primary food source (Galarowicz et al. 2006). Endoparasites exploit food webs to transmit to their host, meaning that infections often require the consumption of an intermediate host infected with a larval stage. In the case of *B. cuspidatus*, larval procercooids develop in copepods and transmit to a definitive host through intermediate host ingestion. Thus, the degree of exposure to the infective stage of *B. cuspidatus* is tied to the number of infected copepods a host ingests. If copepods trend more towards a secondary food source as walleye grow and alter their diet, perhaps declining *B. cuspidatus* abundance in large fish is reflective of this. It should be noted that parasite longevity is an important covariate to this assumption. If tapeworms have a long lifespan, the continual addition of more individuals would suggest that intensity should increase the longer a host has been alive. If tapeworms have a short life span, then as individuals die off the acquisition of new individuals may decline as a host grows and changes its dietary preference.

An alternative interpretation to *B. cuspidatus* infections existing at lower abundance in larger fish could be that walleye are acquiring parasites while targeting forage fish. This means that infected copepods are either ingested accidentally, or perhaps forage fish are serving as suitable paratenic hosts through post cyclical infection. Essex (1928) speculated this to be a potential route of transmission for *B. cuspidatus* in larger walleye. Furthermore, the tendency for larger walleye to have a more piscivorous diet supports this possibility (Scott and Crossman 1973).

Diet

Rainbow smelt likely serve as the most important prey item for Black Bay walleye based on Myers et al. (2009) report on smelt abundance. This observation was supported by my study, as the stomach contents of Black Bay walleye contained primarily rainbow smelt. The drawback of stomach

content analysis is that it provides information on prey items that were consumed during a short time frame prior to capture. By contrast, the parasite community can provide information on the dietary habits of a host over the course of its life history. Considering the adult endoparasites in Black Bay, life cycles indicate that in addition to rainbow smelt, walleye are consuming copepods and amphipods (Essex 1928, Amin 1978). Thus, while rainbow smelt and other forage fish can be considered the most important component of walleye diet in Black Bay, copepods and amphipods should be considered important secondary prey items. This information is not indicated in the stomach contents.

Stomach contents from Circle Lake revealed that walleye have a much more diverse diet in comparison to Black Bay. In Circle Lake, the invasive rainbow smelt are absent and thus walleye rely on forage species that exist naturally in the water body such as yellow perch, white sucker, and logperch. Further, it appears as though the invertebrate community provides a much more important component of the walleye diet in Circle Lake. This is supported through both the stomach contents and the parasite community. The presence of *B. cuspidatus*, *Proteocephalus* sp., and *T. nodulosus* all require the ingestion of a copepod, a prey item which was also found in the stomach contents. With that said, only one individual had a copepod in its stomach contents—but 43 walleye had parasites associated with copepod intermediate hosts.

Diversity

Circle Lake parasite diversity is much greater than in Black Bay. Species richness at the component community level in Circle Lake was double that of Black Bay (14 and 7, respectively). Poulin (2003) found that geographic proximity could be used as a tool to predict component community similarity in yellow perch and northern pike. The hypothesis of Poulin (2003) predicts that as communities become more distant, their similarity will decay exponentially. Poulin (2010) also showed that similarity in parasite communities exists within percids as phylogenetically similar hosts. Circle Lake was chosen as a surrogate under an assumption that waterbodies close in geographic proximity could

exhibit similar parasite species composition in percids. Poulin (2003) also found that there was no pattern of parasite community similarity in white sucker meaning that rules dictating similarity do not applied universally.

Undoubtedly, biogeography is still an important factor in dictating the local parasite species in a region. While it does not apply to my study entirely, there are a number of additional factors that should also be considered in comparing parasite diversity across samples. First, Black Bay is part of an open system that is predominantly oligotrophic. By contrast, the physical features of Circle Lake are far more comparable to a large number of inland lakes of Northwestern Ontario; that is, they are relatively shallow, warm, and unaltered. Marcogliese and Cone (1996) highlighted the influence of lake characteristics in determining the parasite community structure in salmonids, and concluded that the lake depth is an important factor contributing to parasite community structure, especially with regards to larval digeneans. A general assumption is that shallow water supports the gastropod intermediate hosts required for allogenic life cycles. Considering allogenic species, Black Bay walleye were only infected with *Diplostomum* sp., whereas Circle Lake walleye was infected by *Diplostomum* sp., *Neascus* sp., and *C. complanatum*. All three of these species rely on cercarial penetration to transmit to their host (Schell 1985). Further, there are also autogenic digeneans in Circle Lake relying on cercarial penetration (*A. angusticauda*, *S. occidentalis*; Wootton 1957, Muzzall 2000). High richness of digenetic trematodes suggests that Circle Lake walleye are more prone to infections because they spend more time in shallow water where snail intermediate hosts occur. This places them in proximity to be infected by cercaria being shed by infected snails.

Structure and Assembly

The purpose of performing nestedness analysis is to address whether or not walleye parasites exhibit patterns in their community structure. In the context of parasite communities, infracommunities are said to be nested when species poor infracommunities exist as subsets of progressively richer ones,

or put another way, communities become richer by adding more and more increasingly rare species after the common species are present.

The results of the nestedness analysis revealed that all facets of the parasite communities from both locations exhibited a significant degree of structuring during the time period when they were collected (Table 14). These results are consistent with those of Carney and Dick (2000), although different nestedness metrics were used. Because of ongoing debate on which nestedness metric and null model are most pertinent (Wright et al. 1998, Ulrich et al. 2009, Strona et al. 2014, Strona and Fattorini 2014), careful consideration was taken such that the combination is most appropriate for an antagonistic network (host-parasite). Currently, NODF is considered to be a robust alternative to its predecessors (Strona and Fattorini 2014), namely, N (number of presences and absences; Patterson and Atmar 1986) and T (matrix temperature; Atmar and Patterson 1993). The reason that I chose NODF as a metric is because it is less constrained by matrix size (i.e. sum of rows and columns) and shape (i.e. which component (hosts or parasite species) is represented by columns or rows) (Ulrich et al. 2008). For example, absolute values of T (Atmar and Patterson 1993) have been shown to positively correlate with matrix size and shape (Ulrich and Gotelli 2007). This means that the position of hosts or parasites species within a matrix (columns or rows) has an effect on the measure of nestedness. In a host-parasite array, the number of hosts often exceeds the number of parasite species leaving a matrix of uneven dimensions, and thus, producing inconsistent nestedness values under the T metri. The NODF metric is however influenced by matrix fill (i.e. number of presences) like all metrics previously mentioned. This warrants the use of the relative nestedness (RN) value for cross-sample comparison (Bascompte et al. 2003, Strona et al. 2014).

In order to assess the significance of the nestedness metric, a comparison must be made up against a set of null matrices (Strona et al. 2014). There are a variety of null models that have been developed over the years, with each receiving a fair amount of criticism (Ulrich et al. 2009). Recently, CE

(proportional row totals, proportional column totals) has been recommended as a preferable null model algorithm due to its “ecological realism” (Strona and Fattorini 2014). CE gives each cell in a null matrix a probability to be occupied based on the empirical row and column totals. In the context of parasites, this means that prevalence of a species is maintained from empirical to theoretical matrices.

Significant nestedness results suggest that infracommunities are not assembled at random from species available at the component community level, but are assembled by underlying structuring forces. A number of hypotheses have been suggested to produce a nested subset pattern in free-living species, including passive sampling, selective colonization, and selective extinction (Ulrich et al. 2009). Passive sampling predicts that regional abundance dictates species occupancy or that the community composition can be explained by the relative abundances of the species in the community. Carney and Dick (2000) noted that without abundance information on the infective stage of parasites, passive sampling cannot be ruled out as a process that produces a nested pattern. Patterson and Atmar (1986) attributed nested patterns in mammalian communities to species ability to colonize a habitat. In the context of parasites, this would refer to parasite transmission potential. Investigating transmission potential of specific parasite species is outside this scope of my study, and in the absence of information, transmission rates also cannot be ruled out as important factors contributing to the observed nested structure.

Habitat gradients are also a common theme in nestedness research (Ulrich et al. 2009). In parasite communities, the host is the habitat and the gradients could be the differences among host attributes within a sample. If the presence of a species is correlated to host attributes such as age, size, or sex, then perhaps host heterogeneity can explain a nested structure. Guégan and Hugueny (1994) concluded that host size was likely an important factor in producing a nested structure in marine ectoparasites of fish. This was supported by Poulin and Valtonen (2001), who also found nested subset patterns in the endoparasites of fish, and furthermore found that stronger relationships between host

size and richness existed in nested communities. In my study, most parasites with abundance correlated with host attributes (*E. caeruleus*, *U. aculeatus*, *B. cuspidatus*, *P. pusilla*, glochidia) were in turn the most important (common) species in the infracommunities. These are the species that form the base of the structured (nested) community. The exception with these parasites is that for the most part they do not support the general theory that parasite abundance should increase with host size and age, as *E. caeruleus*, *B. cuspidatus*, and glochidia all exhibited a negative relationship with these host traits. Further, there was no correlation between richness or diversity and host attributes in either sample location. This evidence suggests that the difference in age and size structure within a sample did not contribute to a nested pattern.

The process of conducting a nestedness analysis inherently results in empirical presence-absence matrices becoming “maximally packed” (Strona and Fattorini 2014). This means that species are ordered from common to rare, thus providing insight on the general order in which species are accumulated within a host (Figure 10), in turn allowing for speculation on the mechanisms controlling assembly. Considering the process of assembly for base species in the parasite community of Black Bay walleye—*E. caeruleus* is acquired via direct transmission (Abdelhalim, Lewis and Boxshall 1991), *B. cuspidatus* is acquired via copepod ingestion (Essex 1928), and *P. pusilla* is acquired via ingestion of a forage fish (Woodhead 1930). With the omission of *U. aculeatus* which had extremely low prevalence and intensity, this arrangement suggests that the process of assembly is first dictated by contact with infected hosts (direct transmission), and then by feeding. Further, the sequence of *B. cuspidatus* followed by *P. pusilla* supports the hypothesis that ongoing feeding of copepods is supplemented by fish; or that copepod transmission is higher potential than fish transmission.

In Circle Lake, higher species richness makes for a more complex set of rules governing assembly. However, close examination of the maximally packed matrices revealed a similar pattern of assembly to that in Black Bay. Considering the nested structure of base species in Circle Lake (Figure

10), *E. caeruleus* and *U. aculeatus* represent the first two species acquired, both of which are transmitted directly (Cone and Burt 1981, Abdelhalim et al. 1991). Following are *B. cuspidatus* and *Proteocephalus* sp., which are both acquired by ingestion of a copepod (Essex 1928, Scholz 1999). *Raphidascaris* sp. is then acquired by the ingestion of insect larvae or fish intermediate host (Smith 1984), and lastly, *P. pusilla* is acquired by ingestion of a fish (Woodhead 1930). Aside from base species unique to Circle Lake (*Neascus* sp., glochidia), this pattern of assembly is consistent with that of Black Bay—that is, directly transmitted parasites are acquired first, followed by those using copepod/invertebrate and fish intermediate hosts. This evidence suggests that in general, contact with infested hosts followed by dietary behaviour is the order of processes guiding community assembly. Therefore, although the community structures are not the same, mechanisms contributing to base species assembly are comparable.

The rules governing the assembly of rare species are more speculative. The rare species in Black Bay (*U. aculeatus*, *Echinorhynchus* sp., and *A. angusticauda*) also occur at low intensity (Table 4, 10). Because *U. aculeatus* transmits directly, low intensity is perhaps a reflection of low host abundance within the system. Support for this is seen in the fact that *E. caeruleus* comparatively had high prevalence and also relies on contact events with suitable hosts for transmission. This contrasting pattern suggests that, frequent contact with infested hosts should be excluded as a factor dictating the acquisition of *U. aculeatus*. Further, both prevalence and intensity of *U. aculeatus* were high in Circle Lake walleye, indicating that when present at high abundance within a system colonization potential is high on walleye hosts. This evidence suggests that low abundance of *U. aculeatus* in the Black Bay system is a plausible reasoning for its rarity.

The rarity of *Echinorhynchus* sp. in Black Bay walleye may be associated with amphipods being of lesser dietary importance, or being infected at lower rates. Echinorhynchids are generalist parasites that can infect a wide of array of phylogenetically different fish hosts (Margolis and Kabata 1989). With

the diverse fish community in Black Bay, it is likely that several fish species serve as reservoirs for *Echinorhynchus* sp. This suggests that local abundance of *Echinorhynchus* sp. should only be considered a factor contributing to community assembly if the intermediate amphipod hosts are in low abundance. It is more likely that prey choice in combination with transmission success rates are dictating the presence of *Echinorhynchus* sp. in Black Bay.

In Circle Lake, rare species also occurred at low intensity (Table 4, 10). However, rare species richness was much higher than in Black Bay. As previously mentioned, a likely factor contributing to the higher richness observed in Circle Lake is that walleye may frequent the shallow water where snail intermediate hosts reside. This behaviour would expose walleye to the digenean parasites relying on cercarial penetration as a means of transmission. There are both allogenic (*Neascus* sp., *Diplostomum* sp., *Clinostomum* sp.) and autogenic (*S. occidentalis*) digenean trematodes in Circle Lake using cercarial penetration. Aside from *Diplostomum* sp., all other species employing cercarial penetration are unique to the Circle Lake sample. This suggests that either species absent from Black Bay are: (1) not locally available or (2) walleye are not exposed to them. Relatively high prevalence of *Diplostomum* sp. in Black Bay (33%) contradicts the latter interpretation, thus low abundance of absent species cannot be ruled out. However, the recent research revealing that a proportion of walleye in Black Bay migrate beyond the shallow productive boundaries (UGLMU, unpublished) supports the hypothesis that exposure is much more limited than in Circle Lake. It is also likely that both lake depth and size are important factors influencing walleye exposure to cercaria (i.e. abiotic factors limiting walleye distribution within a waterbody). Therefore, both behaviour and lake characteristics should be considered viable rules governing the assembly of digenean parasites employing cercarial penetration with a community.

Another component of the rare species in Circle Lake walleye transmit to their host via ingestion of a fish secondary intermediate host (*C. oxycephalus*, *A. angusticauda*, *T. nodulosus*). Aside from *C. complanatum*, these species represent those which are added to the community structure last (Figure

9). This evidence supports the hypothesis that ongoing feeding on copepods is supplemented with alternative invertebrates as well as fish.

Comments on host exploitation and parasites

All three hypotheses put forward at the beginning of my study operated under the general assumption that a collapse in the host population should have affected the parasite community. The exploitation of a host has been shown to affect parasite richness, abundance, prevalence, and community structure in studies in marine environments (Lafferty et al. 2008, Wood et al. 2010, Wood et al. 2014, Wood and Lafferty 2015). Wood and Lafferty (2015) proposed a set of non-mutually exclusive hypotheses as to why fishing pressure might depress parasite numbers at both the species and community levels. For instance, if a host population drops below the threshold for parasite transmission, transmission consequently becomes more difficult as the instances of contact between host and parasite become less frequent (Dobson and May 1987, Arnegberg et al. 1998, McCallum et al. 2005). From a community standpoint, changes in host abundance can alter community structure based on the life cycle characteristics of the parasites. A significant reduction in the abundance of an obligatory host could hinder the transmission potential of a species (Wood et al. 2010, Lafferty 2012). This statement applies to both intermediate and definitive hosts.

Parasite species that have a complex life cycle typically have a definitive host of higher trophic level. These higher trophic level hosts are often those sought out commercially (Pauly and Watson 2005). Thus, in a parasite community of an exploited host, the parasites employing trophic transmission are perhaps more likely to be affected than those that transmit directly due to the reduction of hosts and subsequent reduction in transmission potential. Another facet to consider is if a parasite species is a host specialist, meaning that its life cycle is considerably tied to the status of its host population (Wood et al. 2010). By contrast, generalist parasite species can use multiple different host species at different developmental stages and hence a decline in one host may be offset by the use of another.

While the impacts of fishing on marine parasites have been documented, the notion that fishing prompts a decline in parasites has yet to be tested on a freshwater ecosystem. In the absence of historical parasite data from Black Bay, I cannot say for certain that host exploitation has altered the parasite community. However, the increasing amount of studies showing that anthropogenic influence can alter the parasite community certainly warrants the extension of said studies into freshwater ecosystems.

Summary

I quantified and described the parasite community of both Black Bay and Circle Lake walleye. Because there had been no prior parasitological survey conducted in either sample location, the data collected represent baseline information that should be used for future studies. The stomach contents in Black Bay suggest that rainbow smelt serve as the most important prey item. The parasite community revealed that copepods and amphipods potentially serve as important secondary prey items. The stomach contents and parasite community in Circle Lake sample revealed a much more diverse walleye diet, which incorporates multiple species of forage fish and invertebrates.

Both parasite richness and diversity were much higher in Circle Lake walleye. Digeneic trematodes employing cercarial penetration as a means of transmission account for a considerable number of species (*Neascus* sp., *Diplostomum* sp., *S. occidentalis*, *C. complanatum*) contributing to the higher richness and diversity observed in Circle Lake. This evidence suggests that Circle Lake walleye frequent shallow habitat more regularly than Black Bay walleye, or these species are absent in Black Bay. This information indicates that host behaviour as well as waterbody characteristics should be considered important processes influencing the acquisition of digeneic parasites.

Although sample locations had considerable differences in their parasite communities, there were also some apparent similarities. The parasitic copepod *E. caeruleus* existed at high prevalence and similar intensity across sample locations. Furthermore, endoparasites *B. cuspidatus*, *A. angusticauda*, *P.*

pusilla, and *Diplostomum* sp. also exhibited similar prevalence and intensity across sample locations. There are also similarities in comparing Black Bay walleye parasites to those reported from Eastern Lake Superior (Dechtiar and Lawrie 1988). *Bothriocephalus cuspidatus* is the most prevalent and intense endoparasite species reported. Considering ectoparasites, Eastern Lake Superior also had high prevalence of *Ergasilus* sp.—however it also had high prevalence of *U. aculeatus* whereas this species was virtually absent from Black Bay. Overall, the low species richness of walleye parasites across both studies in Lake Superior suggest that this is perhaps characteristic of walleye parasites in Lake Superior at large.

Effects of host attributes on ectoparasite and endoparasite numbers varied among species, and sample location. More definitive answers for observed correlations require species specific studies; however host diet, behaviour, and local abundance of intermediate hosts are likely important factors dictating infrapopulations.

Nestedness analysis revealed significant nested patterns in the presence of species for both sample locations across all facets tested (total parasites, ectoparasites, endoparasites, adult parasites). Despite a significant difference in species richness between locations, the assembly of base parasites followed the same general pattern: directly transmitted parasites are acquired first, followed by parasites using copepod intermediate hosts, and lastly parasites using fish secondary intermediate hosts. This pattern agrees with the ontogenetic shift towards piscivory exhibited by walleye.

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Appendix A – Code used in R programming language

Functions and code for general parasite statistics.

```
#####  
# Draw data in from Access database  
  
library(RODBC)  
## Warning: package 'RODBC' was built under R version 3.1.3  
dbase <- file.path('C:/Users/chiodoan/Documents/R-Tony/Parasites/Walleye Para  
site dBase.accdb')  
DBConnection <- odbcConnectAccess2007(dbase)  
  
circle <- sqlFetch(DBConnection, "circle", colnames=FALSE, rownames=FALSE)  
  
close(DBConnection)  
  
#####  
#PREVALENCE - This function calculates prevalence for an individual species  
  
prev <- function(x){  
  (length(x[x >0])/length(x))*100  
}  
  
# Ex. Usage  
  
# prev(circle$Glochidia)  
#[1] 63.04348  
  
#####  
#INTENSITY - This function calculates intensity for an individual species  
  
int <- function(x){  
  if(is.numeric(x)){  
    x[x==0] <- NA  
    mean(x, na.rm=TRUE)  
  }  
}  
  
# Ex. Usage  
  
# int(circle$Glochidia)  
# [1] 76.75862
```



```

#=====
# This function calculates prevalence, abundance, and intensity for an
individual species

psum <- function(x){
  c(prevalence = prev(x),
    abundance = mean(x),
    intensity = int(x))
}

#Ex. Usage

#psum(circle$Glochidia)
# prevalence abundance intensity
# 63.04348 48.39130 76.75862

#=====
# This code creates a table with prevalence, abundance, and intensity calcula
ted for
# selected species

table <- t(as.data.frame(lapply(circle[,c("Glochidia", "Ergasilus", "Urocleides
"), psum)))

#Ex. Usage

# table <- t(as.data.frame(lapply(circle[,c("Glochidia", "Ergasilus", "Urocleid
es"), psum)))

# prevalence abundance intensity
# Glochidia 63.04348 48.39130 76.75862
# Ergasilus 100.00000 28.34783 28.34783
# Urocleides 97.82609 82.28261 84.11111

```

Code for distribution free comparison of mean intensity

```
#####  
# Draw intensitydata in from Access database  
  
Note: For this script to work, data must be transformed such that it is mean  
centered  
  
library(RODBC)  
  
dbase <- file.path('C:/Users/chiodoan/Documents/R-Tony/Parasites/Walleye Para  
site dBase.accdb')  
DBConnection <- odbcConnectAccess2007(dbase)  
  
# Mean centered Bothriocephalus cuspidatus data.  
Bot_MC <- sqlFetch(DBConnection, "Bot_MC", colnames=FALSE, rownames=FALSE)  
  
close(DBConnection)  
  
#####  
# t.test for the observed values of the parasites  
t <- t.test(Bot_MC$Bothriocephalus, Bot_MC$Bothriocephalus1, var.equal = TRUE  
)  
p <- t$p.value  
  
#####  
# Replicate 1000 t.tests for samples drawn from mean centered values  
a <- replicate(1000, t.test(sample(Bot_MC$X, 43, replace=TRUE), sample(Bot_MC$  
Y, 32, replace=TRUE)))$p.value)  
b <- replicate(1000, t.test(sample(Bot_MC$X, 43, replace=TRUE), sample(Bot_MC$  
Y, 32, replace=TRUE)))$statistic)  
  
#####  
# Number of bootstrap samples > observed p.value  
c <- sum(a >= p)  
d <- sum(a <= p) # Just for extra knowledge, should add up to 1000  
  
#####  
# Bootstrap p.value is defined as  
bpv3 <- c/1000  
bpv3  
## [1] 0.972
```

Code for calculation of Simpson's index, and plot

```
library(RODBC)
library(vegan)

### Connect to Access database, pull data

dbase <- file.path('C:/Users/chiodoan/Documents/R-Tony/Parasites/Walleye Para
site dBase.accdb')
DBConnection <- odbcConnectAccess2007(dbase)

blackbay_PARASITE <- sqlFetch(DBConnection, "blackbay_PARASITE", colnames=FAL
SE, rownames=FALSE)
circle_PARASITE <- sqlFetch(DBConnection, "circle_PARASITE", colnames=FALSE,
rownames=FALSE)

close(DBConnection)

### Calculate Simpson's index for each sample location

#####
### BLACK BAY DIVERSITY ###
#####

bbp_MATRIX <- data.matrix(blackbay_PARASITE)

p.simpson1 <- diversity(bbp_MATRIX, index="simpson")

#####
### CIRCLE LAKE DIVERSITY ###
#####

clp_MATRIX <- data.matrix(circle_PARASITE)

p.simpson2 <- diversity(clp_MATRIX, index="simpson")

### Create functions to assign colour scale to specific sample location

colfunc <- colorRampPalette(c("tomato", "grey"))
colfunc2 <- colorRampPalette(c("dodgerblue", "grey"))

### Creat functions to make an ordered plot of the calculated Simpson's index

make.sorted.plot <- function(x){
  ordered <- sort(x, T)
  plot(
    ordered,
```

```

    col = colfunc2(46),
    xaxt = "n", pch = 16, cex = 2,
    ylim = c(0.05, 0.9),
    xlim = c(0, length(x)+1),
    ylab = "Simpson's Index", xlab = "",
    main = "")
}

make.sorted.plot2 <- function(x){
  ordered <- sort(x, T)
  plot(
    ordered,
    col = colfunc(46),
    xaxt = "n", pch = 16, cex = 2,
    ylim = c(0.05, 0.9),
    xlim = c(0, length(x)+1),
    ylab = "Simpson's Index", xlab = "",
    main = "")
}

### Plot results on the same device

make.sorted.plot(p.simpson1)
par(new=TRUE)
make.sorted.plot2(p.simpson2)
text(4,0.5, "Black Bay", col = "dodgerblue")
text(35,0.8, "Circle Lake", col = "tomato")

```

Appendix B – Output of NED (nestedness) software

Figure 11 Nestedness analysis for all parasite species found in Black Bay walleye.

Nestedness Measures:				
METRIC	INDEX	Z-SCORE	RN	NESTED?
NODF	71.146	7.269	0.516	Yes (p<0.001)
NODF_row	71.355	7.315	0.522	Yes (p<0.001)
NODF_col	62.16	3.911	0.286	Yes (p<0.001)

Null Model: Proportional column and row totals (CE)				
Number of Null Matrices: 100				
METRIC	MEAN	ST.DEV.	MIN	MAX
NODF	46.232	2.894	37.665	53.431
NODF_row	46.191	2.898	37.514	53.415
NODF_col	47.968	3.732	37.209	60.679



Figure 12 Nestedness analysis for all adult parasite species found in Black Bay walleye.

Nestedness Measures:				
METRIC	INDEX	Z-SCORE	RN	NESTED?
NODF	70.06	7.263	0.494	Yes (p<0.001)
NODF_row	70.118	7.298	0.496	Yes (p<0.001)
NODF_col	66.548	4.446	0.367	Yes (p<0.001)

Null Model: Proportional column and row totals (CE)				
Number of Null Matrices: 100				
METRIC	MEAN	ST.DEV.	MIN	MAX
NODF	46.9	3.189	40.529	55.089
NODF_row	46.87	3.186	40.495	55.076
NODF_col	48.677	4.019	38.44	57.16



Figure 13 Nestedness analysis for all ectoparasite species found in Black Bay walleye

Nestedness Measures:				
METRIC	INDEX	Z-SCORE	RN	NESTED?
NODF	10.369	32.674	0.012	Yes (p<0.001)
NODF_row	10.242	50449284587.5	0.0	Yes (p<0.001)
NODF_col	100.0	32.759	6.158	Yes (p<0.001)

Null Model: Proportional column and row totals (CE)				
Number of Null Matrices: 100				
METRIC	MEAN	ST.DEV.	MIN	MAX
NODF	10.247	0.004	10.242	10.259
NODF_row	10.242	0.0	10.242	10.242
NODF_col	13.97	2.626	10.526	22.222

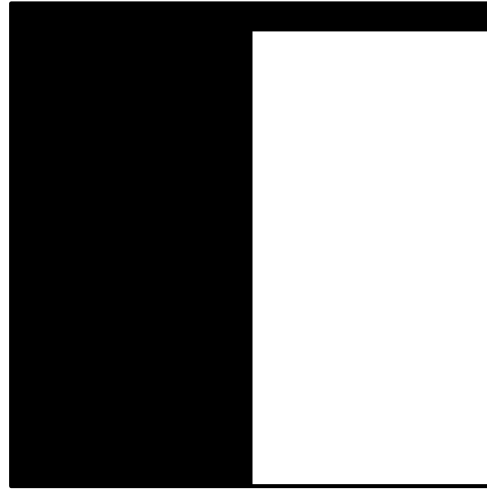
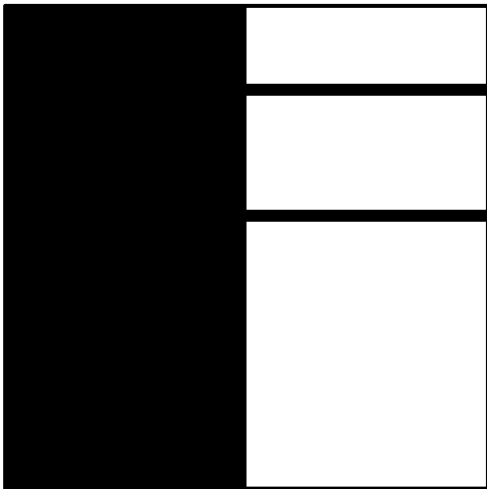


Figure 14 Nestedness analysis for all endoparasite species found in Black Bay walleye.

Nestedness Measures:				
METRIC	INDEX	Z-SCORE	RN	NESTED?
NODF	59.832	5.096	0.257	Yes (p<0.001)
NODF_row	59.647	5.013	0.255	Yes (p<0.001)
NODF_col	64.09	5.622	0.295	Yes (p<0.001)

Null Model: Proportional column and row totals (CE)				
Number of Null Matrices: 100				
METRIC	MEAN	ST.DEV.	MIN	MAX
NODF	47.603	2.4	41.81	53.152
NODF_row	47.521	2.419	41.702	53.046
NODF_col	49.492	2.597	42.456	55.6

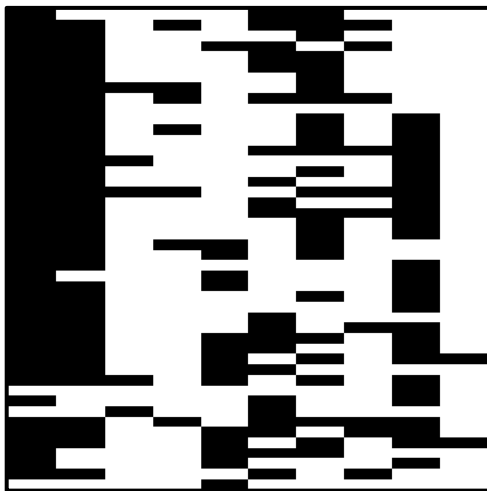


Figure 15 Nestedness analysis for all parasite species found in Circle Lake walleye.

Nestedness Measures:				
METRIC	INDEX	Z-SCORE	RN	NESTED?
NODF	72.621	7.604	0.283	Yes (p<0.001)
NODF_row	72.229	7.302	0.283	Yes (p<0.001)
NODF_col	77.077	8.699	0.28	Yes (p<0.001)

Null Model: Proportional column and row totals (CE)				
Number of Null Matrices: 100				
METRIC	MEAN	ST.DEV.	MIN	MAX
NODF	56.62	2.104	51.505	61.883
NODF_row	56.304	2.181	50.95	61.678
NODF_col	60.216	1.938	54.813	64.208

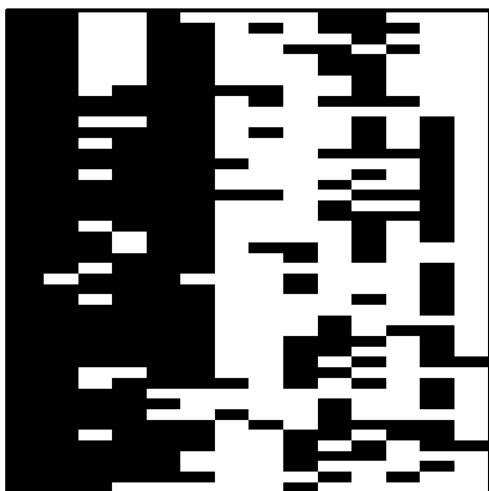


Figure 16 Nestedness analysis for all adult parasite species found in Circle Lake walleye

Nestedness Measures:				
METRIC	INDEX	Z-SCORE	RN	NESTED?
NODF	72.752	5.389	0.249	Yes (p<0.001)
NODF_row	72.435	5.259	0.247	Yes (p<0.001)
NODF_col	81.891	6.914	0.3	Yes (p<0.001)

Null Model: Proportional column and row totals (CE)				
Number of Null Matrices: 100				
METRIC	MEAN	ST.DEV.	MIN	MAX
NODF	58.251	2.691	49.199	64.339
NODF_row	58.086	2.728	48.88	64.379
NODF_col	62.997	2.733	56.034	69.114



Figure 17 Nestedness analysis for all ectoparasite species found in Circle Lake walleye

Nestedness Measures:				
METRIC	INDEX	Z-SCORE	RN	NESTED?
NODF	53.983	-2.228	-0.114	No ($p>0.05$)
NODF_row	53.72	-2.286	-0.117	No ($p>0.05$)
NODF_col	99.425	4.509	0.346	Yes ($p<0.001$)

Null Model: Proportional column and row totals (CE)				
Number of Null Matrices: 100				
METRIC	MEAN	ST.DEV.	MIN	MAX
NODF	60.944	3.125	53.623	67.618
NODF_row	60.869	3.128	53.527	67.536
NODF_col	73.859	5.67	58.57	83.412

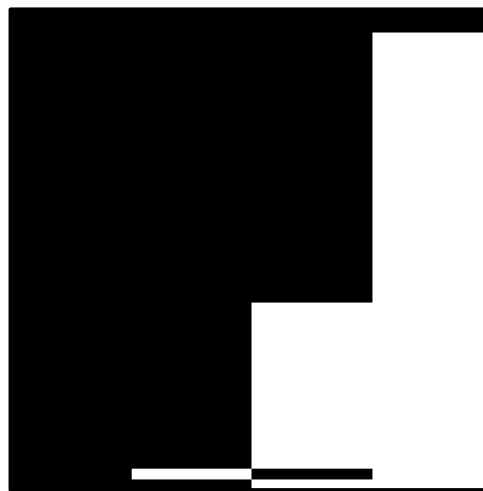


Figure 18 Nestedness analysis for all endoparasite species found in Circle Lake walleye.

Nestedness Measures:				
METRIC	INDEX	Z-SCORE	RN	NESTED?
NODF	59.832	5.247	0.266	Yes (p<0.001)
NODF_row	59.647	5.181	0.264	Yes (p<0.001)
NODF_col	64.09	5.103	0.299	Yes (p<0.001)

Null Model: Proportional column and row totals (CE)				
Number of Null Matrices: 100				
METRIC	MEAN	ST.DEV.	MIN	MAX
NODF	47.27	2.394	41.925	53.86
NODF_row	47.181	2.406	41.647	53.704
NODF_col	49.33	2.892	41.482	57.438

