This is to certify that the
dissertation entitled

ENDOHELMINTH DIVERSITY OF LARGEMOUTH BASS AND
LAKE WHITEFISH IN MICHIGAN

presented by

WALIED MOHAMED ABDELWAHAB FAYED

has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Fisheries and Wildlife

Mohamed Faisal
Major Professor's Signature

08/07/2010
Date

ENDOHELMINTH DIVERSITY OF LARGEMOUTH BASS AND LAKE WHITEFISH IN MICHIGAN

By

Walied Mohamed Abdelwahab Fayed

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Fisheries and Wildlife

2010

ABSTRACT

ENDOHELMINTH DIVERSITY OF LARGEMOUTH BASS AND LAKE WHITEFISH IN MICHIGAN

By

Walied Mohamed Abdelwahab Fayed

In this study, the community composition and structure of gastrointestinal tract (GIT) helminths were investigated in two species of fish: largemouth bass (*Micropterus salmoides*) and lake whitefish (*Coregonus clupeaformis*), both of which are important fish species in the Laurentian Great Lakes basin. The first study was designed to identify the helminth species infecting GIT of largemouth bass (LMB) in 15 inland lakes in Michigan's Lower Peninsula, and to describe their community structure. A total of 16,700 worms were retrieved from the GITs of 641 adult LMB collected between July 2002 and September 2005. Over 75% of the LMB examined harbored at least one helminth species in their GIT, with relatively high intensity (34.72±35.07 worms/fish) and abundance (26.05±35.07 worms/fish). Collected helminths were generalists in nature and represented four phyla and nine species: *Neoechinorhynchus cylindratus*, *Leptorhynchoides thecatus*, *Acanthocephalus parksidei*, *Echinorhynchus salmonis*, *Pomphorhynchus bulbocolli*, *Proteocephalus ambloplitis*, *Contracaecum* sp., *Camallanus oxycephalus*, and *Leuceruthrus micropteri*. The generalized linear mixed model analyses demonstrated the presence of significant effects of the Great Lakes watershed, the presence of inlets, the presence of outlets, and public access on infection parameters of LMB-GIT worms. Diversity was significantly greater in inland lakes with public access.

In the second study, prevalence, intensity, and abundance of swimbladder nematode infections were estimated in 1,272 lake whitefish (*Coregonus clupeaformis*) collected from four sites in northern lakes Huron (near Cheboygan and De Tour Village ports) and Michigan (near Big Bay de Noc and Naubinway ports) from fall 2003 through summer 2006. Morphological examination revealed characteristics consistent with that of *Cystidicola farionis* Fischer 1798. Although *C. farionis* was detected in all four stocks that were examined, Lake Huron stocks generally had higher prevalence, intensity, and abundance of infection than Lake Michigan stocks. A distinct seasonal fluctuation in prevalence, abundance, and intensity of *C. farionis* was observed. Lake whitefish (LWF) heavily infected with *C. farionis* were found to have thickened swimbladder walls.

The third study compliments the second study as it was designed to identify the community composition and structure of GIT helminth infections in LWF stocks. A total of 21,203 helminths were retrieved from the GITs of 1,284 spawning LWF. Collected helminths were generalists in nature and represented two phyla and five species: *Acanthocephalus dirus*, *Neoechinorhynchus tumidus*, *Echinorhynchus salmonis*, *Cyathocephalus truncatus,* and *Bothriocephalus* sp. In order to evaluate the effects of lake, sampling site, year, and season (as well as interactions of these factors), a series of statistical models were fitted to the helminth (all combined and separately for each helminth species) prevalence, abundance, and intensity. LWF from Lake Huron had significantly greater rates of infection than LWF from Lake Michigan. Helminth infection parameters peaked in the spring, while diversity was highest in the winter samples. The findings of this study represent the most comprehensive parasitological study ever conducted on largemouth bass or lake whitefish in the Great Lakes.

DEDICATION

To my mother and father and to my children, Mariam and Yousef.

ACKNOWLEDGEMENTS

My deepest appreciated respect goes to all who have helped me during my Ph.D. study at Michigan State University. First and foremost, I would like to express my sincere gratitude to my major advisor, Dr. Mohamed Faisal, for his guidance, patience, and support that were essential for the completion of this dissertation. "Dr. Faisal, thank you for everything you have done on my behalf."

I would also like to sincerely thank my committee members, Professors Ted Batterson, Scott Fitzgerald, and Donald Garling, for their valuable advice and guidance. I also would like to acknowledge the remarkable support of Professors Abdelaziz Nour and Eglal Omar, Alexandria University, Egypt for their guidance and support.

I would like to thank the Egyptian Government for funding my studies at Michigan State University. I would also like to thank the Great Lakes Fishery Trust, Lansing, Michigan, for funding the lake whitefish study and the Michigan Department of Natural Resources and Environment for funding the largemouth bass study.

The help and support I have received from members of the Michigan State University Aquatic Animal Health Laboratory, particularly Michelle Gunn and Kolina Riley, are also greatly appreciated.

Last, but not least, I am deeply indebted to my mother, Fekria Allam, and my father, Professor Mohamed Abdelwahab Fayed, who always dreamed that I would earn the Ph.D. degree. I would like to thank my sister, Heba Fayed, for her unconditional encouragement. I would also like to endow all my success to my beloved kids, Mariam and Yousef.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Parasites are ubiquitous in all geographical regions and have been found parasitizing organisms from all phyla. Their occurrence in host populations is determined by both host and ecological factors. Therefore, they are ideal to study as models of how the biotic and abiotic factors prevailing in the surrounding environment can influence organisms at the individual, population, and community levels.

This dissertation is focused on identifying parasite infection in two important fish species of the Laurentian Great Lakes: the largemouth bass (*Micropterus salmoides*) and the lake whitefish (*Coregonus clupeaformis*). The two species were selected due to the recent emergence of viral infections in the largemouth bass (e.g., Largemouth Bass Virus, Viral Hemorrhagic Septicemia Virus) and the declining condition and growth of lake whitefish due to diet shifts that may have resulted from dreissenid mussel invasion. Since parasitism is widely recognized as a factor that could influence the composition and structure of wildlife communities (Poulin 1999), the present studies were initiated to fill many gaps of knowledge pertaining to the understanding of parasite infections of largemouth bass (LMB) and lake whitefish (LWF) in the state of Michigan.

This dissertation is divided into four major chapters. Chapter 1 reviews the available literature describing parasitic infection in fish, the assemblies that parasites form, and the factors that may determine the composition and structure of their communities.

Chapter 2 describes the composition and structure of gastrointestinal tract (GIT) helminths in LMB residing in 15 inland lakes in Michigan's Lower Peninsula. Several

analyses were performed to determine if host or environmental factors play a role in shaping the LMB-GIT helminth parasites and their assemblages, such as: fish gender; watershed characteristics; public access; and the lake's connections to other waterbodies through inlets or outlets.

Chapter 3 deals with highly pathogenic swimbladder nematodes (*Cystidicola* spp.) of salmonid fish species that were observed in adult LWF collected from four sites in northern lakes Huron and Michigan. The objectives of this study were to: 1) identify the species of swimbladder nematodes in LWF; 2) measure their prevalence, abundance, and intensity in LWF stocks; 3) evaluate variations in larval stage development and maturation among the stocks; and 4) assess the damage to LWF swimbladders caused by the nematode infection.

Studies described in Chapter 4 were designed to evaluate the spatio-temporal dynamics of GIT helminths infecting the four LWF stocks in northern lakes Michigan and Huron. This information would constitute baseline information that can be followed to determine if GIT helminths can be implied as a potential cause for poor LWF condition. Specific objectivities for Chapter 4 research were to: 1) identify the GIT helminth species found in the LWF in lakes Huron and Michigan; 2) assess the GIT helminth community structure in LWF spawning stocks in northern lakes Michigan and Huron; and 3) to evaluate the spatial and temporal changes on LWF-GIT helminth infection parameters and community structure in these stocks over a three year period.

The thesis is concluded by brief synopses of the major conclusions of this research and recommended directions for future research.

CHAPTER 1

LITERATURE REVIEW

I. Parasites and parasitic infections

From as early as medical archives were kept, the harms inflicted by parasites have been described. Despite centuries of research, details of the interactions between parasites and their hosts remain far from being unraveled since little is known about the nature of parasite assemblages within their hosts. During their evolution, parasites went through a gradual yet progressive adaptation to become partially or totally dependent on another organism, the host, utilizing its energy and nutrients. Parasites are ubiquitous in all geographical regions and have been found parasitizing organisms from all phyla; therefore, they represent the surrounding ecosystem and its biodiversity (Minchella and Scott 1991). The occurrence of a parasite in a host population is determined by both genetic and ecological factors (Janovy et al. 1992; Combes 1996), while the population structure of the parasite is also affected by ecological factors of which the exposure of the host to parasites is primary (Janovy and Kutish 1988). In this chapter, an emphasis will be given to parasites of fish and shellfish.

Parasites are classified in a number of ways (Roberts and Janovy 2008). Depending on their nature, parasites are either unicellular (e.g., protozoa) or multicellular (e.g., worms, annelids and crustaceans). Depending on their site of attachment, parasites are ectoparasites, i.e., those attached to skin and gills (e.g., monogeneans), endoparasites, i.e., those that live inside the host (e.g., mostly worms), or both (e.g., *Ophelia* spp.).

Endoparasites are further divided into gastrointestinal tract (GIT) (e.g., acanthocephalans, trematodes, cestodes, and nematodes), muscle (e.g., *Triaenophorus crassus* and *Heterosporis* spp.), eye (e.g., *Diplostomus* spp.), blood (e.g., *Trypanosoma* spp.), or swimbladder (e.g., *Cystidicola* spp.) parasites. Depending on their feeding habits, parasites are often classified into intermittent (e.g., leeches) and permanent parasites (e.g., worms). According to the number of host species they can parasitize, parasites are either generalists (i.e., can infect many host species) or specialists (i.e., infect only one host species). Moreover, a parasite species that is regionally common, locally abundant, and found in high numbers within a locale is called a core species. Most parasitologists consider a parasite a core species if its abundance exceeds two parasites per fish. On the other hand, a parasite species that is regionally uncommon, locally rare, or is found in low numbers is called a rare or satellite species (Bush et al. 1997).

Many parasites require multiple hosts to complete their life cycles and rely on predator-prey or other stable ecological interactions to get from one host to the other. In many instances, larval stages of endoparasites exist outside the GIT or blood. In these circumstances, larval stages require their host to be consumed by the following host in the parasite's life cycle in order to survive and reproduce. For example, the bass tapeworm (*Proteocephalus ambloplitis*) infects a crustacean (first intermediate host), which when ingested by a fish (second intermediate host) encysts in its visceral cavity. The infected fish is then ingested by a piscivorous fish or bird (final host) where the worm develops into adulthood in the GIT. In this example, transmission can only occur if infected crustaceans are in sufficient proximity with the second intermediate host, which in turn must be consumed by the fish or bird final host, which must defecate, shedding

worm eggs sufficiently close to the crustaceans. In other words, this worm cannot survive without the functioning of several ecological links. For this reason, GIT helminths shed light on the diversity of the surrounding environment (Roberts and Janovy 2008).

In the last two decades, a continuous line of studies have provided clear indications that parasites, among other infectious agents, can regulate wildlife populations by increasing the mortality rates or reducing the fecundity of their hosts (Esch 1994; Gulland 1995; Hudson and Greenman 1998; Tompkins and Begon 1999). Parasites harm their hosts in a number of ways, including deprivation of nutrients (Dezfuli et al. 2000) and destruction of vital tissues and organs such as monogeneans to gills, *Triaenophorus nodulosus* to liver (Brinker 2007), *Myxobolus cerebralis* to cranial cartilages (El-Matbouli et al. 1992), and *Proteocephalus ambloplitis* to gonads (Gillilland and Muzzall 2004). Some parasites threaten species survival by damaging early life stages such as *Ichthyodinium chabelardi,* which causes mechanical rupture of the yolk sac of fry of the Atlantic sardine (*Sardina pilchardus*), thereby leading to their death (Stratoudakis et al. 2000). Parasites can interfere with vital physiological functions such as swimbladder nematodes with buoyancy and acanthocephalans with nutrient absorption (Gollock et al. 2004; McDonough and Gleason 1981). Parasites can also suppress immune functions (Scott 1988) and interfere with host growth and development (e.g., *Gyrodactylus salaris* in salmon, Clers 1993). One of the classic examples on how parasites interfere with normal functions is the Rhizocephalan parasite *Loxothylacus panopei*, which develops an extensive network of branches inside its host crab (*Rhithropanopeus harrisii)*, altering its respiration, reproduction, and molting processes (Hùeg 1995).

The degree of pathological damage due to parasitic infection depends largely upon the nature of the host-parasite interactions, the prevailing environmental conditions, the host nutritional status, as well as other conditions that either stress the host or affect the survival of the parasite (Scott 1988). Parasites and the harms inflicted by them can shape the host population and consequently the community among which it lives (Johnson et al. 1999). The classic example for this case is the trematode *Curtuteria australis* (Digenea: Echinostomatidae), which encysts in the foot of its second intermediate host, the New Zealand cockle (*Austrovenus stutchburyi),* an important member of the intertidal community of the South Island of New Zealand. In heavily infected cockles, the foot becomes stunted and cockles lose their ability to burrow through the sediment and thereby become vulnerable to predation. The increased predation rates the cockle populations experience alter their abundance relative to other bivalves. The substrate niches vacated by affected cockles become available for colonization by other epibionts, thereby altering the bivalve community at this particular locale (McFarland et al. 2003). In order to complete their life cycles, some parasites modify host behavior to make transmission to other hosts more likely. For example, in California salt marshes, the fluke *Euhaplorchis californiensis* (Digenea: Heterophyidae) reduces the ability of its host, the killifish (*Fundulus parvipinnis*), to avoid predators. The parasite matures in egrets, which are more likely to feed on infected killifish than on uninfected fish (Lafferty and Morris 1996). From these examples and many more, one can conclude that the abundance of predator and prey species would be different if some of these parasites were absent from the ecosystem.

II. Parasite assemblage into populations and communities

Parasites are diverse as they vary in anatomy, feeding nature, topological or spatial location within a host, home and host ranges, and life cycle. These factors created enormous difficulties in describing and comparing parasite assemblages in the ecosystem, within their host, and among a variety of hosts and geographic localities. As a result, various descriptive terms have been used that were often misleading (Margolis et al. 1982; Holmes and Price 1986; Simberloff and Moore 1997). Throughout this dissertation, the terminology recommended by Bush et al. (1997) will be used unless otherwise mentioned. Moreover, the term "infection" will be used throughout to denote both infection and infestation. In general, within their ecosystems, parasites assemble together forming populations and communities.

1) Parasite populations and terms used for their description

A population consists of all individuals of a single parasite species at a particular place at a particular time (Bush et al. 1997). The mere presence of a parasite in a host population divides individuals into two categories: infected and non-infected. The term commonly used to describe the presence or absence of a parasite is prevalence (P), which is calculated by dividing the number of hosts infected by a particular parasite species (or a taxonomic group) by the number of hosts tested for the presence of that parasite species. It is expressed as a percentage when used descriptively and as a proportion when incorporated into mathematical models. Prevalence as a term should be demarcated from incidence, which refers to the number of new hosts that become infected with a particular parasite during a specified time interval divided by the number of uninfected hosts

present at the start of the time interval. Both prevalence and incidence do not take into account the enumeration of individual parasites present (parasite load or body burden).

To quantitate parasite burdens, the term "density" is used. It is defined as the number of individuals of a particular parasite species in a measured sampling unit taken from a host or habitat, e.g., in units of area, volume, or weight. Density can be quantitatively described by one of two terms: intensity or abundance. Intensity is the number of individuals of a particular parasite species in a single infected host. For comparative studies, intensity is usually expressed as "mean intensity," which is calculated by dividing the total number of parasites of a particular species found in a sample by the number of hosts infected with that parasite. Since intensity as a measurement did not take into consideration the distribution of the parasite load in both infected and non-infected individual hosts, the term "abundance" was introduced. Abundance is the number of individuals of a particular parasite in a host population, regardless of whether or not each host individual is infected (Bush et al. 1997). Mean abundance is calculated by dividing the total number of individuals of a particular parasite species in a sample of a particular host species by the total number of hosts of that species examined (including infected and uninfected hosts).

To describe assemblages of parasites within a population, there are two schools of thought. Earlier studies (reviewed in Margolis et al. 1982) restricted their definitions to parasites present in or on host species (or a group of host species). More recent studies, however, account for free living stages of the parasite within an ecosystem in the definitions (Bush et al. 1997). Terms that are commonly used to describe parasite populations include:

a. **Infrapopulation:** all individuals of one parasite species in a single individual host at a particular time and in a certain locality.

b. **Component population:** all individuals of a specified life history phase of a parasite species at a particular place and time.

c. **Metapopulation:** all individual parasites belonging to one species in a host population.

d. **Suprapopulation:** developmental phases of a parasite species at a particular place and time.

2) Parasite communities and terms used for their description

A parasite community is comprised of more than one parasite population living together in a spatiotemporal unit (Bush et al. 1997). Terms used to describe parasite communities include:

a. **Infracommunity:** the assemblage of all parasite species in an individual host.

b. **Component community:** all infrapopulations of parasites associated with some subset of a host species or a collection of free-living phases associated with some subset of the abiotic environment.

c. **Supracommunity:** comprises parasite suprapopulations.

3) Parasite community structure

For decades, parasitologists have analyzed the structure of parasite communities in fish and found the obtained information useful in understanding host-parasite-environment interactions (Mouillot et al. 2005). In general, the structure of a parasite community is shaped by a number of factors, such as parasite species present at the locale, richness, evenness, species diversity, and dominance:

a. **Richness:** the number of parasite species found within the community (Wilsey et al. 2005). It is important to emphasize that richness relies on the number of parasite species and not their intensity or abundance. Communities with higher numbers of parasite species are considered more diverse.

b. **Evenness:** defined as the even distribution of abundance among parasite species. Since it is a measure of disparity in the number of individuals that represent each species, communities with higher evenness are considered more diverse.

c. **Diversity:** describes the composition of a community in terms of richness and evenness of each parasite species (Bush et al. 1997). Diversity is expressed by one or more diversity indices, such as Shannon's and Simpson's indices, which are composite measures and are designed in a way so that richness and evenness are mathematically independent. Both diversity indices take into account some important information, such as rarity and commonness of species in a community (Smith and Wilson 1996). Most comparisons of parasite communities include comparisons of diversity indices (assuming the same index is used). Many ecologists prefer to calculate a number of diversity indices to ascertain the robustness of the diversity ordering.

    - The Simpson's Diversity Index measures the probability that two individuals that are randomly selected from a sample will belong to the same species. The Simpson's Diversity Index is computed as the sum of the square of the proportion of the parasite species found in the sample belonging to each species. Simpson's Diversity Index (D) (Simpson 1949) is calculated by the formula: $D = \sum P_i^2$, where $p_i =$

proportion of total sample belonging to *i* species. Using this formula, the bigger the value of D, the lower the diversity, with 0 representing infinite diversity, and 1 representing no diversity. To avoid confusion, D is often subtracted from 1 to give the "Simpson's Index of Diversity" which is 1–D (adopted in Chapter 2 of this dissertation). The value of this index also ranges between 0 and 1, however, the greater the value, the greater the sample diversity. Other ecologists use the so-called Simpson's Reciprocal Index, which is 1/D (adopted in Chapter 4 of this dissertation). The value of this index starts with 1 as the lowest possible figure. This figure would represent a community containing only one species. The higher the value, the greater the diversity. The maximum value is the number of species (or other category being used) in the sample. For example, if there are five species in the sample, then the maximum value is 5.

- The Shannon-Wiener's Diversity Index takes into consideration the number of individuals as well as the number of species in a community. Shannon-Wiener's Diversity Index is computed by the total sum of the multiplication of the proportion of the parasite species found in the sample belonging to each species by the logarithm of this proportion (Shannon 1948). Shannon-Wiener's Diversity Index (H') is computed as follows:

$$H' = \sum_{i=1}^{s} (p_i)(\log_2 p_i)$$, where s is the number of parasite species found in locality and p_i is the number of a specific parasite species (i) divided by the total number of parasites found in the

sample; i.e., p_i is the proportion of total sample belonging to *i* species. High values of H' would be representative of more diverse communities. A community with only one species would have an H' value of 0 because P_i would equal 1 and be multiplied by ln P_i, which would equal zero. If the species were evenly distributed, then the H' value would be high. So the H' value allows us to know not only the number of species, but also how the abundance of the species is distributed among all the species in the community.

d. Species dominance: Species dominance determines the relative importance of a parasite species contributing the most to the total abundance of a parasite community and is measured by the Berger-Parker Dominance Index (Berger and Parker 1970). The Berger-Parker Dominance Index (d) is computed as the mean abundance divided by the total number of individuals in the sample by the formula: $d = \frac{N_{max}}{N_T}$. Where N_{max} = total number of most abundant species in a sample and N_T = total number of individuals (species) in the sample.

e. Similarity: To compare the similarity between two parasite communities within the same host species but in different waterbodies, the Jaccard Similarity Index is used (Cheetham and Hazel 1969). The Jaccard Similarity Index (S_j), which measures the proportion of the joint occurrence of parasites between two

communities to the total number of parasites, is calculated from the formula:

$$S_j = \frac{a}{b+c+d}$$, where

a=number of parasites shared between waterbodies A and B (joint occurrences)

b=number of species in waterbody B but not in waterbody A

c=number of species in waterbody A but not in waterbody B

d=number of species absent in both samples

4) Factors affecting parasite community structure

Studying the structure of parasite communities is of paramount importance as their composition and diversity mirror the surrounding ecosystem. Moreover, since parasite communities are dissimilar in composition and structure with respect to their host species, they have often been used in host species identification. Indeed, parasite communities have a great influence on their hosts to the extent that they can change their abundance as explained above. The structure of a parasite community is, however, not static, rather dynamic and is influenced by a number of factors. Unfortunately, a very limited number of studies have been performed in fish to determine the dynamics of changes of parasite community structures in relation to host factors, anthropogenic effects and prevailing environmental factors. In the few studies performed, it was often impossible for investigators to determine the effects of only one factor since the multiple aspects of the aquatic environment are intertwined.

Factors that affect parasite community structure include:

a. **Geographic location:** The geographic location is considered by many parasitologists to be the most important factor in shaping a parasite community.

Price and Clancy (1983), who modeled helminth communities of monogeneans, acanthocephalans, cestodes, and trematodes in British freshwater fish species, estimated the geographic locations to cause two-thirds of the changes in the parasite community structure. The remaining third of the changes was attributed to type of food, since the intermediate stages of parasites are found in certain prey species but not in others. In the same vein, the studies of Rohde and Heap (1998) found a latitude gradient in parasite species diversity in 108 bony fish species residing in Argentina, Chile, Wadden Sea, White Sea, North West Atlantic, Scotian Shelf, Barents Sea, and the Antarctic. The findings indicated that ectoparasites, but not endoparasites, significantly increased as the latitude decreased and from deep to surface waters. The authors attributed these discrepancies among parasites to the exposure of ectoparasites to more extreme environmental conditions (in particular, strong water currents and high temperatures). Moreover, Barger and Esch (2001) noted that certain structures within the area of study could alter the parasite community structure. The authors studied the spatial distribution of six species of parasites: the protozoan (*Myxobolus* sp.), the monogenean (*Dactylogyrus* sp.), the nematode (*Sterliadochona ephemeridarum),* and three digeneans (*Plagioporus sinitsini, Allopodocotyle chiliticorum,* and *Allocreadium lucyae*) in five species of fish: the rainbow trout (*Oncorhynchus mykiss),* the rosyside dace (*Clinostomus funduloides*), the redlip shiner (*Notropis chiliticus*), the sandbar shiner (*Rhinichthys atratulus*), and the blacknose dace (*Semotilus atromaculatus*) in Basin Creek, North Carolina, USA. They noticed that the position along Basin

Creek was significantly related to parasite community structure since breaks in parasite community composition were observed when waterfalls were present at upstream areas of Basin Creek. These waterfalls restricted the distribution of *C. funduloides, N. chiliticus,* and *S. atromaculatus*. Similarly, the authors noticed that at the downstream study area there was a break in the distribution of *S. ephemeridarum* that coincided with the existence of a dam.

b. **Type of diet:** Choudhury and Dick (2000), who studied the importance of type of diet on parasite community structure, tested the assumption that helminth parasite communities of freshwater fishes are richer and more diverse in the tropics than in temperate regions. The analysis in their study did not support this assumption. While the authors demonstrated that host body size and the number of susceptible hosts present correlated positively with the number of species found in a helminth community, they found that temperate helminth communities had higher richness scores than those from the tropics. The authors concluded that host diet was the major determinant of helminth community richness, regardless of other prevailing environmental factors.

c. **Host factors:** Kennedy and Hartvigsen (2000) tested the hypothesis that intestinal helminth communities in freshwater brown trout (*Salmo trutta*) are dissimilar in composition and structure to those in the European eel (*Anguilla anguilla*) in the same waterbody. Altogether, 17 species were recorded from the 72 localities. Composition of helminth communities differed considerably between the two host species as a group of four parasite species occurred commonly in trout but not in eels. By contrast, all measures of community

structure and indices of richness and diversity indicated that helminth communities in trout were species poor and exhibited low diversity at both component and infracommunity levels compared to those of eels. Despite the fact that all of the parasites were generalists, some factors in the host played a role in shaping the community in each species.

A common belief among fish parasitologists is that abundance and species richness of parasites increase as the host population density increases. This assumption was tested by Begge et al. (2003), who studied populations of the crucian carp, *Carassius carassius*, in nine isolated ponds in Finland. Across ponds, the fish size, not density, was found to play the most significant role on the mean total abundance of monogeneans/fish.

Host sex also seems to affect the parasite community structure. Alves and Luque (2001) studied the parasites of 100 specimens of white croaker (*Micropogonias furnieri*) collected from Pedra de Guaratiba, State of Rio de Janeiro, Brazil, from September 1997 to August 1999. The authors found that the majority of the fish (95%) were parasitized by up to 28 species of metazoan parasites. The monogenean *P. mexicanum* exhibited differences in prevalence and abundance that correlated with sex of the host.

d. Interspecific competition: Interspecific competition among parasite species for food and site of attachment is another important factor that greatly influences the structure of the parasite communities within their hosts. Dobson (1986) collected data from published surveys and used them to estimate the impact of competition on parasite populations within host populations. The analysis included factors

such as host fecundity and recruitment, rate of natural mortality, death rate of host due to parasitism, rate of parasite infective stage development, and rate of parasite death. The author found two distinct general levels of interactions: the joint utilization of a host species by two or more parasite species and the antagonistic mechanism by one parasite species either to reduce the survival rate or fecundity of the second species or to displace it from the site of attachment. The analysis conducted by Dobson (1986) suggested that the most important factor allowing competing species of parasites to coexist is how the parasites are distributed within the host population.

Given the many direct and indirect ways in which a parasite species can modulate the abundance of other species, it is likely that some parasite species have functionally important roles in a community, and that their removal would change the relative composition of the whole community (Poulin 1999). In this context, some studies were performed to investigate the relationship and correlation among different parasite species. Dezfuli et al. (2001) studied the correlation and interspecific competition within a helminth community in the gastrointestinal tract of brown trout from San Giorgio stream in northern Italy. In each individual host, the authors observed pairwise negative correlations between the intensities of the cestode *Cyathocephalus truncatus* and the two species of acanthocephalans: *Acanthocephalus anguillae* and *Echinorhynchus truttae*.

e. **Environmental factors:** Seasonal fluctuations of environmental factors seem to affect the parasite community structure (Kennedy 1990). As an example of the seasonal effects, Fellis and Esch (2004) studied the community structure and

seasonal dynamics of 16 helminth species infecting green (*Lepomis cyanellus*) and bluegill (*L. macrochirus*) sunfishes in Charlie's Pond, North Carolina. A total of 154 fishes (including 90 green sunfish and 64 bluegill sunfish) were collected between March and November 2000 and examined for the presence of helminth parasites. The authors found that worms such as *Capillaria* sp., *Clinostomum complanatum, Spinitectus carolini, S. contorta,* and larval *Diplostomum scheurengi* underwent significant changes in prevalence and abundance in green sunfish infracommunities that correlated well with the season. The results revealed that abundance of *S. carolini* gradually increased through spring, peaking in midsummer, and then slowly declined throughout fall. *Capillaria* sp. peaked in early spring and then gradually declined throughout the remainder of the year. *Diplostomum scheurengi* had its greatest abundance in March, followed by a sharp decline in April, after which it remained at a low, constant level. The authors attributed the seasonal fluctuation of the parasite infracommunity to the availability of the intermediate host and type of host diet.

As another example of environmental conditions, Marcogliese and Cone (1997) showed that parasite communities of the American eels, *Anguilla rostrata,* in Nova Scotia changed in response to the acid conditions (low pH) that prevailed in rivers in this locale. Parasite species richness was greater and there were more multiple infections in eels from a river that was treated to increase its pH level compared to that of an adjacent acidified river. The authors reported that digeneans disappeared completely in acidified rivers. The authors expanded their study to include 28 sites in the Southern Upland and adjacent regions of Nova

Scotia, encompassing a pH gradient increasing from southwest to northeast. Their results showed that parasite diversity in eels, as measured by species richness, Shannon-Wiener Index, decreased when pH was less than 5.4. However, digeneans were absent from the southwest when pH was less than 4.7. Parasite distributions among rivers in adjacent watersheds corresponded to fluctuations in pH in those rivers. Marcogliese and Cone (1997) results suggested that parasite communities are a good reflection of variations in environmental conditions.

Environmental factors can be even more detrimental in shaping parasite communities than phylogenetic relationships. Lile (1998) analyzed 18 species of helminths (ten digeneans, one cestode, six nematodes, and one acanthocephalan) in relation to host–parasite specificity and the effect of host ecological preferences on the establishment of the parasite fauna in the alimentary tract of four pleuronectid flatfish: flounder (*Pleuronectes flesus)*, witch flounder (*Glyptocephalus cynoglossus)*, American plaice (*Hippoglossoides platessoides)*, and Atlantic halibut (*Hippoglossus hippoglossus)* in northern Norway. The author found that 13 parasite species were generalists and the diversity of the parasite faunas decreased with increasing depths inhabited by the host. The author found that the larval nematode *Anisakis simplex* was the dominant species, followed by the digenean *Derogenes varicus.* Lile (1998) calculated the prevalence and abundance of the helminths found and measured the diversity using Shannon-Wiener Index. Similarities were greatest between the parasite faunas of flounder and American plaice and least between flounder and witch flounder. Lile (1998) suggested that host ecology, rather than phylogenetic relationships of the hosts, is

the main influence of the composition and diversity of the parasite communities of flatfishes in northern Norway.

f. **Parasite biology and complexity of life cycle:** A life cycle of a parasite may typically include the fish as a definitive host and one or more intermediate invertebrate hosts, and for the parasite to survive, all hosts must exist (Marcogliese and Cone 1997). Therefore, the fauna of a waterbody dictates the prevalence and abundance of certain parasite species and therefore can alter the community structure. Also, any changes in the environmental conditions such as temperature, food availability, and other factors that could directly or indirectly affect any of the hosts will significantly affect the prevalence and intensity of infection by affecting the rate of parasite development and transmission among the susceptible hosts.

III. GIT and its endoparasites

The GIT is the section of the digestive system that extends between the mouth cavity and the anal opening and includes the pharynx, esophagus, stomach, pyloric caeca and diverticuli, and intestine. The digestive glands (liver, pancreas, and intestinal glands) are a part of the digestive system, but not of the GIT. A number of Protozoa are known to infect the GIT, such as flagellates (e.g., *Hexamita* spp.), sporozoa (e.g., *Eimeria* spp. and *Gussia* spp.), and ciliates (e.g., *Protoopalina* spp.) (Hoffman 1999). The GIT is also a site of attachment of many subadult and adult stages of helminths belonging to trematodes, cestodes, nematodes, and acanthocephalans. Quite often, worms present in high numbers cause severe local damage at the attachment sites of the GIT of their hosts, such as in the

case of acanthocephalans (Bullock 1963; Buron and Nickol 1994), cestodes (Hugghins 1959), and trematodes (Yamaguti 1971).

Examples of GIT helminths include:

1) Acanthocephalans

The acanthocephalans (spiny-headed worms) form a major group of GIT worms of marine and freshwater fish worldwide including more than 1,000 species (Hoffman 1999; Amin et al. 2004). These groups of parasites are characterized by the presence of a cylindrical trunk and an anterior proboscis covered with many hooks whose arrangement is important in species identification. Acanthocephalans require two hosts to complete their life cycle, which begins when eggs are ejected from adult worms into the intestine of their final host and then with feces to the outer environment. The eggs are then ingested by a particular invertebrate where they hatch and undergo several developmental changes (Sparkes et al. 2006). The first intermediate host used by a parasite larval stage is often a crustacean amphipod, such as *Gammarus* spp. When infected intermediate hosts are eaten by a definitive host, mostly fish, the larvae migrate to their specific site of attachment (Amin 1986; Sparkes et al. 2006) where they attach and develop further. There is a controversy regarding the taxonomy of acanthocephalans and the variations in morphological phenotypes within one species. For example, Amin (1975*a*), based exclusively on morphological criteria, reported that *Acanthocephalus parksidei* and *A. dirus* were two separate, yet closely related, species. Later studies, however, considered both worms as synonyms (Amin 1975*b*; Hoffman 1999).

In North America, GIT acanthocephalans are predominant among centrarchids (Amin 1986), salmonids (Muzzall and Bowen 2000; Dezfuli et al. 2002), and cyprinids

(Amin 1975*a*). Indeed, there are a number of published studies demonstrating that acanthocephalans form the majority of the gastrointestinal metazoan parasites. For example, Muzzall and Bowen (2000), who studied parasite fauna of the lake trout, *Salvelinus namaycush*, in Lake Huron, found that the acanthocephalan *Echinorhynchus salmonis* is much higher in number than all other worms combined. Similarly, Kennedy and Hartvigsen (2000) found that *Echinorhynchus truttae* dominated all GIT helminths in the brown trout collected from 72 localities in the United Kingdom and Norway.

While in their attachment sites, acanthocephalans retract and contract their proboscis in a drilling-like movement, which damages the epithelial lining of the intestine (Bullock 1963; McDonough and Gleason 1981; Buron and Nickol 1994) and allows opportunistic microorganisms to invade the body of the host. A description of the damage caused by the acanthocephalan *Leptorhynchoides thecatus* is given by Esch and Huffins (1973), who found widespread necrosis and ulceration of the GIT at the site of attachment in heavily infected fish. In lighter infections, *L. thecatus* caused thickening of the mucosa and underlying muscle layer along with an increased number of goblet cells in affected LMB, *Micropterus salmoides*. *Neoechinorhynchus rutili* is another GIT acanthocephalan that causes mucosa damage in the rainbow trout, *Oncorhynchus mykiss* (Steinstrasser 1936). In 1981, McDonough and Gleason reported epithelial lining damage, connective tissue hyperplasia and granulocytic infiltration in the rainbow darter (*Etheostoma caeruleum*) as a response to the acanthocephalan *Pomphorhynchus bulbocolli* infection.

There are some studies that reported the presence of competitive inhibition between cestodes and acanthocephalans in several fish species. For example, in 1966,

Dogiel found that acanthocephalans were absent in the intestines of the northern pike, *Esox lucius*, that were infected with cestodes. In the same context, Cloutman (1975) demonstrated the presence of negative correlations between metacercariae of the trematode *Posthodiplostomum minimum* and plerocercoids of the bass tapeworm *Proteocephalus ambloplitis* and suggested the presence of antagonism between these two parasites. Durborow et al. (1988) noted that the numbers of plerocercoids of *P. ambloplitis* in the visceral cavity of wild LMB negatively correlate with the numbers of mature *Neoechinorhynchus* sp. present in the GIT of the same fish. The authors suggested that a competitive inhibition between the two parasites might exist. Similarly, the authors found that the *Neoechinorhynchus* sp. in free ranging LMB decreased when adult *P. ambloplitis* were present in the intestine.

2) Cestodes

Cestodes are characterized by their long flattened bodies known as strobila, which are divided into segments called proglottids where eggs are made and stored. In each proglottid, cross or self-fertilization takes place (Schmidt 1986). Therefore, they are considered monoecious, as in each proglottid exist the male and female reproductive organs (Karen et al. 1998). The plerocercoid larvae have a well-developed form of scolex at the anterior end like the adults. The scolex bears either suckers, hooks, or other means of attachment that help the worm attach to the wall of the gut. Cestodes use at least one intermediate host for their life cycle to be completed (Schmidt 1986). The intermediate host serves as a transport host where the larval stage of the worm is found and localized in the viscera, muscles, or any organ other than the intestine (Schmidt 1986). Within the visceral organs and peritoneum of fish, larval cestode stages occur, encysted or free, and

may cause damage of varying degrees; however, adult cestodes inhabit the GIT of their final fish host.

Cestodes induce their pathogenic effects in a variety of ways. First, larval or adult worms cause inflammatory reactions within the infected tissues and GIT (Sotelo and Del Brutto 2002). Second, although these tapeworms normally lack a digestive system, they feed by absorbing digested food from their hosts, thereby depriving the host of important nutritive elements. Third, they lower the pH around them to a level that inhibits and causes dysfunction of the digestive enzymes of their hosts and thereby affect the host's growth and normal functioning (Sotelo and Del Brutto 2002). Last, tapeworms can physically damage internal organs and stimulate peritoneal adhesions (Gillilland and Muzzall 2004).

One of the well-studied fish cestodes is the bass tapeworm (*Proteocephalus ambloplitis),* which is commonly found in a number of freshwater fish species. This worm is considered the most destructive tapeworm of freshwater fishes in North America (Hugghins 1959). The presence of the worm in the bass reproductive organs causes fibrosis of the organs and may reduce the fecundity of the fish (McCormick and Stokes 1982; Joy and Madan 1989; Gillilland and Muzzall 2004). Moreover, low egg development and production due to ovary infection by *P. ambloplitis* could cause sterility of infected individuals that might affect the population coexistence.

3) Nematodes

Nematodes are roundworms with tapered ends, and have a slender unsegmented bilaterally symmetrical body (Hoffman 1999). The outer body layer is a thick cuticular collagen protecting the parasite from the host's digestive enzymes and mechanical

damage (Black and Lankester 1980). These worms require an intermediate amphipod or copepod host to complete their life cycle (Moravec 1978). The fish act as both intermediate and definitive hosts. The female parasite releases eggs to the outside water along with the fish feces and development continues only if eggs are eaten by a susceptible amphipod or copepod (Moravec 1978). Fish become infected when consuming infected copepods or infected prey fish. For example, *Camallanus oxycephalus,* a common nematode widely distributed among freshwater fish of North America, depends on an intermediate host, either *Cyclops bicuspidatus* or *C. vernalis*, and may include a fish as a paratenic host (Stromberg and Crites 1975). Thus, the life cycle may be completed directly via the copepod to the definitive host or indirectly through ingestion of a small forage infected fish. *Contracaecum* sp. is another nematode species which is commonly seen in freshwater fishes, mammals, and piscivorous birds. The larval stage is found in the mesentery, viscera, and intestine of freshwater fishes, including the LMB, while adult worms are found in the intestine of the fish eating-birds (Bauer 1987). Sometimes this nematode is found coiled in the visceral fat. While most nematodes reside in fish intestines, some species parasitize other organs, like the swimbladder in the case of *Cystidicola* spp. (Miscampbell et al. 2004).

4) Trematodes

Digenetic trematodes are a large group of parasitic organisms with over 8,000 species. The adults are endoparasitic with both metacercaria and adult stages in fish. All trematode species require at least one intermediate host. These parasites are hermaphrodites with two large suckers on their body. Suckers are used for attachment: one oral sucker at the anterior end, and one ventral on the first of the three parts of the

body. *Leuceruthrus micropteri* is one of the trematodes that is commonly seen in the stomach of many freshwater fishes of North America, especially centrarchids. These trematodes use copepods where the cercarial stages develop. Fish become infected when ingesting an infected copepod (Yamaguti 1971). Trematodes, such as the black grub (*Uvulifer ambloplitis*) and the yellow grub (*Clinostomum complanatum*), cause economic losses in the aquacultural production of fish species due to consumers' rejection.

IV. The swimbladder and its parasites

The swimbladder is a vital organ that possesses a highly vascularized wall structure that is used heavily by fish in gas diffusion. The inflation and deflation of the swimbladder is essential for fish to attain the neutral buoyancy needed during foraging and predator avoidance (Moyle and Cech 2000). There are two types of swimbladders in teleosts, an open type known as physostomous, which is present in salmonids such as the LWF, and the closed type, known as physoclistous, present in centrarchids such as the LMB. A physostomous swimbladder is connected to the GIT via the pneumatic duct. Swimbladders are vulnerable to parasitism by many unicellular and multicellular parasites. For example, *Myxobolus cycloides* (Myxozoa: Myxobolidae) form cysts within the swimbladder wall of the chub (*Leuciscus cephalus*), thereby interfering with its normal functions (Molnar et al. 2006). In Norway, the flagellate *Hexamita* sp. attacks the swimbladder wall of the Atlantic salmon (*Salmo salar*) causing severe inflammation (Poppe et al. 1992). Metazoan parasites also inhabit fish swimbladders, such as larval *Didymozoid* sp. (Trematoda), which parasitizes the Red Sea coral-reef fish (*Anthias squamipinnis)* (Lengy and Fishelson 1972).

Among swimbladder parasites, two genera of nematodes have been thoroughly studied: *Anguillicola* spp. and *Cystidicola* spp. The nematode *Anguillicola crassus* is widespread in the swimbladder of the European eel (*Anguilla anguilla)*. Kirk (2003), who studied the life cycle, transmission dynamics, and pathogenicity of *A. crassus* in European eel populations, found that *A. crassus* can severely impair vital functions of the swimbladder leading to mortalities in both farmed and wild populations. *A. crassus* causes thickening of the swimbladder walls in infected eels along with hemorrhages, a matter that affects the ability of eels to migrate to the Sargasso Sea where they develop and mature, and thereby may contribute to the decline in this species' fisheries worldwide (Kennedy 2007). Knopf and Mahnke (2004) reported the presence of differences in resistance to *A. crassus* between two species of eels. The Japanese eel (*Anguilla japonica*) seems to possess more effective defense mechanisms against *A. crassus* than does the European eel. In infected *A. japonica*, most worms found were dead or encapsulated within the swimbladder wall. In contrast, no dead larvae were found in *A. anguilla*. Furthermore, the development of the worms was shown to be significantly slower in *A. japonica* compared with *A. anguilla*. The lower survival rate of the worms, together with their slower development, resulted in a significantly lower adult worm burden in *A. japonica* compared with *A. anguilla.* The reason for the heightened resistance of the Japanese eels is currently unknown.

Most of the studies performed on *A. crassus* reported on the status of infection at a given point in time, and did not deal with changes in the swimbladders of infected eels over time. For this reason, Szekely et al. (2005) followed *A. crassus in vivo* using radiological methods. The authors monitored the pathological changes caused by

A. crassus in 78 spontaneously infected European eels collected from Lake Balaton, Hungary, and kept in the laboratory for three months. By the end of the observation period, the status of the swimbladder had deteriorated in 55%, remained the same in 37%, and improved in 1%, while variable findings were obtained in the remaining fish examined. In another study, the dynamics of *A. crassus* infection in *A. anguilla* was monitored over two decades in an oligohyaline canal in southern France (Camargue, Mediterranean coast) by Lefebvre et al. (2002). Since the first detection of the parasite in this canal in 1985, the authors observed a phase of rapid spread of infection followed by stabilization around peak levels. The authors demonstrated that the health of infected eels varies seasonally, with maximum damage and mortalities occurring in the warmest months.

Nematodes of the genus *Cystidicola* (Spirurida: Cystidicolidae) are commonly found parasitizing physostomous swimbladders of Salmonidae and Osmeridae in Eurasia and North America. Unlike the extensive studies performed on *Anguillicola* spp., little is known about the taxonomy, phylogeny, and morphological details of *Cystidicola* spp. Indeed, only a handful of studies were published on the morphology of the parasite (Lankester and Smith 1980; Black 1983*b*), life cycle (Black and Lankester 1980, 1984), and phylogeny (Miscampbell et al. 2004). Forty years ago, it was believed that there were 21 *Cystidicola* spp., however, Ko and Anderson (1969) recognized only three species as valid: *C. farionis* Fischer 1798, *C. stigmatura* Leidy 1886, and *C. cristivomeri* White 1941. This classification was revisited by Black (1983*a*), who examined the worm and egg samples originally deposited by Leidy in 1886 and demonstrated that *C. stigmatura* and *C. cristivomeri* are synonymous. Currently, only two *Cystidicola* species are

acknowledged: *C. stigmatura* infecting *Salvelinus* species in North America and *C. farionis* infecting a number of fish species in Europe and North America, including LWF (*Coregonus clupeaformis*), cisco (*C. artedii*), bloater (*C. hoyi*), blackfin cisco (*C. nigripinnus*), round whitefish (*Prosopium cylindraceum)*, pink salmon (*Oncorhynchus gorbuscha*), coho salmon (*O. kisutch*), rainbow trout (*O. mykiss*), chinook salmon (*O. tshawytscha*), brown trout (*Salmo trutta*), brook trout (*Salvelinus fontinalis*), lake trout (*S. namaycush*), and rainbow smelt (*Osmerus mordax*) (Hoffman 1999).

Fertilized eggs of *Cystidicola* spp. pass through the pneumatic duct to the gastrointestinal tract and through the feces to the surrounding water. Benthic amphipods of several species, such as *Gammarus* sp., *Hyalella* sp., or *Pontoporeia* sp., (Smith and Lankester 1979) ingest the eggs, which after hatching, molt twice inside the amphipod hemocoel to become the third larval stage (L_3), which is the infective stage (Smith and Lankester 1979). Fish become infected when feeding on L_3–infected amphipods. L_3 reaches the swimbladder through the pneumatic duct and molts for the third time to become L_4. L_4 then molts for the fourth and final time. The post- L_4 worms, often referred to as the fifth stage larvae or subadults (Black and Lankester 1980), grow further, mature, and become adult worms. The diameter of the pneumatic duct allows the passage of eggs and L_3 only, while other larval and adult stages remain in the bladder until the host dies. The two moltings within the final host and sexual maturation process of *C. farionis* can take several months, and once matured, the worms live within their final host for several years laying eggs (Black and Lankester 1980, 1981, 1984; Giæver et al. 1991). Amundsen et al. (2003), who studied parasites of the Arctic char (*Salvelinus alpinus*) in Fjellfrøsvatn Lake in northern Norway, reported that *C. farionis* has a relatively long life

inside its final host. Studies from the Lithuanian Bay performed on a number of fish species also estimated that adult *C. farionis* have a life span of several years (Valtonen and Valtonen 1978).

The experimental studies of Black and Lankester (1980), who infected healthy fish with L_3 extracted from infected fish via a stomach tube, is the only account for the development of *Cystidicola* spp. within its final host. The authors determined that L_3 stays exclusively in the GIT and does not migrate to any other internal organ of infected fish. The authors also concluded that intervals between molts are variable and can differ from one fish species to the other and are probably temperature dependent. Within 6-8 hrs post infection (pi), L_3 worms were found only in the stomach, and by 12 hr pi they appeared in the esophagus where they started their migration through the pneumatic duct. As early as 16 hrs pi, L_3 reached the swimbladder cavity. In the case of *C. farionis* (worms collected from infected LWF and given to rainbow trout), it took 1-12 days for the first L_4 to appear in the swimbladder, while some L_3 remained without molting for up to three months. The fourth molt took place at the 74^{th} day pi for male larvae and the 111^{th} day for female larvae. The subadults, L_5 stage worms, did not reach maturation until the 112^{th} day for males and the 235^{th} day for females. The experiment was performed at a water temperature that fluctuated from 4-10 °C.

Differentiation between the two *Cystidicola* spp. relies primarily on key morphological features of the eggs and worms (Black 1983*a*). Eggs exhibiting polar and/or lateral filaments are those of *C. farionis* Fischer 1798. The eggs of *C. stigmatura* Leidy 1886 carry two lateral lobes but no filaments. The lip projection in the pseudolabia within the buccal cavity is a morphological criterion that was used by some authors to

differentiate between the two North American *Cystidicola* spp., being obvious in the case of *C. farionis*, while in *C. stigmatura* it is fused with the pseudolabia (Ko and Anderson 1969; Black 1983*b*). This difference in the buccal morphology as a criterion to differentiate between the two species was later refuted by Miscampbell et al. (2004), who concluded that the presence of a lip projection could vary from one *C. farionis* to the other depending upon the host fish species and the geographic area in which the worms developed. Moreover, sequences of segments of the ribosomal RNA gene segments: the spacer regions (ITS1 and ITS2), D3 expansion loop of the large subunit (28S), and 5.8S rDNA performed on seven species of fish and 11 locations in Canada and Finland found that while there has been some variation in the ITS2 region, the rDNA spacer regions may not be useful for distinguishing between *C. farionis* and *C. stigmatura (*Miscampbell et al. 2004). Currently, there is a consensus that *Cystidicola* spp. isolates exhibit a continuum of morphological and molecular variations that makes taxonomy of this genus extremely difficult (Miscampbell et al. 2004).

Within *C. farionis*, larval and adult stages can be distinguished from each other through their morphological criteria as suggested by Black and Lankester (1980), Lankester and Smith (1980), and Dextrase (1987). In general, there are four stages within the swimbladder cavity of the final fish host; L_3, L_4, post-fourth molt (subadult) worms which are sexually immature, mature male, and mature female worms. Males are distinct from females even during larval stages as their tails are spirally twisted and they possess a pair of unequal spicules and numerous preanal and postanal papillae. The sexually mature males are characterized by the presence of spermatozoa in their vas deferens, whereas sexually mature females have straight blunt tails, vulva in the middle

or anterior part of the body, and two uteri laden with eggs with thick shells surrounded by filaments (polar and/or lateral). The infective stage is L_3 and has a prominent cuticular protrusion at the tail and dumb-bell shaped oral opening. The fourth larval stage exhibits circumoral teeth and convoluted gonads with the tail protrusion beginning to fuse with the body. Following the fourth molt, the tail protrusion totally disappears and worms become substantially larger, yet their gametes are invisible until reaching sexual maturity.

C. farionis-induced effects on the fish are dose dependent, as the long life span of *Cystidicola* spp. permits the aggregation of several hundred worms in a single fish (Black and Lankester 1984; Knudsen and Klemetsen 1994). During and after the molting and maturation process, the delicate swimbladder epithelium and walls are damaged by mechanical irritation. *C. farionis* also secretes a number of hydrolytic enzymes to facilitate larval molting. These enzymes were shown to block blood coagulation and destroy host tissues (Zółtowska et al. 2001; Kenyon and Knox 2002). As a result, the swimbladder membranes become extremely thickened and inflamed (Willers et al. 1991) and hemorrhages are often seen (Rynko et al. 2003). The tissue alteration inflicted by *C. farionis* is intensity dependent (Stromberg and Crites 1975; Anderson and Gordon 1982; Knudsen et al. 2004). In a long-term field study performed in the Takvatn Lake in northern Norway, Knudsen et al. (2002) demonstrated that *C. farionis* induces mortality in the Arctic char (*Salvelinus alpinus)*. Over a period that extended from 1987 to 1999, the authors found that the cumulative numbers of L_3 steadily increased with increasing host age, indicating a continuous exposure to infection throughout the life of the target fish host. When the data was pooled over years along with a long-term cohort analysis, it was concluded that most parasite-induced host mortality occurs in hosts older than 10

years. Additionally, the short-term cohort analysis, adjusted for worm recruitment, demonstrated that the parasite-induced mortality occurs even in younger age groups. The degree to which *C. farionis* induced mortalities in *S. alpinus* populations in Takvatn Lake is, however, uncertain (Knudsen et al. 2002). Earlier studies performed in the same waterbody demonstrated that *S. alpinus* excess mortality related to *C. farionis* infection occurs mostly during the winter season and during spawning (Giæver et al. 1991), which were following peaks of intensity of infection that occurred in samples collected August to November. Both Giæver et al. (1991) and Knudsen et al. (2002) concluded that *C. farionis* parasitizing *S. alpinus* have a relatively long life.

V. LMB and its parasites

Order: Perciformes

Family: Centrarchidae

Genus: *Micropterus*

Species: *salmoides*

LMB is native to the eastern USA, though it has spread to other regions in North America. As one of the most popular sport fishes, LMB was intentionally introduced into many areas all over the world. Currently, LMB exists in North America, Africa, Europe, Asia, and New Zealand. According to U.S. Fish and Wildlife Service statistics, 43% of freshwater anglers fish for LMB. Apart from its recreational fisheries importance, as a predator, LMB plays an important role in the stability of the food web. In the USA, there are two LMB subspecies: the northern LMB (*Micropterus salmoides salmoides*) and the Florida LMB (*Micropterus salmoides floridanus*). LMB may also form hybrid fish by

spawning with other centrarchids such as the smallmouth bass, rock bass, bluegill, warmouth, and black crappie (Hubbs 1964; Carlander 1977; Page and Burr 1991).

Following the absorption of the yolk sac, LMB fry feed on zooplankton, and as the young LMB grow, their diet changes to small fish, frogs, turtles, salamanders, mice, insects, worms, mollusks, crayfish, and snails. The presence of well-developed pharyngeal jaws consisting of six major pads of caniform teeth in the upper pharynx and two pads in the lower pharynx allows LMB to firmly catch its prey. The average length of mature LMB is 18 inches, but LMB may attain a length of 24 inches or more. Males live a maximum of six years, while females can live up to nine years. Due to its position in the food web, LMB is vulnerable to many threats, particularly toxic chemicals and parasites. Parasites such as protozoans, copepods, roundworms, tapeworms, flatworms and leeches are common in LMB. A list of LMB parasites and their morphological criteria are found in Hoffman (1999). Externally, LMB harbors *Trichodina* spp., *Costia* spp., and *Ichthyophthirius multifiliis* (Heidinger 2000; Huh et al. 2005). The leech *Myzobdella lugubris* causes severe mouth ulcerations in LMB in North Carolina (Noga et al. 1990). Gill trematodes from the family Bucephalidae (Cloutman 1975) were reported to cause inflammation of gill lamellae. Parasitic copepods of the genus *Achtheres* are widespread in LMB in North America, often causing mortalities (Hoffman 1999). The GIT of LMB is known to harbor a number of acanthocephalans, cestodes, nematodes, and trematodes such as:

1) *Neoechinorhynchus cylindratus* Van Cleave, 1913

This acanthocephalan has been reported from almost every study that was performed in North America on LMB endoparasites, such as those from Texas (Sparks

1951), Michigan (Esch 1971; Muzzall and Gillilland 2004), Arkansas (Cloutman 1975), South Carolina (Eure 1976), and Belton Reservoir (Ingham and Dronen 1980). A pronounced seasonal cycling pattern in the intensity of infection has been observed, which was attributed to changes in temperature and fish feeding behavior (Eure 1976; Ingham and Dronen 1980). Some authors noted that this helminth attaches itself to the lining of the intestine of bass, inflicting considerable damage to mucosa (Sparks 1951).

2) *Leptorhynchoides thecatus* Linton, 1891

This acanthocephalan parasite inhabits the pyloric caeca primarily, but can be rarely found in the small intestine. This parasite seems to be very common among LMB populations from most regions of the USA (Esch 1971; Muzzall and Gillilland 2004; Steinauer et al. 2006). It was believed that females of *L. thecatus* could not reach sexual maturity in LMB, however, in a mesocosm study, Olson and Nickol (1996) demonstrated that LMB can maintain *L. thecatus* suprapopulations. Leadabrand and Nickol (1993) followed the establishment, survival and distribution of *L. thecatus* in LMB following feeding naïve LMB with cystacanths. The authors noticed that the worms established widely in the alimentary tracts of LMB, but by 5 weeks pi they had localized in the pyloric caeca and intercaecal region. Steinauer et al. (2006) observed geographic patterning within the variable traits of *L. thecatus* across a range of the species. They attributed this distribution pattern to ecological factors or that *L. thecatus* may be comprised of multiple cryptic species.

3) *Echinorhynchus salmonis* Müller, 1784

This parasite has a cylindrical body with a long cylindrical proboscis of many circles of hooks (Hoffman 1999). Its life cycle involves amphipods (*Gammarus* sp.) and

the adult stage is found in final host fish such as LMB and other centrarchids and several salmonid species (Hoffman 1999). The parasite was also found in burbot (*Lota lota*) in Lake Huron (Muzzall et al. 2003) and in the slimy sculpin, *Cottus cognatus* (Muzzall and Bowen 2002).

4) *Pomphorhynchus bulbocolli* Van Cleave, 1919

This species is found in a variety of freshwater fishes in North America and Mexico. Its life cycle requires an amphipod and a small fish for the larval stage before it reaches the final fish host for the adult stage (Hoffman 1999). The parasite has been reported from LMB and smallmouth bass *M. dolomieu* of Gull Lake, Michigan (Esch 1971; Muzzall and Gillilland 2004), and in rainbow darter *Etheostoma caeruleum* from Kentucky (McDonough and Gleason 1981). *P. bulbocolli* is believed to have been introduced to Canada along with LMB introduction (Szalai and Dick 1990).

5) *Proteocephalus ambloplitis* Leidy, 1887

This cestode is known as bass tapeworm as it has been found in the intestine and peritoneum of bass in most North American lakes (Hoffman 1999). In Michigan, the worm has been reported from LMB and smallmouth bass in Gull Lake (Esch 1971; Muzzall and Gillilland 2004). It has also been reported from the Lower Atchafalaya River Basin, Louisiana, and Boundary Reservoir, Saskatchewan (Szalai and Dick 1990). Szalai and Dick (1990) determined that the prevalence and mean intensity of *P. ambloplitis* plerocercoids in bass were low until age two; older bass harbored significantly more plerocercoids. Analysis of stomach contents indicates that *P. ambloplitis* prevalence and intensity increase as LMB starts feeding on aquatic insects and cannibalizing after age two.

6) *Contracaecum* spp.

These species are commonly seen parasites in freshwater fish species, mammals, and piscivorous birds. The larval stage is found in the viscera and intestine of freshwater fishes, including LMB, while adult worms are found in the intestine of the fish eating birds. Quite often these worms are found in a coiled position embedded in the fatty tissues of the visceral cavity or visceral organs (Szalai and Dick 1990). The gravid females release the eggs into the intestinal tract of the final host, and the eggs are released from the digestive tract with the host fecal material to continue the life cycle (Szalai and Dick 1990). These nematodes are generalists and were found in pond reared walleye fingerlings, *Sander vitreus*, in Wisconsin (Muzzall et al. 2006), and the channel catfish of Little Colorado River, Grand Canyon, Arizona (Choudhury et al. 2004). Aloo (1999), who studied the parasites of 541 LMB over a period of 12 months in Lake Naivasha in Kenya and its bay, demonstrated that LMB from Lake Naivasha serve as paratenic hosts of *Contracaecum* sp. While some authors found no more than a single worm/fish (Warren and Wilson 1978), others found up to 90 worms/fish (Lowe et al. 1977).

7) *Camallanus oxycephalus* Ward and Magath, 1917

This species is a common and widely distributed parasite of freshwater fish in North America, including LMB (Hoffman 1999). *C. oxycephalus* life cycle depends on an intermediate host, *Cyclops bicuspidatus* or *C. vernalis*, a fish as a paratenic host, and a piscivorous fish as a final host. A paratenic host is an intermediate host whose presence may be required for the completion of the parasite's life cycle, but in which no development of the parasite occurs (Stromberg and Crites 1975). Thus, the life cycle

may be completed directly via the copepod to the final host or indirectly through ingestion of a small forage infected fish (Stromberg and Crites 1975).

8) *Leuceruthrus micropteri* Marshall and Gilbert, 1905

This stomach trematode is not as widespread in LMB as *N. cylindratus* or *P. ambloplitis*; however, Hazen and Esch (2006), who followed its prevalence for a 15 month period, reported a prevalence that can reach up to 30%. It is believed that *L. micropteri* is found in LMB primarily in the southern states of USA such as Alabama (Hubert and Warner 1975), where prevalence rates are >35%.

Apart from parasite description and prevalence data, very little has been reported on LMB parasite communities and their structure. In 1975, Cloutman studied fish parasite community structure in LMB in Lake Fort Smith, Arkansas, among other centrarchids, and found that diversity did not fluctuate noticeably on a seasonal basis. He also found that there was no significant difference in parasite community structure between sexes or ages. Szalai and Dick (1990) studied parasites of LMB in its new habitat in Canada and found four parasite species only: *Diplostomum* sp., *Proteocephalus ambloplitis*, *Pomphorhynchus bulbocolli*, and *Contracaecum* sp. Banks and Ashley (2000) conducted a survey of the helminth fauna of LMB to examine helminth biodiversity and community structure in a northwestern Missouri reservoir. Seven species of helminths were recovered: *Proteocephalus ambloplitis*, *Spinitectus* sp., *Contracaecum sp.*, *Camallanus* sp., *Posthodiplostomum minimum*, *Crepidostomum* sp., and *Neoechinorhynchus cylindratus*. The acanthocephalan *N. cylindratus* was the most prevalent parasite in fish sampled and its prevalence reached up to 95%. A study on habitat influences on parasite assemblages of young-of-the-year LMB in the Lower Atchafalaya River Basin, Louisiana

was conducted by Landry and Kelso (2000). The authors found that physicochemical characteristics of Basin habitats may significantly influence parasite assemblages of young-of-the-year LMB.

VI. LWF and its GIT parasites

Order: Salmoniformes

Family: Salmonidae

Genus: *Coregonus*

Species: *clupeaformis*

The LWF, one of the most economically valuable freshwater species, feeds primarily on benthic macroinvertebrates (Bernatchez et al. 1991; Nalepa et al. 2005*b*). Following the Wisconsin glaciation during the Pleistocene, several members of the genus *Coregonus*, native to northern Europe and Asia, reached North America (Bernatchez et al. 1991) and formed sustainable colonies in the Great Lakes (Bailey and Smith 1981; Stott et al. 2004). Because LWF primarily lives along the shorelines of lakes in relatively shallow water (15-55 m in depth) (Selgeby and Hoff 1996), LWF constituted the first commercial fisheries in the Great Lakes (Cleland 1982; Spangler and Peters 1995; Brown et al. 1999). By the end of the 19th century, LWF fisheries started a long, steady decline from 11 million kg in 1879 to 701,000 kg by 1959 (Fleischer 1992; Spangler and Peters 1995). Habitat degradation, excessive exploitation by commercial fisheries, sea lamprey invasion, and the influx of toxic chemicals to the lakes have been blamed as causes for the decline. As a result, tribal, state, federal, and binational agencies undertook a number of managerial measures that allowed LWF populations to recover (Fleischer 1992; Spangler and Peters 1995). Unfortunately, the condition of LWF has worsened with the

invasion of the Great Lakes basin by dreissenid mussels, which have moved into lakes Erie, Huron, Michigan, and Ontario. When the zebra (*Dreissena polymorpha*) and quagga (*D. bugensis*) mussels' abundance increased in water <75 m deep in the four lower Great Lakes in the early 1990s, abundance of LWF prey, such as indigenous benthic macroinvertebrates and especially the amphipod *Diporeia* spp., significantly declined (Pothoven et al. 2001; Pothoven 2005; Mills et al. 2005; Nalepa et al. 2005*a*). As *Diporeia* spp. declined, LWF diets shifted to dreissenid mussels, gastropods, opossum shrimp (*Mysis relicta)*, ostracods, oligochaetes, and zooplankton (Hoyle et al. 1999; Pothoven et al. 2001; Pothoven 2005; Hoyle 2005). This diet shift was accompanied by a severe decline in LWF condition and growth (Hoyle et al. 1999; Pothoven et al. 2001; Mohr and Ebener 2005).

Few reports have described the parasites of LWF or the structure of their communities. Watson and Dick (1979), who studied the metazoan parasites of LWF and cisco (*Coregonus artedii)* from the Southern Indian Lake, Manitoba, found 19 species. They noticed that the parasites exhibited definite patterns of abundance with host age and season, due to dietary and behavioral causes. There have been no differences in parasite abundance between host sexes. The authors suggested that the increase in the abundance of copepod-vectored cestodes with the decrease in abundance of amphipod-vectored parasites has influenced the structure of the parasite community. Leong and Holmes (1981) described and compared the communities of metazoan parasites in salmonid fish species from Cold Lake, Alberta, Canada. Parasite communities in LWF were relatively rich in species and diversity compared with other salmonid species in the lake such as *Salvelinus* spp.

Examples of LWF-GIT worms include:

1) *Neoechinorhynchus tumidus* Van Cleave and Bengham, 1949

This species has a short proboscis, with six hooks arranged in spiral, circular, or diagonal rows. This species infects LWF in North America (Petrochenko 1956). The same worm also infects other salmonids worldwide, such as the pink salmon (*Oncorhynchus gorbuscha*, Muzzall and Peebles 1986).

2) *Acanthocephalus dirus* Van Cleave, 1931

This acanthocephalan is known by its short neck, which lacks a bulbous expansion. The body is cylindrical, containing six cement glands (Amin 1989). *A. dirus* life cycle involves one intermediate host, the freshwater isopod *Caecidotea intermedius,* where it develops through larval stages (Bierbower and Sparkes 2007) and then becomes an adult in the intestine of the final fish host. *A. dirus* is widely distributed in North America in a number of fish species, including salmonid species (e.g., lake trout *Salvelinus fontinalis* (Muzzall 2007)), centrarchids, and rainbow darter *Etheostoma caeruleum* (McDonough and Gleason 1981).

3) *Cyathocephalus truncatus* Pallas, 1781

This tapeworm, *C. truncatus*, is common in many fish species, including coregonids. It is known for its funnel-shaped apical adhesive scolex, with a slight constriction separating scolex from strobila. In North America, it is mostly seen in salmonids and whitefish, in particular. Adult tapeworms have a large front bell-shaped end (scolex) that attaches to the intestines (Petersson 1971). It is found in coregonids in Sweden (Petersson 1971) and Norway (Amundsen et al. 2003), burbot (*Lota lota*) in Lake

Huron, Michigan (Muzzall et al. 2003), and in brown trout (*Salmo trutta*) of Italy (Dezfuli et al. 2001).

4) *Bothriocephalus* sp. Rudolphi, 1808

These species are common parasites of LWF (Camp et al. 1999; Stewart and Bernier 1999). The parasite is also found worldwide infecting other fish species such as the burbot, *Lota lota*, in Ontario, Canada (Anthony 1987), and Maine (Meyer 1954).

VII. Basic information on the study area

With a total surface of 208,610 km^2 and a total volume of 22,560 km^3, the Laurentian Great Lakes (Huron, Superior, Erie, Michigan and Ontario) compose one of the planet's largest freshwater ecosystems. The Great Lakes were formed by the retreat of the mile-thick glaciers in Wisconsin during the Ice Age, which was between 10,000 and 7,000 years ago. In addition to the five Great Lakes, the basin encompasses tens of thousands of inland lakes, embayments, rivers, and littoral zones, forming one of the largest watersheds in the world. Despite the fact that the different components of the Great Lakes Basin are interconnected, obvious physical, chemical, and hydrobiological variations exist among different regions, thereby creating a unique ecosystem. Natural habitats in the Great Lakes watershed include wetlands, sand dunes, islands, and streams. The unique basin of the Great Lakes is in the center of 40 million people in eight U.S. states and the Canadian province of Ontario.

Unfortunately the Great Lakes basin has been plagued with a number of problems. Industrial waste and agricultural runoffs have negatively impacted the health of the Great Lakes ecosystem and its fauna. The invasion of the Great Lakes by invasive species severely disrupted the food web, resulting in large economic impacts. Moreover,

over exploitation has devastated economically and ecologically important fish species such as LWF, lake trout (*Salvelinus namaycush*), and lake sturgeon (*Acipenser fulvescens*). In this study, parasites of two important Great Lakes fish species, LWF and LMB, were studied. Samples were collected from the watersheds of three of the Great Lakes within the State of Michigan: Lake Erie, Lake Huron, and Lake Michigan.

1) Lake Erie Watershed

The portion of the Lake Erie watershed within Michigan is located in the southeastern section of the lower peninsula. This area contains all waters that flow east or southeast into the Lake Erie drainage, including the connecting waterways of the St. Clair and Detroit rivers and Lake St. Clair. Agriculture is dominant in the southern and northern portions of the watershed. The Lake Erie watershed is 15,042 km^2 and includes the major watersheds of the Black, Pine, Belle, Clinton, Rouge, Huron, and Raisin rivers. Only 5% of the area is currently classified as wetlands, while the majority of the land area (58%) is mainly agricultural and urban parks, 19% forest, and 15% urban areas. Urbanization is gathered between these areas and includes the metropolitan Detroit area and its expanding suburbs. Large urban parks are found along the Huron and Clinton rivers, both in urban areas and on the fringes. Dredging, channelization, macrophyte removal, thermal changes, and nutrient inflow alterations are results of wetland modifications, urban and riparian modifications, and municipal and industrial pollution. (www.michigan.gov/dnr)

2) Lake Huron Watershed

The Lake Huron watershed is located in the northeastern part of Michigan. This area contains all waters, including the connecting waterway of the St. Mary's River, that flow east or southeast into the Lake Huron drainage. This watershed spans both the Lower and Upper peninsulas of Michigan. The Lake Huron watershed is 41,823 km^2 and includes the major watersheds of the Munuscong, Carp, Cheboygan, Thunder Bay, Au Sable, Rifle, Saginaw (tributaries: Tittabawassee, Shiawassee, Flint, and Cass rivers), Sebewaing and Pigeon rivers, besides some small coastal watersheds. Wetlands comprise 18% of the watershed, while the majority of the land cover is forested (40%), primarily in the northern portions, then agricultural areas (33%), which are mostly in the southern part, and urban areas, comprising only 2%. Altered hydrologic regimes, altered sediment loads, social attitudes, thermal changes and wetland modifications are the major threats to these watershed areas. (www.michigan.gov/dnr)

3) Lake Michigan Watershed

The Lake Michigan watershed is the largest in Michigan. It contains all waters that flow into Lake Michigan from the western half of the Lower Peninsula of Michigan and all flow heads south from the Upper Peninsula. The Lake Michigan watershed is 73,837 km^2 and includes the major Upper Peninsula watersheds of the Menominee, Cedar, Ford, Escanaba, Rapid, Whitefish, Sturgeon, and Manistique rivers, among several small coastal watersheds. In the Lower Peninsula, the major watersheds are the Pine, Elk, Boardman, Platte, Betsie, Manistee, Pere Marquette, White, Muskegon, Grand, Kalamazoo, and St. Joseph rivers, as well as some small coastal watersheds. This

watershed is the most developed in the southern section with dominant agricultural areas (37%), forestry (36%), and wetlands (19%). (www.michigan.gov/dnr)

CHAPTER 2

DIVERSITY AND COMMUNITY STRUCTURE OF GASTROINTESTINAL TRACT HELMINTHS OF THE LARGEMOUTH BASS, *MICROPTERUS SALMOIDES,* COLLECTED FROM INLAND LAKES OF MICHIGAN'S LOWER PENINSULA, USA

ABSTRACT

Largemouth bass (*Micropterus salmoides*; LMB) is an important sportfish species in the Laurentian Great Lakes that is critical for the stabilization of their ecosystems. This study was designed to identify the helminth species infecting the gastrointestinal tract (GIT) of LMB in 15 Michigan inland lakes and describe their community structure. A total of 16,700 worms were retrieved from the GITs of 641 adult LMB collected from 15 inland lakes between July 2002 and September 2005. Over 75% of the LMB examined harbored at least one helminth species in their GIT, with relatively high intensity (34.72 $\pm$ 35.07 worms/fish) and abundance (26.05 $\pm$ 35.07 worms/fish). Collected helminths were generalists in nature and represented four phyla and nine species: *Neoechinorhynchus cylindratus*, *Leptorhynchoides thecatus*, *Acanthocephalus parksidei*, *Echinorhynchus salmonis*, *Pomphorhynchus bulbocolli*, *Proteocephalus ambloplitis*, *Contracaecum* sp., *Camallanus oxycephalus*, and *Leuceruthrus micropteri*. *N. cylindratus* dominated the GIT helminth community with a prevalence of 57.88%, was found in all of the lakes examined, and was the dominant species in 13 lakes. *L. thecatus* infected 27.3% of LMB

and was the dominant species in two inland lakes. Based on their abundance, *N. cylindratus* and *L. thecatus* were considered the core species in the LMB-GIT helminth community. The generalized linear mixed model analyses demonstrated the presence of significant effects of the Great Lakes watershed in which the inland lake lies (P=0.0003), the presence of inlets (P =0.0005, 63 DF), the presence of outlets (P <0.0001, 63 DF), and the accessibility of the inland lake to the public (P <0.0001, 63 DF) on infection parameters of LMB-GIT worms. On the contrary, fish gender showed no significant effects on infection parameters. Diversity, as measured by Simpson's Index of Diversity (SID) and Shannon-Wiener Index (SWI), was significantly greater in inland lakes with public access (P<0.04). Inland lakes in the Lake Huron watershed exhibited higher diversity than their counterparts in the Lake Michigan or Lake Erie watersheds. Despite the obvious dominance of *N. cylindratus* and the low species richness of LMB-GIT helminths, only one pair of lakes was 100% similar and only 18 out of 105 pairwise comparisons of inland lakes exhibited ≥75% similarity. In this study, significant positive correlations were found among three pairs of LMB-GIT helminths: *N. cylindratus* and *Contracaecum* sp.; *L. thecatus* and *P. bulbocolli;* and *A. parksidei* and *E. salmonis*. The data in this study represent the most comprehensive investigation ever conducted on LMB gastrointestinal tract helminths in the Great Lakes basin. Due to their indirect life cycles, often employing a number of intermediate and final host species, the structure of the GIT helminth communities is important for fishery managers as it reflects the biodiversity and ecosystem health in the surrounding aquatic environment.

INTRODUCTION

The centrarchid largemouth bass (*Micropterus salmoides*; LMB) is native to North America, where its original habitat extends from southern Canada to northern Mexico and from the Atlantic coast to the central region of the United States (Hubbs 1964; Carlander 1977; Page and Burr 1991). As a predator, LMB plays an important role in maintaining balance in ecosystems (Olson and Young 2003). In the Great Lakes basin, LMB populations have suffered from epizootic infections, in particular, those caused by the Largemouth Bass Virus (Faisal and Hnath 2005). The potential that pathogens may negatively affect the Great Lakes basin has raised serious concern and emphasized the need to study the ecology of LMB pathogens and diseases.

Following absorption of the yolk sac, LMB fry feed on zooplankton, and as they grow, their diet changes to amphipods, worms, mollusks, crayfish, small fish, frogs, turtles, salamanders, and rodents (Clady 1974). As a result of this diverse feeding regime, LMB is continuously exposed to the infective stages of numerous parasitic helminth species (Hoffman 1999; León et al. 2000). Due to their indirect life cycles, often employing a number of intermediate and final host species, helminths parasitizing the gastrointestinal tract (GIT) have often been used as a mirror of biodiversity and ecosystem health in the surrounding environment, and as an indication of diet, parasite biology, and prevailing hydrobiological factors (Mouillot et al. 2005; Kennedy 2009).

LMB-GIT helminths have been described from fish collected from Arkansas (Cloutman 1975), Louisiana (Landry and Kelso 2000), Michigan (Muzzall and Gillilland 2004), Missouri (Banks and Ashley 2000), South Carolina (Eure 1976), Tennessee River

(Hubert and Warner 1975), Wisconsin (Amin 1975*c*; Amin 1986), Canada (Szalai and Dick 1990), and Kenya (Aloo 1999). Most of these studies have been limited in scope and have not addressed the role hydrobiological factors may play in shaping the GIT helminth community structure, creating knowledge gaps on the distribution and spread of individual parasites along with the nature of the assemblages they form in LMB. Despite the common belief, not based on published evidence, that GIT helminths cause little or no harm to their hosts, a number of reports demonstrated pathological lesions associated with the site of attachment, such as erosion and ulceration of the intestinal epithelial lining along with connective tissue formation (McDonough and Gleason 1981; Adel-Meguid et al. 1995). Perforation of the intestinal wall and physical damage to visceral organs has also been reported (Aloo 1999). The severity of the lesions is dependent on the helminth species and its intensity in the GIT of infected LMB.

To this end, this study was designed to determine the prevailing GIT worms and their community structure in LMB residing in 15 inland lakes in Michigan's Lower Peninsula. Several analyses were performed to determine if host or environmental factors play a role in shaping the LMB-GIT helminth parasites and their assemblages such as: fish gender, watershed, public access, and the lake's connections to other waterbodies through inlets or outlets.

MATERIALS AND METHODS

1) Fish and sampling sites

A total of 641 (368 females and 273 males) adult LMB with a mean total length of 29.35 cm±5.88 cm and weight of 373.96 g±230.32 g were collected in summer months from 15 inland lakes in Michigan's Lower Peninsula between July 2002 and September 2005. The lakes were selected so as to represent each of the watersheds of lakes Michigan, Huron, and Erie (Figure 2.1). Inland lakes within the Lake Huron (LH) watershed included Woodland, Nepessing, Shupac, Pine and Budd lakes. Inland lakes within the Lake Erie (LE) watershed included Orion, Independence, and Big lakes, while inland lakes within the Lake Michigan (LM) watershed included Randall, Eagle, Jordan, Ovid, Duck, Nichols, and Ruppert lakes. Information on each of the lakes is provided in Table 2.1. The lakes ranged in area from 0.11 to 2.55 km^2. Pine and Ovid lakes are not accessible to the public. Eight inland lakes have both inlets and outlets, while five lakes have neither inlets nor outlets. Lake Nepessing and Eagle Lake have no inlets but have outlets. The Lower Peninsula of Michigan was selected for study of LMB-GIT helminths for three reasons. First, each inland lake of the Lower Peninsula of Michigan lies within the watershed of one of three Great Lakes: Erie, Huron, or Michigan, and thereby provides a variety of hydrobiological factors that may influence the infection parameters of parasites. Second, the LMB is one of the most popular sport fisheries in the Lower Peninsula of Michigan. Last, a comprehensive health survey on LMB in the Lower Peninsula of Michigan to determine the causes of fish kills and the distribution of LMB pathogens, in particular Largemouth Bass Virus, was conducted between 2002 and 2005,

thereby providing a unique opportunity to study parasite community structure using a relatively large number of LMB.

The number, gender, length, and weight of fish sampled from each of the inland lakes are provided in Table 2.2. Fish were collected primarily by electro-fishing, hook and line angling, and trap nets by biologists from the Michigan Department of Natural Resources and Environment. Fish were transported alive in tanks with aerators to the Aquatic Animal Health Laboratory at Michigan State University, East Lansing, Michigan for sample collection and processing.

2) Parasite examination

Fish were sacrificed with an overdose of Tricaine Methanesulfonate (MS-222, Argent Laboratories, Redmond, Washington). Each GIT with attached mesentery was removed from the esophagus to the anus and kept in tap water for about 24 - 48 hours at 4 °C to allow for parasite relaxation before further processing. GIT helminths were retrieved manually and preserved in 70% ethanol for later identification and counting. Nematodes were cleared in a mixture of glycerol and 70% ethanol (1:1) and then examined microscopically. Worm species were identified according to morphological criteria and the identification keys of Yamaguti (1971), Aliff et al. (1977), Moravec (1980), Ingham and Dronen (1982), Amin (1985*a*) and Hoffman (1999).

3) Measurements of LMB-GIT helminth assemblage

Measurements of parasites and terms used to describe parasite individuals and communities throughout this study were adopted from Bush et al. (1997) unless otherwise indicated. Prevalence denotes the percentage of host individuals infected with one or more parasites of a particular species. Intensity is defined as the number of

individual parasites from a certain species found in an infected host and hence does not include uninfected fish, whereas abundance is defined as the number of individual parasites of a certain species found in both infected and uninfected hosts. Species richness is the number of parasite species found in a fish population. Diversity indices were used to determine GIT helminth diversity within each of the inland lakes. Both Shannon-Wiener's (Ricklefs 1993) and Simpson's diversity indices were used. The Shannon-Wiener's Diversity Index was calculated as detailed in Shannon (1948). The Simpson's Index of Diversity was calculated by first determining Simpson's Diversity Index (D) according to the equation developed by Simpson (1949), and then subtracting D from 1. Increasing values of the Shannon–Wiener's and Simpson's diversity indices indicate an increase in diversity. The dominance of a particular parasite species was expressed as the Berger-Parker Dominance Index, which measures the proportion of the total number of parasites due to dominant parasite species (Berger and Parker 1970). To compare between two inland lakes for similarity of GIT helminths, the Jaccard Similarity Index was used (Cheetham and Hazel 1969).

4) Statistical analysis

Data on abundance was analyzed separately for each helminth species using generalized linear mixed model (GLMM) analyses. For this, the procedure PROC GLIMMIX in the software SAS® was used based on a negative binomial distribution and a log link. Similarly, intensity data was analyzed in the same manner as that for abundance except that the distributional and link specifications were, respectively, truncated negative binomial and log using the SAS® procedure PROC NLMIXED. Prevalence data was analyzed separately for each species using another set of GLMM

analyses based again on PROC GLIMMIX, but using the binary distribution and the logit link specification. For all three types of GLMM analyses, the same risk factors were investigated, specifically: the presence of a water inlet into the lake (Yes vs. No); presence of an outlet to the lake connecting it to other waterbodies (Yes vs. No); access of the lake to public boating and fishing (Yes vs. No); the Great Lakes watershed within which the inland lake is physically present (Lake Erie vs. Lake Huron vs. Lake Michigan); and fish gender (Male vs. Female). For all factors except gender, lake was defined as the experimental unit or replicate, whereas fish was defined as the experimental unit for gender. Estimated means for levels of each potential risk factor were expressed on the scale of measurement adjusted for all other risk factors, while their corresponding standard errors were based on the use of the delta method (Oehlert 1992). Differences in abundance, intensity, and prevalence by the potential risk factors were assessed by pairwise comparisons of adjusted means. Because of the convenient specification of the logit link in the GLMM analysis of prevalence data, these comparisons were expressed as odds ratios.

Comparisons for differences among watersheds for the diversity indices and the Berger-Parker Dominance Index were performed using standard analysis of variance (ANOVA) based on the same risk factors described above. Data was log-transformed when necessary. Watersheds were also compared for richness scores based on the nonparametric Wilcoxon test. Unless otherwise noted, a statistical test with $P<0.05$ was considered statistically significant. Cluster analysis was performed based on the prevalence data in order to combine parasite species into major groups. All cluster

analyses were based on the squared Euclidean distance according to Ward's method using the SAS PROC CLUSTER procedure (Johnson 1998).

The correlation between LMB-GIT helminth species based on prevalence data was computed by the Pearson correlation coefficient, while the cluster analysis, as described by Aldenderfer and Blashfield (1984), was used to determine the degree of association of inter- and intraclusters. Pearson's correlation coefficient was also used as an indicator for the relationship among different parasite species found in each watershed and to compare lakes and watersheds for the incidence of LMB-GIT worms. The cluster analysis was performed based on the prevalence data in order to combine all similar, or close, locations into major groups. The cluster analysis was conducted using the Euclidean distance and the weighted pair groups mean average method. This analysis and the corresponding Dendogram were done using the Minitab software (Minitab Inc., State College, Pennsylvania).

RESULTS

Out of the 641 LMB examined, 368 were females and 273 were males. Females (419.81 g±49.9g) were significantly heavier (P=0.01 at 625 DF) than males (376.02±50g). The overall prevalence of the infection was 75%, with LM-Eagle Lake being the lowest in prevalence (18%). Three lakes exhibited 100% infection: LM-Nepessing Lake, LE-Big Lake, and LM-Ruppert Lake (Table 2.3). Based on the three infection parameters of GIT helminths combined, LMB residing in LM-Ovid Lake have an odds ratio of 0.261 to be infected with at least one GIT helminth, followed by LM-Eagle Lake (0.286), LH-Budd Lake (0.457), LE-Independence Lake (0.515), LH-Nepessing Lake (0.711), LE-Orion Lake (0.806), LM-Jordan Lake (0.804), LH-Woodland Lake (0.865), LH-Shupac Lake (0.932), LM-Duck Lake (1.429), LM-Randall Lake (1.930), LM-Nichols Lake (2.2361), LE-Big Lake (2.452), LM-Ruppert Lake (2.834), and LH-Pine Lake (3.912).

A total of 16,700 worms were retrieved from GITs of 641 LMB, representing four phyla and nine species. Acanthocephalans constituted an overwhelming majority of LMB-GIT helminths, with a total of 16,062 worms (96.18%). Five species of acanthocephalans were identified: *Neoechinorhynchus cylindratus* Van Cleave 1913, *Leptorhynchoides thecatus* Linton 1891, *Acanthocephalus parksidei* Amin 1975, *Echinorhynchus salmonis* Müller 1784, and *Pomphorhynchus bulbocolli* Van Cleave 1919. The only cestode found was the bass tapeworm, *Proteocephalus ambloplitis* Leidy 1887, which constituted 1.5% (250 worms) of LMB-GIT helminth community. Two nematode species were identified in LMB-GIT: *Contracaecum* sp., and *Camallanus oxycephalus* Ward and Magath 1917. The number of *Contracaecum* sp. found in the GIT

was 312 worms, accounting for 1.9% of the total worm population. *C. oxycephalus* was present in one fish from LE-Lake Orion. The only other GIT parasite was a trematode, *Leuceruthrus micropteri* Marshall and Gilbert 1905, which constituted 0.37% (62 worms) of the LMB-GIT helminth community.

Analyses of infection parameter data of all helminth species combined revealed that the odds of LMB being infected with *N. cylindratus* was 23.3 times higher than *Contracaecum* sp., 95.5 higher than *P. ambloplitis* ($P<0.0001$), and 125 times higher than *L. thecatus*. Additionally, the odds ratios of LMB becoming infected with *Contracaecum* sp. was 5.5 times higher than the odds of being infected with *L. thecatus* ($P<0.0062$) and 4.1 times higher than with *P. ambloplitis* ($P<0.001$).

1) Infection parameters and effects of risk factors

Analyses clearly demonstrated the presence of significant effects of the watershed ($P=0.0003$, 63 DF), the presence of inlets ($P=0.0005$, 63 DF), the presence of outlets ($P<0.0001$, 63 DF), and the accessibility of the inland lake to the public ($P<0.0001$, 63 DF) on infection parameters of LMB-GIT worms combined. On the contrary, fish gender showed no significant effects of potential risk factors on infection parameters. Infection parameters of each of the GIT helminths found in this study, as well as statistically significant effects of risk factors on infection parameters of each of the worms, are given below.

a. *Neoechinorhynchus cylindratus*

N. cylindratus dominated the acanthocephalan populations of LMB-GIT helminths. This worm was found in all of the inland lakes examined with an overall prevalence of 57.88% (371 fish infected out of 641), with LM-Ruppert

Lake being the highest prevalence (96.77%) and LM-Lake Eagle being the lowest prevalence (18.18%, Table 2.3). *N. cylindratus* inhabited the intestine and was the dominant GIT helminth species in 13 of the 15 lakes examined and was the second most dominant worm in the two remaining lakes. None of the potential risk factors examined exhibited any significant effects on *N. cylindratus* prevalence. The odds of LMB being infected with *N. cylindratus* are 95.5 times higher than *P. ambloplitis* ($P<0.0001$), 125 times higher than *L. thecatus* ($P<0.0001$), and 23.3 times higher than *Contracaecum* sp. ($P<0.0001$).

N. cylindratus also exhibited the highest intensity among all worms with 11,827 worms from 641 fish, constituting 73.63% of all acanthocephalans and 70.82% of the total worm count. The overall mean intensity was 31.88 worms per fish. The number of worms per fish reached up to 275 worms in an individual fish caught from LE-Big Lake. A significant difference ($P<0.001$) was noticed in the *N. cylindratus* mean intensity among the 15 lakes, with Big Lake being the highest (65.5$\pm$63.72 worms/fish) and Shupac Lake being the lowest (8.42$\pm$5.87 worms/fish) (Table 2.4). The intensity of *N. cylindratus* varied among lakes within each watershed (Table 2.4). Analyses revealed that LMB collected from inland lakes with no public access had an estimated intensity (55.02$\pm$21.21worms/fish) of *N. cylindratus* that was significantly greater ($P=0.03$) than that of lakes with public access (21.07$\pm$3.66 worms/fish). Analyses also showed that other potential risk factors tested exhibited no significant effects on *N. cylindratus* intensity.

Similarly, *N. cylindratus* mean abundance varied greatly among the 15 inland lakes, with LM-Eagle Lake being the lowest (1.64±4.36 worms/fish) and LE-Big Lake the highest (63.07±63.72 worms/fish, Table 2.5). The overall mean abundance was 18.45±30.26 worms/fish. The presence of public access exerted significant (P=0.0411) effects on *N. cylindratus* abundance, with LMB in inland lakes with no public access having five times greater abundance of *N. cylindratus* compared to LMB in lakes with public access (48.5±29.6 vs. 10.5±2.7 worms/fish).

b. *Leptorhynchoides thecatus*

L. thecatus was found primarily in the pyloric caeca in LMB collected from 10 of the 15 lakes examined. *L. thecatus* accounted for 21.89% of total worms and 22.76% of acanthocephalans. Unlike *N. cylindratus*, *L. thecatus* had a much lower prevalence (27.3%) in LMB (175 out of 641) examined in this study, and was the dominant worm in LM-Duck Lake and LH-Nepessing Lake, with prevalence values of 91.43% and 88.89%, respectively. The absence of inlets to the lake significantly increased the prevalence of *L. thecatus* (P<0.01 at 9 degrees of freedom). That is, LMB residing in lakes with no inlets have 12 fold higher odds of contracting the infection as opposed to LMB residing in lakes with inlets. In infected LMB, the number of worms/fish ranged from 3.55±2.91in LM-Jordan Lake to 50±8.11 in LH-Pine Lake (average 20.89±18.24). It was found that LMB residing in lakes with no inlet had an estimated intensity (33.51±24.64 worms/fish) of *L. thecatus* that was significantly greater (P=0.0003) than lakes with inlets (7.81±5.86 worms/fish). Abundance varied from zero to 40.26±41.63

worms/fish (average 5.7±18.24 worms/fish). The presence of an outlet tended to increase the abundance of *L. thecatus*; however, the increase was not statistically significant (P=0.089).

c. *Acanthocephalus parksidei*

A. parksidei was present in the intestine of 4.37% of LMB examined, and its presence was limited to five inland lakes only. The watershed in which the inland lake lies exhibited a significant effect on the prevalence of *A. parksidei* (P=0.0017 at 9 DF) with LH higher than either LM (P<0.003 at 9 DF) or LE (P<0.001 at 9 DF). Mean intensity varied greatly between lakes but never exceeded 4, except in Woodland Lake, where it was >24 worms/fish. It was determined that lakes with outlets had an estimated intensity (5.36±3.09 worms/fish) of *A. parksidei* that was significantly greater (P=0.04) than lakes without outlets. A similar trend was observed with the abundance data, where significant differences were observed among the three watersheds (P=0.0336 at 9DF) with LH greater than LM (P=0.0234) and LE (P=0.014).

d. *Echinorhynchus salmonis* Müller, 1784

E. salmonis was also found in the LMB intestine with a prevalence of 1.25%. Its presence was confined to two inland lakes only: LH-Woodland Lake and LM-Randall Lake. Despite the limited distribution, statistical analysis showed that LH watershed is significantly higher than LM and LE (P<0.0014 at 9 DF) in prevalence. As displayed in Table 2.4, the mean intensity was 5.5±1.97 worms/fish in LH-Woodland Lake, which is significantly less than in LM-Randall Lake (14.5±4.08 worms/fish; P=0.04).

e. *Pomphorhynchus bulbocolli* Van Cleave, 1919

P. bulbocolli was found in the pyloric caeca of LMB from two lakes: LH-Budd Lake and LM-Duck Lake. This worm has a relatively low overall prevalence (0.31%), intensity (11.00±0.76 worms/fish) and abundance (0.03±0.76 worms/fish). Statistical analyses failed to find significant effects of potential risk factors on infection parameters of *P. bulbocolli*.

f. *Contracaecum* sp.

Contracaecum sp. was present in 10 lakes with a prevalence that ranged from 1.72 in LH-Shupac Lake to 51.85% in LE-Big Lake (11.23% among all LMB examined). Statistical analyses revealed that the odds ratios of LMB becoming infected with *Contracaecum* sp. are 5.5 times higher than the odds of being infected with *L. thecatus* ($P<0.0062$) and 4.1 times higher than that of *P. ambloplitis* ($P<0.001$). None of the risk factors seem to influence *Contracaecum* sp. prevalence. Despite the relatively wide distribution of this nematode, its mean intensity was relatively low (4.51±3.52 worms/fish). The number of *Contracaecum* sp. found in the intestine was 312 worms, accounting for 1.9% of the total worm population. It is noteworthy that *Contracaecum* sp. was also present in the mesentery and abdominal cavity of infected fish; however, numbers presented in this study refer to the immature worms in the GIT only. It was found that lakes with outlets had an intensity (5.36±3.09 worms/fish) of *Contracaecum* sp. that was significantly greater ($P=0.04$) than lakes without outlets. It was also found that inland lakes in LE watershed had an intensity (10.60±7.86 worms/fish)

that was significantly greater than in LM (2.85±1.27 worms/fish; P=0.02), and tended to be greater than in LH (2.97±1.43 worms/fish, P=0.06). Mean abundance also varied greatly from one lake to the other; however, there have been no significant differences noted among watersheds and with any of the risk factors. Other potential risk factors examined exhibited no statistically significant effects on *Contracaecum* sp. infection parameters.

g. *Camallanus oxycephalus*

C. oxycephalus was present as a single specimen in one fish caught from LE-Lake Orion. Since *C. oxycephalus* was present as a single specimen in one fish only, it was excluded from the statistical analyses of infection parameters and the effects of risk factors on them.

h. *Proteocephalus ambloplitis*

The cestode *P. ambloplitis* was found attached to the intestinal wall of 82 LMB out of 641 (12.8%) with the prevalence ranging from 0-59% (Table 2.3). The worm was widespread as it was present in all lakes except Eagle Lake. The total number of tapeworms found was 250, accounting for 1.5% of the total GIT worm population, and the mean intensity varied from 1.5-5.0 worms/fish with an average of 3.05±1.57 worms/fish (Table 2.4). As displayed in Table 2.5, the mean abundance exceeded 1.0 in two lakes only, with an average of 0.39±1.57 worms/fish. No significant effects of any of the tested risk factors were found on the prevalence, intensity, or abundance of *P. ambloplitis*.

i. ***Leuceruthrus micropteri***

This fluke was present in the stomach of 0.93% of all fish examined, and its numbers accounted for 0.37% of the total LMB-GIT worm community. Its distribution was limited to Randall, Ovid, Nichols, and Ruppert lakes, all within the Lake Michigan watershed. Public access increased prevalence ($P<0.01$, 9 DF). It was found that lakes with no inlet had an estimated intensity (10.33 ± 7.93) of *L. micropteri* that was significantly greater ($P=0.0063$) than lakes with inlets (1.19 ± 0.71). It was also found that lakes with public access had a smaller mean abundance of *L. micropteri* ($P<0.07$), albeit not statistically significant. Moreover, males had higher mean abundance of *L. micropteri* ($P<0.0186$ at 625 DF) than females.

2) Measurements of LMB-GIT community structure

The 15 inland lakes varied widely in the numbers of LMB-GIT helminth species that they carried, ranging from one in LM-Eagle Lake to seven in LM-Randall Lake. Statistical analysis showed no significant differences in richness scores connected to any of the potential risk factors. The Berger-Parker Dominance Index (B-P) ranged from 0.499 (meaning the dominant species accounts for ~49.9% of GIT worm composition) in LH-Woodland Lake to 1.0 in LM-Eagle Lake (meaning that the dominant species accounts for 100% of the GIT worm composition) reflecting the depauperate nature of LMB-GIT helminth community being dominated by one species of acanthocephalan. Overall, *N. cylindratus* was the most dominant species in all LMB examined in this study, being the dominant species in 13 out of the 15 lakes, and was the second most dominant in LH-Nepessing Lake and LM-Duck Lake after *L. thecatus* (Table 2.6).

Statistical analysis of B-P scores showed marginally significant evidence for a watershed effect with *P*-values greater than 0.05 (0.079 at 9 DF). Specifically, the difference between LH and LM watersheds was significant, with LM having higher B-P mean scores ($P<0.0202$). Differences between LE and either of the other two watersheds were not statistically significant. Lakes with no public access tend to have higher values than those with public access; this tendency was, however, marginally significant ($P=0.0656$).

Both Simpson's Index of Diversity (SID) and Shannon-Wiener Index (SWI) used in this study yielded more or less identical results. As displayed in Table 2.6, LMB residing in LM-Eagle Lake had the lowest diversity, being infected with one species only, while LMB residing in LH-Woodland Lake exhibited the highest diversity. ANOVA statistical analyses revealed that the presence of public access leads to significant increases ($P<0.04$ at 9 DF) in SWI ($P<0.0449$ at 9 DF) and SID ($P<0.048$ at 9 DF). Watershed also exerted effects on both diversity indices, which was significant in the case of SWI ($P<0.0441$ at 9 DF) and less significant in the case of SID ($P<0.0528$ at 9 DF). LH watershed exhibited higher diversity than the other two watersheds, with its values being significantly higher than LM for SWI ($P<0.0167$ at 9 DF) and SID ($P<0.0183$ at 9 DF).

3) Similarity among the 15 lakes and three watersheds

The Jaccard's Similarity Index of the 15 lakes varied greatly from 0.14-1.0. Only 18 out of 105 pairwise comparisons were ≥ 0.75 (i.e., $\geq$ 75% similarity between two inland lakes in GIT helminths composition). LM-Eagle Lake exhibited the lowest similarity indices when compared to each of the other 14 lakes. On the other hand, LH-Lake Shupac and LE-Big Lake exhibited a similarity index of 1.0, meaning that they are

100% similar. In general, statistical analysis of the similarity index did not show any significant trends associated with the potential risk factors tested in this study (Table 2.7). When the data was combined within watersheds, inland lakes in LM and LH watersheds shared 88% similarity, while inland lakes in LE watershed shared a 56% and 63% similarity in parasite composition with lakes in LM and LH watersheds, respectively.

4) Correlation between LMB-GIT helminths and odds ratio of infection

Pearson correlation coefficient demonstrated the presence of positive correlation among some of LMB-GIT helminths. As displayed in Table 2.8, when data from inland lakes of the three watersheds was analyzed combined, three positive correlations were determined: namely, *E. salmonis* with *A. parksidei* ($P<0.001$), *P. bulbocolli* with *L. thecatus* ($P<0.001$), and *N. cylindratus* with *Contracaecum* sp. ($P<0.029$). When the same analysis was conducted on inland lakes within each of the watersheds, the positive correlation between *A. parksidei* and *E. salmonis* was obvious in LM ($P<0.004$) and LH ($P<0.00$), but not in LE. The positive correlation between *L. thecatus* and *P. bulbocolli*, however, was determined in LM ($P<0.000$) only, while the positive correlation between *Contracaecum* sp. and *N. cylindratus* was determined in LH only ($P<0.002$). Additionally, a positive correlation between *Contracaecum* sp. and *P. ambloplitis* was evident in LM watershed only ($P<0.000$).

The analysis also demonstrated that watersheds can play a role in the odds ratio of infection by a particular GIT helminth species versus another. For example, the odds ratios of LMB becoming infected with *N. cylindratus* were 34.5 times higher in LE ($P<0.0001$), 14.5 times higher in LH ($P<0.0001$), and 25.6 times higher in LM ($P<0.0001$) watersheds when compared to *Contracaecum* sp. In the same context,

L. micropteri was 4.8 times more likely to be found in LMB in LM watershed than *P. ambloplitis* ($P<0.0218$). In the inland lakes of the other two watersheds, the increased likelihood of infection by *L. micropteri* versus *P. ambloplitis* did not exist. Table 2.9 displays other statistically significant odds ratio comparisons among GIT helminth species and the potential role of the watershed in influencing the odds ratios.

The presence of an inlet to the lake increased the odds ratio of infection of *N. cylindratus* and *Contracaecum* sp. over *L. thecatus* and *P. ambloplitis.* For example, in lakes with inlets, the odds of *N. cylindratus* infecting LMB versus *L. thecatus* rose from 28.6 ($P<0.0001$) to 500 ($P<0.0001$) in favor of *N. cylindratus*. Similarly, in lakes with inlets, the odds of *N. cylindratus* infecting LMB versus *P. ambloplitis* rose from 56.4 ($P<0.0001$) to 161.7 ($P<0.0001$) in favor of *N. cylindratus*. Regarding *Contracaecum* sp., the odds of its infection versus *P. ambloplitis* doubled in lakes with inlets (5.9, $P<0.0014$) as opposed to lakes without inlets (2.9, $P<0.0396$). The odds of *Contracaecum* sp. infecting LMB were 20.8 ($P<0.0001$) times those of *L. thecatus* in lakes with inlets, while the odds ratio of the two species was not significant in lakes without inlets. The same trend was observed between *N. cylindratus* and *Contracaecum* sp., with an odds ratio of 27.8 ($P<0.0014$) in favor of *N. cylindratus* in the presence of an inlet that rose from 19.6 ($P<0.0001$) in lakes without inlets (Table 2.10). On the contrary, the presence of an outlet to the lake seems to have reduced the odds ratio of infection by *N. cylindratus* versus *Contracaecum* sp., *P. ambloplitis*, and *L. thecatus.* In other comparisons, variable results were obtained (Table 2.11). Last, in lakes where public access was permitted, the odds ratio of infection of *N. cylindratus* and *Contracaecum* sp. over *L. thecatus* and *P. ambloplitis* were reduced dramatically (Table 2.12).

DISCUSSION

1) Composition of LMB-GIT helminths

Findings of this study clearly demonstrate the widespread infection of LMB by GIT helminths. Over 75% of the LMB examined harbored at least one helminth species in their GIT, with relatively high intensity (34.72±35.07 worms/fish) and abundance (26.00±35.07 worms/fish). Helminth species forming the LMB-GIT community reported from this study are generalists in nature, as they have been reported in a number of freshwater fish species from North America, including centrarchids (Hoffman 1999). Although nine species of helminths were identified, the overwhelming dominance of *N. cylindratus* and *L. thecatus* left negligible niches to be colonized by other helminth species. The number of LMB-GIT helminth species seems to be relatively low, particularly when compared with other fish species (Kennedy et al. 1997; Zander 2007).

Dominance by a single species is not uncommon in GIT helminth communities of freshwater fish species; however, it is believed that acanthocephalans are the dominant species in cold zones (Kennedy 1993), while trematodes are dominant in warmer areas (Salgado-Maldonado and Kennedy 1997). In the case of LMB, however, acanthocephalans dominate the GIT helminth community not only in cold areas such as Michigan (Muzzall and Gillilland 2004), Wisconsin (Amin 1986), Missouri (Banks and Ashley 2000), and Canada (Steinauer et al. 2006), but also in warmer areas such as Kenya (Aloo 1999), Florida (Bangham 1939), and Texas (Sparks 1951). The high dominance of acanthocephalans in LMB-GIT is probably the result of their ability to survive within their hosts, as well as their use of novel strategies that ensure completion

of their life cycle. For example, *L. thecatus* eggs release filaments of the fibrillar coat upon contact with water, which entangle in filamentous algae, the major food item of the amphipod intermediate host *Hyalella azteca* (Uznanski and Nickol 1976; Barger and Nickol 1998). In the same context, acanthors penetrate the gut wall of amphipod intermediate hosts immediately after they hatch and live and consequently grow in the body cavity making the amphipod more visible to LMB (Taraschewski 2000). While in the body cavity of their intermediate hosts, Cornet et al. (2009) demonstrated that the developing acanthocephalan larvae are capable of suppressing the prophenoloxidase system, a major defense mechanism in the gammarid intermediate hosts, limiting their ability to encapsulate and immobilize the larval helminths. In the final host (e.g., LMB), acanthocephalans strongly attach to the deep layers of the intestinal walls making their physical removal, by repeated peristaltic movements or through connective tissue formation, almost impossible.

Comparing findings of this study to other published reports on LMB-GIT helminths, similarities and differences have been observed. The nine helminth species found in this study were all found in LMB-GIT from other geographical areas (Hoffman 1999), albeit with different infection parameters. For example, while in this study *N. cylindratus* was the overall dominant species with a prevalence of 57.88%, LMB from other North American locales such as Aiken, South Carolina (Eure 1976), Missouri (Banks and Ashley 2000), and Gull Lake, Michigan (Muzzall and Gillilland 2004), had prevalence values of *N. cylindratus* that consistently exceeded 95%. Moreover, *L. thecatus* accounted for 21.9% of the total worm population and 22.8% of the acanthocephalans with a prevalence of 27.3%. This differs from what Muzzall and

Gillilland (2004) reported for *L. thecatus* in LMB from Gull Lake in Michigan, which was 52%. Similarly, *A. parksidei* was present in 4.4% of LMB in this study, which was far lower than what Amin (1975*c*) found in the Pike River, Wisconsin, which had a prevalence of 100%. This acanthocephalan, however, was not found in Gull Lake LMB (Muzzall and Gillilland 2004) or in other surveys performed on LMB-GIT helminths prior to 1975.

Discrepancies among findings of this study and those of previous studies also extended to non-acanthocephalan helminth species. For example, *Contracaecum* sp. was found in 11.23% of LMB examined and was absent in five inland lakes. This is surprising since larval nematodes belonging to this genus are widespread in LMB (Aloo 1999; Szalai and Dick 1990; Banks and Ashley 2000; Landry and Kelso 2000). This discrepancy can be attributed to the fact that the figures of *Contracaecum* sp. prevalence and intensity reported in this study refer only to the nematodes found inside the GIT cavity and not to those in the mesentery. The adult bass tapeworm, *Proteocephalus ambloplitis*, was found in the intestine of 82 out of the 641 LMB examined (12.7%), accounting for 1.5% of the total gastrointestinal worm population. Similar results on the LMB from Gull Lake in Michigan showed low prevalence of *P. ambloplitis* in the intestine (Muzzall and Gillilland 2004). Again, these figures are of adult *P. ambloplitis* found within the GIT, where LMB is a final host, and are much less than figures given in earlier studies which focused on the more widespread visceral infection with the larval *P. ambloplitis*, where LMB also acts as an intermediate host, due to the severe lesions it causes (Eure 1976). In this study, the trematode *Leuceruthrus micropteri* was present occasionally in the stomach of LMB from LM inland lakes and was absent in the other

two watersheds, with an overall prevalence of <1%. On the contrary, Hubert and Warner (1975) observed a high prevalence of the trematode with 36% in LMB-GIT from the Tennessee River. Similarly, Cloutman (1975) found heavy infection with *C. oxycephalus* in LMB caught from Arkansas, while in this study, a single worm of this species was encountered in a single LMB caught from LE-Orion Lake. On the contrary, Banks and Ashley (2000) reported the presence of *Crepidostomum* sp. in LMB-GIT in abundance in Missouri, which was not found in this study.

Factors that determine the presence of a certain helminth in a locale as well as its infection parameters are multiple and include the biological, chemical, and physical components of each waterbody, such as the presence and density of susceptible intermediate and final hosts, the prevailing temperature, and the presence of dominant helminth species (Kennedy 2009). The composition of the fish community in a waterbody is also believed to play a key role in the presence and intensity of a particular helminth. For example, Steinauer et al. (2006) attributed the geographic patterning of *L. thecatus* in LMB to the abundance of *Lepomis* spp. (sunfishes) in the waterbody. In the Lower Mississippi and South Atlantic regions, where *Lepomis* spp. are abundant, *L. thecatus* tends to infect fewer largemouth or smallmouth bass and vice versa. Based solely on findings of this study, it is impossible to attribute the discrepancy in the composition and infection parameters of LMB-GIT helminths to a particular factor(s); however, it is likely that the physical characteristics of each of the inland lakes and watersheds are potential major determinants of the composition of the LMB-GIT helminth community.

Regardless of the factors that led to the significant variations in infection parameters of the nine helminth species found in this study, it is obvious that the nine parasites can be divided into core species (with an abundance of >2) such as *N. cylindratus* and *L. thecatus*, and secondary species (with an abundance of 0.6-2) such as *A. parksidei*, while the remaining six species (with an abundance of <0.6) are rare species. Prevalence values coincide well with this classification except for *P. ambloplitis* and *Contracaecum* sp., whose prevalence values are >11%; however, since their abundance is relatively low, they are considered rare species (Zander et al. 1999). The odd ratios of LMB infection by each parasite is as important to know as its absolute value. Such data was unavailable before this study and can definitely function as a baseline and powerful tool for prediction in future studies on LMB-GIT parasites in the same or similar sampling sites.

Despite the fact that tissue damage to or mortality of LMB by its GIT helminths were not addressed in this study, there are a number of published reports indicating that the nine species detected in this study can cause substantial harm to their hosts. The lesions caused by acanthocephalans, in particular, can be significant, as the spiny proboscis that penetrates deep into the intestinal walls often causes perforation (Aloo 1999). *N. cylindratus* was shown to penetrate deeply into the intestinal wall of infected fish leading to the formation of excessive connective tissue around the proboscis at the expense of the functional intestinal epithelium, thereby affecting the proper function of the intestine (Adel-Meguid et al. 1995). In the GIT of the rainbow darter (*Etheostoma caeruleum*), *P. bulbocolli* was found to initiate severe inflammatory response leading to widespread erosions and deep ulcerations (McDonough and Gleason 1981). *P. bulbocolli*

inserts not only its proboscis in the intestinal wall, but also its bulb and neck, eliciting an intense host response. Moreover, *Camallanus oxycephalus* causes complete destruction of the columnar epithelium with extensive fibrosis in the intestine of another centrarchid, the green sunfish (*Lepomis cyanellus*) (Meguid and Eure 1996). The extent to which these parasites affect LMB populations in Michigan's inland lakes remains to be investigated.

2) Effects of potential risk factors on infection parameters

Throughout the course of this study, it was apparent that the watershed within which an inland lake is located plays an important role on its helminth composition and odds ratio of infection. For example, inland lakes in the LE watershed lacked *P. bulbocolli*, *E. salmonis*, and *L. micropteri,* but LE was the only watershed in which *C. oxycephalus* existed. Similarly, *L. micropteri* existed in the LM watershed only, and not in the LE or LH watersheds. Such discrepancies were also noticed among inland lakes within the same watershed. For example, within the LM watershed, LMB from Lake Randall harbored seven species of GIT helminths, while GIT of LMB caught from Eagle Lake harbored only one species. In the absence of hydrobiological data on the inland lakes of this study, it is extremely difficult to determine the contributing factor(s) in helminth distribution. Inferences from other studies performed on LMB parasites are also difficult to draw, since most of these studies were primarily of descriptive nature and used a relatively small number of fish and sampling localities.

Studies on other freshwater fish species were able to pinpoint certain factors as the driving forces in determining the helminth species existing in a particular waterbody; e.g., other resident fish species, lake size, anthropogenic activities, et cetera. Other

parasitologists suggested that host genetic predisposition is the major driving force for colonization success of a particular parasite in a specific host. This assumption, however, does not explain why the trematode, *L. micropteri*, was present in <1% of LMB in this study, while its prevalence in LMB caught from the Tennessee River reached up to 36% (Hubert and Warner 1975). A more plausible explanation came from the studies of parasites of fishes of the River Danube, which emphasized the role of the invertebrate fauna dominating in sections of the river in determining parasite species in resident fish species (Nachev and Sures 2009). This assumption, however, does not provide explanations on why the watersheds had no influence on any of the infection parameters of the two core species, *N. cylindratus* and *L. thecatus*, while they affected other rare species such as *A. parksidei* and *E. salmonis* (more abundant in lakes within the LH watershed) or *Contracaecum* sp. (more abundant in lakes of the LE watershed), which use the same invertebrate intermediate hosts for their development. Indeed, why certain helminths are present in a distinct habitat but absent in another continues to be a paradoxical dilemma among parasite ecologists.

This study also demonstrated lake connectivity to other waterbodies through an inlet, outlet, or public access can influence certain infection parameters of LMB-GIT helminths. Limitations of this connection seem to be in favor of the core species, while findings regarding rare species were inconclusive, probably due to their limited distribution and smaller numbers. In a pioneering study, Karvonen and Valtonen (2004) demonstrated that hydrobiological ecological factors surpass geographical connection (or separation) in determining the composition of the parasite community. Regardless of the minimal degree of pathology caused by GIT helminths, the role public access may

play in structuring animal communities should be better understood, as it is vital for the development of sound management strategies of inland lakes. Fish sex seems to be the least influential among the potential risk factors examined. That males had higher mean abundance of *L. micropteri* ($P<0.0186$ at 625 DF) than females seems to be independent of the fact that females are significantly heavier than males. In another study performed on LMB parasites, Cloutman (1975) reported that the host sex did not show any significant difference in the intensity of infection or in the diversity of any of the parasites.

3) Community structure: Main characteristics and effects of potential risk factors

In this study, the diversity of the LMB-GIT helminth community in each inland lake was determined not only by species richness, but also with Simpson Index of Diversity (SID) and Shannon-Wiener Index (SWI), both of which take abundance and evenness of the species present into consideration, together with the Berger-Parker Dominance Index (B-P) which measures the proportion occupied by the dominant species. This approach was successful in shedding light on the important characteristics of the GIT helminth community, which was relatively poor in diversity and controlled by the dominant acanthocephalan, *N. cylindratus*.

Subtle differences among the inland lakes regarding their LMB-GIT helminth community structures were observed, yet they should be interpreted not only by their absolute values, but also in context of what each of the four values measured emphasizes. For example, LMB of LE-Orion Lake harbored four species of GIT helminths, yet its community is more diverse (as measured by SID and SWI) when compared to other helminth communities whose species richness is equal (e.g., LH-Budd Lake or LM-

Nichols Lake) or even exceeds (e.g., LM-Ovid Lake and LM-Duck Lake) that of LE-Orion Lake. This is primarily because of the relatively low proportion of the dominant species in LE-Orion Lake (based on their B-P value), which permitted better evenness of the other LMB-GIT helminth species.

As expected, the factors that favor any of *N. cylindratus* infection parameters tend to have positive effects on B-P value, such as the absence of public access. On the contrary, in lakes with public access, both SID and SWI significantly increased. Similarly, LMB residing in lakes within the LH watershed have more diverse GIT helminth communities when compared to lakes in the LM watershed, which is primarily because lakes in the LM watershed have higher B-P values.

Interestingly, when the lakes were ranked based on the values of their SID (from high to low) and B-P (from low to high), the ranks were almost identical; that is, the lower the B-P value, the higher the SID value. This is primarily because calculation of SID amplifies the dominant species by using the squared value of its frequency. Since the square of a frequency <1 is much smaller, rare species contribution to diversity was minimized. On the other hand, SWI calculation weights species exactly by their frequencies, without amplifying the contribution of the dominant species at the expense of the rare species. Therefore, SWI can detect minor differences in helminth diversity. In summary, these results suggest that *N. cylindratus* has shaped the structure of LMB-GIT helminth communities and marginalized the contribution of the rare helminth species to the diversity of their community. This kind of dominance is considered a key factor in determining the similarity and predictability of parasite assemblages (Kennedy and Bush 1994; Choudhury and Dick 1998).

4) Similarity

It is known that, if parasite assemblages of one particular host species are dominated by one parasite species, this species is likely to promote similarity between populations (Kennedy 2009). Despite the obvious dominance of *N. cylindratus* and the low species richness of LMB-GIT helminths, it was surprising to find that only one pair of lakes was 100% similar and only 18 out of 105 pairwise comparisons exhibited ≥75% similarity. Indeed, based on the similarity index values in Eagle Lake, where *N. cylindratus* is the only GIT helminth found, and other lakes, it became obvious that the contribution of *N. cylindratus* to similarity is no more than 33% (range 14-33%). Examining the >75% similar lakes, one could not observe any pattern for their distribution among the three watersheds, close geographic distance, public access, or connection to other waterbodies through inlets or outlets. Factors that determine similarities or variations in parasite community structure among populations of the same host species remain one of the least understood aspects of parasite community ecology (Timi and Poulin 2003). Logically, one would expect that adjacent, interconnected waterbodies should theoretically have identical parasite communities. However, the elegant studies of Karvonen and Valtonen (2004), provided evidence that the combination of biotic and abiotic factors prevalent at the waterbody determines the success of colonization by a particular parasite in a particular waterbody, or a region within that waterbody.

In a series of studies performed in the United Kingdom, it was demonstrated that individual characteristics of lakes lead to stochastic nature of parasite assemblages in fish (Kennedy 1978, 1990; Hartvigsen and Kennedy 1993). Among the lake-related factors

affecting parasite assemblages of freshwater fish are lake size, altitude, trophic status, availability and abundance of intermediate hosts (Wisniewski 1958; Chubb 1970; Esch 1971; Kennedy 1978; Marcogliese and Cone 1991), pollution, and anthropogenic activities (Applegate and Mullan 1967). As mentioned above, these factors, alone or combined, are likely to have led to the qualitative (prevalence) and quantitative (intensity and abundances) variations noticed in LMB-GIT helminths of this study.

5) Correlations among LMB-GIT helminths

One of the factors that may have led to the qualitative and quantitative variations among lakes in GIT helminth communities is the presence of association or competition among the helminth species. Cloutman (1975), who studied LMB parasites, suggested that one parasite species might influence the abundance of another, which affects community structure. Similarly, Durborow et al. (1988) demonstrated the presence of an inverse relationship between *Neoechinorhynchus* sp. and *P. ambloplitis* in LMB collected from southern USA. Data of the present study did not support these findings, which may be attributed to the different hydrobiological factors between the study sites in the different studies.

In this study, significant positive correlations were found among three pairs of LMB-GIT helminths: *N. cylindratus* and *Contracaecum* sp.; *L. thecatus* and *P. bulbocolli;* and *A. parksidei* and *E. salmonis*. It was obvious that watersheds play an important role in determining the correlations between the worms and odds of infection. For example, the correlation between *L. thecatus* and *P. bulbocolli* was found in the LM watershed, yet was strong enough to continue to be significant when the data of the 15 lakes were analyzed together. Similar trends were observed with the correlation between

A. parksidei and *E. salmonis* (significant in both LH and LM but not LE) and *N. cylindratus* and *Contracaecum* sp. (significant in LH only). On the contrary, the correlation between *Contracaecum* sp. and *P. ambloplitis*, which was significant in the LM watershed only, became insignificant when analyzed with the data from the lakes of the other two watersheds. This data clearly suggests that the watershed has a strong effect on LMB-GIT community structure.

Further support for this assumption came from the odds ratio of infection data, which clearly demonstrated the watershed effects not only on the rare species, but also on the core species. For example, while *N. cylindratus* has strong odds ratio advantage of infecting LMB against *L. micropteri* in both LM and LE watersheds, it loses this advantage in the LH watershed. As mentioned earlier, the clear effects of watershed can be attributed to a number of hydrobiological factors pertaining to the waterbodies in this study and host ecological traits (e.g., density, diet, body size) (Poulin 1997), many of which were not addressed in this study. Among these, the presence and abundance of either the invertebrate intermediate host or the final host (e.g., birds for *Contracaecum* sp.) and the ability of more than one helminth species to share the same host for life cycle completion, seem to be the most plausible explanations for the correlation and odds ratio variations. Unfortunately, hydrobiological data on Michigan's inland lakes included in this study do not exist.

Data presented in tables 2.10 through 2.12 demonstrate that connection to other waterbodies and public access influence the probability of infection of LMB-GIT helminths against each other, *N. cylindratus* and *Contracaecum* sp. in particular. It is worth mentioning that the odds ratio values should not be confused with the prevalence

and intensity data, based on absolute numbers of each of the worm species and not as an odds ratio to another species. The fact that the presence or absence of inlets or outlets can influence the infection odds ratios versus another worm species sheds light on the dynamic nature of the host-parasite relationship and requires further research into factors affecting the recruitment and longevity of invertebrate intermediate hosts in a given waterbody. Without this knowledge, it will be hard to interpret why *N. cylindratus* has better chances of infecting LMB in the presence of inlets or the absence of outlets versus *Contracaecum* sp., *L. thecatus*, or *P. ambloplitis*. In the same context, when in lakes with no public access, the dominant species *N. cylindratus* and *Contracaecum* sp. gained advantages to infect resident LMB over *L. thecatus* or *P. ambloplitis*. This suggests that anthropogenic activities (e.g., recreational fishing) significantly affect the intricacies among biotic components of the waterbody. Again, the scientific explanation to this finding requires additional research on the types of parasites that bait carry and their ability to colonize the new environment. This information is currently not available.

In conclusion, the data generated in this study is of importance to fishery managers as it deals with one of the most popular sportfish in the state of Michigan. Although the effects of several important risk factors on infection parameters were analyzed, it is important to recognize that numerous other biotic and abiotic factors found important in other studies might also be operating in the present system. The presented data represents the most comprehensive parasitological study ever conducted on LMB gastrointestinal tract worms in the Great Lakes basin. The inland lakes from which LMB samples were collected were never examined previously for GIT worms. Therefore, most of these findings should be considered as new geographical range

extensions for the nine parasite species.

6) Declaration of new geographic range for the following helminths:

a. *Neoechinorhynchus cylindratus* Van Cleave, 1913

***Prevalence*:** Varied from 18.18-96.77 (average 57.88%)

***Site of infection*:** Intestine

Type host: Largemouth bass

***Other reported hosts*:** *Ambloplites* spp., *Amblyopsis* spp., *Amia* spp., *Anguilla* spp., *Carpiodes* spp., *Catostomus* spp., *Chaenobryttus* spp., *Coregonus* spp., *Erimyzon* spp., *Esox* spp., *Etheostoma* spp., *Fundulus* spp., *Gambusia* spp., *Ictalurus* spp., *Lepomis* spp., *Lota* spp., *Micropterus* spp., *Morone* spp., *Moxostoma* spp., *Notemigonus* spp., *Notropis* spp., *Perca* spp., *Petromyzon* spp., *Pornoxis* spp., *Richardsonius* spp., *Salvelinus* spp., and *Stizostedion* spp.

***New location(s) based on the present study*:** The following inland lakes in Michigan's Lower Peninsula: Lakes Randall, Eagle, Jordan, Ovid, Nichols, Duck, Ruppert, Woodland, Nepessing, Shupac, Budd, Pine, Orion, Independence and Big.

Other reported localities in North America: The US states of Alabama, Arkansas, Florida, Georgia, Kansas, Kentucky, Massachusetts, Maine, Michigan, Minnesota, New York, Ohio, Oklahoma, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Virginia, Wisconsin and Wyoming. It was also reported from the Canadian province of Ontario.

Representative publications: Bangham 1926; Eure 1976; Cloutman 1975; Fischer and Kelso 1990; Banks and Ashley 2000; Muzzall and Gillilland 2004.

***Specimen deposited*:** Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1364

b. *Leptorhynchoides thecatus* Linton, 1891

***Prevalence*:** Ranged from 0-91.73 (average 27.3%)

***Site of infection*:** Pyloric caeca and intestine

***Type host*:** Largemouth bass

***Other reported hosts*:** *Ambloplites* spp., *Amia* spp., *Anguilla* spp., *Aplodinotus* spp., *Carpiodes* spp., *Catostomus* spp., *Coregonus* spp., *Cottus* spp., *Culaea* spp., *Cyprinus* spp., *Enneacanthus* spp., *Esox* spp., *Etheostoma* spp., *Fundulus* spp., *Hiodon* spp., *Hybopsis* spp., *Ictalurus* spp., *Ictiobus* spp., *Lepisosteus* spp., *Lepomis* spp., *Lota* spp., *Microgadus* spp., *Micropterus* spp., *Minytrema* spp., *Morone* spp., *Moxostoma* spp., *Nocomis* spp., *Notropis* spp., *Oncorhynchus* spp., *Osmerus* spp., *Perca* spp., *Percina* spp., *Percopsis* spp., *Pomoxis* spp., *Pungitius* spp., *Rhinichthys* spp., *Salmo* spp., *Salvelinus* spp., *Semotilus* spp., *Stizostedion* spp., and *Umbra* spp.

***New location(s) based on the present study*:** Lakes Randall, Jordan, Duck, Woodland, Ruppert, Nepessing, Budd, Pine, Orion, and Independence, all in Michigan's Lower Peninsula.

Other reported localities in North America: US records from Alabama, Florida, Georgia, Illinois, Kansas, Louisiana, Massachusetts, Maine, Michigan,

Minnesota, North Dakota, New York, Ohio, Pennsylvania, Tennessee, Texas, Washington, and Wisconsin. It was also reported in the Canadian province of Ontario.

Representative publications: Bangham 1926; Howard and Aliff 1980; Amin 1988; Muzzall and Gillilland 2004.

***Specimen deposited*:** Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1363

c. ***Pomphorhynchus bulbocolli* Van Cleave, 1919**

***Prevalence*:** 0.31% (range 0-2.86)

***Site of infection*:** Pyloric caeca

Type host: Largemouth bass

***Other reported hosts*:** larvae were reported from *Ambloplites* spp., *Catostomus* spp., *Cottus* spp., *Etheostoma* spp., *Ictalurus* spp., *Micropterus* spp., *Notropis* spp., *Osmeras* spp., *Perca* spp., *Percina* spp., *Percopsis* spp.

***New location(s) based on the present study*:** Lake Nepessing and Duck Lake, both in Michigan's Lower Peninsula.

***Other reported localities*:** US records from Georgia, Illinois, Kansas, Kentucky, Louisiana, Maine, Michigan, Montana, North Dakota, New Hampshire, New York, Ohio, Tennessee, Texas, Utah, Washington, Wisconsin, and Wyoming. Canadian records from British Columbia and Saskatchewan.

Representative publications: Fischer and Kelso 1990; Szalai and Dick 1990; Banks and Ashley 2000; Muzzall and Gillilland 2004.

***Specimen deposited*:** Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1366

Comments: All *P. bulbocolli* found in the present study were immature larvae.

d. *Acanthocephalus parksidei* Amin, 1975

***Prevalence*:** 4.37% (range from 0-31.15)

***Site of infection*:** Intestine

***Type host*:** Largemouth bass

***Other reported hosts*:** *Aplodinotus grunniens, Ictalurus punctatus, Lepomis macrochirus, Micropterus salmoides, Catostomus commersoni, Ictalurus melas, Lepomis cyanellus, L. macrochirus, M. salmoides, Notemigonus crysoleucas, Oncorhynchus mykiss, Phnephales promelas, Semotilus atromaculatus, S. margarita.*

***New location(s) based on the present study*:** Lakes Randall, Jordan, Ovid, Woodland, and Independence, located in Michigan's Lower Peninsula.

Other reported localities: Pike River in Wisconsin.

Representative publications: Amin 1975*c*

***Specimen deposited*:** Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1362

Comments: Hoffman (1999) considered this acanthocephalan synonymous with *A. dirus*. Despite the heterogeneity in dimensions, worms of this study fitted the description detailed in Amin (1975*c*) and *A. parksidei* and differed from those of *A. dirus*.

e. ***Echinorhynchus salmonis*** **Müller, 1784**

Prevalence**:** 1.25% (range 0-9.84)

Site of infection**:** Intestine

Type host: Largemouth bass

Other reported hosts**:** *Osmerus mordax, Oncorhynchus kisutch, 0. tshawytscha, O. gorbuscha, O. mykiss, S. namaycush, Petromyzon marinus, Acipenser fulvescens, Ambloplites rupestris ,Catostomus catostomus, C. commersoni, Couesius plumbeus, Coregonus* spp., *Lepomis gibbosus, Lota lota, Micropterus dolomieu, M. salmoides, Notropis hudsonius, Perca flavescens, Percopsis omiscomaycus, Stizostedion canadense* ,and *Trigonopsis thompsoni.*

New location(s) based on the present study**:** Lakes Randall and Woodland, located in Michigan's Lower Peninsula.

Other reported localities: Europe, Ontario, Canada, Lake Huron, Michigan and Wisconsin, USA.

Representative publications: McLain 1951; Applegate 1950; Bangham 1955; Tedla and Fernando 1969; Amin 1981, 1985*b*; Muzzall and Peebles 1986, 1988; Arai 1989; Hoffman 1999; Muzzall and Bowen 2000.

Specimen deposited**:** Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1365

f. ***Proteocephalus ambloplitis*** **Leidy, 1887**

Prevalence**:** 12.79% (range from 0-59.26)

Site of infection**:** Intestine

Type host: Largemouth bass

***Other reported hosts*:** *Micropterus dolomieu, M. salmoides*, and *Amia calva.*

***New location(s) based on the present study*:** Lakes Randall, Ovid, Nichols, Duck, Woodland, Nepessing, Shupac, Budd, Pine, Orion, Independence and Big, located within Michigan's Lower Peninsula.

Other reported localities: The adult has been reported from Connecticut, Kansas, Louisiana, Massachusetts, Maine, Gull Lake in Michigan's Lower Peninsula, Minneapolis, New Hampshire, South Dakota, Tennessee, Washington, Wisconsin, and West Virginia. Adults were also reported from British Columbia, Canada.

Representative publications: Sogandares-Bernal 1955; Gillilland and Muzzall 2004.

***Specimen deposited*:** Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1367

Comments: Only adult *P. ambloplitis*, attached to the intestinal walls and exhibiting fully developed gonads in their strobilas, were considered in this study.

g. *Contracaecum* sp.: Unidentified member (s) of the Genus *Contracaecum* Railliet and Henry 1915

Prevalence: 11.23% (range from 0-51.85%)

Site of infection: Intestine

Type of host: Largemouth bass

Other reported host: The majority of wild freshwater fish species (some are listed in Hoffman 1999).

New location(s) based on the present study: Lakes Woodland, Shupac, Pine, Independence, Big, Randall, Ovid, Duck, Nichols, and Ruppert, located within Michigan's Lower Peninsula.

Other reported localities: Cosmopolitan in their distribution. In LMB in North America reports are available from Saskatchewan and Gull Lake, Michigan.

Specimen deposited: Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1368

Representative publications: Szalai and Dick 1990; Gillilland and Muzzall 2004.

Comments: These immature nematodes were impossible to identify to the species level.

h. *Camallanus oxycephalus* Ward and Magath, 1917

***Prevalence*:** 0.16% (only one specimen in a single lake)

***Site of infection*:** The hind portion of the intestine

Type host: Largemouth bass

***Other reported hosts*:** *Alosa* spp., *Ambloplites* spp., *Amia* spp., *Ammocrypta* spp., *Anguilla* spp., *Aplodinotus* spp., *Carpiodes* spp., *Chaenobryttus* spp., *Cottus* spp., *Culaea* spp., *Ericymba* spp., *Esox* spp., *Etheostoma* spp., *Hadrepterus* spp., *Hiodon* spp., *Ictalurus* spp., *Labidesthes* spp., *Lepomis* spp., *Micropterus* spp., *Minytrema* spp., *Morone* spp., *Moxostoma* spp., *Notropis* spp., *Noturus* spp., *Perca* spp., *Percina* spp., *Polyodon* spp.,

Pomoxis spp., *Pylodictis* spp., *Rheocrytpa* spp., *Rhinichthys* spp., *Semotilus* spp., and *Stizostedion* spp.

***New location(s) based on the present study*:** Lake Orion, located in Michigan's Lower Peninsula.

***Other reported localities*:** In North America: Georgia, Texas, Wisconsin, Ohio, Arkansas, Colorado, Louisiana, Missouri, Pennsylvania, North Dakota, Kansas, Kentucky, Montana, Massachusetts, and New York.

***Representative publications*:** Steinauer and Font 2003; Banks and Ashley 2000; Aliff et al. 1977; Baker and Crites 1976; Bangham and Venard 1942; Cloutman 1975; Deutsch 1977; Forstie and Holloway 1984; Gash and Gash 1973.

***Specimen deposited*:** Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1369

i. ***Leuceruthrus micropteri* Marshall and Gilbert, 1905**

Prevalence: 0.94% (range 0-5.45%)

Site of infection: Stomach

Type host: Largemouth bass

***Other reported hosts*:** *Lepomis macrochirus, L. megalotis, Micropterus dolomieu,* and *Amia calva.*

***New location(s) based on the present study*:** Lakes Randall, Ovid, Nichols and Ruppert, located in Michigan's Lower Peninsula.

Other reported localities: in North America: Wisconsin, Lake Erie, Arkansas, Minnesota, and Tennessee.

Representative publications: Hubert and Warner 1975; Aliff 1977; Bangham 1939; Becker 1978; Becker et al. 1966.

Specimen deposited: Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1370

Lake	County	Latitude	Longitude	Nature	Area (km^2)	Public Access	Presence of Inlet	Presence of Outlet	Maximum Depth (m)
Lake Huron watershed									
Woodland	Livingston	42° 33' 19" N	83° 47' 02" W	Natural with dam	1.17	Yes	Yes	Yes	10.67
Nepessing	Lapeer	43° 01' 03" N	83° 22' 18" W	Natural	1.68	Yes	No	Yes	7.62
Shupac	Crawford	44° 49' 18" N	84° 28' 34" W	Natural	0.43	Yes	No	No	29.57
Pine	Genesee	42° 47' 45" N	83° 46' 06" W	Natural	0.52	No	No	No	7.92
Budd	Clare	44° 01' 13" N	84° 47' 39" W	Natural	0.71	Yes	No	No	9.14
Lake Erie watershed									
Orion	Oakland	42° 46' 56" N	83° 15' 01" W	Natural with dam	1.90	Yes	Yes	Yes	17.68
Independence	Washtenaw	42° 24' 21" N	83° 48' 10" W	Natural	0.78	Yes	Yes	Yes	10.36
Big	Oakland	42° 43' 22" N	83° 31' 11" W	Natural with dam	0.87	Yes	No	Yes	4.27
Lake Michigan watershed									
Randall	Branch	41° 58' 24" N	85° 01' 53" W	Natural	0.89	Yes	Yes	Yes	10.67
Eagle	Van Buren	42° 10' 12" N	85° 58' 32" W	Natural	0.80	Yes	No	No	19.81
Jordan	Barry	42° 46' 12" N	85° 08' 27" W	Natural with dam	1.74	Yes	Yes	Yes	17.68
Ovid	Clinton	42° 56' 42" N	84° 25' 10" W	Reservoir	1.67	No	Yes	Yes	6.10
Duck	Calhoun	42° 23' 09" N	84° 47' 09" W	Natural	2.55	Yes	No	Yes	15.24
Nichols	Newaygo	43° 43' 35" N	85° 54' 22" W	Natural	0.64	Yes	No	No	17.37
Ruppert	Kalamazoo	42° 24' 27" N	85° 44' 01" W	Natural	0.11	Yes	Yes	Yes	8.84

Table 2.1. Inland lakes in Michigan's Lower Peninsula from which largemouth bass (*Micropterus salmoides*) were obtained for this study. Largemouth bass were collected from July 2002 to September 2005. Information on each of the lakes was obtained from the Michigan Department of Natural Resources and Environment.

Lake	Number of Fish Sampled			Length (cm) $\bar{x}$ ± SD	Weight (g) $\bar{x}$ ± SD
	Female	Male	Total		
Lake Huron watershed					
Woodland	37	24	61	30.91 ± 4.53	417.26 ± 186.45
Nepessing	16	11	27	33.26 ± 7.29	573.01 ± 439.79
Shupac	30	28	58	24.81 ± 5.35	222.42 ± 127.58
Pine	18	20	38	29.05 ± 8.50	448.19 ± 270.42
Budd	32	24	56	31.65 ± 3.64	455.10 ± 164.62
	133	107	240	29.58 ± 6.37	401.42 ± 251.79
Lake Erie watershed					
Orion	38	41	79	30.37 ± 5.39	392.28 ± 266.90
Independence	13	13	26	32.17 ± 5.72	457.68 ± 286.49
Big	17	10	27	30.34 ± 6.34	375.44 ± 212.31
	68	64	132	30.72 ± 5.66	401.72 ± 260.46
Lake Michigan watershed					
Randall	34	7	41	29.11 ± 2.98	313.20 ± 102.38
Eagle	18	15	33	25.89 ± 2.65	194.48 ± 54.22
Jordan	12	18	30	32.23 ± 4.10	499.24 ± 183.08
Ovid	23	21	44	30.94 ± 4.27	412.92 ± 209.89
Duck	18	17	35	29.53 ± 3.67	329.77 ± 117.21
Nichols	38	17	55	29.48 ± 5.89	389.11 ± 214.82
Ruppert	24	7	31	20.21 ± 3.99	161.04 ± 40.92
	167	102	269	28.47 ± 5.39	335.84 ± 185.65
Total	368	273	641	29.35 ± 5.88	373.96 ± 230.32

Table 2.2. Sex, length, and weight of largemouth bass (*Micropterus salmoides*) collected from 15 inland lakes in Michigan's Lower Peninsula from July 2002 to September 2005.

Lake	Acanthocephalans					Nematodes		Cestodes	Trematodes	GIT Worm Prevalence
	Neoechino-rhynchus cylindratus	*Leptorhy-nchoides thecatus*	*Acantho-cephalus parksidei*	*Echinor-hynchus salmonis*	*Pomphor-hynchus bulbocolli*	*Contra-caecum* sp.	*Camallanus oxycephalus*	*Proteo-cephalus ambloplitis*	*Leucer-uthrus micropteri*	
Lake Huron Watershed										
Woodland	50.82	45.90	31.15	9.84	0	16.39	0	9.84	0	93.44
Nepessing	66.67	88.89	0	0	0	0	0	7.41	0	100
Shupac	32.76	0	0	0	0	1.72	0	31.03	0	51.72
Pine	94.74	2.63	0	0	0	50.00	0	5.26	0	97.37
Budd	30.36	1.79	0	0	1.79	0	0	3.57	0	33.93
Prevalence	50.42	22.50	7.92	2.50	0.42	12.50	0	12.50	0	70.83
Lake Erie Watershed										
Orion	65.82	48.10	0	0	0	0	1.27	3.80	0	83.54
Independence	42.31	26.92	11.54	0	0	3.85	0	3.85	0	53.85
Big	96.30	0	0	0	0	51.85	0	59.26	0	100
Prevalence	67.42	34.09	2.27	0	0	11.36	0.76	15.15	0	81.06
Lake Michigan Watershed										
Randall	58.54	63.41	9.76	4.88	0	2.44	0	12.20	2.44	82.93
Eagle	18.18	0	0	0	0	0	0	0	0	18.18
Jordan	50.00	36.67	3.33	0	0	0	0	3.33	0	76.67
Ovid	68.18	0	2.27	0	0	11.36	0	9.09	2.27	72.72
Duck	37.14	91.43	0	0	2.86	20.00	0	28.57	0	94.29
Nichols	78.18	0	0	0	0	3.64	0	7.27	5.45	81.82
Ruppert	96.77	22.58	0	0	0	38.71	0	25.81	3.23	100
Prevalence	59.85	28.25	2.23	0.74	0.37	10.04	0	11.90	2.23	75.84
Total										
Total Fish Prevalence	57.88	27.30	4.37	1.25	0.31	11.23	0.16	12.79	0.94	75.04

Table 2.3. Prevalence of largemouth bass (*Micropterus salmoides*) gastrointestinal tract (GIT) helminths. Largemouth bass were collected from 15 inland lakes in Michigan's Lower Peninsula from July 2002 to September 2005.

Lake	Acanthocephalans					Nematodes		Cestodes	Trematode	Mean Intensity
	Neoechino-rhynchus cylindratus	*Leptorhy-nchoides thecatus*	*Acantho-cephalus parksidei*	*Echinor-hynchus salmonis*	*Pomphor-hynchus bulbocolli*	*Contra-caecum* sp.	*Camallanus oxycephalus*	*Proteo-cephalus ambloplitis*	*Leucer-uthrus micropteri*	
Lake Huron Watershed										
Woodland	31.06±24.78	14.68±12.80	24.68±20.29	5.50±1.97	0	2.60±1.24	0	4.67±2.59	0	33.86±31.49
Nepessing	19.72±12.40	36.00±41.77	0	0	0	0	0	3.50±0.94	0	45.41±38.60
Shupac	8.42±5.87	0	0	0	0	1.00±0.13	0	2.89±1.86	0	7.10±6.22
Pine	49.58±37.86	50.00±8.11	0	0	0	3.26±2.91	0	3.50±0.80	0	51.46±37.70
Budd	26.29±22.02	19.00±2.54	0	0	3.00±0.40	0	0	2.50±0.55	0	24.95±22.16
Mean Intensity ± SD	30.66±26.89	24.89±18.40	24.68±10.70	5.50±1.02	3.00±0.19	2.97±1.43	0	3.30±1.69	0	33.81±32.94
Lake Erie Watershed										
Orion	16.38±11.82	9.74±12.88	0	0	0	0	1.00±0.20	5.00±1.00	0	18.76±16.46
Independ-ence	19.55±18.96	16.71±12.56	2.67±0.93	0	0	1.00±0.20	0	2.00±0.39	0	24.50±22.90
Big	65.50±63.72	0	0	0	0	11.29±15.08	0	2.63±2.10	0	70.48±63.85
Mean Intensity ± SD	31.12±37.65	10.82±11.51	2.67±0.42	0	0	10.60 ±7.86	1.00±0.20	2.95±1.35	0	32.56±39.69
Lake Michigan Watershed										
Randall	27.13±22.05	11.88±12.64	2.75±1.00	14.50±4.08	0	1.00±0.16	0	1.80±0.65	3.00±0.47	29.79±27.85
Eagle	9.00±4.36	0	0	0	0	0	0	0	0	9.00±4.36
Jordan	9.07 ±6.95	3.55±2.91	4.00±0.73	0	0	0	0	3.00±0.55	0	7.91±7.21
Ovid	58.73±41.54	0	3.00±0.45	0	0	1.80±0.79	0	2.25±0.82	1.00±0.15	55.75±41.87
Duck	11.38±9.26	44.03±41.63	0	0	19.00±3.21	4.86±2.88	0	3.50±3.12	0	49.85±47.35
Nichols	31.81±24.77	0	0	0	0	2.00±0.42	0	1.50±0.46	18.33±5.13	31.84±26.06
Ruppert	40.93±33.38	9.71±6.70	0	0	0	2.42±1.55	0	3.75±2.64	3.00±0.54	43.81±33.13
Mean Intensity ± SD	33.21±28.90	24.01±20.61	3.00±0.50	14.50±1.60	19.00±1.16	2.85±1.27	0	2.88±1.55	10.33±2.35	36.61±34.54
Total										
Mean Intensity ± SD	31.88±30.26	20.89±18.24	17.68±6.62	7.75±1.21	11.00±0.76	4.51±3.52	1.00±0.12	3.05±1.57	10.33±1.53	34.72±35.07

Table 2.4. Intensity of largemouth bass (*Micropterus salmoides*) gastrointestinal tract helminths. Largemouth bass were collected from 15 inland lakes in Michigan's Lower Peninsula from July 2002 to September 2005.

Lake	Acanthocephalans					Nematodes		Cestodes	Trematodes	Total
	Neoechino-rhynchus cylindratus	*Leptorhyn-choides thecatus*	*Acantho-cephalus parksidei*	*Echino-rhynchus salmonis*	*Pompho-rhynchus bulbo-colli*	*Contra-caecum* sp.	*Camal-lanus oxyceph-alus*	*Proteo-cephalus amblo-plitis*	*Leucer-uthrus micropteri*	
Lake Huron Watershed										
Woodland	15.79±24.78	6.74±12.80	7.69±20.29	0.54±1.97	0	0.43±1.24	0	0.46±2.59	0	31.64±31.49
Nepessing	13.15±12.40	32.00±41.77	0	0	0	0	0	0.26±0.94	0	45.41±38.60
Shupac	2.76±5.87	0	0	0	0	0.02±0.13	0	0.90±1.86	0	3.67±6.22
Pine	46.97±37.86	1.32±8.11	0	0	0	1.63±2.91	0	0.18±0.80	0	50.11±37.70
Budd	7.98±22.02	0.34±2.54	0	0	0.05±0.40	0	0	0.09±0.55	0	8.46±22.16
Mean Abundance ± SD	15.46±26.89	5.60±18.40	1.95±10.70	0.14±1.02	0.01±0.19	0.37±1.43	0	0.41±1.69	0	23.95±32.94
Lake Erie Watershed										
Orion	10.78±11.82	4.68±12.88	0	0	0	0	0.01±0.20	0.19±1.00	0	15.67±16.46
Independence	8.27±18.96	4.50±12.56	0.31±0.93	0	0	0.04±0.20	0	0.08±0.39	0	13.19±22.90
Big	63.07±63.72	0	0	0	0	5.85±15.08	0	1.56±2.10	0	70.48±63.85
Mean Abundance ± SD	20.98±37.65	3.69±11.51	0.06±0.42	0	0	1.20±7.86	0.008±0.20	0.45±1.35	0	26.39±39.69
Lake Michigan Watershed										
Randall	15.88±22.05	7.54±12.64	0.27±1.00	0.71±4.08	0	0.02±0.16	0	0.22±0.65	0.07±0.47	24.71±27.85
Eagle	1.64±4.36	0	0	0	0	0	0	0	0	1.64±4.36
Jordan	4.53±6.95	1.30±2.91	0.13±0.73	0	0	0	0	0.10±0.55	0	6.07±7.21
Ovid	40.05±41.54	0	0.07±0.45	0	0	0.20±0.79	0	0.20±0.82	0.02±0.15	40.55±41.87
Duck	4.23±9.26	40.26±41.63	0	0	0.54±3.21	0.97±2.88	0	1.00±3.12	0	47.00±47.35
Nichols	24.87±24.77	0	0	0	0	0.07±0.42	0	0.11±0.46	1.00±5.13	26.05±26.06
Ruppert	39.61±33.38	2.19±6.70	0	0	0	0.94±1.55	0	0.97±2.64	0.10±0.54	43.81±33.13
Mean Abundance ± SD	19.88±28.90	6.78±20.61	0.07±0.50	0.11±1.60	0.07±1.16	0.29±1.27	0	0.34±1.55	0.23±2.35	27.77±34.54
Total										
Mean Abundance ± SD	18.45±30.26	5.70±18.24	0.77±6.62	0.10±1.21	0.03±0.76	0.51±3.52	0.002±0.12	0.39±1.57	0.10±1.53	26.05±35.07

Table 2.5. Abundance of largemouth bass (*Micropterus salmoides*) gastrointestinal tract helminths. Largemouth bass were collected from 15 inland lakes in Michigan's Lower Peninsula from July 2002 to September 2005.

Sample Category / Lake	Simpson's Index of Diversity (1 – D)	Shannon-Wiener Index	Richness	Berger-Parker Dominance Index (*d*)	Dominant Species
Lake Huron Watershed					
Woodland	0.646	1.209	6	0.499	*N. cylindratus*
Nepessing	0.420	0.635	3	0.705	*L. thecatus*
Shupac	0.378	0.584	3	0.751	*N. cylindratus*
Pine	0.119	0.288	4	0.938	*N. cylindratus*
Budd	0.109	0.264	4	0.943	*N. cylindratus*
Total	0.521	0.995	7	0.646	*N. cylindratus*
Lake Erie Watershed					
Orion	0.437	0.677	4	0.688	*N. cylindratus*
Independence	0.492	0.794	5	0.627	*N. cylindratus*
Big	0.192	0.390	3	0.895	*N. cylindratus*
Total	0.346	0.684	6	0.795	*N. cylindratus*
Lake Michigan Watershed					
Randall	0.493	0.863	7	0.643	*N. cylindratus*
Eagle	0.000	0.000	1	1.000	*N. cylindratus*
Jordan	0.397	0.699	4	0.747	*N. cylindratus*
Ovid	0.024	0.081	5	0.988	*N. cylindratus*
Duck	0.257	0.563	5	0.857	*L. thecatus*
Nichols	0.087	0.209	4	0.955	*N. cylindratus*
Ruppert	0.179	0.421	5	0.904	*N. cylindratus*
Total	0.427	0.776	8	0.716	*N. cylindratus*
All Total	0.449	0.872	9	0.708	*N. cylindratus*

Table 2.6. Community structure measurements of the gastrointestinal tract helminths in largemouth bass (*Micropterus salmoides*) collected from 15 inland lakes in Michigan's Lower Peninsula from July 2002 to September 2005.

	LH-Woodland	LH-Nepessing	LH-Shupac	LH-Pine	LH-Budd	LE-Orion	LE-Indep-endence	LE-Big	LM-Randall	LM-Eagle	LM-Jordan	LM-Ovid	LM-Duck	LM-Nichols
LH-Nepessing	0.5													
LH-Shupac	0.5	0.5												
LH-Pine	0.67	**0.75**	**0.75**											
LH-Budd	0.43	**0.75**	0.4	0.6										
LE-Orion	0.43	**0.75**	0.4	0.6	0.6									
LE-Indep-endence	**0.83**	0.6	0.6	**0.8**	0.5	0.5								
LE-Big	0.5	0.5	**1**	**0.75**	0.4	0.4	0.6							
LM-Randall	**0.86**	0.43	0.43	0.57	0.38	0.38	0.71	0.43						
LM-Eagle	0.17	0.33	0.33	0.25	0.25	0.25	0.2	0.33	0.14					
LM-Jordan	0.67	**0.75**	0.4	0.6	0.6	0.6	**0.8**	0.4	0.57	0.25				
LM-Ovid	0.57	0.33	0.6	0.5	0.29	0.29	0.67	0.6	0.71	0.2	0.5			
LM-Duck	0.57	0.6	0.6	**0.8**	**0.8**	0.5	0.67	0.6	0.5	0.2	0.5	0.43		
LM-Nichols	0.43	0.4	**0.75**	0.6	0.33	0.33	0.5	**0.75**	0.57	0.25	0.33	**0.8**	0.5	
LM-Ruppert	0.57	0.6	0.6	**0.8**	0.5	0.5	0.67	0.6	0.71	0.2	0.5	0.67	0.67	**0.8**

Table 2.7. Comparison among 15 inland lakes in Michigan's Lower Peninsula in terms of their similarity of largemouth bass (*Micropterus salmoides*) gastrointestinal tract helminths. Largemouth bass were collected from July 2002 to September 2005. Data is presented as Jaccard Similarity Index based on prevalence data with a value of 1 meaning identical parasite composition and 0 meaning no similarity. Letters in bold indicate similarity of >75% between two lakes. (LH=Lake Huron watershed, LE=Lake Erie watershed, LM=Lake Michigan watershed)

Species	*Neoechino-rhynchus cylindratus*	*Leptorhynchoides thecatus*	*Acanthocephalus parksidei*	*Echinorhynchus salmonis*	*Pomphorhynchus bulbocolli*	*Contracaecum* sp.	*Camallanus oxycephalus*	*Proteocephalus ambloplitis*
Leptorhynchoides thecatus	-0.344 **0.209**							
Acanthocephalus parksidei	0.070 **0.804**	0.113 **0.689**						
Echinorhynchus salmonis	0.018 **0.949**	0.120 **0.669**	0.744 **0.001****					
Pomphorhynchus bulbocolli	-0.299 **0.279**	0.776 **0.001**** *	-0.088 **0.756**	-0.121 **0.668**				
caecum sp.	0.562 **0.029***	-0.064 **0.820**	0.021 **0.940**	-0.071 **0.801**	0.059 **0.835**			
Camallanus oxycephalus	0.027 **0.923**	0.087 **0.758**	-0.076 **0.789**	-0.105 **0.711**	-0.083 **0.770**	-0.143 **0.611**		
Proteocephalus ambloplitis	0.085 **0.764**	0.185 **0.509**	0.182 **0.517**	0.060 **0.831**	0.278 **0.316**	0.479 **0.071**	-0.028 **0.921**	
Leuceruthrus micropteri	0.266 **0.337**	-0.176 **0.530**	-0.084 **0.765**	-0.078 **0.781**	-0.094 **0.740**	-0.124 **0.660**	-0.081 **0.774**	-0.178 **0.525**

*Statistically significant correlation (P<0.05), ** Statistically significant correlation (P<0.01).

Table 2.8. Pearson's correlation coefficients (r) values with the corresponding *P-values* used to evaluate possible relationship among largemouth bass (*Micropterus salmoides*) gastrointestinal tract helminths combined. Largemouth bass were collected from 15 inland lakes in Michigan's Lower Peninsula from July 2002 to September 2005.

	LE	LH	LM
Neoechinorhynchus cylindratus vs. *Contracaecum* sp.	34.5 (P <0.0001)	14.5 (P <0.0001)	25.6 (P <0.0001)
Neoechinorhynchus cylindratus vs. *Proteocephalus ambloplitis*	118 (P <0.0001)	55 (P <0.0001)	134 (P <0.0001)
Neoechinorhynchus cylindratus vs. *Leuceruthrus micropteri*	24.4 (P <0.0038)	-	27.8 (P <0.0001)
Neoechinorhynchus cylindratus vs. *Leptorhynchoides thecatus*	200 (P<0.0001)	62.5 (P <0.0001)	143 (P <0.0001)
Contracaecum sp. vs. *Leptorhynchoides thecatus*	6.4 (P<0.0115)	4.5 (P<0.014)	5.9 (P<0.0088)
Contracaecum sp. vs. *Proteocephalus ambloplitis*	3.4 (P <0.0386)	3.8 (P <0.0072)	5.3 (P <0.0009)
Leuceruthrus micropteri vs. *Leptorhynchoides thecatus*	-	-	5.3 (P <0.0274)
Leuceruthrus micropteri vs. *Proteocephalus ambloplitis*	-	-	4.8 (P <0.0218).

*Odd ratio not significant

Table 2.9. Significant effects of the watershed on odd ratios of prevalence by individual gastrointestinal tract helminth species versus each other. Largemouth bass (*Micropterus salmoides*) were collected from 15 inland lakes in Michigan's Lower Peninsula from July 2002 to September 2005. Ratios not included in this table were not significant. (LE=Lake Erie watershed, LH=Lake Huron watershed, LM=Lake Michigan watershed)

	Inlet	
	Absent	**Present**
Neoechinorhynchus cylindratus vs. *Contracaecum* sp.	19.6 (P<0.0001)	27.8 (P<0.0001)
Neoechinorhynchus cylindratus vs. *Proteocephalus ambloplitis*	56.4 (P<0.0001)	161.7 (P<0.0001)
Neoechinorhynchus cylindratus vs. *Leptorhynchoides thecatus*	28.6 (P<0.0001)	500.0 (P<0.0001)
Contracaecum sp. vs. *Leptorhynchoides thecatus*	- *	20.8 (P<0.0001)
Contracaecum sp. vs. *Proteocephalus ambloplitis*	2.9 (P<0.0396)	5.9 (P<0.0014)

*Odd ratio not significant

Table 2.10. Significant effects of the presence/absence of an inlet to the inland lake on the odd ratios of infection of largemouth bass (*Micropterus salmoides*) by gastrointestinal tract helminths compared to each other. Largemouth bass were collected from 15 inland lakes in Michigan's Lower Peninsula from July 2002 to September 2005. Ratios not included in this table were not significant.

	Outlet	
	Absent	**Present**
Neoechinorhynchus cylindratus vs. *Contracaecum* sp.	52.6 (P<0.0001),	10.3 (P<0.0001)
Neoechinorhynchus cylindratus vs. *Proteocephalus ambloplitis*	122.8 (P<0.0001)	74.3 (P<0.0001)
Neoechinorhynchus cylindratus vs. *Leptorhynchoides thecatus*	1000 (*P*<0.0001)	3.9 (P<0.0305)
Contracaecum sp. vs. *Leptorhynchoides thecatus*	80.2 (P<0.0001),	-
Leptorhynchoides thecatus vs. *Proteocephalus ambloplitis*	34.5 (P<0.0005)	18.9 (P<0.0002)
Contracaecum sp. vs. *Proteocephalus ambloplitis*	-	7.2 (*P*<0.0004)

*Odd ratio not significant

Table 2.11. Significant effects of the presence/absence of an outlet to the inland lake on the odd ratios of infection of largemouth bass (*Micropterus salmoides*) by gastrointestinal tract helminths compared to each other. Largemouth bass were collected from 15 inland lakes in Michigan's Lower Peninsula from July 2002 to September 2005. Ratios not included in this table were not significant.

	Public Access	
	Not Permitted	**Permitted**
Neoechinorhynchus cylindratus vs. *Contracaecum* sp.	21.8 (P <0.0001)	25.0 (P <0.0001)
Neoechinorhynchus cylindratus vs. *Proteocephalus ambloplitis*	949 (P <0.0001)	9.6 (P <0.0001)
Neoechinorhynchus cylindratus vs. *Leptorhynchoides thecatus*	1000 (P<0.0001)	18.9 (P <0.0001)
Contracaecum sp. vs. *Leptorhynchoides thecatus*	40.4 (P<0.0113)	-
Contracaecum sp. vs. *Proteocephalus ambloplitis*	43.8 (P <0.0001)	0.38 (P <0.0017)

*Odd ratio not significant

Table 2.12. Significant effects of permitting the public to access the inland lake on the odd ratios of infection of largemouth bass (*Micropterus salmoides*) by gastrointestinal tract helminths compared to each other. Largemouth bass were collected from 15 inland lakes in Michigan's Lower Peninsula from July 2002 to September 2005. Ratios not included in this table were not significant.

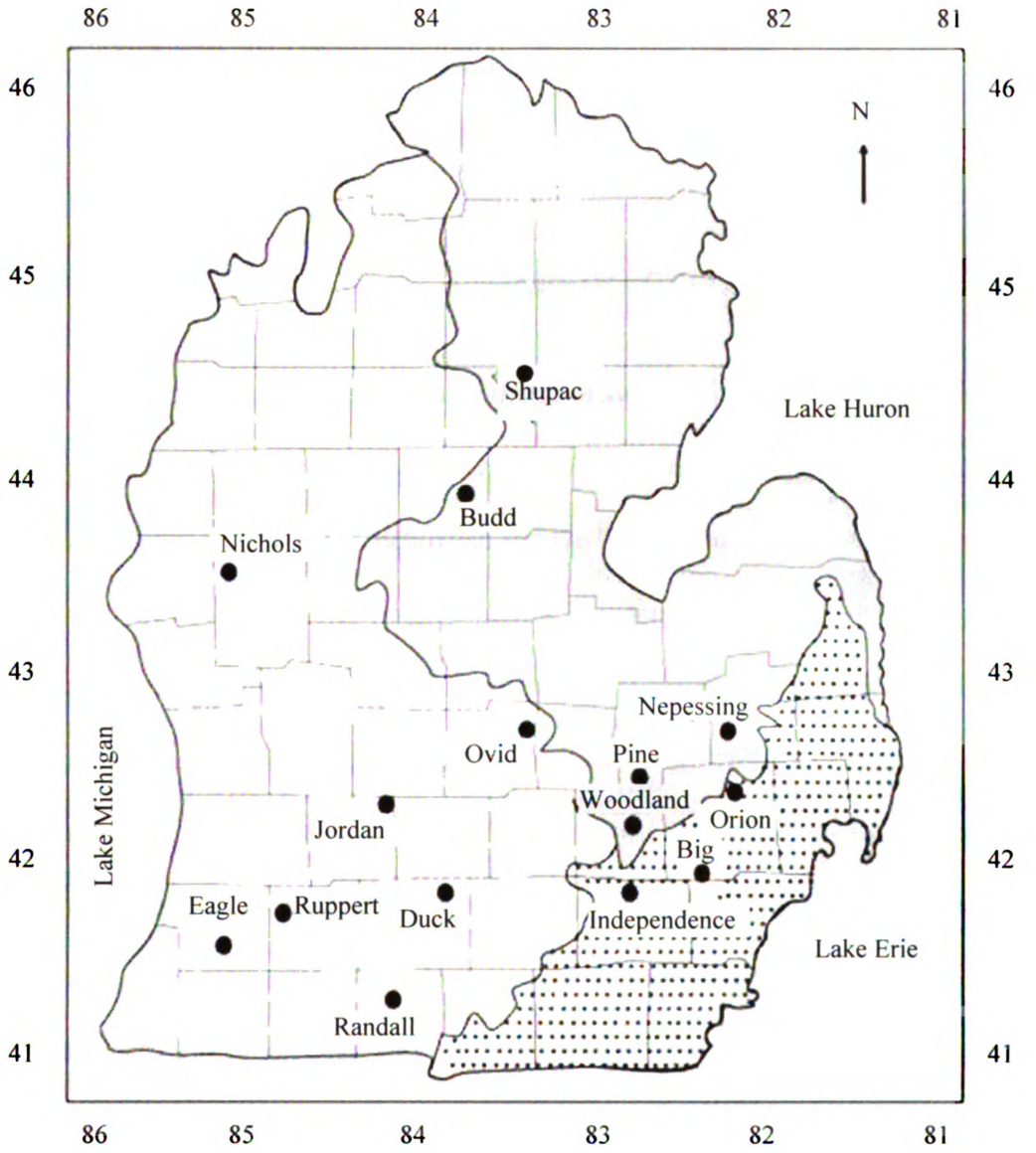


Figure 2.1. Inland lakes (filled black circles) in Michigan's Lower Peninsula from which largemouth bass (*Micropterus salmoides*) samples were obtained. Inland lakes were selected randomly to represent Lake Huron (dark gray), Lake Erie (dotted), and Lake Michigan (light gray) watersheds. Latitude and longitude of each of the lakes are listed in Table 2.1. Borders of Michigan counties are displayed in the background, and lake names given by the counties are written next to the lake location.

CHAPTER 3

WIDESPREAD INFECTION OF LAKE WHITEFISH (*COREGONUS CLUPEAFORMIS)* WITH THE SWIMBLADDER NEMATODE *CYSTIDICOLA FARIONIS* IN NORTHERN LAKES MICHIGAN AND HURON, USA

ABSTRACT

Prevalence, intensity, and abundance of swimbladder nematode infection were estimated in 1272 lake whitefish (*Coregonus clupeaformis*; LWF) collected from four sites in northern lakes Huron (near Cheboygan and De Tour Village ports) and Michigan (near Big Bay de Noc and Naubinway ports) from fall 2003 through summer 2006. Morphological examination of nematode egg, larval, and mature stages through light and scanning electron microscopy revealed characteristics consistent with that of *Cystidicola farionis* Fischer 1798. Total *C. farionis* prevalence was 26.94%, while the mean intensity and abundance of infection was 26.72 and 7.21 nematodes/fish, respectively. Although *C. farionis* was detected in all four stocks that were examined, Lake Huron stocks generally had higher prevalence, intensity, and abundance of infection than Lake Michigan stocks. A distinct seasonal fluctuation in prevalence, abundance, and intensity of *C. farionis* was observed, which does not coincide with reported *C. farionis* development in other fish species. LWF that were heavily infected with *C. farionis* were found to have thickened swimbladder walls with deteriorated mucosa lining, which could affect swimbladder function. Whether *C. farionis* infection may be negatively impacting LWF stocks in the Great Lakes is unclear; continued monitoring of *C. farionis* infection should be conducted to measure responses of LWF stocks to infection levels.

INTRODUCTION

Nematodes of the genus *Cystidicola* Fischer 1798 (Habronematoidea: Cysticolidae) are parasitic in the swimbladders of physostomous fishes in the Northern Hemisphere. Presently, two species of *Cystidicola* are recognized: *C. farionis* and *C. stigmatura*. *C. farionis* Fischer 1798 parasitizes the swimbladders of rainbow smelt (*Osmerus mordax*) and *Coregonus*, *Oncorhynchus*, and *Salvelinus* spp. from Eurasia and North America, while *C. stigmatura* Leidy 1886 parasitizes *Salvelinus* spp. from North America (Black 1983*b*). Through their physical movements and production of toxic metabolites, *Cystidicola* spp. cause destruction of the highly vascularized swimbladder walls, which can affect swimming performance and buoyancy control of infected fish (Lankester and Smith 1980; Black 1984; Willers et al. 1991; Dzeikonska-Rynko et al. 2003). The infection rate of *Cystidicola* spp. in fish populations depends on several factors, including fish age, parasite and intermediate host abundance, and water temperature (Knudsen et al. 2002, 2004).

Lake whitefish (*Coregonus clupeaformis*; LWF) in North America have been found to be susceptible to infection with *C. farionis* (Lankester and Smith 1980). However, maturation of *C. farionis* in LWF in North America is regarded as atypical, which has led some researchers to theorize that it is a new *Cystidicola* sp. infecting LWF and not *C. farionis* (Lankester and Smith 1980; Dextrase 1987). In Lake Nipigon, Canada, LWF have been found to be commonly infected with large numbers of immature *C. farionis*, but no mature nematodes have been found in infected individuals

(Lankester and Smith 1980). Immature nematodes are also common in Lake Superior LWF, but only small numbers of mature nematodes have been found (Lankester and Smith 1980). Buccal cavity structure and eggs from *C. farionis* found in LWF exhibit morphological characteristics that are slightly different when compared to eggs collected from other susceptible species in the same environment (Dextrase 1987; Miscampbell et al. 2004). Despite these atypical characteristics, extensive genetic studies using samples from both Canada and Finland have failed to find sequence differences in ribosomal DNA among mature *C. farionis* nematodes in LWF and other susceptible species, which has led researchers to conclude that it is indeed *C. farionis* that is infecting LWF (Miscampbell et al. 2004).

In the Laurentian Great Lakes of North America, LWF is a commercially, ecologically, and culturally important species (Fleischer 1992; Ebener et al. 2008). The invasion and spread of zebra (*Dreissena polymorpha*) and quagga mussels (*D. bugensis*) in the Great Lakes have been associated with significant declines in LWF condition, growth, and recruitment (Hoyle et al. 1999; Pothoven et al. 2001; Mohr and Ebener 2005). These declines are believed to have been caused primarily from declines in indigenous benthic macroinvertebrates, *Diporeia* spp. in particular, as a result of dreissenid invasion in the Great Lakes (Pothoven et al. 2001; Mills et al. 2005; Nalepa et al. 2005*a*). The absence of *Diporeia* spp. in large areas of the Great Lakes has resulted in LWF increasing consumption of other benthic macroinvertebrates, including dreissenid mussels, gastropods, opossum shrimp (*Mysis relicta*), ostracods, oligochaetes, and zooplankton (Hoyle et al. 1999; Pothoven et al. 2001; Hoyle 2005; Pothoven 2005). Because benthic macroinvertebrates are known to be immediate hosts for *C. farionis*, a

question has been raised whether changes in feeding habits of LWF may have led to changes in the extent of swimbladder nematode infection or exacerbated its pathologic impacts.

The objectives of this study were to: (1) identify the species of swimbladder nematodes in LWF collected from four LWF stocks in northern lakes Huron and Michigan; (2) measure the prevalence, abundance, and intensity of the swimbladder nematodes in these stocks; (3) evaluate variations in larval stage development and maturation among the stocks; and (4) assess the damage to LWF swimbladders caused by the nematode infection.

MATERIALS AND METHODS

1) Study area and fish sampling

In order to better manage LWF stocks, the State of Michigan's Department of Natural Resources and Environment (MDNRE), the five tribes of the Chippewa/Ottawa Resource Authority (CORA), and the United States Department of Interior's U.S. Fish and Wildlife Service divided Michigan waters into LWF management units and negotiated an agreement (Consent Decree) to resolve issues of allocation, management, and regulation of fishing in 1836 Treaty waters of lakes Superior, Michigan, and Huron (U.S. v. Michigan 2000). In general, there are eight LWF units in each of lakes Superior, Huron, and Michigan. In this study, LWF were collected from four management units; two in Lake Huron (WFH-01 and WFH-02) and two in Lake Michigan (WFM-01 and WFM-02) (Figure 3.1). CORA has had exclusive fishing rights for the four units since 1985. WFH-01 lies in the northwest section of Lake Huron and is relatively shallow (<150 ft deep). There are several reproductively isolated stocks of LWF in this unit, one of which is located near Cheboygan, Michigan. WFH-02 is located along the northern shore of Lake Huron, with water deeper than 150 ft. Due to its irregular shoreline; the unit is heavily inhabited by spawning stocks of LWF. In Lake Michigan, WFM-01 is located in 1836 Treaty waters of northern Green Bay and includes two large bays (Big and Little Bays de Noc), several embayments, and a number of islands. The Big Bay de Noc is relatively shallow with depths ranging from 30-70 ft and is considered an important area for LWF reproduction and as a nursery ground for larvae and fry. WFM-03 is also located in northern Lake Michigan, east of WFM-01. WFM-03 is shallow (<90

ft deep) and is inhabited by large spawning aggregations of LWF. For simplicity, the four LWF stocks included in the study will be referred to by the names of their closest fishing ports to which they were brought after catching: Big Bay de Noc (BBN), Naubinway (NAB), Cheboygan (CHB), and De Tour Village (DET). As described above, each of these areas has large spawning aggregations of LWF, and although < 50 kilometers separates some of these locations, individuals have been found to display strong fidelity to these areas during the spawning season (Ebener and Copes 1985; Ebener et al. 2010).

Collection of LWF from each of the stocks began in fall 2003, and continued seasonally through summer 2006. For the purpose of studies described in Chapters 3 and 4 of this dissertation, fall is considered to encompass the months of October through December, winter encompasses the months of January through March, spring encompasses the months of April through June, and summer encompasses the months of July through September. Additionally, for the purpose of this study, fall 2003 through summer 2004 is identified as the 2004 sampling year; fall 2004 through summer 2005 is identified as the 2005 sampling year; and fall 2005 through summer 2006 is identified as the 2006 sampling year. Because of inclement weather conditions, no LWF were collected from the CHB stock in fall 2006 or from the NAB stock in winter 2004. Total numbers of LWF collected and examined for swimbladder nematodes during each sampling period ranged from 15-35 fish/stock (Table 3.1). Sampling locations were typically chosen by contract fishermen based on prior commercial catches. LWF were collected using a combination of commercial traps and gill nets.

2) Fish examination

Captured LWF were transferred (alive or recently dead and shipped on ice) to the Michigan State University Aquatic Animal Health Laboratory in East Lansing, Michigan for immediate processing. Once at the laboratory, live fish were sacrificed with an overdose (300 mg/liter) of tricaine methanesulfonate (MS-222, Argent Laboratories, Redmond, Washington). Before processing, LWF were thoroughly examined for external lesions, then measured (to 0.1 cm), weighed (to 0.1 g) and sexed. The swimbladder from each LWF was removed intact, dissected, and swimbladder walls examined for the presence of macroscopic lesions. In this study, a total of 1272 LWF were analyzed.

3) Parasite identification and swimbladder pathology

Swimbladder nematodes were retrieved manually and preserved in 70% ethanol for later identification and enumeration. Nematodes were cleared in a mixture of glycerol and 70% ethanol (1:1) at room temperature and examined microscopically. Mature and larval-stage nematodes were identified using the dichotomous keys of Ko and Anderson (1969), Smith and Lankester (1979), Black and Lankester (1980), Lankester and Smith (1980), Black (1983*b*), Dextrase (1987), Hoffman (1999), and Miscampbell et al. (2004). Total numbers of nematodes, as well as maturation stage and sex of mature nematodes, were recorded for each LWF.

4) Scanning electron microscopy

Scanning electron microscopy (SEM) was used to confirm the light microscopical identification of the species within the genus *Cystidicola* as recommended by Dextrase (1987) and Miscampbell et al. (2004). Eggs were extruded from gravid females and their morphology examined as described by Dextrase (1987). Briefly, the mid-sections of

female nematodes were excised, covered with a drop of glycerin, and then mounted with a cover slip with gentle pressure to permit the extrusion of eggs from uteri. Extruded eggs as well as the anterior portion of the mature nematodes (including the lips) were dehydrated through an ethanol gradient (35–95%), followed by three washes of 100% ethanol, critical point-dried with carbon dioxide, gold coated, and then examined.

5) Histopathology

To evaluate the damage to swimbladders that may have been caused by nematode infection, swimbladders were examined both visually and histologically. Swimbladders from LWF were fixed in 10% buffered formalin, dehydrated, and paraffin-embedded. The embedded tissues were then sectioned (5 μm thick) and stained with haematoxylin and eosin as described by Prophet et al. (1992) and examined microscopically.

6) Data analysis

The prevalence, abundance, and intensity, as defined by Bush et al. (1997), of *Cystidicola* spp. were calculated for each stock. Prevalence was the percent of LWF infected with *Cystidicola* spp. Abundance was the number of *Cystidicola* spp. found in a LWF regardless of whether the particular fish was infected or not (zero counts possible). Intensity was the number of *Cystidicola* spp. nematodes found in infected LWF (zero counts not possible). Generalized estimating equations (GEEs) were used to test whether prevalence, abundance, and intensity of *Cystidicola* spp. infection differed among stocks, seasons, and years. Given that the LWF for this study were collected with commercial fishing nets, it was felt that it was likely that measurements within sampling occasion would be correlated. As a result, fish that were collected together were considered to have an exchangeable correlation structure, meaning that the correlations among individuals

within a sampling event were the same for all individuals. Because of the very low infection rate of the NAB stock (see Results below), this stock was excluded from the testing of infection parameters as it was obviously different from the others and the low level of variability in infection parameters caused problems when fitting the GEEs. A binomial error structure was assumed to test differences in *Cystidicola* spp. prevalence. To test differences in *Cystidicola* spp. abundance, a negative binomial error structure was assumed. To test differences in *Cystidicola* spp. intensity, intensity counts were first log-transformed and a normal error structure for the GEE was then assumed. First-order interactions were included between stocks, seasons, and years in the GEEs to determine if there were significant interactions among the variables. Differences in prevalence, abundance, and intensity by stocks, years, seasons, or the first-order interactions between these factors were assessed using pairwise comparisons of least squares means. Because of the potentially large number of comparisons to be performed, a Bonferroni adjustment to control the pairwise test error rate was used.

For infected LWF, differences in maturation of *Cystidicola* spp. between stocks were tested by modeling the maturation probabilities for the stocks through multinomial logistic regression and then comparing the odds ratios of being in one maturation stage versus a reference maturation stage between the stocks (Agresti 2007). The maturation stages were modeled through multinomial logistic regression rather than a cumulative logit model because the data did not meet the proportional odds assumption required for the cumulative logit model (Agresti 2007). The earliest development stage was used as the reference category, thus the calculated odds were that of a LWF being infected with a later development stage compared to the earliest development stage. Differences in

development and maturation levels of *Cystidicola* spp. were chosen to be tested in this manner as it was simpler than conducting additional multi-factor tests on individual maturation stages and because it was felt that such tests would be redundant with the tests conducted on overall prevalence, abundance, and intensity. A similar test was used to determine whether the stocks differed in the sex ratios of *Cystidicola* spp. in LWF infected with adult nematodes.

Because swimbladder function may be an important factor affecting foraging of LWF, the condition factor (K) of both infected and non-infected LWF was calculated. Condition factor was calculated by dividing a fish's weight (in grams) by the cube of its length (in millimeters) and multiplying the resulting quotient by 100,000 (Anderson and Neumann 1996). It was then evaluated whether prevalence, abundance, and intensity of *Cystidicola* spp. infection affected LWF condition after accounting for the effects of stock, year, and season of sampling by calculating the residuals from the GEEs described above and then using simple linear regression to relate the residuals to K. All analyses were conducted in SAS using the GENMOD or GLM procedures. In most cases, a statistical test with $P < 0.05$ was regarded as statistically significant. The one exception to this was when testing first-order interactions between stock, year, and season of *Cystidicola* spp. infection characteristics in which case a $P<0.10$ was considered statistically significant. This exception was made because of the concern that even weak variable interactions could mask comparisons of main factor levels.

RESULTS

1) Morphological examination of swimbladder nematodes of LWF

Nematodes were found in the swimbladders of LWF collected from each of the four stocks. Total number of infected fish collected during a sampling period ranged from 1 to 16, 0 to 3, 0 to 30, and 0 to 27, for the BBN, NAB, CHB, and DET stocks, respectively (Table 3.1). Total number of nematodes recovered from fish for each site ranged from 13 for the NAB stock to more than 6100 for the CHB stock.

Most nematodes were found free within the swimbladder cavity, although a few were attached to the bladder walls. Based on morphology, nematodes were identified as larval and adult stages of *Cystidicola* spp. Additional light and scanning electron microscopy on extruded eggs and mouth parts of collected individuals indicated the presence of polar and lateral filaments on eggs (Figure 3.2) and the absence of a lip projection in the pseudolabia (Fig. 3.3). Based on these characteristics, swimbladder nematodes were identified as *C. farionis* Fischer 1798.

Total *C. farionis* prevalence in LWF was 26.78% (SE=1.24%). Overall, *C. farionis* prevalence in the stocks ranged from 2.24% (SE=0.84%) for the NAB stock to 50.00% (SE=2.89%) for the CHB stock. Prevalence in the BBN and DET stocks was 14.68% (SE=1.96%) and 41.44% (SE=2.70%), respectively. Prevalence across all stocks tended to increase during the winter and spring sampling periods (Table 3.1; Figure 3.4). Overall, mean abundance of *C. farionis* was 7.21 (SE=0.78) nematodes. For the individual stocks, mean abundance of *C. farionis* equaled 0.85 (SE=0.20), 0.04 (SE=0.02), 20.51 (SE=3.20), 8.18 (SE=1.24) nematodes/fish for the BBN, NAB, CHB,

and DET stocks, respectively. Abundance generally peaked during the spring sampling period for most of the stocks; the CHB stock in particular had very large increases in *C. farionis* abundance during the spring (Figure 3.4). Of those LWF that were infected with *C. farionis*, mean intensity of the infection was 26.72 (SE=4.63) nematodes/fish, with stock level intensity of infection ranging from 1.86 (SE=1.93) and 5.75 (SE=2.92) nematodes/fish for the NAB and BBN stocks to 19.74 (SE=3.82) and 41.04 (SE=5.41) nematodes/fish for the DET and CHB stocks, respectively. *C. farionis* intensity of infection had greater sampling period variability than did prevalence or abundance; however, for most stocks intensity of infection peaked during the spring sampling period and then declined (Figure 3.4).

When testing differences in *C. farionis* prevalence among stocks (excluding the NAB stock), seasons, and years, the first-order interactions between year and season ($\chi 2$=18.29, df=6, P=0.006), stock and year ($\chi 2$=8.10, df=4, P=0.088), and stock and season ($\chi 2$=10.91, df=6, P=0.091) were found to be statistically significant. Pairwise comparisons of least squares means for the stock-by-year interaction indicated that prevalence in 2004 was significantly lower than in 2005 and 2006 for the CHB and DET stocks, but prevalence in 2004 was greater than in 2006 for the BBN stock (Table 3.2). Additionally, prevalence in most sampling years was lower for the BBN stock than for the CHB and DET stocks; the exception was in 2004 when there was no difference in the BBN and CHB stocks and the prevalence in the BBN stock was significantly greater than in the DET stock (Table 3.2). In 2004, prevalence in the CHB stock was significantly greater than in the DET stock (Table 3.2).

For the stock-by-season interaction, pairwise comparisons of least squares means indicated that for the BBN, CHB, and DET stocks, *C. farionis* prevalence in the spring was significantly greater than in any other season (Table 3.3). The only exception to this was the spring-versus-summer comparison for the BBN stock in which no difference was found. For the CHB and DET stocks, prevalence of *C. farionis* in the fall was significantly less than in summer and winter.

During the fall, prevalence in the BBN stock was significantly greater than in the CHB and DET stocks; however, during all other seasons prevalence in the BBN stock was significantly lower than in the CHB and DET stocks (Table 3.3). Spring prevalence in the CHB stock was significantly greater than in the DET stock (Table 3.3). For the year-by-season interaction, pairwise comparisons of least squares means indicated that during most years, prevalence of *C. farionis* in LWF was significantly greater in the spring than in the other seasons, and that in 2004 and 2006 fall prevalence was significantly lower than in winter or summer (Table 3.4). For individual seasons, whether *C. farionis* prevalence differed among the year of sampling was highly variable (Table 3.4). For example, fall prevalence in 2005 was significantly greater than fall prevalence in 2004 and 2006, but winter prevalence in 2005 was not different than in 2004 or 2006 (Table 3.4). When testing differences in *C. farionis* abundance among stocks (excluding the NAB stock), seasons, and years, the first-order interactions between stock and year ($\chi 2$=11.36, df=4, P=0.023), stock and season ($\chi 2$=11.46, df=6, P=0.075), and year and season ($\chi 2$=17.52, df=6, P=0.008) were found to be significant. Pairwise comparisons of least squares means for the stock-by-year interaction indicated that abundance of *C. farionis* in the BBN stock in 2004 was significantly greater than in 2005 and abundance

in 2005 was significantly greater than in 2006 (Table 3.2). For the CHB and DET stocks, however, abundance in 2005 was significantly greater than in 2004 and 2006 (Table 3.2). In all sampling years, abundance in the BBN stock was significantly lower than in the CHB and DET stocks. In 2005 and 2006, there were no differences in abundance for the CHB and DET stocks, but in 2004, *C. farionis* abundance in the CHB stock was significantly greater than in the DET stock. For the stock-by-season interaction in *C. farionis* abundance, abundance in the spring was greater than in the fall for the BBN, CHB, and DET stocks, and was greater than in the summer for the BBN and CHB stocks and greater than in winter for the BBN and DET stocks (Table 3.3). For both the CHB and DET stocks, abundance in the summer and winter was greater than in the fall. Abundance in summer was greater than in winter for the DET stock (Table 3.3). Although there were no differences in fall abundance of *C. farionis* among the BBN, CHB, and DET stocks, for all other seasons, abundance in the BBN stock was significantly lower than in the CHB and DET stocks (Table 3.3). There were no differences in abundance between the CHB and DET stocks in fall, winter, and summer, but the spring abundance in the CHB stock was significantly greater than in the DET stock (Table 3.3).

Differences in abundance for the year-by-season interaction were similar to those found for prevalence. Abundance was greater in the spring than in other seasons, and abundance in the fall was generally less than in summer or winter (Table 3.4). Additionally, like prevalence, differences in abundance in individual years depended on which seasons were tested. Fall abundance in 2005 was significantly greater than in 2004 and 2006, but winter abundance in 2005 was not different than in 2004 or 2006 (Table

3.4). When testing differences in *C. farionis* intensity among stocks (excluding the NAB stock), seasons, and years, the first-order interactions between stock and year ($\chi 2$=10.00, df=4, P=0.040) and year and season were found significant (Year×Season: $\chi 2$=11.82, df=6, P=0.066). However, the interaction between stock and season was not significant (Year×Season: $\chi 2$=9.22, df=6, P=0.16). As a result, pairwise comparisons of the least squares means for the stock-by-year and year-by-season interactions were conducted, but differences among the levels of the stock-by-season interactions were not tested. Compared to prevalence and abundance, there were fewer differences in intensity among the stock-and-year combinations. For the BBN and DET stocks, there were no differences in intensity of infection between years; however, for the CHB stock intensity of infection was significantly lower in 2006 than in 2005 and 2004 (Table 3.2). For all sampling years, intensity of infection was significantly lower in the BBN stock than in the CHB and DET stocks, but there were no differences in intensity of infection between the CHB and DET stocks (Table 3.2).

For the year-by-season interaction in infection intensity, spring intensity in most years was significantly greater than in other seasons (Table 3.4). Additionally, in 2004 and 2006 summer intensity of infection was significantly greater than in fall and winter. For both fall and spring, intensity of infection in 2004 and 2005 was significantly greater than intensity of infection in 2006 (Table 3.4). Winter infection intensity was also greater in 2005 than in 2006 (Table 3.4).

2) Maturation stages and sex of nematodes in individuals

Larval stages of *C. farionis* were identified according to their morphological criteria. The third larval stage (L_3), which is the infective stage, was identified by its

dumbbell shaped oral opening, pseudolabia, and the prominent tail protrusion (Figure 3.5a). The fourth larval stage (L_4) showed gonadal primordia, with the tail protrusion starting its fusion with the nematode body (Figure 3.5b). Following the fourth and final molt, nematodes exhibited fully formed buccal cavity with circumoral teeth and the tail protrusion disappeared, however, they were sexually immature as no gametes (eggs or spermatozoa) could be seen. This stage is referred to as the sub-adult (SA) stage. Males were recognized by their twisted tail and two speculae that were present throughout their development (Figure 3.5c). Sexually mature males (M) were identified by the presence of spermatozoa in their vas deferens. Mature females (F) were recognized by the presence of shelled eggs filling a considerable portion of their bodies (Figure 3.5d).

In general, LWF from Lake Michigan were infected with a larger fraction of adult *C. farionis* than LWF from Lake Huron (Table 3.2). For the NAB stock, which had the lowest levels of prevalence, abundance, and intensity among the stocks, only adult nematodes were collected from infected LWF. For the BBN stock, which often had significantly lower infection parameters in particular seasons and years than the CHB or DET stocks, 64.5% of collected nematodes were in the adult stage. In comparison, approximately 43% and 45% of collected nematodes from the CHB and DET stocks, respectively, were in the adult stage (Table 3.5). Adult *C. farionis* prevalence, abundance, and intensity of infection exhibited sharp seasonal fluctuations similar to overall prevalence, abundance, and intensity, with peaks generally observed during the spring sampling period.

Sex ratios of *C. farionis* in infected individuals varied considerably by sampling period within the stocks (Table 3.5). Female to male sex ratios ranged from 0 to 3.00 in

the BBN stock, 0.23 to 1.78 in the CHB stock, and 0.82 to 3.22 DET stock. In the NAB stock, female to male ratios ranged from 0.33 to 1.00, with an additional sampling in which only one adult female was collected from all sampled LWF resulting in an undefined sex ratio for that sampling period.

From the multinomial logistic regression model of *C. farionis* maturation stages in infected LWF, it was determined that the odds of LWF from the BBN stock being infected with L_4, SA, and adult developmental stages versus the L_3 stage were 1.7 (95% CI: 1.1–2.7), 2.1 (95% CI: 1.3–3.3), and 4.5 (95% CI: 3.0–6.7) times the odds of fish from the CHB stock, and were 2.0 (95% CI: 1.3–3.1), 3.0 (95% CI: 1.8–4.9), and 2.0 (95% CI: 1.9–4.4) times the odds of fish from the DET stock. Thus, LWF from the BBN stock were more likely to be infected with later developmental stage *C. farionis* than the Lake Huron stocks relative to the L_3 stage. The odds of LWF from CHB stock being infected with L_4, SA, and adult developmental stages versus the L_3 stage were 1.2 (95% CI: 1.0–1.3), 1.4 (95% CI: 1.2–1.7), and 0.6 (95% CI: 0.5–0.7) times the odds of those from the DET stock, meaning that the LWF from the CHB stock were more likely than the DET to be infected with L_4 and SA *C. farionis* but were less likely to be infected with adult nematodes relative to the L_3 stage.

As for sex of *C. farionis*, the odds of LWF from the BBN stock being infected with female versus male *C. farionis* were 1.19 (95% CI: 0.8–1.7) times the odds of fish from the CHB stock, meaning the odds for both stocks were relatively equal. Conversely, the odds of LWF from the BBN stock being infected with female versus male *C. farionis* were 0.6 (95% CI: 0.4–0.9) times the odds of those from the DET stock. The odds of fish

from the CHB stock being infected with female versus male nematodes were 0.7 (95% CI: 0.6–0.9) times the odds of those from the DET stock.

3) Gross and histopathological alterations in LWF swimbladders

Examination of LWF infected and not infected with *C. farionis* revealed the presence of several gross pathological changes due to *C. farionis* infection. Non-infected LWF swimbladders had glistening outer membranes and transparent inner membranes with blood vessels apparent within the membranes (Figure 3.6a). Conversely, in LWF (252 fish) with low to medium infection intensity (1–100 nematodes/fish), nematodes could be visualized through the membrane that appeared opaque and thickened (Figure 3.6b). There were 21 LWF with relatively high infection (>100 nematodes/fish). In terms of gross appearance, nematode-filled swimbladder walls appeared extremely opaque and thickened (Figures 3.6c, d), with the lumen often containing yellowish turbid fluid.

Histologically, healthy LWF swimbladder walls consisted of serosa, tunica fibrosa, tunica muscularis, and epithelial mucosa with a well-developed vascular system supplying blood to the organ. The mucosal layer was folded with the most outer epithelial cells, cuboidal to low-columnar in appearance (Figure 3.7a). In infected LWF, a number of focal lymphocytic and histocytic infiltrates in the subepithelial connective tissue along with erosion of mucosal lining were observed; intensity of infiltrates increased with intensity of infection (Figure 3.7b). Blood vessels in the deep connective tissues were often congested (Figure 3.7c). In heavily infected fish, multifocal lymphocytic and histocytic infiltrates were apparent in the deep connective tissue (Figure 3.7d) with widespread erosion of the mucosal lining. In a number of heavily infected fish, the swimbladder and lumen were filled with nematodes and fibrinous proteinaceous exudate

that contained inflammatory cells (Figure 3.7e). Rarely, the swimbladder tunica fibrosa connective tissue was restructured, appearing like a granulation tissue (Figure 3.7f).

4) Relationship between condition factor and infection parameters

A positive relationship was found between K and GEE residuals for *C. farionis* abundance (Slope=26.46, SE=9.42) and intensity (Slope=47.17, SE=24.04), suggesting that individuals with greater than average *C. farionis* abundance and intensity had larger condition factors than individuals with lower than average abundance and intensity. The test for whether the model coefficient was different from zero was statistically significant for *C. farionis* abundance (t=2.81, P=0.005) but was statistically insignificant, although only marginally so, for *C. farionis* intensity (t=1.96, P=0.051). It is important to note, however, that the total amount of variability explained in the *C. farionis* GEE residuals by K was low (R^2<0.02) for both models.

DISCUSSION

1) Nematode identification

Examination of the morphology of nematodes at both larval and mature stages through light and scanning electron microscopy revealed characteristics consistent with that of *Cystidicola* spp. (Hoffman 1999). The presence of filaments that were either lateral or polar in arrangement on eggs collected from the nematodes suggested that the nematodes collected in this study were *Cystidicola farionis* Fischer 1798, while the absence of lateral lobes on the eggs excluded *C. stigmatura* as the agent of infection (Lankester and Smith 1980; Black 1983*b*; Dextrase 1987; Hoffman 1999; Miscampbell et al. 2004). Additional confirmation of *C. farionis* as the swimbladder nematode infecting LWF in northern lakes Huron and Michigan came from examination of the nematode mouth parts, which showed the absence of a prominent lip projection in the pseudolabia (Figures 3.2 and 3.3).

Based on filament arrangement of eggs collected from LWF nematodes, it appears that the *C. farionis* collected in this study are identical to those collected in LWF from lakes Superior, Huron, and Ontario (Lankester and Smith 1980; Dextrase 1987), but are possibly different from those collected in Finland, Lake Nipigon (Ontario), and British Columbia (Miscampbell et al. 2004). Contrary to previous studies that have found LWF to be an unsuitable host for *C. farionis* maturation, this study clearly found that LWF from lakes Huron and Michigan were able to support the development of *C. farionis* to adult, sexually mature stages. Although *C. farionis* was detected at all four sampling sites, the Lake Huron spawning stocks generally had higher rates of infection than the

Lake Michigan spawning stocks. Despite the fact that *C. farionis* has been previously reported in LWF from other locations within Lake Huron (Lankester and Smith 1980), this is the first study to have found the nematode in LWF from CHB and DET stocks in Lake Huron, or from any site within Lake Michigan, despite prior parasitological examination of LWF from these systems (Amin 1977*b*; Hoffman 1999). Therefore, these findings are considered new geographic range expansion of LWF *C. farionis* in these four sites, as well as the first report in Lake Michigan.

Considering the relatively short distance separating the collection sites, it was somewhat surprising to find significant differences in *C. farionis* infection parameters among the four stocks. Based on differences in the L_3 infective stage, it would appear that both Lake Huron spawning stocks are continuously exposed to *C. farionis*, while the low number of L_3 stage nematodes in LWF from the Lake Michigan sites suggests that recruitment is relatively low and that the expansion of *C. farionis* into LWF in Lake Michigan may be a recent event (Amin 1977*b*).

One explanation for the site-associated differences in *C. farionis* infection parameters that were observed is that feeding habits of LWF differ between the sites. The benthic invertebrate communities of lakes Huron and Michigan have undergone drastic changes since dreissenids first invaded the Great Lakes (Pothoven et al. 2001; McNickle et al. 2006; Nalepa et al. 2007, 2009*a*, 2009*b*). Many areas of the Great Lakes are now devoid of *Diporeia* spp., which has resulted in LWF elevating their consumption of other food items. In Lake Michigan, LWF now primarily consume dreissenid mussels, clams and snails (Pothoven and Madenjian 2008). Conversely, in Lake Huron, there are areas where *Diporeia* spp. are still present, and LWF in Lake Huron have been found to still

consume amphipods, including *Diporeia* spp. (Nalepa et al. 2007, 2009*a*). Amphipods are known intermediate hosts for *C. farionis* that become infected with the nematode by consuming the feces of infected fish containing *C. farionis* eggs (Smith and Lankester 1979; Lankester and Smith 1980).

The seasonal, often dramatic, fluctuations in *C. farionis* infection parameters in LWF within the study area are rather puzzling. While seasonal fluctuation in L_3 abundance can be related to increased abundance of intermediate hosts during the spring and summer months (Watson and Dick 1979; Dextrase 1987; Giæver et al. 1991; Knudsen et al. 2002), the dramatic fluctuations in the numbers of mature nematodes observed in this study are difficult to explain. The experimental study of Black and Lankester (1980), who infected rainbow trout (*Oncorhynchus mykiss*) with L_3 nematodes extracted from infected LWF, is the only recorded account for the development of *Cystidicola* spp. within their final host. Black and Lankester (1980) determined that L_3 stages undergo two moltings and reach sexual maturation in the swimbladder within 4 to 7 months post infection. Once matured, the nematodes live within their final host until the host's death (Black and Lankester 1980, 1981). Although the size of the pneumatic duct in swimbladders of LWF permits the passage of egg and L_3 stage nematodes (Genten et al. 2009), it is too small to permit passage of adult nematodes, which frequently were as large as 30 mm in length and 0.2 to 0.5 mm in width. Consequently, steady or increasing levels of infection in LWF over time like those observed in Europe in Arctic char (*Salvelinus alpinus*) (Amundsen et al. 2003; Knudsen et al. 2004) and broad whitefish (*Coregonus nasus*) (Valtonen and Valtonen 1978) were expected. On the contrary, a dramatic fluctuation in the prevalence, intensity, and abundance of *C. farionis* was

observed, with abundance of mature nematodes often fluctuating from high to low levels within a few months, which does not coincide with *C. farionis* development as presented by Black and Lankester (1980).

One explanation for the seasonal difference in *C. farionis* that was observed is that the *C. farionis* strain infecting LWF has a shorter life span than other strains affecting other fish species. Since the fluctuations, whether increasing or decreasing, involved both larval and adult nematodes and no dead or lysed nematodes were observed in swimbladders of infected LWF, this explanation is believed to be unlikely. A second explanation could be that the heavily infected fish died. This explanation would be in line with the results of Knudsen et al. (2002), who found that Arctic char that were heavily infected with *C. farionis* frequently died during the winter and during spawning when stress levels are high. However, Wagner et al. (2010) did not find a relationship between stock-level estimates of natural mortality and indicators of fish health, including *C. farionis* infection intensity, which is contradictory to this hypothesis. Another explanation for the differences in infection parameters among the sites relates to dispersal and movement differences among the stocks. Analysis of tag-recapture data showed that the stocks were primarily segregated during the spawning season, which lasts from fall to midwinter, but that fish from the four stocks (particularly the DET and CHB stocks) were mixed during the remainder of the year (Ebener et al. 2010). While differences in dispersal and movement may help explain the seasonal fluctuations in infection parameters, it does not necessarily explain the much lower *C. farionis* intensity and abundance in the two Lake Michigan stocks, or the lack of *C. farionis* recruitment in the NAB stock that was observed. Whether the seasonal fluctuation of *C. farionis* in LWF is

due to one or a combination of the above-mentioned explanations requires additional long term monitoring.

Examination of LWF swimbladders as part of this study showed that *C. farionis* induces pathological effects on swimbladders that are commensurate with the degree of infection. With increased infection intensity, swimbladder walls become thickened and lose their transparency, which will affect swimbladder vital functions, including gas exchange. Similar signs were described by Snoj et al. (1986) in brown trout (*Salmo trutta*) heavily infected with *C. farionis.* Most of the histopathological changes in lake LWF swimbladders noticed in this study were in the form of focal to multifocal inflammatory cell infiltration, loss of mucosal folding, as well as mucosal erosion. These lesions are probably due to mechanical irritation by the highly mobile nematodes or the lytic enzymes produced by *C. farionis* (Zółtowska et al. 2001). Similar effects were described by Willers et al. (1991) in ciscoes infected with *C. farionis*.

No studies have been performed to determine if the *C. farionis* induced pathological change can negatively impact its final host at the population level. Extensive studies have been conducted, however, on another swimbladder nematode, *Anguillicola crassus*, that infects the European eel (*Anguilla anguilla*). *A. crassus* caused swimbladder wall thickening that affected the ability of eels to migrate to the Sargasso Sea where they develop and mature, and therefore may be contributing to the worldwide decline of the European eel (Kirk 2003; Kennedy 2007). Using advanced radiolabeling methods, Szekely et al. (2005) followed *A. crassus* infection in captive eels and demonstrated that the swimbladder nematode can cause dramatic deterioration in swimbladder condition of infected eels.

The finding of a weak, yet statistically significant, positive relationship between condition factor and *C. farionis* abundance and intensity in this study is likely attributable to several factors that may be increasing the weight of infected fish, such as the weight of the nematodes, the accumulation of fluid in the swimbladder of infected fish, or an increase in the water content of fish as a result of impaired swimbladder functions. In a parallel study to this one, Wagner et al. (2010) found variations in percent water content among stocks that positively correlated with *C. farionis* intensity of infection, with Lake Michigan stocks (BBN and NAB) having a lower percentage of water content and lower *C. farionis* intensity of infection, and Lake Huron stocks (CHB and DET) having a higher percentage of water content and higher *C. farionis* intensity of infection.

In conclusion, this study sheds light on the spread and potential negative impacts of swimbladder nematodes in LWF stocks in lakes Huron and Michigan, including a range expansion of *C. farionis* to four new geographical locations. Whether *C. farionis* will have a long-term negative impact on LWF stocks in the Great Lakes basin remains unclear, but this study emphasizes the need for continued monitoring and analysis of *C. farionis* infection throughout the lakes and additional studies to determine how infection intensity may affect stock health.

2) Range expansion report of *Cystidicola Farionis* Fisher 1798

Prevalence: 0-100%

Site of infection: Swimbladder

Type host: Lake whitefish

Other reported hosts: *Coregonus* spp., *Salvelinus* spp., *Oncorhynchus* spp., *Salmo* spp., *Osmerus mordax*, *Prosopium cylindraceum*, *P. williamsoni*, *Thymallus articus*.

New location(s) based on the present study: LWF spawning grounds in northern lakes Huron and Michigan served by the fishing ports of Big Bay de Noc and Naubinway in Lake Michigan and Cheboygan and De Tour Village in Lake Huron.

Other reported localities: Several other sites in Lake Huron, Lake Ontario (including inland lakes in its watershed), Lake Superior, and British Colombia, Norway, Green Lake and Sparkling Lake, Wisconsin, Takvatn, northern Norway, and Bothnian Bay.

Representative publications: Valtonen and Valtonen 1978; Muzzall and Peebles 1986; Dextrase 1987; Giæver et al. 1991; Willers et al. 1991; Knudsen et al. 2002; Miscampbell et al. 2004.

Specimen deposited: Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1361

Year	Season	Fish	Infected	Worms	Fish	Infected	Worms
				Lake Michigan			
			Big Bay de Noc			**Naubinway**	
2004	Fall	28	2	7	30	0	0
	Winter	24	2	4	NS	NS	NS
	Spring	30	16	142	30	0	0
	Summer	22	1	3	20	0	0
2005	Fall	26	7	49	30	3	8
	Winter	16	1	1	30	0	0
	Spring	30	6	37	30	0	0
	Summer	30	4	7	30	0	0
2006	Fall	30	1	2	30	3	4
	Winter	30	3	5	23	0	0
	Spring	30	3	15	30	1	1
	Summer	30	2	4	30	0	0
				Lake Huron			
			Cheboygan			**De Tour Village**	
2004	Fall	30	0	0	34	0	0
	Winter	32	6	39	10	1	1
	Spring	20	17	1233	30	11	296
	Summer	30	7	80	20	2	37
2005	Fall	26	3	82	30	9	86
	Winter	15	0	0	30	13	125
	Spring	30	30	2556	30	27	834
	Summer	28	20	935	30	17	280
2006	Fall	NS	NS	NS	30	0	0
	Winter	30	19	233	30	14	77
	Spring	29	27	825	29	20	261
	Summer	30	21	169	30	24	727

Table 3.1. Number of lake whitefish (*Coregonus clupleaformis*) examined (Fish), number of fish infected with *Cystidicola farionis* (Infected), and total number of *C. farionis* found in infected individuals (Worms) collected from four different spawning stocks in Lakes Michigan and Huron from the fall of 2003 to the summer of 2006. The spawning stocks are referenced by the names of their closest fishing ports: Lake Michigan –Big Bay de Noc (BBN) and Naubinway (NAB) and in Lake Huron – Cheboygan (CHB) and De Tour Village (DET). NS = no sampling conducted during that season for that spawning stock.

				Prevalence			Abundance			Intensity		
Stock	Year	Stock	Year	Diff.	$\chi 2$	*P*	Diff.	$\chi 2$	*P*	Diff.	$\chi 2$	*P*
BBN	2004	BBN	2005	0.23	0.6	0.4515	-0.23	1.18	0.2766	0.10	1.03	0.3091
BBN	2004	BBN	2006	0.96	13.8	**0.0002**	0.97	24.36	**<0.0001**	0.17	2.16	0.1414
BBN	2005	BBN	2006	0.72	4.0	0.0467	1.20	26.77	**<0.0001**	0.07	0.32	0.5700
CHB	2004	CHB	2005	-1.84	13.8	**0.0002**	-2.53	70.98	**<0.0001**	-0.40	9.90	0.0017
CHB	2004	CHB	2006	-1.41	20.1	**<0.0001**	-0.52	4.13	0.0421	0.66	40.92	**<0.0001**
CHB	2005	CHB	2006	0.43	0.6	0.4416	2.01	29.36	**<0.0001**	1.06	34.57	**<0.0001**
DET	2004	DET	2005	-3.29	156.4	**<0.0001**	-2.89	153.28	**<0.0001**	-0.47	10.73	0.0011
DET	2004	DET	2006	-2.39	74.7	**<0.0001**	-1.50	30.29	**<0.0001**	0.10	0.16	0.6856
DET	2005	DET	2006	0.90	8.0	0.0046	1.39	21.76	**<0.0001**	0.57	6.86	0.0088
BBN	2004	CHB	2004	-0.13	0.2	0.6930	-1.18	45.95	**<0.0001**	-1.07	152.36	**<0.0001**
BBN	2005	CHB	2005	-2.20	25.9	**<0.0001**	-0.09	0.23	0.6286	-0.74	29.94	**<0.0001**
BBN	2006	CHB	2006	-2.49	76.4	**<0.0001**	-3.47	193.50	**<0.0001**	-1.57	185.73	**<0.0001**
BBN	2004	DET	2004	1.24	27.1	**<0.0001**	-2.75	136.01	**<0.0001**	-1.31	75.48	**<0.0001**
BBN	2005	DET	2005	-2.28	47.1	**<0.0001**	-2.66	150.56	**<0.0001**	-0.58	16.29	**0.0001**
BBN	2006	DET	2006	-2.11	68.8	**<0.0001**	-2.56	130.10	**<0.0001**	-0.82	19.59	**<0.0001**
CHB	2004	DET	2004	1.37	16.9	**<0.0001**	1.09	42.81	**<0.0001**	0.33	7.57	0.0059
CHB	2005	DET	2005	-0.08	0.0	0.8506	0.72	6.63	0.0100	0.26	2.48	0.1151
CHB	2006	DET	2006	0.39	1.4	0.2397	0.11	0.14	0.7105	-0.23	1.56	0.2122

Table 3.2. Pairwise differences in least-squares means for levels of the stock-by-year interaction in *Cystidicola farionis* prevalence, abundance, and intensity of lake whitefish (*Coregonus clupeaformis*) collected from the Big Bay de Noc, Cheboygan, and De Tour Village spawning stocks in lakes Huron and Michigan from the fall of 2003 to the summer of 2006. The difference in the least-squares means (Diff.) and the chi-square test static ($\chi 2$) and *P* for whether the differences are significantly different from 0 are shown. A total of 78 pairwise comparisons were conducted for infection prevalence and abundance; thus statistical significance was based on a Bonferroni corrected Type-I error rate of 0.0006. A total of 48 pairwise comparisons were conducted on infection intensity; thus statistical significance was based on a Bonferroni corrected Type-I error rate of 0.0010. Bold-face *P*s indicate a comparison that was statistically significant.

				Prevalence			Abundance		
Stock	Season	Stock	Season	Diff.	$\chi 2$	*P*	Diff.	$\chi 2$	*P*
BBN	F	BBN	S	-1.38	27.96	**<0.0001**	-1.63	31.51	**<0.0001**
BBN	F	BBN	U	-0.16	0.13	0.7210	0.45	1.87	0.1714
BBN	F	BBN	W	0.09	0.05	0.8220	0.68	4.74	0.0295
BBN	S	BBN	U	1.23	10.74	0.0010	2.09	126.46	**<0.0001**
BBN	S	BBN	W	1.47	22.25	**<0.0001**	2.32	245.14	**<0.0001**
BBN	U	BBN	W	0.24	0.35	0.5562	0.23	1.22	0.2688
CHB	F	CHB	S	-7.08	129.87	**<0.0001**	-6.34	296.85	**<0.0001**
CHB	F	CHB	U	-4.48	100.80	**<0.0001**	-4.50	111.74	**<0.0001**
CHB	F	CHB	W	-3.51	26.84	**<0.0001**	-3.84	65.44	**<0.0001**
CHB	S	CHB	U	2.60	40.02	**<0.0001**	1.84	54.48	**<0.0001**
CHB	S	CHB	W	3.57	54.10	**<0.0001**	2.50	83.26	0.0000
CHB	U	CHB	W	0.97	5.93	0.0149	0.66	4.45	0.0348
DET	F	DET	S	-4.57	187.25	**<0.0001**	-5.08	224.85	**<0.0001**
DET	F	DET	U	-3.47	58.86	**<0.0001**	-4.56	101.21	**<0.0001**
DET	F	DET	W	-2.78	44.96	**<0.0001**	-2.81	49.60	**<0.0001**
DET	S	DET	U	1.10	12.84	**0.0003**	0.52	3.21	0.0734
DET	S	DET	W	1.79	46.27	**<0.0001**	2.27	102.95	**<0.0001**
DET	U	DET	W	0.69	2.73	0.0987	1.75	21.68	**<0.0001**
BBN	F	CHB	F	1.80	20.92	**<0.0001**	1.11	7.88	0.0050
BBN	F	DET	F	1.29	18.72	**<0.0001**	1.19	9.21	0.0024
BBN	S	CHB	S	-3.90	93.32	**<0.0001**	-3.60	727.95	**<0.0001**
BBN	S	DET	S	-1.90	198.63	**<0.0001**	-2.25	580.85	**<0.0001**
BBN	U	CHB	U	-2.53	43.18	**<0.0001**	-3.84	187.23	**<0.0001**
BBN	U	DET	U	-2.02	18.30	**<0.0001**	-3.82	127.22	**<0.0001**
BBN	W	CHB	W	-1.80	24.52	**<0.0001**	-3.41	221.48	**<0.0001**
BBN	W	DET	W	-1.57	14.70	**0.0001**	-2.30	78.18	**<0.0001**
CHB	F	DET	F	-0.50	1.69	0.1939	0.08	0.06	0.8127
CHB	S	DET	S	2.00	29.10	**<0.0001**	1.34	108.75	**<0.0001**
CHB	U	DET	U	0.51	2.04	0.1534	0.02	0.00	0.9604
CHB	W	DET	W	0.22	0.20	0.6566	1.11	9.24	0.0024

Table 3.3. Pairwise differences in least-squares means for levels of the stock-by-season interaction in *Cystidicola farionis* prevalence and abundance of lake whitefish (*Coregonus clupeaformis*) collected from the Big Bay de Noc, Cheboygan, and De Tour Village spawning stocks in lakes Huron and Michigan from the fall of 2003 to the summer of 2006. The difference in the least-squares means (Diff.) and the chi-square test static ($\chi 2$) and *P* for whether the differences are significantly different from 0 are shown. A total of 78 pairwise comparisons were conducted for infection prevalence and abundance; thus statistical significance was based on a Bonferroni corrected Type-I error rate of 0.0006. Bold-face *P*s indicate a comparison that was statistically significant. No results are shown for infection intensity as the interaction between stock and season was not significant for this infection parameter.

				Prevalence			Abundance			Intensity		
Yr	Season	Yr	Season	Diff.	$\chi 2$	*P*	Diff.	$\chi 2$	*P*	Diff.	$\chi 2$	*P*
04	F	04	S	-5.55	293.2	**<0.0001**	-6.10	255.46	**<0.0001**	-1.34	302.08	**<0.0001**
04	F	04	U	-2.90	53.6	**<0.0001**	-3.40	73.72	**<0.0001**	-0.51	11.24	**0.0008**
04	F	04	W	-2.89	61.4	**<0.0001**	-2.41	35.44	**<0.0001**	0.33	4.23	0.0396
04	S	04	U	2.65	65.2	**<0.0001**	2.70	529.50	**<0.0001**	0.83	38.55	**<0.0001**
04	S	04	W	2.66	62.3	**<0.0001**	3.68	466.45	**<0.0001**	1.67	126.67	**<0.0001**
04	U	04	W	0.01	0.0	0.9723	0.99	27.58	**<0.0001**	0.84	21.62	**<0.0001**
05	F	05	S	-2.73	107.2	**<0.0001**	-1.65	91.89	**<0.0001**	-1.15	596.27	**<0.0001**
05	F	05	U	-0.92	8.5	0.0036	-0.45	2.03	0.1545	-0.23	1.16	0.2818
05	F	05	W	0.38	0.5	0.4630	0.55	2.04	0.1536	-0.15	0.69	0.4074
05	S	05	U	1.81	17.3	**<0.0001**	1.20	16.34	**0.0001**	0.92	15.42	**0.0001**
05	S	05	W	3.11	32.4	**<0.0001**	2.20	35.80	**<0.0001**	1.00	26.81	**<0.0001**
05	U	05	W	1.30	4.6	0.0323	1.00	4.54	0.0332	0.09	0.08	0.7841
06	F	06	S	-4.76	50.8	**<0.0001**	-5.30	76.91	**<0.0001**	-1.44	58.09	**<0.0001**
06	F	06	U	-4.29	36.3	**<0.0001**	-4.75	54.26	**<0.0001**	-1.12	31.36	**<0.0001**
06	F	06	W	-3.70	28.3	**<0.0001**	-4.09	44.45	**<0.0001**	-0.47	10.35	0.0013
06	S	06	U	0.47	3.2	0.0723	0.55	4.31	0.0380	0.32	2.24	0.1344
06	S	06	W	1.06	29.6	**<0.0001**	1.21	57.96	**<0.0001**	0.98	46.08	**<0.0001**
06	U	06	W	0.59	3.7	0.0545	0.66	5.43	0.0198	0.65	16.24	**0.0001**
04	F	05	F	-3.65	160.2	**<0.0001**	-4.47	108.75	**<0.0001**	-0.25	11.50	0.0007
04	F	06	F	-0.60	0.8	0.3758	0.21	0.10	0.7569	0.69	31.01	**<0.0001**
05	F	06	F	3.06	20.6	**<0.0001**	4.68	54.78	**<0.0001**	0.94	48.93	**<0.0001**
04	S	05	S	-0.83	8.7	0.0031	-0.02	0.05	0.8196	-0.06	1.68	0.1945
04	S	06	S	0.20	1.2	0.2741	1.01	95.85	**<0.0001**	0.58	19.77	**<0.0001**
05	S	06	S	1.03	18.1	**<0.0001**	1.03	66.34	**<0.0001**	0.65	18.78	**<0.0001**
04	U	05	U	-1.67	21.6	**<0.0001**	-1.52	25.25	**<0.0001**	0.02	0.01	0.9367
04	U	06	U	-1.99	39.9	**<0.0001**	-1.14	17.91	**<0.0001**	0.08	0.13	0.7222
05	U	06	U	-0.32	0.7	0.4198	0.38	0.96	0.3279	0.05	0.03	0.8565
04	W	05	W	-0.38	0.4	0.5507	-1.51	11.49	0.0007	-0.73	7.33	0.0068
04	W	06	W	-1.41	25.6	**<0.0001**	-1.47	51.22	**<0.0001**	-0.11	0.60	0.4393
05	W	06	W	-1.03	3.4	0.0660	0.05	0.01	0.9077	0.62	12.09	**0.0005**

Table 3.4. Pairwise differences in least-squares means for levels of the year-by-season interaction in *Cystidicola farionis* prevalence, abundance, and intensity of lake whitefish (*Coregonus clupeaformis*) collected from the Big Bay de Noc, Cheboygan, and De Tour Village spawning stocks in lakes Huron and Michigan from the fall of 2003 to the summer of 2006. The difference in the least-squares means (Diff.) and the chi-square test static ($\chi 2$) and *P* for whether the differences are significantly different from 0 are shown. A total of 78 pairwise comparisons were conducted for infection prevalence and abundance; thus statistical significance was based on a Bonferroni corrected Type-I error rate of 0.0006. A total of 48 pairwise comparisons were conducted on infection intensity; thus statistical significance was based on a Bonferroni corrected Type-I error rate of 0.0010. Bold-face *P*s indicate a comparison that was statistically significant.

Year	Season	L3	L4	SA	AD	F:M	L3	L4	SA	AD	F:M
						Lake Michigan					
			Big Bay de Noc						**Naubinway**		
04	Fall	0.00	0.00	0.00	1.00	1.33	--	--	--	--	--
	Winter	0.00	0.00	0.00	1.00	0.33	NS	NS	NS	NS	NS
	Spring	0.14	0.44	0.19	0.23	1.75	--	--	--	--	--
	Summer	0.00	0.00	0.00	1.00	0.50	--	--	--	--	--
05	Fall										
		0.02	0.00	0.04	0.94	1.00	0.00	0.00	0.00	1.00	0.33
	Winter	0.00	0.00	1.00	0.00	--	--	--	--	--	--
	Spring	0.00	0.00	0.16	0.84	1.07	--	--	--	--	--
	Summer	0.00	0.00	0.57	0.43	2.00	--	--	--	--	--
06	Fall										
		0.00	0.50	0.00	0.50	0.00	0.00	0.00	0.00	1.00	1.00
	Winter	0.00	0.20	0.20	0.60	0.00	--	--	--	--	--
	Spring										
		0.60	0.20	0.00	0.20	0.00	0.00	0.00	0.00	1.00	UDF
	Summer	0.00	0.00	0.00	1.00	3.00	--	--	--	--	--
						Lake Huron					
			Cheboygan						**De Tour Village**		
04	Fall	--	--	--	--	--	--	--	--	--	--
	Winter	0.08	0.13	0.15	0.64	1.78	0.00	0.00	0.00	1.00	UDF
	Spring	0.18	0.29	0.18	0.35	1.57	0.59	0.28	0.02	0.11	2.00
	Summer	0.05	0.10	0.18	0.68	1.08	0.00	0.05	0.00	0.95	2.89
05	Fall	0.01	0.00	0.00	0.99	1.38	0.08	0.29	0.31	0.31	2.00
	Winter	--	--	--	--	--	0.39	0.15	0.15	0.30	3.22
	Spring	0.45	0.36	0.08	0.12	1.19	0.38	0.42	0.09	0.11	1.44
	Summer	0.09	0.26	0.50	0.14	0.82	0.06	0.17	0.53	0.24	1.79
06	Fall	NS	NS	NS	NS	NS	--	--	--	--	--
	Winter	0.17	0.31	0.45	0.07	0.23	0.12	0.27	0.35	0.26	0.82
	Spring	0.05	0.45	0.00	0.49	1.24	0.25	0.41	0.00	0.34	1.57
	Summer	0.00	0.21	0.00	0.79	1.33	0.03	0.11	0.00	0.87	1.72

Table 3.5. Proportion of *Cystidicola farionis* at different maturation stages collected by year and season for lake whitefish (*Coregonus clupleaformis*) collected from Big Bay de Noc (Lake Michigan), Naubinway (Lake Michigan), Cheboygan (Lake Huron), and De Tour Village (Lake Huron) spawning stocks (L3 =third larval stage, L4 = fourth larval stage, SA = sub-adult stage, AD = adult stage). Also shown is the female to male ratio of *Cystidicola farionis* adults collected from infected lake whitefish. A "--" in each of the maturation stage categories indicates that no *C. farionis* were found during that sampling period. A "--" in the F:M category indicates that no *C. farionis* adults were collected during the sampling period, while a "UDF" in the F:M category indicates that only *C. farionis* adult females were collected during the sampling period. NS = no sampling conducted during that season.

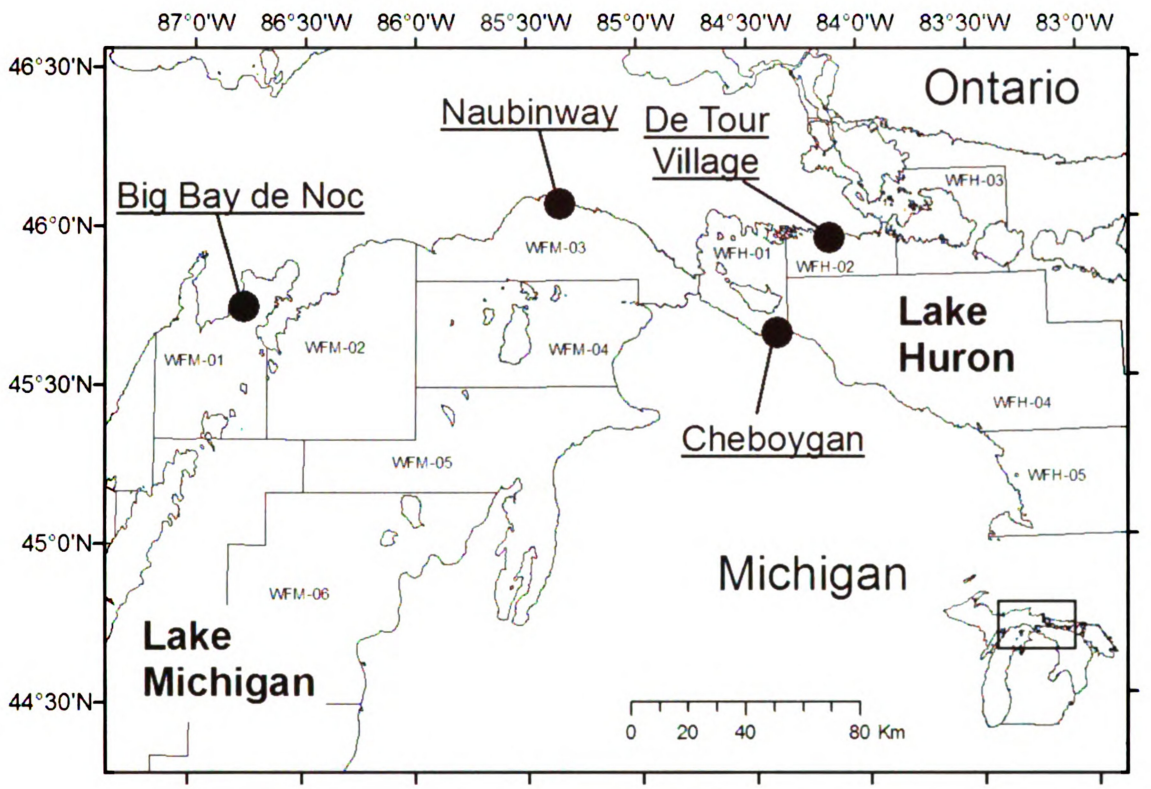


Figure 3.1. Map of northern lakes Huron and Michigan showing the lake whitefish (*Coregonus clupeaformis*) management units in Lake Huron (WFH) and Lake Michigan (WFM). The map also shows the locations of the four fishing ports, Big Bay de Noc, Naubinway, Cheboygan, and De Tour Village where lake whitefish caught from the surrounding waters were delivered.

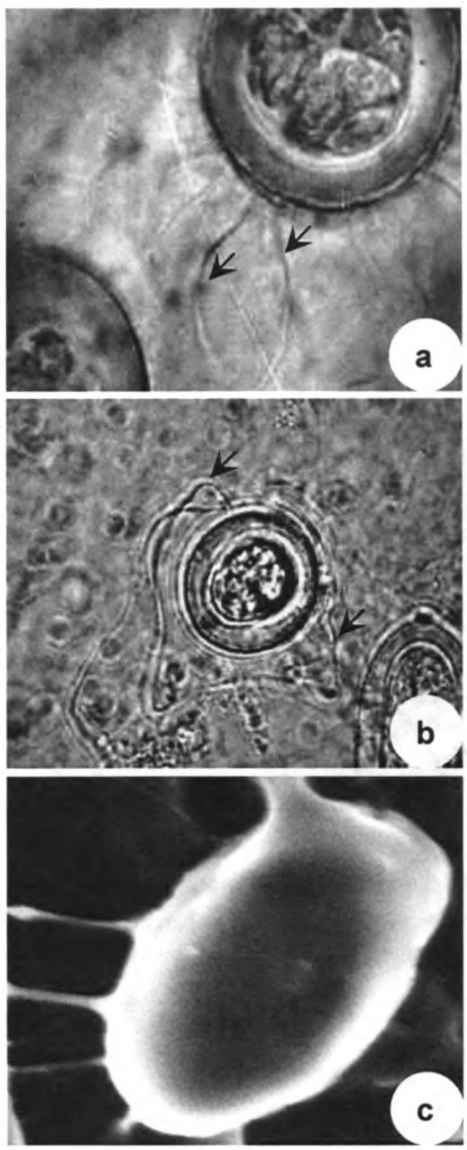


Figure 3.2. Light microscopy (a and b) and scanning electron microscopy (c) of eggs extruded from females of *Cystidicola farionis*: (a) an egg exhibiting lateral filament arrangement (arrows, 1000X); (b) an egg with polar filaments (400X); and (c) an egg with lateral filaments.

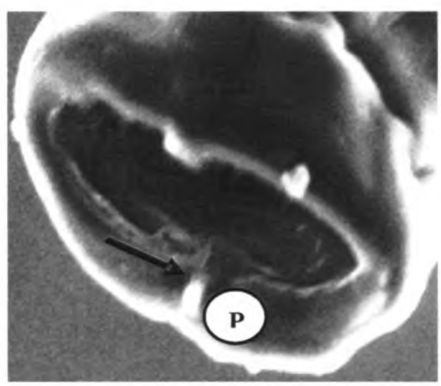


Figure 3.3. Scanning electron microscopy of the buccal cavity of *Cystidicola farionis* showing pseudolabia (p) associated with projecting lip.

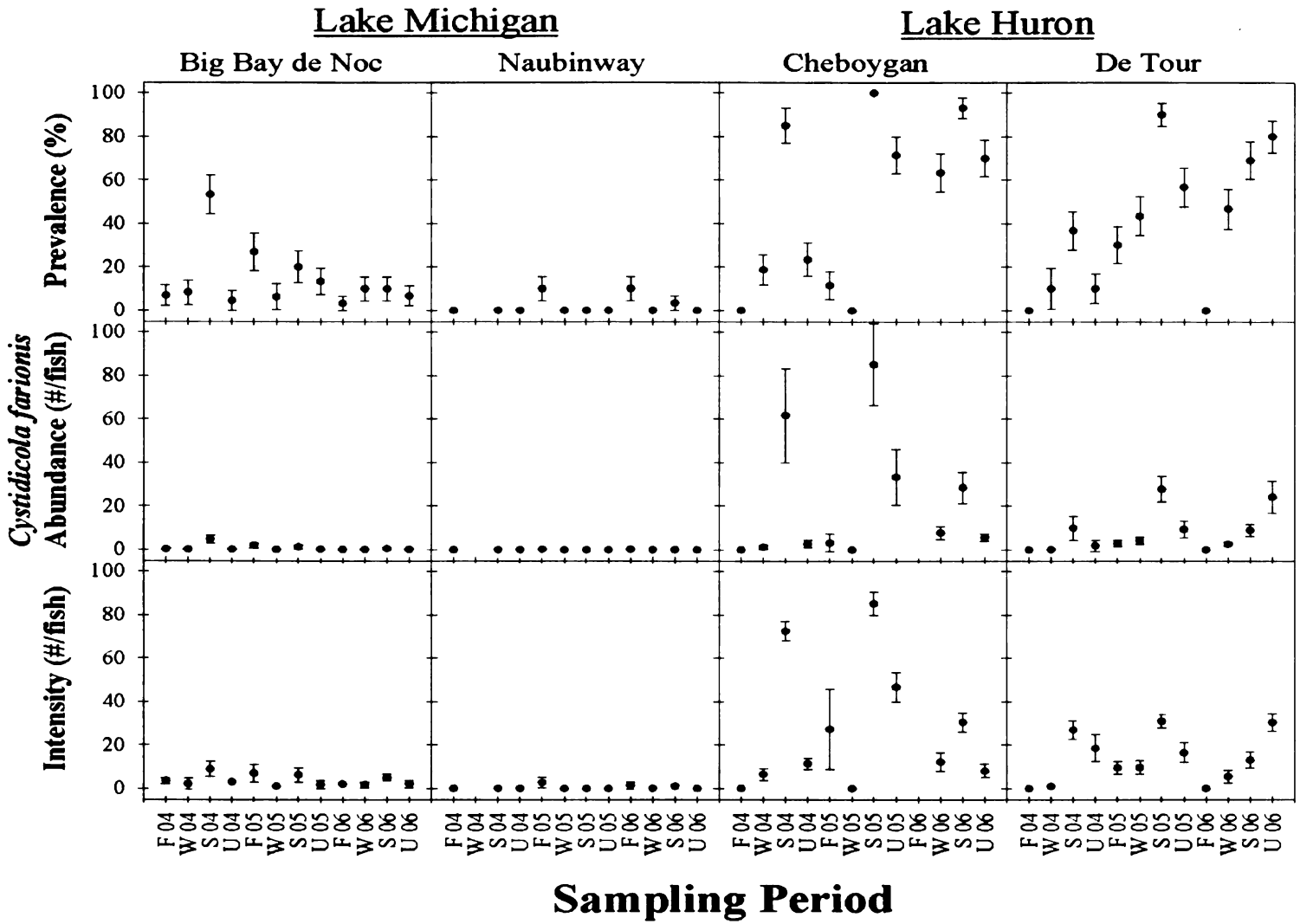


Figure 3.4. ***Cystidicola farionis*** **prevalence, abundance, and intensity (± SE) by sampling occasion for lake whitefish (*Coregonus clupeaformis*) collected from the Big Bay de Noc, Cheboygan, De Tour Village, and Naubinway spawning stocks.**

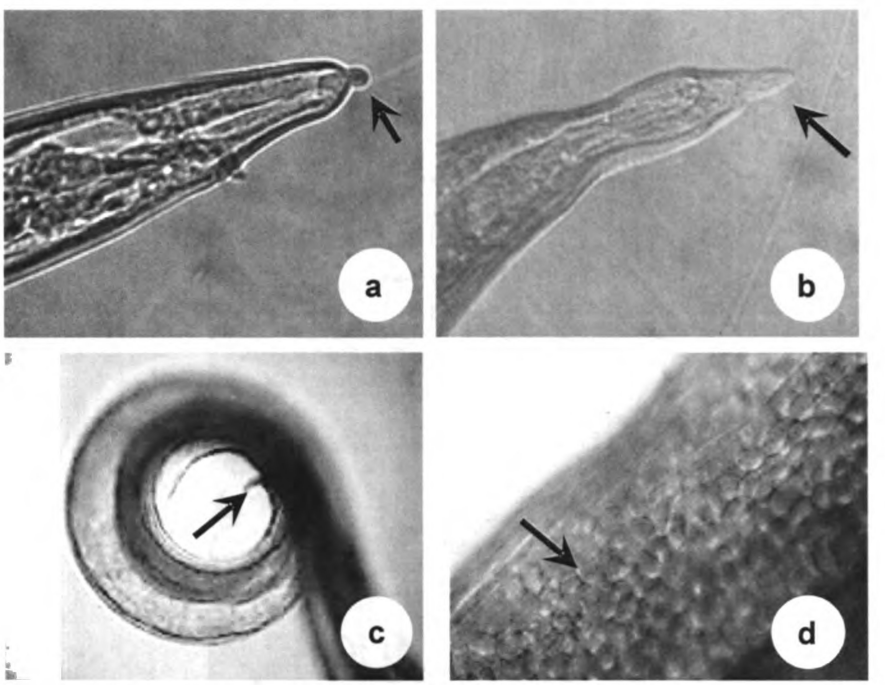


Figure 3.5. Light microscopy showing larval stages of *Cystidicola farionis* found in the swimbladder of infected lake whitefish (*Coregonus clupleaformis*): (a) third larval stage (L3, 400X), the infective stage, exhibiting a prominent tail papilla (arrow, 400X); (b) fourth larval stage (L4) with the tail papilla expanding and fusing with the body (arrow); (c) male nematode showing spicules (arrow, 200X); (d) adult gravid female with eggs (arrow, 1,000X).

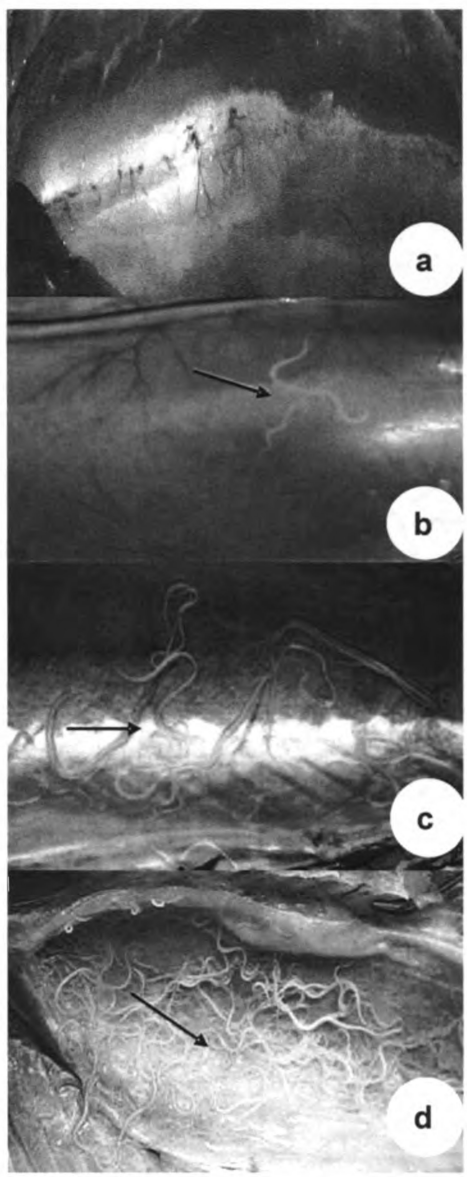


Figure 3.6. Morphological examination of the swimbladder of lake whitefish (*Coregonus clupeaformis*): (a) normal swimbladder; (b) swimbladder infected with a few nematodes (arrow) without affecting the transparency of the membrane; (c) swimbladder with slightly opaque membrane showing moderate number of nematodes (arrow); (d) swimbladder with very thick membrane showing heavy nematode infection (arrow).

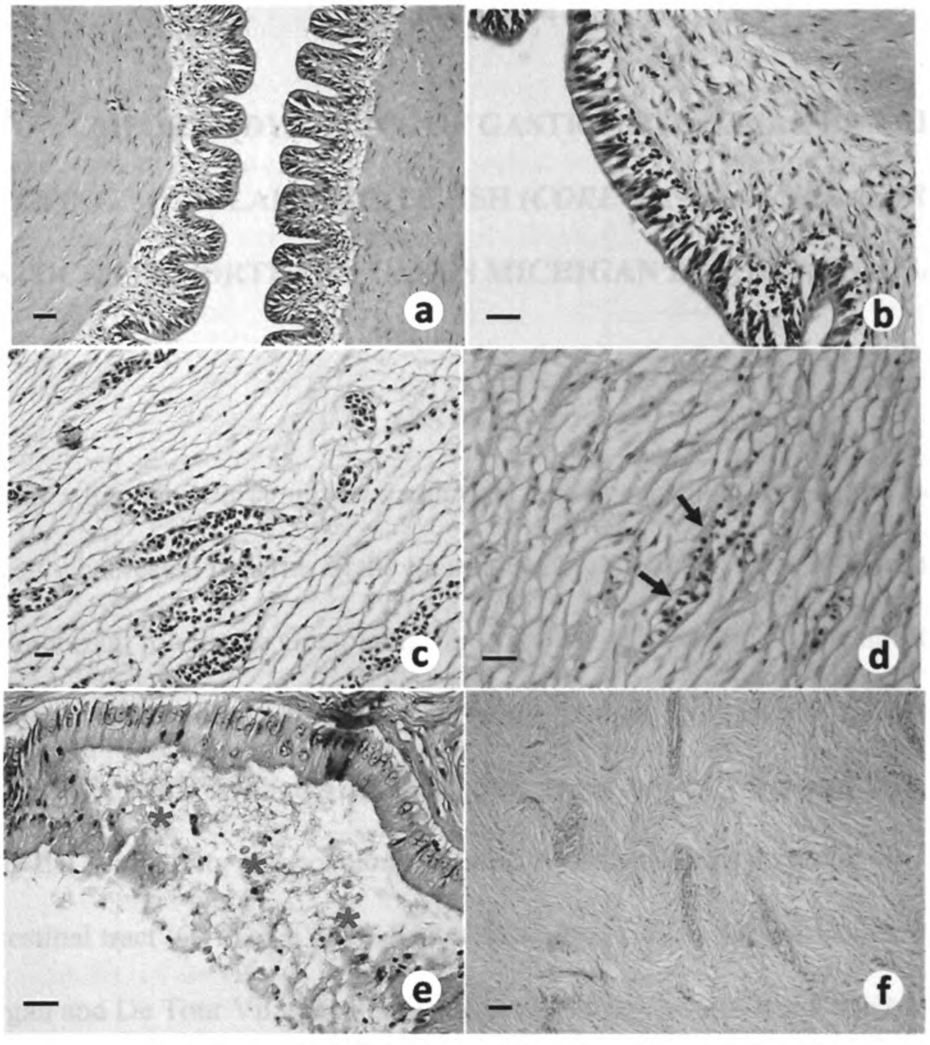


Figure 3.7. Light microscopy of lake whitefish (*Coregonus clupeaformis*) swimbladder wall sections stained with hematoxylin & eosin: a) healthy mucosal lining of a non-infected fish; b) focal lymphocytic infiltrates in the subepithelial tissue; c) blood vessels in the deep connective tissues engorged with red blood cells; d) multifocal lymphocytic and histocytic infiltrates (arrows); e) fibrinous proteinaceous exudates (asterix) containing inflammatory cells; and f) tunica fibrosa of a heavily infected fish taking the appearance of a granulation tissue. All scale bars= 25 μm except in a and f = 50 μm.

CHAPTER 4

SPATIO-TEMPORAL DYNAMICS OF GASTROINTESTINAL HELMINTHS INFECTING FOUR LAKE WHITEFISH (*COREGONUS CLUPEAFORMIS*) STOCKS IN NORTHERN LAKES MICHIGAN AND HURON, USA

ABSTRACT

Lake whitefish (*Coregonus clupeaformis*; LWF) constitutes one of the most commercially harvested fisheries in the Laurentian Great Lakes. As a benthivore, LWF plays an important role in the Great Lakes food webs due to its remarkable ability to transfer energy from lower to higher trophic levels. Despite its economic and ecologic importance, little is known about LWF pathogens and parasites. This study was designed to identify the community composition and structure of helminths infecting the gastrointestinal tract (GIT) of LWF collected from four sites in northern lakes Huron (Cheboygan and De Tour Village) and Michigan (Big Bay de Noc and Naubinway) from fall 2003 through summer 2006. A total of 21,203 helminths were retrieved from the GITs of 1284 spawning LWF. Approximately 41% of the LWF examined harbored at least one helminth species in their GIT, with relatively high mean intensity of 39.37 worms/fish (SE±3.28) and mean abundance of 16.37 worms/fish (SE±1.47). Collected helminths were generalists in nature and represented two phyla and five species: *Acanthocephalus dirus*, *Neoechinorhynchus tumidus*, *Echinorhynchus salmonis*, *Cyathocephalus truncatus,* and *Bothriocephalus* sp. Based on their abundance, *A. dirus* and *C. truncatus* were considered the core species in the LWF-GIT helminth community, while the remaining three worm species were considered rare species. In order to

evaluate the effects of lake, sampling site, year, and season (as well as interactions of these factors), a series of statistical models were fitted to the helminth (all helminth combined and separately for each helminth species) prevalence, abundance, and intensity. LWF from Lake Huron had significantly greater rates of infection than LWF from Lake Michigan. Infection parameters for each of the helminth species generally followed the same pattern observed for the combined data. *A. dirus* was the most prevalent and abundant helminth in LWF-GIT. Differences in infection parameters between the sexes appeared relatively minor. Helminth infection parameters peaked in the spring, while diversity was highest in the winter samples. Despite the spatial and temporal variations, the GIT helminth community composition was almost identical in the Big Bay de Noc, De Tour Village, and Cheboygan spawning stocks. The factors that led to the observed spatial and temporal variations in the LWF-GIT helminth community remain to be elucidated. The findings of this study represent the most comprehensive parasitological study ever conducted on LWF in the Great Lakes and will be an ideal baseline for future studies aiming at investigating the effects of GIT helminths on LWF health, growth and condition.

INTRODUCTION

Lake whitefish, *Coregonus clupeaformis,* is indigenous to the Laurentian Great Lakes. Ecologically, lake whitefish (LWF) plays an important role in the Great Lakes food webs due to its remarkable ability to transfer energy from lower to higher trophic levels (reviewed in Ebener et al. 2008). Following a century of decline, LWF fishery populations in the Great Lakes have shown a strong recovery since their historical low level in 1959, partially because of the success in sea lamprey control and partly due to reduced phosphorus loading following the implementation of the 1972 Great Lakes Water Quality Act (Spangler and Collins 1980; Spangler et al. 1980; Ebener 1997; Ebener et al. 2008). Recent declines in condition and size at age of harvested LWF, along with elevated environmental contaminant tissue levels and changes in fecundity and egg lipid content, have raised serious concerns among Great Lakes fishery managers and scientists as to the sustainability of LWF stocks in the basin (Frank et al. 1978; Mikaelian et al. 1998; Hoyle et al. 1999; Pothoven et al. 2001; Mikaelian et al. 2002; Nalepa et al. 2005*a*; Kratzer et al. 2007; Pothoven and Madenjian 2008).

There is a general consensus among scientists that the declines in condition of LWF are at least partly due to abundance declines in nearshore waters of amphipods of the genus *Diporeia* (Nalepa et al. 2007). Compared to other benthic macroinvertebrates in the Great Lakes, *Diporeia* spp. have high lipid content and historically have been a favored prey item of LWF (Pothoven et al. 2001). As a result of declining *Diporeia* spp. abundance, LWF have been forced to forage in deeper water where the amphipods may still be available (Pothoven 2005), or to consume lower quality food items such as the

invasive zebra (*Dreissena polymorpha*) and quagga (*D. bugensis*) mussels (Pothoven et al. 2001; Nalepa et al. 2009*a*, 2009*b*). Concomitant with declines in *Diporeia* spp. abundance have been the explosive expansion of dreissenid invasion into the Great Lakes and dramatic changes in phytoplankton and benthic macroinvertebrate community composition (Nalepa et al. 1998; Bierman et al. 2005; Higgins et al. 2005; McNickle et al. 2006; Nalepa et al. 2007). Whether these factors have directly or indirectly contributed to the decline in LWF condition and growth remains unclear.

Previous studies have demonstrated that LWF can be a host for numerous helminth parasites (Hoffman 1999). Lawler (1970) published a comprehensive list of parasites affecting *Coregonus* spp., including LWF worldwide. Since then, there have been minimal surveys performed on parasite populations and communities of LWF in the USA. Such studies can be important as they can provide indications of recent or ongoing alterations in benthic macroinvertebrate communities given the role of macroinvertebrates in completing endohelminth life cycles. It has repeatedly been shown through terrestrial models that the gastrointestinal tract (GIT) helminth community structure mirrors the biotic components and the prevailing environmental conditions in the surrounding ecosystem, particularly temporal variations that arise through seasonal and annual fluctuations (Langley and Fairley 1982; O'Sullivan et al. 1984; Haukisalmi et al. 1988; Montgomery and Montgomery 1989). Compared to extrinsic factors, intrinsic factors such as fish age and sex are believed to play a more reduced role in influencing endohelminth community structure (O'Sullivan et al. 1984; Abu-Madi et al. 2000), although there are reports of some parasite species showing a significant sex bias (Behnke et al. 1999).

An additional reason as to why it can be useful to study parasitic infection is that such infections can have major affects on growth and survival rates of host populations (Hudson and Dobson 1997; Behnke et al. 1999). Consequently, it is at least somewhat plausible that recent declines in LWF condition and growth rates in the Great Lakes may be related to helminth parasitism. The overall goal of this research was to quantify the spatio-temporal dynamics of GIT helminths infecting four LWF stocks in northern lakes Michigan and Huron, USA. This information would constitute baseline information that can be followed to determine if GIT helminths can be implied as a potential cause for poor LWF condition. Specific objectivities for this research were to 1) identify the GIT helminth species found in the LWF in lakes Huron and Michigan; 2) assess the GIT helminth community structure in LWF spawning stocks in northern lakes Michigan and Huron; and 3) to evaluate the spatial and temporal changes on LWF-GIT helminth infection parameters and community structure in these stocks over a three year period.

MATERIALS AND METHODS

1) Fish and sampling sites

This study was performed on four LWF spawning stocks, two located in northern Lake Huron and two located in northern Lake Michigan. The stocks are referenced by the names of the closest fishing ports: Big Bay de Noc (BBN), Naubinway (NAB), Cheboygan (CHB), and De Tour Village (DET). The BBN and NAB stocks are located in northern Lake Michigan, while the CHB and DET stocks are located in northern Lake Huron. Details of sampling frequency by season and location are given in Chapter 3 (Table 3.1 and Figure 3.1). The number of LWF used in this study is given in Table 4.1.

2) Parasite identification

Captured LWF were transferred (alive or recently dead and shipped on ice) to the Michigan State University-Aquatic Animal Health Laboratory in East Lansing, Michigan for processing. Once at the laboratory, live fish were sacrificed with an overdose of tricaine methanesulfonate (MS–222, Argent Laboratories, Redmond, Washington). The GIT with attached mesentery for each fish was removed from the esophagus to the anus and kept in 4 °C tap water for 24 to 48 hours to allow parasite relaxation before further processing. Helminths were retrieved manually and preserved in 70% ethanol for later identification and counting. Nematodes were cleared in a mixture of glycerol and 70% ethanol (1:1) and then examined microscopically. Worms were identified to species based on morphology using the identification keys of Yamaguti (1971), Aliff et al. (1977), Moravec (1980), Ingham and Dronen (1982), Amin (1985*a*), and Hoffman (1999).

3) Measurements of LWF-GIT helminth assemblage

Measurements of parasites and terms used to describe parasite communities throughout this study were adopted from Bush et al. (1997), unless otherwise indicated. Prevalence denotes the percentage of host individuals infected with one or more parasites of a particular species. Intensity is defined as the number of individual parasites from a certain species found in an infected host and hence does not include uninfected fish, whereas abundance is defined as the number of individual parasites of a certain species found in both infected and uninfected hosts. Species richness is the number of parasite species found in a fish population.

Diversity indices were used to determine GIT helminth diversity in each of the LWF spawning stocks sampled seasonally over three years. Both Shannon-Wiener's and Simpson's diversity indices were used. The Shannon-Wiener Diversity Index was calculated as detailed in Shannon (1948). The Simpson Reciprocal Diversity Index was calculated by first determining Simpson Index (D) according to the equation developed by Simpson (1949), and then dividing 1/D. Increasing values of the Shannon–Wiener Diversity Index and of Simpson Reciprocal Diversity Index indicate an increase in diversity. The dominance of a particular parasite species was expressed as the Berger-Parker Dominance Index, which measures the proportion of the total number of parasites due to dominant parasite species (Berger and Parker 1970).

4) Statistical analyses

Prior experience with the *C. farionis* data (Chapter 3) has demonstrated that analysis and interpretation of LWF parasite infections using ANOVA-style approaches are difficult due to occurrences of strong interactions among the sampling sites, years,

and seasons. The existence of these interactions can make it difficult to determine whether there are any overall differences among main factor levels (e.g., individual sampling sites), because the interactions can cause factor level differences to be masked. For example, suppose in one year that prevalence of a particular parasite is high in one stock and low in another stock. But in the following year, the stock with the high prevalence experiences a significant die off as a result of the parasite infection, and the only fish that remain have low rates of infection. At the same time, fish with the low infection rate originally experience an outbreak of the parasite. Overall, there may be no differences in the infection rates for these stocks if infection rates are averaged across years, even though there are obvious factors affecting the stocks. As Chapter 3 demonstrates, thorough examination of multifactor interactions can be a lengthy process. ANOVA-style approaches to studying interactions can also be problematic from an inference-based perspective, as each statistical test that is conducted in theory increases the chances of making a Type-I error. As a result of these issues, in this chapter a more succinct approach for examining the effects of lake, spawning site, year, and season on infection parameters was employed.

Initially, Spearman correlation analyses were conducted on infection prevalences and abundances for the GIT helminth species to determine if there were any associations among the different types. Correlations were conducted with all the sampling data combined, as well as separately for each lake and sampling site.

Generalized linear mixed modeling (GLMM) was used to assess how helminth (combined across helminth species and for each individual helminth species) prevalences, abundances, and intensities differed in relation to lake, sampling site, year, and season.

For infection prevalences, mixed models were fit assuming a binomial distribution and a log link. For infection abundances, mixed models were fit assuming a quasi-Poisson distribution and a log link. For infection intensities, mixed models were fit by first $\log_e$ transforming the intensity values and then fitting the models assuming a Gaussian distribution and an identity link. Because of the schooling behavior of LWF, helminth infections were assumed to be correlated within each site×year×season sampling occasion. The mixed models were all fit in R (R Development Core Team 2010) using the lme4 package (Bates and Maechler 2010). The lme4 package can fit mixed models using either Laplace approximation or Gauss-Hermite quadrature, which are generally regarded as more accurate methods for fitting mixed models than other methods, such as penalized quasilikelihood estimation (Bolker et al. 2009). Fitting mixed models using the methods available in the lme4 package is additionally advantageous in that inferential statistical techniques, such as likelihood ratio tests and information-theoretic criterion model selection, can be validly used (Bolker et al. 2009). In the present study, mixed models were fit by Laplace approximation.

In order to evaluate the effects of lake, sampling site, year, and season (as well as interactions of these factors), a series of models were fit to the helminth (all helminth data combined and separately for each helminth species) prevalence, abundance, and intensity data (Table 4.2). These models ranged in complexity from intercept (i.e., grand-mean) only models to models that contained lake, year, and season or sampling site, year, and season as main factor levels and all possible first-order interaction terms. For the prevalence and intensity data, Akaike's information criterion (AIC) were calculated for each one of the fitted models, and the models with the lowest AIC values were then

identified as those that provided the best models in terms of goodness of fit and model parsimony (Burnham and Anderson 2002). The model terms that were included in these best performing models formed the basis for evaluating how prevalence and intensity varied according to the aforementioned factors. A similar process was used for abundance data, except in the case of abundance models, which were evaluated using quasi AIC, which incorporated the additional overdispersion (extra variability) parameter that resulted from modeling abundance as a quasi-Poisson distributed variable in the AIC calculation (Burnham and Anderson 2002).

GLMM and the model selection process described above were also used to evaluate the effects of lake, sampling site, year, and season on the diversity and dominance indices that were estimated from the helminth data. The mixed models for these indices were fit assuming a Gaussian distribution and an identify link.

Nonmetric multidimensional scaling (NMDS) based on Bray-Curtis distances was used as an ordination method to summarize how the helminth assemblage structure differed among the site×year×season sampling occasions. A two-dimensional NMDS solution was used to facilitate interpretation of the ordination results. Up to 100 random restarts were used in the NMDS analysis to help find the best solution. The NMDS analysis was conducted in R using the vegan package (Oksanen et al. 2010).

RESULTS

1) Identification of GIT helminths

The numbers of LWF collected and examined for GIT helminths during each sampling period ranged from 10 to 35 fish per stock (Table 4.1). Altogether, GIT from 1,284 LWF were examined, from which 21,023 helminths were retrieved from the gastrointestinal tracts 531 fish (41.36%). The majority of LWF examined (750 fish, 58.41%) were non-infected. The majority of infected fish harbored one parasite species only (57.44%), which was significantly higher than those that harbored two (28.25%), three (12.99%), or four (1.13%) parasite species. Only one fish harbored all five species.

Approximately 53% (11,221) of the collected helminths were identified as acanthocephalans, with the remainder identified as cestodes. Based on morphology, a total of five helminth species were identified: *Acanthocephalus dirus* Van Cleave 1931 (Acanthocephala), *Neoechinorhynchus tumidus* Van Cleave and Bangham 1949 (Acanthocephala), *Echinorhynchus salmonis* Muller 1784 (Acanthocephala), *Bothriocephalus* sp. (Cestoda), and *Cyathocephalus truncatus* Pallas 1781 (Cestoda). The majority of collected helminths were identified as *A. dirus* (≈47%), followed by *C. truncatus* (≈43%), *N. tumidus* (≈4%), *Bothriocephalus* sp. (≈4%), and *E. salmonis* (≈1%). Attachment sites generally differed among the various helminth species. Whereas the three acanthocephalan species were generally found within fish intestines, *Bothriocephalus* sp. were generally found attached to the gastric mucosa and *C. truncatus* were generally found in the pyloric caeca.

Across all sampling sites and occasions, the mean abundance of infection was 16.37 (SE=1.47) helminths/fish, while the mean intensity of infection was 39.37 (SE=3.38) helminths/fish. Prevalence and abundance of infection with *A. dirus* was significantly greatest out of all the helminth types, followed by *C. truncatus*, *Bothriocephalus* sp., *N. tumidus*, and *E. salmonis* (Table 4.3). In terms of intensity of infection, *C. truncatus* intensity was the greatest, followed by *A. dirus, N. tumidus*, *Bothriocephalus* sp., and *E. salmonis* (Table 4.3).

Regardless of the helminth type or their infection parameters, LWF from Lake Huron had significantly greater rates of infection than LWF from Lake Michigan (Table 4.3). Infection parameters for each of the lakes generally followed the patterns observed for the combined data; that is, prevalence and abundance of *A. dirus* infections were greater than for the other helminth species, but intensity of infections were the greatest for *C. truncatus* (Table 4.3). Most of the discrepancies that were observed between lakes Huron and Michigan appeared to be caused by the lower infection parameters for LWF collected from the NAB spawning site compared to fish collected from the other three sites (Table 4.3). As for differences in infection between LWF sexes, females typically had higher rates of infection than male LWF, with the one notable exception being that males had higher intensities of infection with *C. truncatus* (Table 4.3). Overall, differences in infection parameters between the sexes appeared relatively minor (Table 4.3).

For the correlations analyses that were conducted based on helminth prevalence, significant positive correlations were detected between *A. dirus* and *Bothriocephalus* sp. (r_S=0.17; P<0.0001), *A. dirus* and *C. truncatus* (r_S=0.28; P<0.0001), *A. dirus* and *E.*

salmonis (r_S=0.11; P<0.0001), *A. dirus* and *N. tumidus* (r_S=0.08; P=0.00024), *Bothriocephalus* sp. and *C. truncatus* (r_S=0.38; P<0.0001), and *E. salmonis* and *N. tumidus* (r_S=0.49; P<0.0001). When the correlation analyses were conducted separately by lake, significant positive correlations were detected between Lake Huron prevalences of *A. dirus* and *Bothriocephalus* sp. (r_S=0.21; P<0.0001), *A. dirus* and *C. truncatus* (r_S=0.33; P<0.0001), *A. dirus* and *N. tumidus* (r_S=0.10; P=0.0109), and *E. salmonis* and *N. tumidus* (r_S=0.59; P<0.0001). For Lake Michigan, significant positive correlations were detected between prevalences of *A. dirus* and *Bothriocephalus* sp. (r_S=0.11; P=0.0051), *A. dirus* and *C. truncatus* (r_S=0.18; P<0.0001), *A. dirus* and *E. salmonis* (r_S=0.13; P=0.0006), *Bothriocephalus* sp. and *C. truncatus* (r_S=0.28; P<0.0001), *Bothriocephalus* sp. and *N. tumidus* (r_S=0.08; P=0.0424), and *E. salmonis* and *N. tumidus* (r_S=0.24; P<0.0001). When the correlations were conducted separately by spawning site, significant positive correlations were detected between Big Bay de Noc prevalences of *A. dirus* and *Bothriocephalus* sp. (r_S=0.13; P=0.0176), *A. dirus* and *C. truncatus* (r_S=0.17; P=0.0014), *A. dirus* and *E. salmonis* (r_S=0.11; P=0.0384), *Bothriocephalus* sp. and *C. truncatus* (r_S=0.34; P<0.0001), and *E. salmonis* and *N. tumidus* (r_S=0.28; P<0.0001). For Cheboygan, significant positive correlations in prevalences were detected between *A. dirus* and *Bothriocephalus* sp. (r_S=0.31; P<0.0001), *A. dirus* and *C. truncatus* (r_S=0.41;

P<0.0001), *A. dirus* and *E. salmonis* (r_S=0.16; P=0.0063), *Bothriocephalus* sp. and *C. truncatus* (r_S=0.48; P<0.0001), and *E. salmonis* and *N. tumidus* (r_S=0.61; P<0.0001). For De Tour Village, significant positive correlations in prevalences were detected between *A. dirus* and *Bothriocephalus* sp. (r_S=0.13; P=0.0144), *A. dirus* and *C. truncatus* (r_S=0.26; P<0.0001), *A. dirus* and *N. tumidus* (r_S=0.13; P=0.0139), *Bothriocephalus* sp. and *C. truncatus* (r_S=0.44; P<0.0001), and *E. salmonis* and *N. tumidus* (r_S=0.60; P<0.0001). For the Naubinway spawning site, significant positive correlations were detected between *Bothriocephalus* sp. and *C. truncatus* (r_S=0.21; P=0.0003).

When the correlations analyses were conducted on helminth abundances, significant positive correlations were detected between *A. dirus* and *Bothriocephalus* sp. (r_S=0.18; P<0.0001), *A. dirus* and *C. truncatus* (r_S=0.30; P<0.0001), *A. dirus* and *E. salmonis* (r_S=0.09; P=0.0009), *A. dirus* and *N. tumidus* (r_S=0.06; P=0.0293), *Bothriocephalus* sp. and *C. truncatus* (r_S=0.39; P<0.0001), and *E. salmonis* and *N. tumidus* (r_S=0.50; P<0.0001). When the correlation analyses on abundance were conducted separately by lake, significant positive correlations in Lake Huron were found between *A. dirus* and *Bothriocephalus* sp. (r_S=0.24; P<0.0001), *A. dirus* and *C. truncatus* (r_S=0.39; P<0.0001), *Bothriocephalus* sp. and *C. truncatus* (r_S=0.47; P<0.0001), and *E. salmonis* and *N. tumidus* (r_S=0.60; P<0.0001). For Lake Michigan, significant positive correlations were found between *A. dirus* and *Bothriocephalus* sp. (r_S=0.11; P=0.0051),

A. dirus and *C. truncatus* (r_S=0.17; P<0.0001), *A. dirus* and *E. salmonis* (r_S=0.14; P=0.0003), *Bothriocephalus* sp. and *C. truncatus* (r_S=0.28; P<0.0001), *Bothriocephalus* sp. and *N. tumidus* (r_S=0.09; P=0.0239), and *E. salmonis* and *N. tumidus* (r_S=0.25; P<0.0001). When the correlations were conducted by spawning site, significant positive correlations were found between Big Bay de Noc abundances of *A. dirus* and *Bothriocephalus* sp. (r_S=0.14; P=0.0113), *A. dirus* and *C. truncatus* (r_S=0.15; P=0.0064), *A. dirus* and *E. salmonis* (r_S=0.13; P=0.0207), *Bothriocephalus* sp. and *C. truncatus* (r_S=0.33; P<0.0001), and *E. salmonis* and *N. tumidus* abundances (r_S=0.29; P<0.0001). For Cheboygan, significant positive correlations were found between *A. dirus* and *Bothriocephalus* sp. (r_S=0.34; P<0.0001), *A. dirus* and *C. truncatus* (r_S=0.46; P<0.0001), *A. dirus* and *E. salmonis* (r_S=0.12; P=0.0415), *Bothriocephalus* sp. and *C. truncatus* (r_S=0.51; P<0.0001), and *E. salmonis* and *N. tumidus* (r_S=0.62; P<0.0001). For De Tour Village, significant positive correlations were found between *A. dirus* and *Bothriocephalus* sp. (r_S=0.16; P=0.0037), *A. dirus* and *C. truncatus* (r_S=0.32; P<0.0001), and *E. salmonis* and *N. tumidus* abundances (r_S=0.62; P<0.0001). For the Naubinway spawning site, significant positive correlations were found between *A. dirus* and *C. truncatus* (r_S=0.13; P=0.0190), *E. salmonis* and *N. tumidus* (r_S=0.62; P<0.0001), *Bothriocephalus* sp. and *C. truncatus* (r_S=0.20; P=0.0004), and *Bothriocephalus* sp. and *N. tumidus* (r_S=0.12; P=0.0379).

2) Spatio-temporal evaluation of infection dynamics

a. All helminths combined

For the combined helminth data, the model with the lowest AIC value for both prevalence and abundance was Model 30, which included sampling site, year, and season as main effects and site ×season and year×season as first-order interaction terms. The occurrence of these interaction terms suggested that any site-to-site or year-to-year differences in infection prevalences or abundances depended on which season the sampling occurred. For intensity of infection, the model with the lowest AIC value was Model 28, which included sampling site, year, and season as main effects and site ×year and site×season as first-order interaction terms.

Similar to the interpretation for the prevalence and abundance models, the occurrence of these interaction terms suggested that any site-to-site differences in infection intensity depended on the year and season for which comparisons might be made. Indeed, plots of the overall helminth infection rates by sampling occasion showed substantial variability in infection dynamics, with prevalences, abundances, and intensities fluctuating between high and low rates across all stocks under study (Figure 4.1).

When the helminth infection data were pooled across sampling years and seasons, the odds of LWF from Lake Huron being infected with GIT helminths was 1.91 (95% CI: 0.54 – 6.78) higher than the odds of fish from Lake Michigan being infected. For the individual spawning sites, the odds of LWF from the DET spawning stock being infected with GIT helminths was roughly equal (95% CI: 0.19 – 5.52) to that of fish from the BBN spawning stock. But LWF from DET had 1.74 (95% CI: 0.30 – 9.90) and 7.01

(95% CI: 1.21 – 41.28) times the odds of being infected compared to that of fish from the CHB and NAB spawning stocks, respectively.

b. *Acanthocephalus dirus* Van Cleave, 1931

As was found with the combined infection data, the model with the lowest AIC value for both *A. dirus* prevalence and abundance was Model 30 (main effects = site, year, and season; first-order interactions = site ×season and year×season). As with the combined infection data, because the model contained site×season and year×season interactions, differences in infection prevalence and abundance among sites and years depended on the season being considered. This was clearly evident in the plots of *A. dirus* prevalence and abundance, which indicated infection parameters fluctuating between high and low levels (Figure 4.2).

For *A. dirus* infection intensity, the model with the lowest AIC value was Model 25 (main effects = site, year, and season; first-order interactions = site×year). Because season in which the sampling occurred was included in the model as a main effect but was not included as part of a first-order interaction, this suggests that there were fairly consistent differences in *A. dirus* infection intensity among seasons regardless of sampling site or year. Across all the stocks, *A. dirus* infection intensity was greater during the spring and summer than during the fall and winter (Figure 4.2).

When *A. dirus* infections were pooled across sampling years and seasons, the odds of LWF being infected in Lake Huron were 3.00 (95% CI: 0.87 – 10.44) times greater than the odds of fish from Lake Michigan. LWF from the DET spawning stock had 1.40 (95% CI: 0.30 – 6.62), 2.15 (95% CI: 0.43 – 10.75), and 20.18 (95% CI: 3.48 –

116.95) greater odds of being infected than fish from the BBN, CHB, and NAB spawning stocks, respectively.

c. *Neoechinorhynchus tumidus* Van Cleave and Bangham, 1949

For *N. tumidus*, the model with the lowest AIC value for both infection prevalence and abundance was Model 31 (main effects = site, year, and season; first-order interactions = site×year, site×season and year×season). For infection intensity, the model with the lowest AIC value was Model 17 (main effects = site, year, and season). Because there were no interaction terms in the model, differences in the model main effects were largely consistent across the levels of the other main effects. For sampling sites, the DET stock appeared to have consistently greater infection intensities compared to the other stocks, while the NAB and CHB stocks had the lowest infection intensities (Figure 4.3). The one notable exception was the infection intensity of the NAB stock from the summer 2004 sampling occasion, which was the highest *N. tumidus* infection intensity observed across all sites and sampling occasions (Figure 4.3). As for year-to-year difference, *N. tumidus* infection intensity generally appeared to be lower during the 2005 sampling year compared to the other sampling years, although admittedly these year-to-year difference did not seem large. As for seasons, *N. tumidus* infection intensity appeared to peak during the spring and summer months.

When the *N. tumidus* infection data were pooled across sampling years and seasons, the odds of LWF from Lake Huron being infected with GIT helminths was 1.60 times greater than the odds for fish from Lake Michigan. Overall, LWF from DET had 1.59 (95% CI: 0.30 – 8.47), 2.06 (95% CI: 0.36 – 11.76), and 3.32 (95% CI: 0.55 –

20.18) greater odds of being infected with *N. tumidus* than fish from BBN, CHB, and NAB, respectively.

d. *Echinorhynchus salmonis* Muller, 1784

Because of the overall low rate of infection with *E. salmonis* (only 59 LWF in total were found to be infected with this species of helminth), mixed models for infection rates were not constructed, as there was concern that the low sample sizes would not yield meaningful results. Plots of infections by sampling occasion suggested that perhaps a sharp rise of *E. salmonis* infections was beginning to occur just as this study ended, as prevalences in three of the stocks appeared to have been on the rise in summer and spring of 2006 (Figure 4.4).

e. *Cyathocephalus truncatus* Pallas, 1781

As was the case for the combined and *A. dirus* infection data, the model with the lowest AIC value for both *C. truncatus* prevalence and abundance was Model 30 (main effects = site, year, and season; first-order interactions = site×season and year×season). Again, this model suggests that site-to-site and year-to-year differences in *C. truncatus* prevalence and abundance depended largely on sampling season (Figure 4.5). For intensity of infection of *C. truncatus*, the model with the lowest AIC value was Model 21 (main effects = lake, year, and season; first-order interactions = lake×year and lake×season. This model suggests that there were larger lake rather than sampling site variations in *C. truncatus* infection intensities, but that the lake-to-lake differences in intensity depended on the year and season being considered.

When infections were pooled across sampling years and seasons, LWF from Lake Huron had 1.91 (95% CI: 0.57 – 6.37) times the odds of being infected with *C. truncatus*

than fish from Lake Michigan. LWF from the DET spawning stock had 1.3 times the odds of being infected compared to the CHB spawning stock. When odds of infection were compared across sampling sites, LWF from DET had 1.65 (95% CI: 0.30 – 9.01), 1.23 (95% CI: 0.22 – 6.80), and 2.81 (95% CI: 0.48 – 16.57) greater odds than fish from BBN, DET, and NAB, respectively.

f. *Bothriocephalus* sp.

For *Bothriocephalus* sp. prevalence, the model with the lowest AIC value was Model 24 (main effects = lake, year, and season; first-order interactions = lake×year, lake×season and year×season). For abundance, the model with the lowest AIC value was Model 31 (main effects = site, year, and season; first-order interactions = site×year, site×season and year×season). The occurrence of these interactions again suggests that differences among sites, lakes, years, and seasons depend heavily on the levels of the other factors.

Like *A. dirus* infection intensity, the model with the lowest AIC value for *Bothriocephalus* sp. infection intensity was Model 25 (main effects = site, year, and season; first-order interactions = site×year). As with *A. dirus* infection intensity, since season did not appear in the model as a first-order interactions term, differences in *Bothriocephalus* sp. infection intensity among season were consistent across sites and years. Plots of infection rates suggested that intensity of infection was generally greatest during the spring than in the other seasons, although infection intensity was also at time high during the summer months (Figure 4.6).

When infections were pooled across sampling years and seasons, LWF from Lake Huron had 1.5 (95% CI: 0.46 – 4.89) times the odds of being infected with

Bothriocephalus sp. than fish from Lake Michigan. As was the case with the other nematode types, LWF from DET spawning had the greatest odds of becoming infected with *Bothriocephalus* sp. The odds of LWF from DET being infected with *Bothriocephalus* sp. were 1.49 (95% CI: 0.28 – 8.06), 1.16 (95% CI: 0.21 – 6.25), and 1.77 (95% CI: 0.31 – 10.03) greater than for those from BBN, CHB, and NAB, respectively.

3) Community structure of LWF-GIT helminth community

Overall richness and diversity of the GIT helminth community in LWF was low. Species richness never exceeded 5 and was as low as 0 on six occasions. Total species richness in fish collected from the BBN, CHB, and DET spawning sites was 5, while richness for fish from NAB spawning stock was 4. As far as seasons, overall species richness during fall and winter was 4, while for spring and summer overall species richness was 5.

For the helminth diversity measures, the mixed models with the lowest AIC values were Model 5 (main effects = season) and Model 7 (main effects = lake and season) for the Simpson and Shannon-Wiener diversity indices, respectively. For the Simpson Reciprocal Diversity Index, winter was the season where diversity was greatest, followed by fall, summer, and spring. For the Shannon-Wiener Diversity Index, Lake Michigan had a greater diversity than Lake Huron. Similar to what was found with the Simpson Reciprocal Diversity Index, winter was the season with the greatest Shannon-Wiener Diversity Index, followed by fall, summer, and spring.

The mean Berger-Parker Dominance Index for the different sampling sites and sampling occasions ranged from a minimum of 0.4 (meaning the dominant species

accounted for approximately 40% of helminth composition) for the BBN spawning stock in spring 2006 to 1.0 (meaning that the dominant species accounts for 100% of the GIT worm composition), which was observed on seven occasions. Dominance in the LWF-GIT helminth community was, to a greater extent, shared between *A. dirus* and *C. truncatus*. *A. dirus* was the most frequently dominant helminth type for the Big Bay de Noc, Cheboygan, and De Tour Village spawning stocks, followed by *C. truncatus* and *N. tumidus*. For the Naubinway spawning stock, *C. truncatus* was the most frequently dominant species, followed by *Bothriocephalus* sp. and *A. dirus*. Sharing of dominance between the two species was also observed when the data was stratified by season. *A. dirus* was the dominant species, with relatively high Berger-Parker Index value, in the fall and summer samples, while *C. truncatus* was dominant in the winter and spring seasons. Dominance sharing continued to be observed when the data was divided by year. In the first year, *C. truncatus* was dominant, while *A. dirus* was the dominant species in the second and third years. Interestingly, by the end of the study, *N. tumidus* emerged as a dominant species. For example, in the 2006 spring samples, *N. tumidus* was dominant in two stocks (NAB and CHB), and in the 2006 summer samples, *N. tumidus* was dominant in the BBN, DET, and CHB stocks with Berger-Parker Dominance Index values that exceeded 0.5. From the mixed models that were constructed for the Berger-Parker Dominance Index, the model with the lowest AIC value was Model 5 (main effects = season). Fall was the season with the largest dominance index, followed in decreasing order by winter, summer, and spring.

4) Similarities in GIT helminth community composition among the four LWF spawning stocks

When the NMDS scores were plotted according to sampling site and occasion, the substantial variability that was observed in the GIT helminth infection parameters for the stocks became evident (Figure 4.7). There was very little indication of a clear grouping structure for the helminth community data, with different sampling sites, years, and seasons often grouped close together indicating similar GIT helminth communities. The one exception to this observation was that there did appear to be a grouping of sampling sites from the 2006 spring and summer sampling periods (Figure 4.7), which was based on the species loadings for the NMDS axes corresponded to high abundances of *E. salmonis* and *N. tumidus.*

Plotting the NMDS scores simply based on sampling site and lake (Figure 4.8), it was clear that the GIT helminth communities from BBN, CHB, and DET were very similar, but that the GIT helminth community from fish from NAB was different compared to the other three stocks. The GIT helminth community from NAB consisted primarily of *C. truncatus*, *Bothriocephalus* sp., and *N. tumidus*, which was different from the other areas. As for differences between lakes, overall lakes Michigan and Huron had similar GIT helminth communities, which could be attributed primarily to the consistency of the BBN spawning site with the DET and CHB spawning sites.

DISCUSSION

1) Composition of LWF-GIT helminths

Findings of this study clearly demonstrate that GIT helminth infections are present in the four LWF spawning stocks examined in this study. The numbers of helminth species found in this study are much lower than those listed for LWF (Lawler 1970; Hoffman 1999). The majority of LWF examined (>58%) harbored no worms in their GITs or were lightly infected. The five helminth species found in the GIT of LWF in this study are generalists in nature, as they have been reported from a number of freshwater fish species from North America, including coregonids (Lawler 1970; Camp et al. 1999; Hoffman 1999; Stewart and Bernier 1999; Muzzall and Bowen 2002; Muzzall et al. 2003).

Although five species of helminths were identified, the majority of infected fish (57.44%) harbored only one species of helminths in their GIT. The number of LWF helminth species is extremely low when compared to other fish species where GIT helminth species can reach up to 36 (Kennedy 2009). Like the case of other freshwater fish species in the northern hemisphere, the LWF-GIT helminth community was dominated by acanthocephalan species (Kennedy 2009) with *Acanthocephalus dirus* being the most prevalent and *Cyathocephalus truncatus* the most abundant. Based on abundance data, *A. dirus* and *C. truncatus* are considered core species (with abundance value >2 worms/fish) in the sampled lake whitefish stocks, while *Echinorhynchus salmonis*, *Neoechinorhynchus tumidis*, and *Bothriocephalus* sp. are considered rare

species, with overall abundances that never exceeded 0.7 helminths/fish (Zander et al. 1999).

Unfortunately, there have been few studies performed on LWF-GIT helminths in the USA that presented detailed infection parameters. There have been, however, a number of studies of LWF-GIT helminths in Canada. For example, *A. dirus* was reported in LWF collected from Lake Huron by Collins and Dechtiar (1974), and in Lake Ontario at a prevalence of 28% (1-9 worms/fish) by Dechtiar and Christie (1988). Bangham (1955) found relatively widespread infections with *E. salmonis* (68 out of 99 fish) and *C. truncatus* (40 out of 99 fish) in LWF collected from South Bay, Ontario, and Lake Huron around South Baymouth. In another study, Dechtiar (1972), who collected 15 LWF from Lake of the Woods, Ontario, found 100% infection with *C. truncatus,* with each fish harboring between 11-50 worms. The author also found a single LWF with light *N. tumidus* infection. In a study conducted on LWF collected from Southern Indian Lake Manitoba, Manitoba, Canada, Watson and Dick (1979) showed that *E. salmonis* and *C. truncatus* are core GIT helminth species in LWF with a prevalence of 37.6% and 21.8%, and mean abundance of 4.79 and 6.56, respectively. These two helminth species were also found in Cold Lake, Alberta, Canada at almost identical prevalence and abundance (Leong and Holmes 1981). These two worms are known for their pathogenicity to coregonids (e.g., *Coregonus albula*) (Lawler 1970).

The above mentioned studies and several additional Canadian studies reported on the presence of GIT helminths in LWF collected from lakes Ontario, Huron, and Superior, and in lakes in northern Alberta. In addition to *E. salmonis* and *C. truncatus*, these reports included *Capillaria salvelini, Crepidostomum farionis, Cystidicoloides*

tenuissima, Diphyllobothrium dendriticum, Neoechinorhynchus crassus, N. rutili, Pomphorhynchus bulbocolli, Proteocephalus longicollis, Raphidascaris acus, and *Spinitectus gracilis* (Hart 1931; Hunter and Bangham 1933; Collins and Dechtiar 1974; Dechtiar and Christie 1988; Dechtiar and Lawrie 1988; Dechtiar et al. 1988; Baldwin and Goater 2003), none of which were found in this study. This difference in GIT helminth species among LWF stocks in North America underscores the role biotic and abiotic ecological components play in determining the parasite community composition.

2) Spatial and temporal variation on the infection parameters of LWF-GIT helminths

Despite the fact that the same helminth species were found in both Lake Huron and Lake Michigan, infection parameters (particularly abundance) in LWF from Lake Huron sites were remarkably higher than those of Lake Michigan. In a parallel study performed on the same LWF stocks (Chapter 3), the swimbladder nematode *Cystidicola farionis* also exhibited higher infection parameters in Lake Huron LWF stocks compared to those from Lake Michigan stocks. Additionally, LMB collected from inland lakes within Lake Huron watershed have a more diverse community of GIT helminths compared to the communities found in LMB collected from watersheds of Lake Michigan or Lake Erie (Chapter 2). Considering the relatively short distance separating the four sampling sites, it was surprising to find that the LWF stock sampled at the NAB site was much less infected with GIT helminths compared to the other three stocks. Moreover, unlike in DET, CHB, and BBN stocks, no *E. salmonis* was found in the NAB stock. Similarly, studies performed on *C. farionis* demonstrated that it was almost nonexistent in the NAB stock indicating that the NAB stock had much fewer parasitic

infections in general. Similar observations on spatial differences in parasite composition have been reported for LWF sampled from other northern lakes in Alberta (Leong and Holmes 1981; Poole 1985) and eastern Canada (Curtis 1988). They are also similar to those reported for the common whitefish (*Coregonus lavaretus*) collected from lakes in Finland (Karvonen and Valtonen 2004).

It has long been recognized that diet plays a major role in the composition of fish helminth communities, particularly those of the gut (Ward 1894). Therefore, one can attribute the spatial-associated differences in GIT helminths noticed in this study to the variations in food items available to LWF at the NAB site versus the three other sites. LWF is a benthivore, and the benthic invertebrate communities of lakes Huron (McNickle et al. 2006; Nalepa et al. 2007, 2009*a*) and Michigan (Pothoven et al. 2001; Nalepa et al. 2009*b*) have undergone drastic changes since dreissenids first invaded the Great Lakes. In this context, Messick et al. (2004), who studied pathology of *Diporeia* spp. from Lake Michigan and Lake Huron, confirmed their role as intermediate hosts for cestodes and acanthocephalans. Therefore, it is logical to think that parasite communities of LWF should be altered in sites where *Diporeia* spp. has disappeared. However, diet may not be the only reason for the low infection of the NAB LWF stock. Goater et al. (2005), who studied the parasite communities in LWF in isolated lakes in the Caribou Mountains, found that, despite the similarities in diet items available to LWF, there have been differences in parasite composition that the authors attributed to variations in limnological features and the diversity and abundance of other fish species at the sampling site.

In the same context, when Kennedy (1978) tested the correlation between selected physicochemical factors and the composition of the parasite fauna of trout in British lakes, he found a positive correlation between the number of parasite species and lake size, and a negative correlation between the number of parasite species and lake altitude. However, he was unable to find a correlation between the occurrence of a specific parasite species with selected physicochemical variables. He concluded that it was individual factors and chance colonization events that determined the species composition, and it was therefore impossible to predict a parasite assemblage at a selected sampling site. Unfortunately, limnological information available on each of the sampling sites of this study is not detailed enough to allow for drawing correlations between the site characteristics and infection parameters of each of the sites.

The findings of this study clearly demonstrate the presence of temporal variations in infection parameters of LWF-GIT helminths, with a peak in the spring and summer seasons. Similar trend was found in the swimbladder nematodes, indicating that the spring peak seem to be not limited to GIT helminths (Chapter 3). Similar findings were reported by Watson and Dick (1979) in LWF collected from the Southern Indian Lake, Manitoba, Canada. The authors found that abundances of *E. salmonis* and *C. truncatus* exhibit seasonal patterns with a peak in the spring. There are a number of interpretations for these findings. First, it is possible that water temperatures control the life cycles of the two helminths either directly or indirectly by affecting the abundance of the amphipod intermediate hosts. Second, it is possible that foraging by LWF increases as the temperature rises and the fish's metabolic activities increase. Third, fluctuation in precipitation can affect parasite infections. For example, Akhtar et al. (1992) reported on

a higher rate of infection by *Heteropneustes fossilis* in the rainy season, while in other helminth species increases in prevalence and intensity were noticed during the dry period with marked oscillations in abundance through the year. It has been suggested that the higher abundance of parasites in the dry months is due to an increase in host density and greater overlap of intermediate and definitive hosts as water bodies shrink (Ezenwaji and Ilozumba 1992) which facilitate transmission. Fourth, spawning condition of the fish could be a confounding factor since fish during the spawning season tend to limit food consumption by ripe adult fish (Gupta et al. 1984). In the case of Great Lakes LWF, spawning takes place in the fall, therefore, spawning stress can be excluded as a potential cause for the rise of GIT helminths in the spring and summer seasons. Last, it is possible that the lives of the GIT worms are relatively short, and therefore, there is a continual recruitment of the parasites' infective stages. Unfortunately, the period that any of the GIT worms can spend within their hosts is unknown. Based only on the data generated in this study and available information on the sampling sites, one cannot attribute the mechanisms leading to increased spring parasitism in LWF examined.

One of the important temporal changes noticed in this study was the overall decline in prevalence of GIT worms in the third year of the study, with absence of all GIT parasites in several samples. Concomitant with this decline was the obvious change in the relative composition of GIT helminth community in the third year; as *A. dirus*, *C. truncatus*, and *Bothriocephalus* sp. prevalence sharply declined by 30-85%, prevalence in *N. tumidus* and *E. salmonis* sharply increased by 4-5 folds. No dramatic changes in the surrounding ecosystem that can explain this phenomenon are known except what has been implicated as the cause for the declining LWF growth and condition: steady decline

of *Diporeia* spp. along with the steady increase in the abundance of invasive dreissenids in lakes Michigan and Huron (Pothoven et al. 2001; McNickle et al. 2006; Nalepa et al. 2007, 2009*a*, 2009*b*). On the contrary, the recent extensive analysis of 69 years of data on LWF in South Bay, Lake Huron refutes the theory that environmental changes could account for growth and condition changes observed in LWF (Rennie et al. 2009).

In general, the dispersal and movement differences among the LWF stocks can explain the spatial and temporal variations in infection parameters noticed in this study. The studies of Ebener et al. (2010) that analyzed tag–recapture data demonstrated that the stocks were primarily segregated during the spawning season, which lasts from fall to midwinter, but that fish from the four stocks (particularly the DET and CHB stocks) were mixed during the remainder of the year (Ebener et al. 2010). While differences in dispersal and movement of the four LWF stocks may help explain the seasonal fluctuations in infection parameters, it does not necessarily explain the absence of *E. salmonis* in the NAB spawning stock throughout the three years, or the decline in infection in the third year.

The results of the present study demonstrated that the infection parameters were higher in LWF females when compared to males throughout the three years of the study. Data analysis, however, demonstrated changes are negligible, which is in accordance with what most of the parasitological studies have proven about the minimal role of the fish sex in infection with GIT worms. Similar conclusions were reported for the GIT helminth community of LMB (Chapter 2).

3) Diversity and community structure

In this study, the diversity of the LWF-GIT helminth community in each

spawning stock was determined not only by species richness, but also with Simpson Reciprocal Diversity Index (SRDI) and Shannon-Wiener Diversity Index (SWI), both of which take abundance and evenness of the species present into consideration, together with the Berger-Parker Dominance Index (B-P), which measures the proportion occupied by the dominant species. This approach was successful in shedding light on the important characteristics of the LWF-GIT helminth community. It is evident that the LWF-GIT helminth community is species poor, with relatively low diversity and two dominant species. No competitions were found among the helminth species as there have been no negative correlations. On the contrary, there have been numerous positive correlations among the helminth species that varied by year, season, and sampling sites. This is probably due to sharing of the macroinvertebrate intermediate hosts.

It seems that the LWF-GIT helminth community structure is not constant, rather in a dynamic status as it changes both spatially and temporally. Thus, while the diversity and dominance indices can determine the community structure at the time of sampling, the generated data cannot be used in predicting the composition of the GIT helminth community in the same species and sampling site. The findings of the present study corroborate with those of Goater et al. (2005), who studied LWF parasite community structure in North America, and those of Karvonen and Valtonen (2004), who studied parasite community structure in the common whitefish (*Coregonus lavaretus*).

Diversity also exhibited spatial and temporal variations. Winter samples of this study had higher diversity when compared to spring or summer samples. Likewise, samples collected in the third year of the study had higher diversity than those of the first and second years. This increased diversity can be explained by the decline of the

prevalence of the two core species (*A. dirus* and *C. truncatus*), thereby increasing the relative contribution of the rare species in the calculations of both diversity indices. This same explanation applies to the increased SWI value for Lake Michigan LWF stocks, as these stocks had lower prevalence of the two core species compared to Lake Huron stocks, thereby increasing the contribution of the rare species to the SWI value. Since the SWI does not amplify the contribution of the core species to the index value like Simpson Diversity Index does, the increased diversity in Lake Michigan was demonstrated by the former but not by the latter index.

The NMDS scores clearly demonstrated the absence of clear trends on how the GIT helminth community structure and species composition vary spatially or temporally (Figure 4.7), a matter that support the notion that community structure of fish parasite is rather stochastic and not the result of a predictable patterns (Kennedy 2009). When all of the generated data were pooled together in the NMDS score plot (Figure 4.8), it was clear that BBN, DET, and CHB are identical as they group together, while the NAB stock was different than the other three stocks. This is surprising considering the close proximity of the sampling sites and the fact that the helminth species identified in this study were all generalists in nature, existing in multiple species in lakes Huron and Michigan (Hoffman 1999).

In conclusion, the data generated in this study is of importance to fishery managers as they deal with one of the most economically important fisheries in the Great Lakes. The presented data represents the most comprehensive parasitological study ever conducted on LWF in the Great Lakes and will be an ideal baseline for future studies aiming at studying the role of GIT helminths in LWF growth and condition. The sites

from which LWF were collected were never examined previously for GIT worms. Therefore, most of these findings should be considered as new geographical range extensions for the five parasite species.

4) Declaration of new geographic range

a. *Acanthocephalus dirus* Van Cleave, 1931

Prevalence: 28.43 (SE=1.26)

Site of infection: Intestine

Type host: Lake whitefish

Other reported hosts: *Catostomus commersoni, Ictalurus melas, Lepomis cyanellus, L. macrochirus, Micropterus salmoides, Notemigonus crysoleucas, Oncorhynchus mykiss, Phnephales promelas, Semotilus atromaculatus, S. margarita, Fundulus notatus, Notropis atherinoides, and Pomoxis annularis*

New location(s) based on the present study: Samples were collected from the areas served by the fishing ports of Big Bay de Noc and Naubinway (Lake Michigan) and Cheboygan and De Tour Village (Lake Huron)

Other reported localities: Wisconsin, Indiana, Illinois, Kansas, and Kentucky

Representative publications: Camp et al. 1999; Sparkes et al. 2004

Specimen deposited: Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1361

b. *Neoechinorhynchus tumidus* Van Cleave and Bangham, 1949

Prevalence: 8.57% (SE=0.78)

Site of infection: Intestine

Type host: Lake whitefish

Other reported hosts: *Coregonus artedii, Prosopium cylindraceum, Salvelinus namaycush X Salvelinus fontinalis*

New location(s) based on the present study: Samples were collected from the areas served by the ports of Big Bay de Noc and Naubinway (Lake Michigan) and Cheboygan and De Tour Village (Lake Huron)

Other reported localities: Aishihik Lake in the Yukon Territory, Canada

Representative publications: Arthur et al. 1976

Specimen deposited: Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1357

c. ***Echinorhynchus salmonis*** **Müller, 1784**

***Prevalence*:** 4.60% (SE=0.58)

***Site of infection*:** Intestine

Type host: Lake whitefish

***Other reported hosts*:** *Osmerus mordax, Oncorhynchus kisutch, O. tshawytscha, O. gorbuscha, O. mykiss, Salvelinus namaycush, Petromyzon marinus, Acipenser fulvescens, Ambloplites rupestris, Catostomus catostomus, C. commersoni, Couesius plumbeus, Coregonus* spp., *Lepomis gibbosus, Lota lota, Micropterus dolomieu, M. salmoides, Notropis hudsonius, Perca flavescens, Percopsis omiscomaycus, Stizostedion canadense ,and Trigonopsis thompsoni*

***New location(s) based on the present study*:** Samples were collected from the areas served by the ports of Big Bay de Noc and Naubinway (Lake Michigan) and Cheboygan and De Tour Village (Lake Huron)

Other reported localities: Europe, Ontario, Canada, Lake Huron, Michigan and Wisconsin, USA

Representative publications: McLain 1951; Applegate 1950; Bangham 1955; Tedla and Fernando 1969; Amin 1981, 1985*b*; Muzzall and Peebles 1986, 1988; Arai 1989; Hoffman 1999; Muzzall and Bowen 2000; Muzzall et al. 2003

***Specimen deposited*:** Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1358

d. *Cyathocephalus truncatus* Pallas, 1781

Prevalence: 13.16% (SE=0.94)

Site of infection: Pyloric caeca and anterior portion of the intestine

Type host: Lake whitefish

Other reported hosts: *Coregonus* spp. including LWF, *Cottus asper, Esox lucius, Gasterosteus aculeatus, Lota lota, Oncorhynchus clarki, O. gorbuscha, O. kisutch, O. mykiss, Osmerus mordax, Perca flavescens, Prosopium cylindraceum, Salmo trutta, Salvelinus alpinus, S. namaycush*

New location(s) based on the present study: Samples were collected from the areas served by the ports of Big Bay De Noc and Naubinway (Lake Michigan) and Cheboygan and De Tour village (Lake Huron)

Other reported localities: Alaska, Montana, Oregon, Michigan, Wisconsin, British Columbia, and Ontario

Representative publications: Alexander 1960; Amin 1977*a*, 1977*b*; Arthur et al. 1976; Bangham 1955; Bangham and Adams 1954; Dechtiar 1972; Leong and Holmes 1981; Mudry and McCarty 1976; Neiland 1952; Pearse 1924; Wardle 1932*a*, 1932*b*; Watson and Dick 1980

Specimen deposited: Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1359

e. *Bothriocephalus* sp.

Prevalence: 10.98 (SE=0.87)

Site of infection: Intestine

Type host: Lake whitefish

Other reported hosts: *Apollonia melanostoma* (formerly *Neogobius melanostomus*), *Cyprinus carpio*

New location(s) based on the present study: Samples were collected from the areas served by the ports of Big Bay De Noc and Naubinway (Lake Michigan) and Cheboygan and De Tour village (Lake Huron)

Other reported localities: Southern upland, Nova Scotia, Canada

Representative publications: Marcogliese and Cone 1997

Specimen deposited: Parasite Collection of the Department of Zoology, Michigan State University, accession # MSUIZ 1360

Year	Season	Fish	Infected	Helminths	Fish	Infected	Helminths
				Lake Michigan			
			Big Bay de Noc			**Naubinway**	
2004	Fall	35	19	306	30	0	0
	Winter	29	10	184	NS	NS	NS
	Spring	30	24	1261	30	12	179
	Summer	22	17	507	20	5	78
2005	Fall	26	21	412	30	12	248
	Winter	16	5	103	30	1	5
	Spring	30	30	1099	30	21	316
	Summer	30	3	54	28	2	9
2006	Fall	30	12	68	30	3	4
	Winter	30	0	0	23	0	0
	Spring	30	18	333	30	9	65
	Summer	30	6	18	30	0	0
				Lake Huron			
			Cheboygan			**De Tour Village**	
2004	Fall	30	4	51	34	3	56
	Winter	32	5	154	10	3	92
	Spring	20	17	2465	30	23	1597
	Summer	30	21	363	20	17	476
2005	Fall	26	0	0	30	21	363
	Winter	15	3	85	30	0	0
	Spring	30	28	3896	30	29	3006
	Summer	28	23	1415	30	25	471
2006	Fall	NS	NS	NS	30	20	273
	Winter	30	0	0	30	0	0
	Spring	30	21	264	30	13	420
	Summer	30	5	31	30	23	296

Table 4.1. Number of lake whitefish (*Coregonus clupleaformis*) examined (Fish), number of lake whitefish infected with gastrointestinal tract helminths (Infected), and total number of helminths found in infected individuals (Helminths) in lake whitefish by year and season for lake whitefish collected from Big Bay de Noc (Lake Michigan), Naubinway (Lake Michigan), Cheboygan (Lake Huron), and De Tour Village (Lake Huron) spawning stocks. NS = no sampling conducted from that site during that particular year and season.

Model	Main effects	First-order interactions
1	Intercept Only	(none)
2	Lake	(none)
3	Site	(none)
4	Year	(none)
5	Season	(none)
6	Lake, Year	(none)
7	Lake, Season	(none)
8	Site, Year	(none)
9	Site, Season	(none)
10	Year, Season	(none)
11	Lake, Year	Lake×Year
12	Lake, Season	Lake×Season
13	Site, Year	Site×Year
14	Site, Season	Site×Season
15	Year, Season	Year×Season
16	Lake, Year, Season	(none)
17	Site, Year, Season	(none)
18	Lake, Year, Season	Lake×Year
19	Lake, Year, Season	Lake×Season
20	Lake, Year, Season	Year×Season
21	Lake, Year, Season	Lake×Year, Lake×Season
22	Lake, Year, Season	Lake×Year, Year×Season
23	Lake, Year, Season	Lake×Season, Year×Season
24	Lake, Year, Season	Lake×Year, Lake×Season, Year×Season
25	Site, Year, Season	Site×Year
26	Site, Year, Season	Site×Season
27	Site, Year, Season	Year×Season
28	Site, Year, Season	Site ×Year, Site×Season
29	Site, Year, Season	Site ×Year, Year×Season
30	Site, Year, Season	Site ×Season, Year×Season
31	Site, Year, Season	Site×Year, Site×Season, Year×Season

Table 4.2. Listing of main effects and first-order interactions between main effects for each of the 31 models that were fit to the lake whitefish (*Coregonus clupleaformis*) gastrointestinal tract helminth data (Lake = Lake Huron or Lake Michigan; Site = sampling site; Year = Year in which sampling occurred; Season = Season in which sampling occurred).

		Lake		Sampling Site				Sex	
	Total	Michigan	Huron	BBN	NAB	CHB	DET	Female	Male
Combined									
Prevalence (%)	41.59 (1.38)	35.44 (1.88)	48.87 (1.98)	48.82 (2.72)	20.90 (2.31)	42.19 (2.85)	52.99 (2.74)	45.69 (2.09)	37.67 (1.91)
Abundance (#/fish)	16.37 (1.47)	8.09 (0.92)	24.84 (2.77)	12.86 (1.63)	2.91 (0.61)	28.98 (4.81)	21.11 (3.00)	18.46 (2.30)	13.47 (1.84)
Intensity (#/infected fish)	39.37 (3.28)	22.82 (2.29)	51.88 (5.39)	26.33 (3.00)	13.91 (2.49)	68.69 (10.44)	39.83 (5.27)	40.40 (4.69)	35.75 (4.53)
A. dirus									
Prevalence (%)	28.43 (1.26)	20.80 (1.59)	36.22 (1.91)	34.32 (2.59)	6.11 (1.36)	30.90 (2.67)	41.02 (2.70)	31.81 (1.95)	25.12 (1.71)
Abundance (#/fish)	7.82 (0.78)	3.84 (0.57)	11.89 (1.46)	6.70 (1.02)	0.73 (0.34)	14.16 (2.45)	9.84 (1.68)	9.61 (1.39)	6.13 (0.93)
Intensity (#/infected fish)	27.50 (2.48)	18.44 (2.33)	32.82 (3.65)	19.51 (2.58)	11.89 (4.93)	45.84 (6.91)	23.99 (3.77)	30.20 (3.96)	24.42 (3.31)
N. tumidus									
Prevalence (%)	8.57 (0.78)	6.16 (0.94)	11.02 (1.24)	8.28 (1.50)	3.86 (1.09)	8.31 (1.59)	13.47 (1.87)	8.08 (1.14)	9.30 (1.14)
Abundance (#/fish)	0.70 (0.10)	0.46 (0.10)	0.94 (0.16)	0.64 (0.16)	0.26 (0.13)	0.59 (0.14)	1.27 (0.28)	0.76 (0.18)	0.66 (0.11)
Intensity (#/infected fish)	8.16 (0.85)	7.45 (1.25)	8.57 (1.14)	7.71 (1.31)	6.68 (2.91)	7.08 (0.98)	9.40 (1.68)	9.37 (1.77)	7.10 (0.74)
E. salmonis									
Prevalence (%)	4.60 (0.58)	1.39 (0.46)	7.87 (1.07)	2.66 (0.88)	0.00 (0.00)	9.30 (1.68)	6.59 (1.36)	5.27 (0.94)	4.50 (0.82)
Abundance (#/fish)	0.22 (0.04)	0.07 (0.03)	0.38 (0.07)	0.13 (0.06)	0.00 (0.00)	0.44 (0.11)	0.33 (0.08)	0.25 (0.06)	0.22 (0.05)
Intensity (#/infected fish)	4.83 (050)	5.00 (1.44)	4.80 (0.53)	5.00 (1.44)	0.00 (0.00)	4.68 (0.77)	4.95 (0.73)	4.80 (0.65)	4.86 (0.77)
C. truncatus									
Prevalence (%)	13.16 (0.94)	10.17 (1.19)	16.22 (1.46)	12.72 (1.82)	7.40 (1.49)	14.95 (2.06)	17.37 (2.08)	14.94 (1.50)	11.01 (1.23)
Abundance (#/fish)	6.98 (0.97)	3.21 (0.64)	10.83 (1.84)	4.66 (1.15)	1.63 (0.47)	13.25 (3.23)	8.65 (1.94)	7.05 (1.34)	5.92 (1.28)
Intensity (#/infected fish)	53.01 (6.34)	31.56 (5.14)	66.76 (9.65)	36.66 (7.40)	22.04 (4.70)	88.62 (17.99)	49.79 (9.51)	47.20 (7.70)	53.80 (10.00)
***Bothriocephalus* spp.**									
Prevalence (%)	10.98 (0.87)	10.48 (1.20)	11.50 (1.27)	11.54 (1.74)	9.32 (1.65)	9.97 (1.73)	12.87 (1.84)	13.01 (1.41)	8.68 (1.11)
Abundance (#/fish)	0.66 (0.08)	0.51 (0.09)	0.80 (0.14)	0.72 (0.16)	0.29 (0.08)	0.55 (0.14)	1.03 (0.24)	0.79 (0.14)	0.53 (0.10)
Intensity (#/infected fish)	5.98 (0.60)	4.91 (0.67)	6.97 (0.98)	6.28 (0.98)	3.07 (0.72)	5.50 (1.02)	8.00 (1.49)	6.07 (0.90)	6.16 (0.88)

Table 4.3. Prevalence, abundance, and intensity of infection for individual lake whitefish (*Coregonus clupleaformis*) gastrointestinal tract helminth species and combined across helminth species by lake, site, and sex (pooled across year and season). The numbers in the parentheses are standard errors. (BBN = Big Bay de Noc spawning site, CHB = Cheboygan spawning site, DET = De Tour Village spawning site, NAB = Naubinway spawning site).

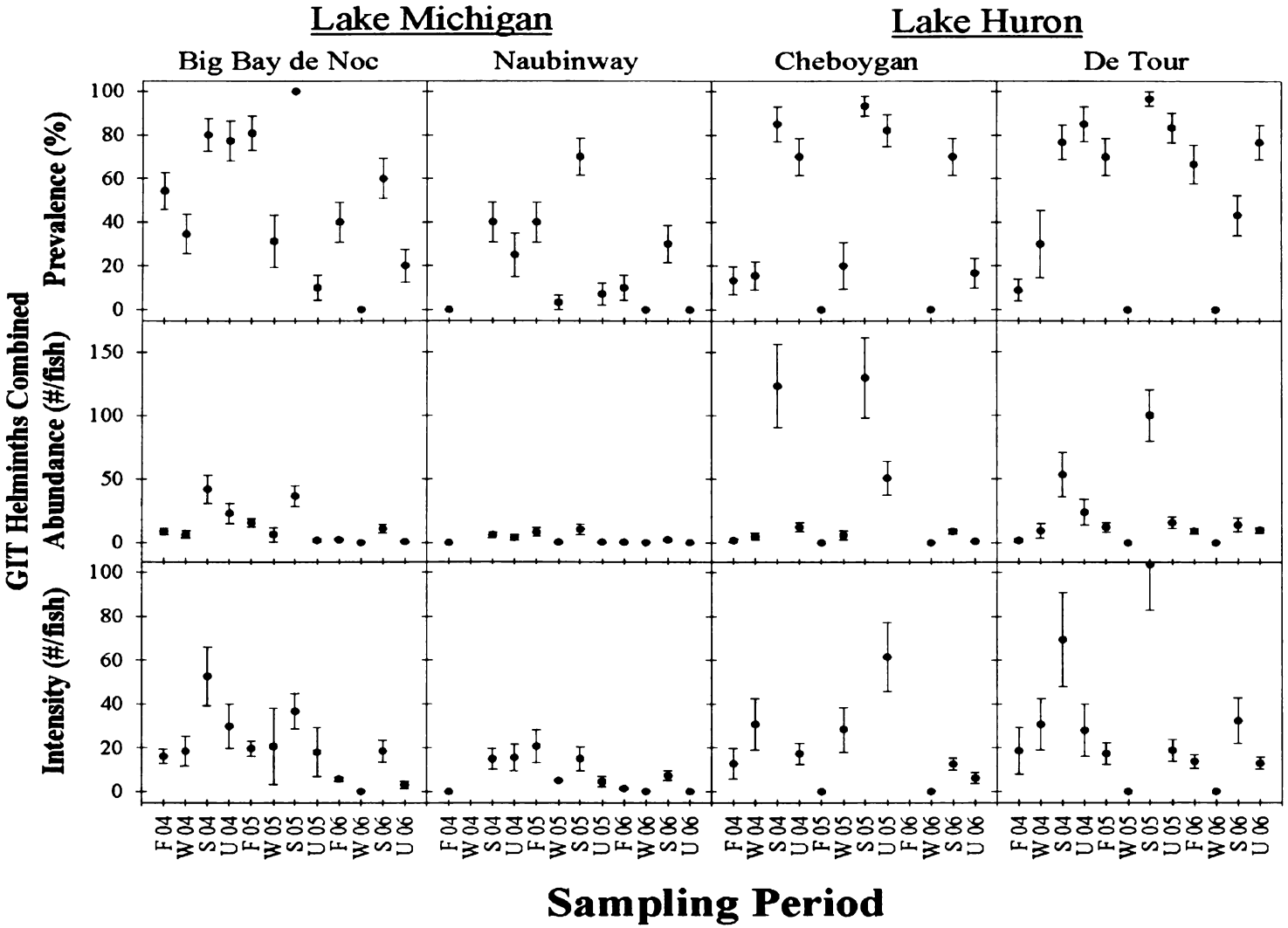


Figure 4.1. Combined gastrointestinal tract helminth prevalence, abundance, and intensity (± SE) by sampling occasion for lake whitefish (*Coregonus clupeaformis*) collected from the Big Bay de Noc, Naubinway, Cheboygan, and De Tour Village sampling sites (04 = 2004, 05 = 2005, 06 = 2006, F = fall, W = winter, S = spring, U = summer).

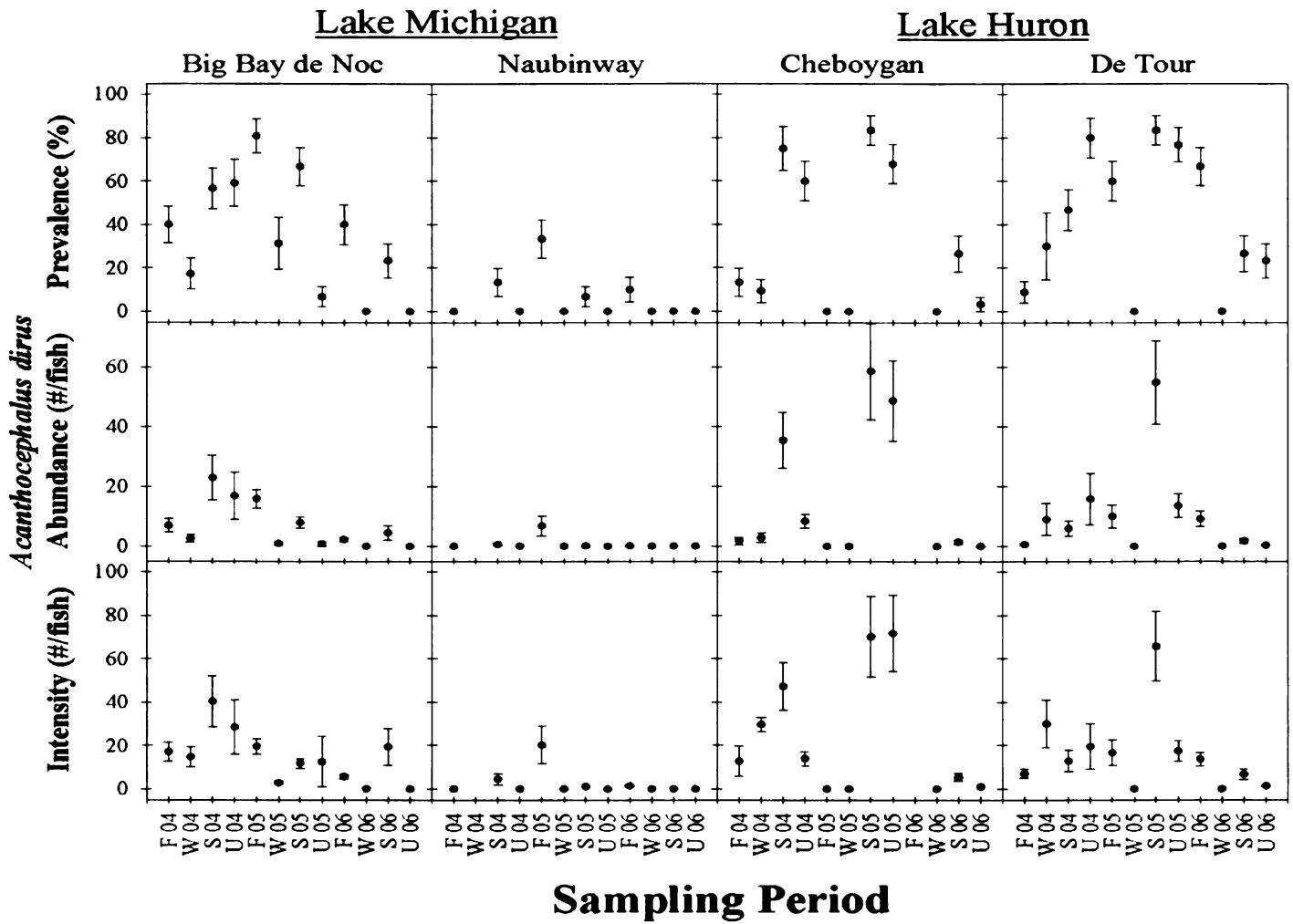


Figure 4.2. ***Acanthocephalus dirus*** **prevalence, abundance, and intensity (± SE) by sampling occasion for lake whitefish (*Coregonus clupeaformis*) collected from the Big Bay de Noc, Naubinway, Cheboygan, and De Tour Village sampling sites (04 = 2004, 05 = 2005, 06 = 2006, F = fall, W = winter, S = spring, U = summer).**

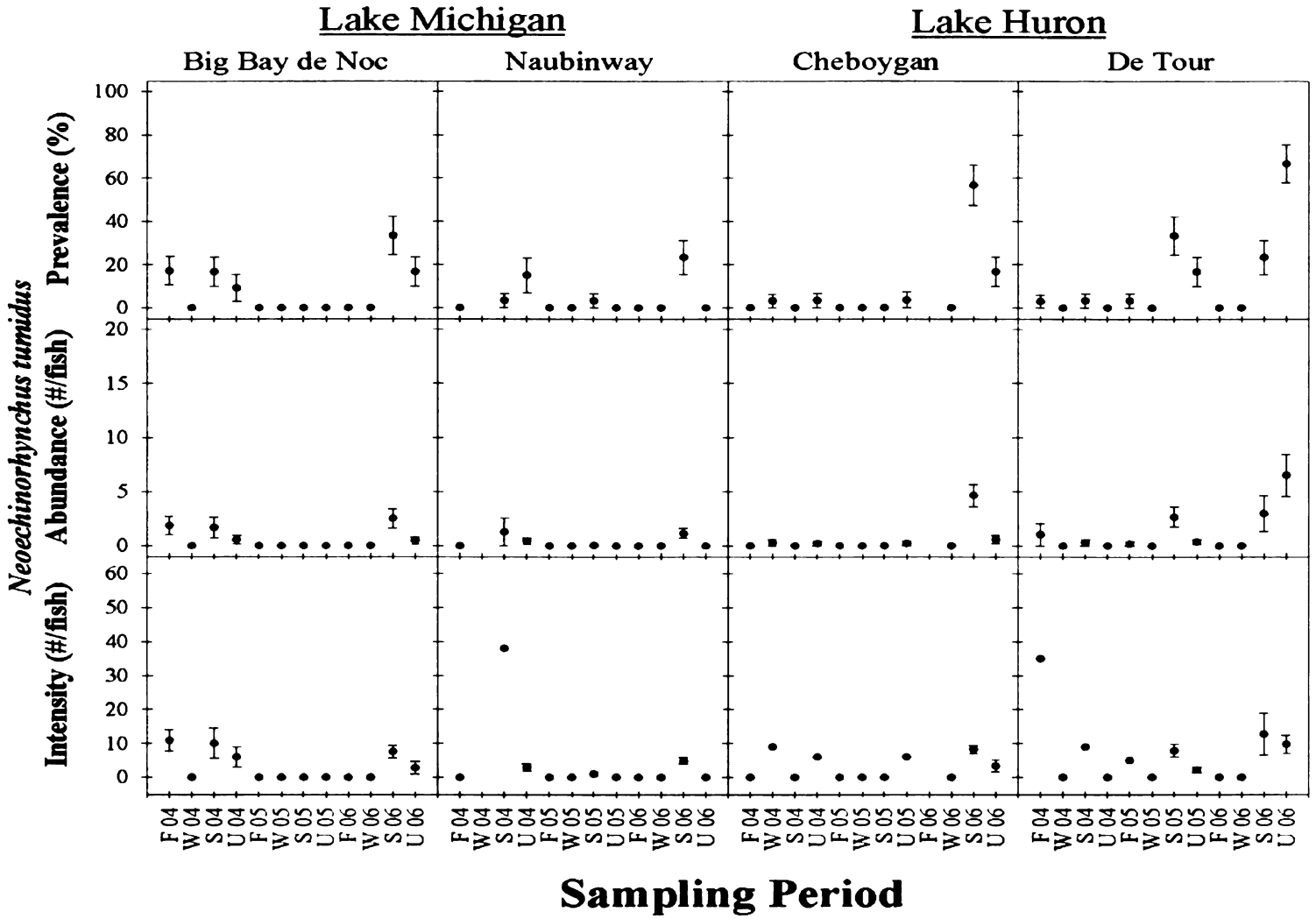


Figure 4.3. ***Neoechinorhynchus tumidus*** **prevalence, abundance, and intensity (± SE) by sampling occasion for lake whitefish (*Coregonus clupeaformis*) collected from the Big Bay de Noc, Naubinway, Cheboygan, and De Tour Village sampling sites (04 = 2004, 05 = 2005, 06 = 2006, F = fall, W = winter, S = spring, U = summer).**

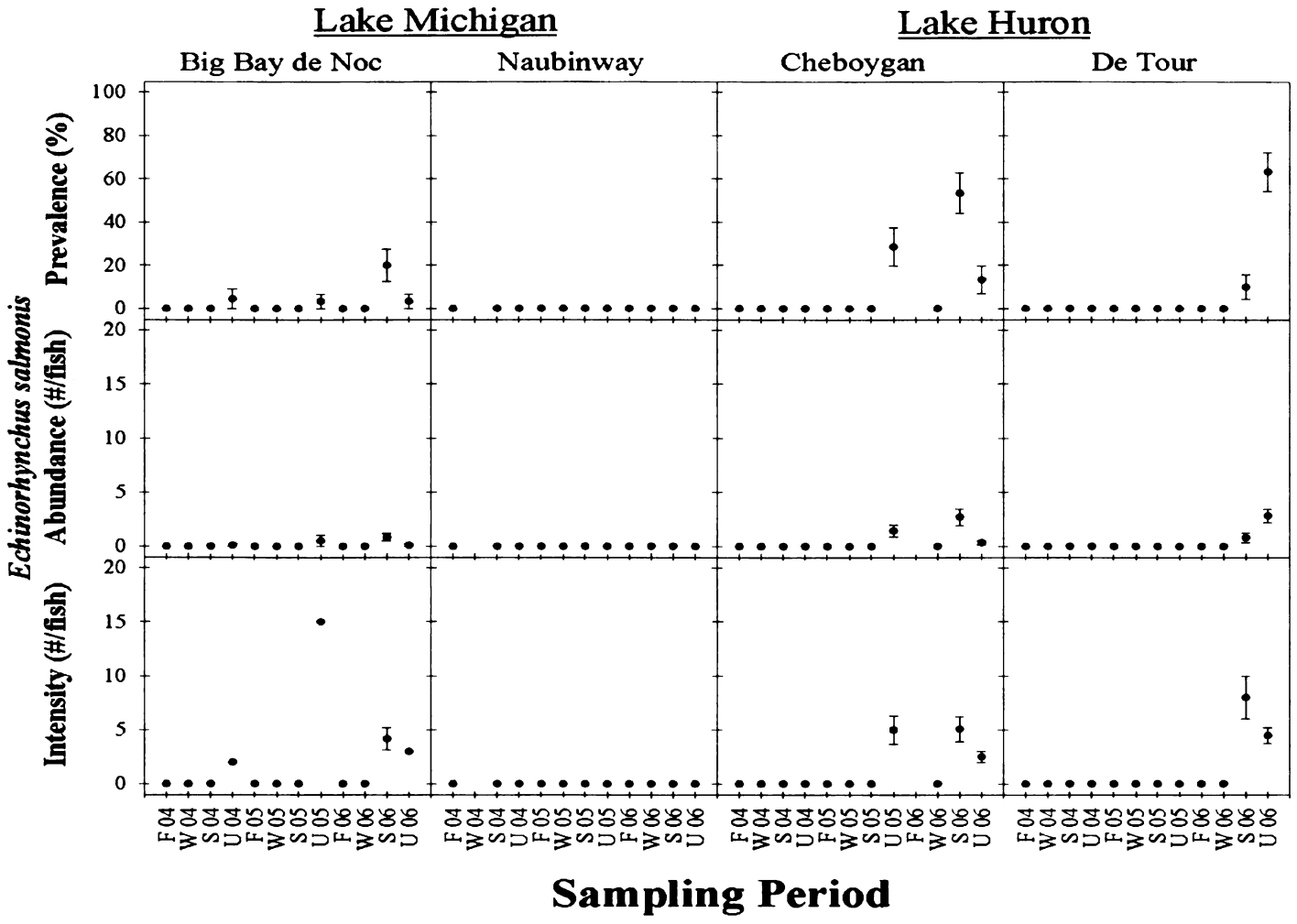


Figure 4.4. ***Echinorhynchus salmonis*** **prevalence, abundance, and intensity (± SE) by sampling occasion for lake whitefish (*Coregonus clupleaformis*) collected from the Big Bay de Noc, Naubinway, Cheboygan, and De Tour Village sampling sites (04 = 2004, 05 = 2005, 06 = 2006, F = fall, W = winter, S = spring, U = summer).**

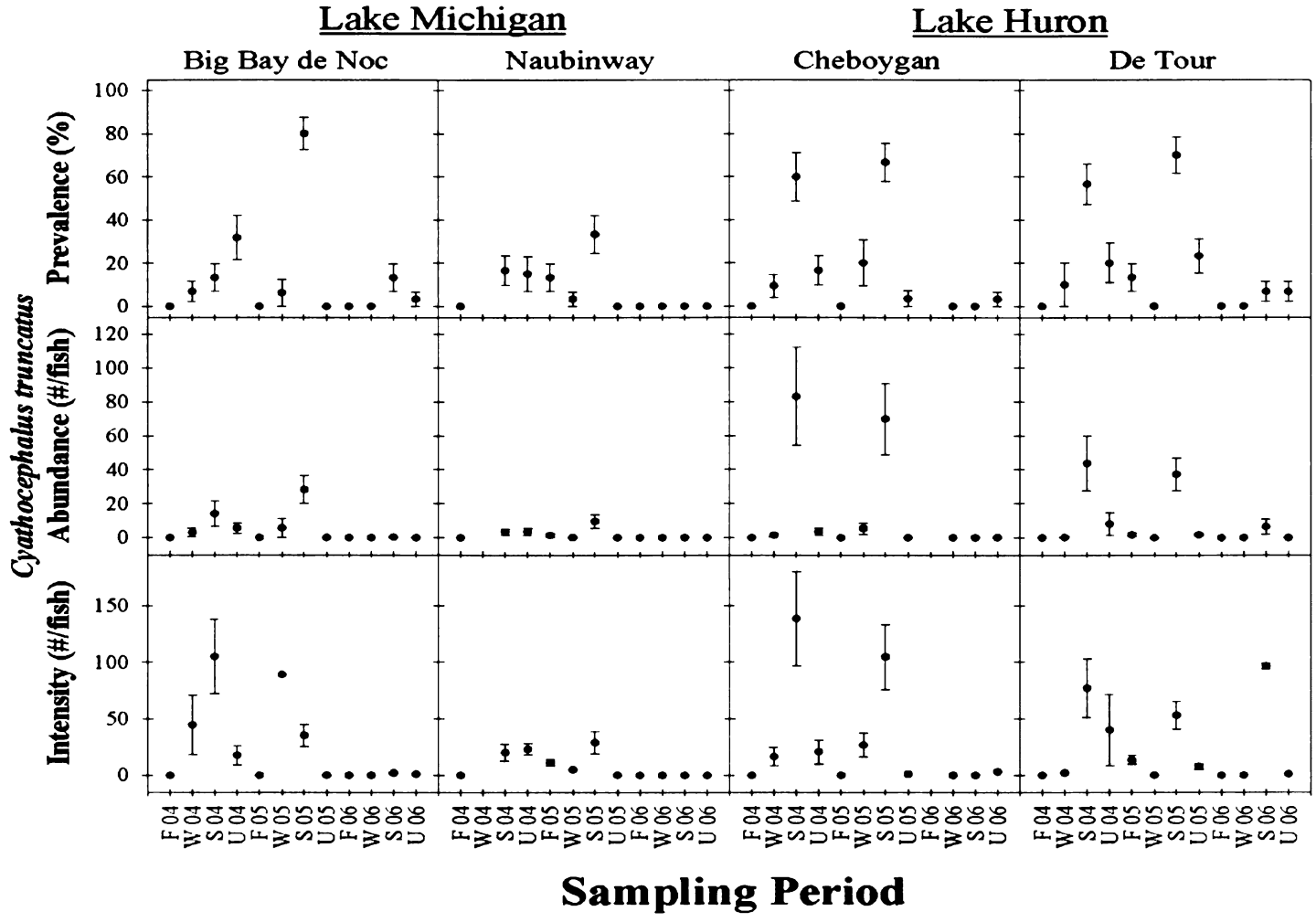


Figure 4.5. ***Cyathocephalus truncatus*** **prevalence, abundance, and intensity (± SE) by sampling occasion for lake whitefish (*Coregonus clupeaformis*) collected from the Big Bay de Noc, Naubinway, Cheboygan, and De Tour Village sampling sites (04 = 2004, 05 = 2005, 06 = 2006, F = fall, W = winter, S = spring, U = summer).**

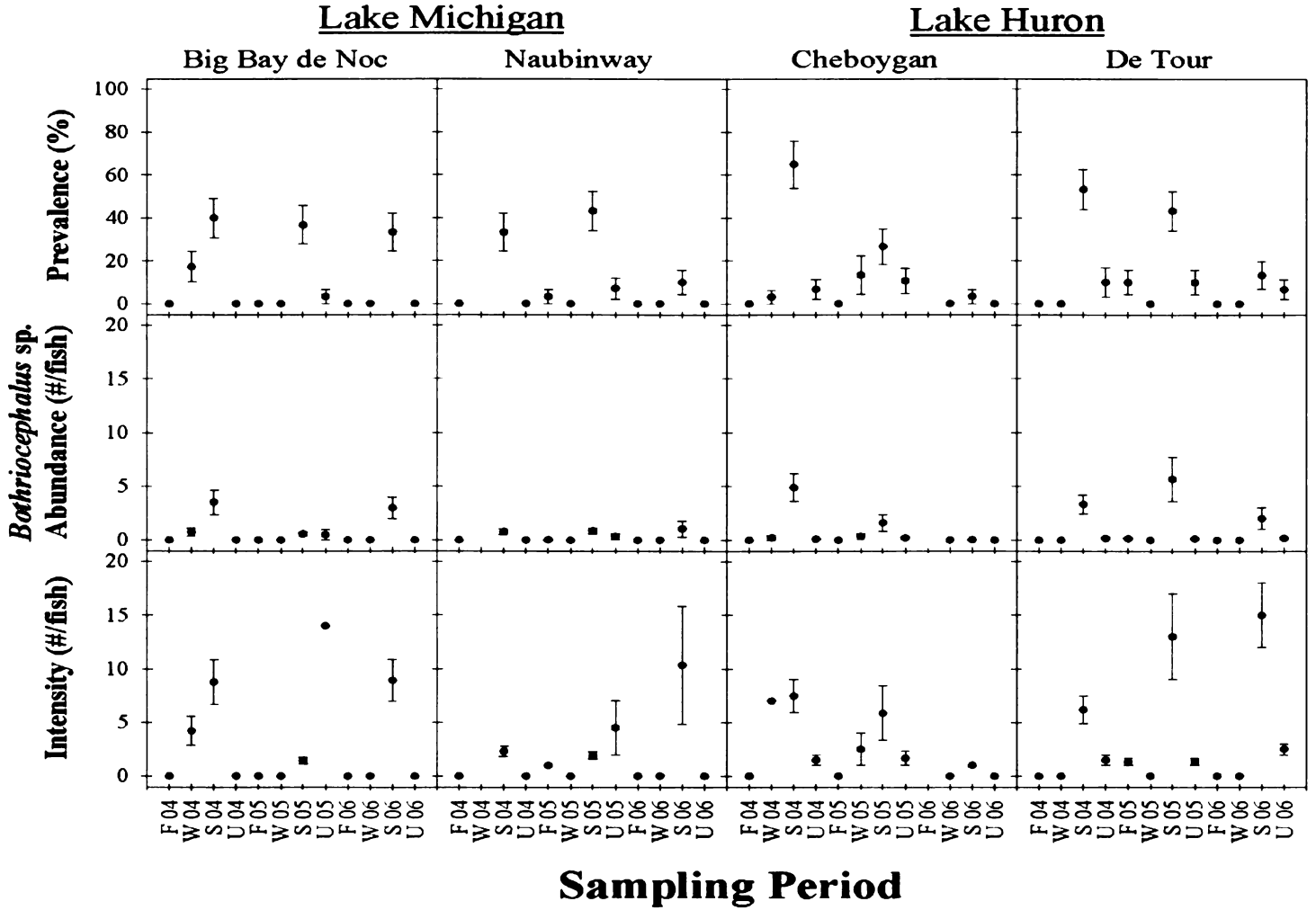
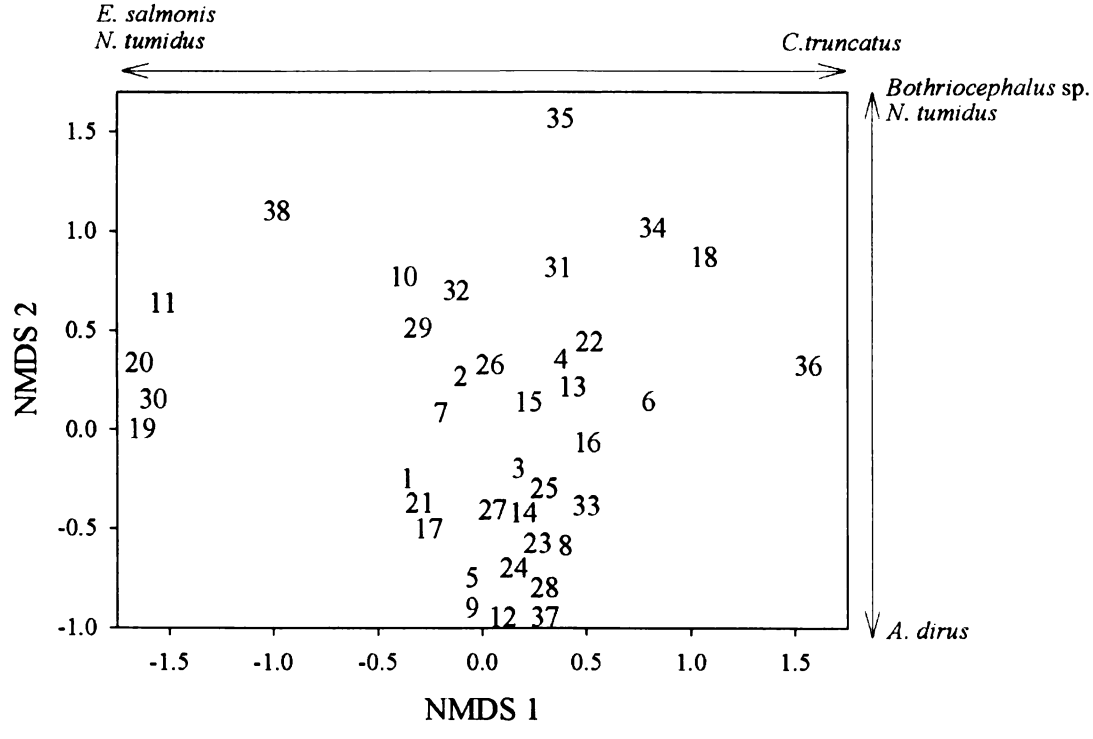


Figure 4.6. ***Bothriocephalus*** **sp. prevalence, abundance, and intensity (± SE) by sampling occasion for lake whitefish (*Coregonus clupeaformis*) collected from the Big Bay de Noc, Naubinway, Cheboygan, and De Tour Village sampling sites (04 = 2004, 05 = 2005, 06 = 2006, F = fall, W = winter, S = spring, U = summer).**



1 = B4F 7 = B5U 13 = C4S 19 = C6S 25 = D5F 31 = N4S 37 = N6F
2 = B4S 8 = B5W 14 = C4U 20 = C6U 26 = D5S 32 = N4U 38 = N6S
3 = B4U 9 = B6F 15 = C4W 21 = D4F 27 = D5U 33 = N5F
4 = B4W 10 = B6S 16 = C5S 22 = D4S 28 = D6F 34 = N5S
5 = B5F 11 = B6U 17 = C5U 23 = D4U 29 = D6S 35 = N5U
6 = B5S 12 = C4F 18 = C5W 24 = D4W 30 = D6U 36 = N5W

Figure 4.7. Ordination from nonmetric multidimensional scaling (NMDS) analysis of gastrointestinal tract helminth structure from collected lake whitefish (*Coregonus clupleaformis*) by spawning site and sampling occasion. Plotted labels identify the score centroids for the spawning sites and sampling occasions (B = Big Bay de Noc, C = Cheboygan, D = De Tour Village, N = Naubinway), sampling year (04 = 2004, 05 = 2005, 06 = 2006) and sampling season (F= fall, W = winter, S = spring, U = summer).

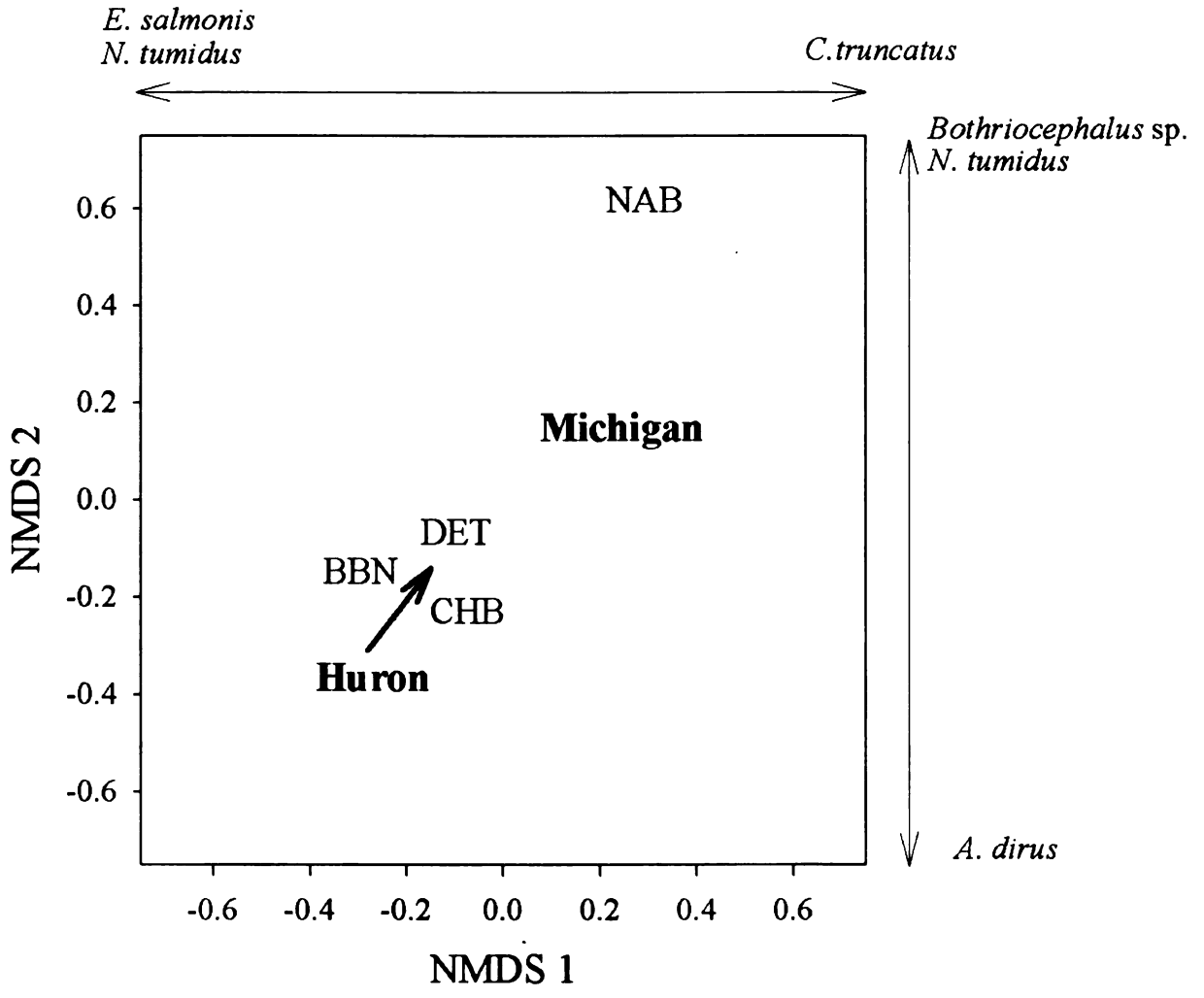


Figure 4.8. Ordination from nonmetric multidimensional scaling (NMDS) analysis of gastrointestinal tract helminth structure from collected lake whitefish (*Coregonus clupeaformis*) by spawning site and lake. Plotted labels identify the score centroids for the spawning sites (BBN = Big Bay de Noc, CHB = Cheboygan, DET = De Tour Village, NAB = Naubinway) and lakes sampling year (Huron = Lake Huron, Mich = Lake Michigan). Individual helminths with the most strongly positive and negative loadings on the NMDS axes are shown.

CONCLUSIONS AND FUTURE RESEARCH

For over a century, researchers from Great Lakes U.S. states and Canadian provinces have generated valuable information regarding the taxonomy of parasites of Great Lakes fish species and their abundance. Unfortunately, relatively little attention has been given to the assemblages these parasites form in their hosts at the population and community levels, details of the life cycles of many of these parasites, and their impacts on infected hosts. A part of this lack of knowledge is due to the absence of strong infrastructure in fish health diagnostics and research, as well as relative lack of funds to support research on fish health in general and parasites in particular.

Similarly, Great Lakes fishery agencies, scientists, biologists, and managers have done an excellent job in identifying fish species, understanding their population dynamics, and estimating fishing pressure and natural mortality. Unfortunately, there have been many challenges of unprecedented magnitude that ravaged the Great Lakes and their fisheries: habitat degradation, pollution, parasitism by sea lamprey, invasion by non-native dreissenid mussels, and emerging pathogens are just a few examples. The problem is compounded by the limited knowledge on limnological details of the Great Lakes basin. Such details are needed to better understand the interactions between the biotic and abiotic components of the ecosystem. Without this knowledge, effective management strategies for the Great Lakes cannot be developed.

Pioneering studies have recently focused on understanding the factors that affect the pattern and processes of fish parasite community structure and diversity as they mirror the surrounding ecosystem. Unfortunately, in the Great Lakes basin this line of

research is in its infancy. In this context, findings of this study clearly fill several gaps of knowledge regarding parasitism in two important Great Lakes fish species: the largemouth bass (LMB) and the lake whitefish (LWF).

The studies on LMB gastrointestinal tract (GIT) helminths demonstrated a species-poor helminth community that is of relatively low diversity and high dominance. The GIT helminth structure and diversity has varied, however, from one watershed to the other and from one inland lake to the other. A number of factors have been analyzed for their potential effects on the GIT helminth community structure. The statistical models used were sensitive enough to show the slightest trend. The generated information was novel despite some shortcomings. For example, the study focused on the GIT helminth community only, ignoring the community of other parasites, such as those associated with the skin, gills, eyes, and peritoneum. Also, the study was performed on 15 inland lakes only, which are not enough to generalize any trend in data. It was hoped that more limnological data would be available on each of the inland lakes. The dearth of limnological data on the inland lakes in the Great Lakes basin has been an impediment for this study that one cannot overcome. As mentioned, the data generated in this study is filling many gaps and showing trends to be followed on future research on a larger number of inland lakes.

The history of LWF in the Great Lakes embodies both success and failure. While the binational, federal, tribal, and regulatory agencies were able to achieve an increase in LWF abundance after its historic low in 1959, they failed to improve its declining growth and condition. One of the important factors that is missing from the relatively rich body of literature on LWF is the role of pathogens and parasites on the recruitment and growth

of LWF. This is particularly important as the LWF diet is changing from the nutrient-rich *Diporeia* spp. to other items such as dreissends and mollusks. How this diet shift has affected the helminth community and other pathogens is a question that needs to be addressed, yet it is impossible to follow as the baseline information does not exist.

One such helminth is the swimbladder nematode *Cystidicola farionis*. The nematode was believed to never develop to maturity in LWF, yet this study showed LWF can support the transformation of the *C. farionis* infective stage, L_3, into sexually mature male and female worms. The fact that the adult worms cannot find their way out of the pneumatic duct due to its size, allowing passage of the smaller L_3 and eggs only, led to the assumption that the disappearance of heavily infected fish from the same site in two adjacent sampling events is probably due to their death. To prove that this is the case in the four LWF stocks, additional radiological studies seem necessary. Statistical analyses performed in this study were extremely difficult and cumbersome, as the data was accumulated from four sites, at four seasons, and three years. Under this scenario, there have been many overlapping factors that complicated the process of finding out the significant trends in infection parameters. Infection with swimbladder nematodes should be taken seriously, as studies have shown that swimbladder nematodes have devastated eels in Europe. Needless to say, a comprehensive study on LWF and swimbladder nematodes is urgently needed, particularly in assessing their roles on deteriorating fish growth.

Determining the spatial and temporal variations of the GIT helminth community in LWF composed of five species proved not to be an easy task. This complexity necessitated the development of multiple models to find out the best fit for analyses.

Despite the overlapping factors, it was possible to determine that sites in Lake Huron are more infected than those in Lake Michigan, and that spring was the peak season of infection. Most importantly, it showed that the third year of the study witnessed changes not only in the community structure of LWF-GIT helminths, but also in their diversity with three helminth species declining and two species on the rise. The most plausible explanation for this phenomenon is the fact that there are dynamic changes taking place in the macroinvertebrate community in the same sites. The absence of these data, as well as other limnological details at the four sampling sites, has weakened the ability to decipher the mechanisms leading to the changes in parasite diversity.

In general, it is anticipated that the findings of these studies will guide future research in Great Lakes fish diseases and parasites, which will ultimately lead, through better informed management strategies, into a more balanced ecosystem of the bountiful Laurentian Great Lakes.

REFERENCES

Abu-Madi, M.A., J.M. Behnke, J.W. Lewis, and F.S. Gilbert. 2000. Seasonal and site specific variation in the component community structure of intestinal helminths in *Apodemus sylvaticus* from three contrasting habitats in south-east England. Journal of Helminthology, 74: 7-16.

Adel-Meguid, M., G.W. Esch, and H.E. Eure. 1995. The distribution and pathobiology of *Neoechinorhynchus cylindratus* in the intestine of green sunfish, *Lepomis cyanellus*. Parasitology, 111(2): 221-231.

Agresti, A. 2007. An introduction to categorical data analysis, 2nd edition. Hooken, New Jersey: John Wiley & Sons, Inc.

Akhtar, H.K., Z. Zaman, and N. Begum. 1992. Metazoan parasites of *Heteropneustes fossilis* (Bloch). Bangladesh Journal of Zoology, 20: 103-112.

Aldenderfer, M.S. and R.K. Blashfield. 1984. Cluster analysis. Sage Publications, Newbury Park, C.A.

Alexander, C.G. 1960. A survey of parasites of Oregon trout. Report of the Oregon State Game Commission, 1-34.

Aliff, J.V. 1977. Digenetic trematodes from Kentucky fishes. Transactions of the Kentucky Academy of Science, 38: 1-14.

Aliff, J.V., D. Smith, and H. Lucas. 1977. Some metazoan parasites from fishes of middle Georgia. Transactions of the American Microscopical Society, 96: 145-148.

Aloo, P.A. 1999. Ecological studies of helminth parasites of the largemouth bass, *Micropterus salmoides*, from Lake Naivasha and the Oloidien Bay, Kenya. Onderstepoort Journal of Veterinary Research, 66(2): 73-79.

Alves, D.R. and J.L. Luque. 2001. Community ecology of the metazoan parasites of white croaker, *Micropogonias furnieri* (Osteichthyes: Sciaenidae), from the coastal zone of the State of Rio de Janeiro, Brazil. Memórias do Instituto Oswaldo Cruz, Rio de Janeiro, 96: 145-153.

Amin, O.M. 1975*a*. Variability in *Acanthocephalus parksidei* Amin, 1974 (Acanthocephala: Echinorhynchidae). The Journal of Parasitology, 61: 307-317.

Amin, O.M. 1975*b*. Host and seasonal associations of *Acanthocephalus parksidei* Amin, 1974 (Acanthocephala: Echinorhynchidae) in Wisconsin fishes. The Journal of Parasitology, 61: 318-329.

Amin, O.M. 1975*c*. *Acanthocephalus parksidei* sp. n. (Acanthocephala: Echinorhynchidae) from Wisconsin fishes. The Journal of Parasitology, 61(2): 301-306.

Amin, O.M. 1977*a*. Distribution of fish parasites from two southeast Wisconsin streams. Wisconsin Academy of Science, 65: 225-230.

Amin, O.M. 1977*b*. Helminth parasites of some southwestern Lake Michigan fishes. Proceedings of the Helminthological Society of Washington, 44(2): 210-217.

Amin, O.M. 1981. The seasonal distribution of *Echinorhynchus salmonis* (Acanthocephala: Echinorhynchidae) among rainbow smelt, *Osmerus mordax* Mitchell, in Lake Michigan. Journal of Fish Biology, 19: 467-474.

Amin, O.M. 1985*a*. Classification. Pages 27-72 *in* D.W.T. Crompton and B.B. Nickol, editors. Biology of the Acanthocephala. Cambridge University Press, Cambridge.

Amin, O.M. 1985*b*. The relationship between the size of some salmonid fishes and the intensity of their acanthocephalan infections. Canadian Journal of Zoology, 63: 924-927.

Amin, O.M. 1986. Acanthocephala from lake fishes in Wisconsin: Host and seasonal distribution of species of the genus *Neoechinorhynchus* Hamann, 1892. The Journal of Parasitology, 72: 111-118.

Amin, O.M. 1988. Acanthocephala from lake fishes in Wisconsin: On the ecology of *Leptorhynchoides thecatus* (Rhadinorhynchidae). Proceedings of the Helminthological Society of Washington, 55: 252-255.

Amin, O.M. 1989. Abnormalities in some helminth parasites of fish. Transactions of the American Microscopical Society, 108: 27-39.

Amin, O.M., R.A. Heckmann, and N.V. Ha. 2004. On the immature stages of *Pallisentis (Pallisentis) celatus* (Acanthocephala: Quadrigyridae) from occasional fish hosts in Vietnam. The Raffles Bulletin of Zoology, 52(2): 593-598.

Amundsen, P.A., R. Knudsen, A.M. Kuris, and R. Kristoffersen. 2003. Seasonal and ontogenetic dynamics in trophic transmission of parasites. Oikos, 102: 285-293.

Anderson, R.M. and D.M. Gordon. 1982. Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. Parasitology, 85: 373–398.

Anderson, R.O. and R.M. Neumann. 1996. Length, weight, and associated structural indices. Pages 353-383 *in* B.R. Murphy and D.W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Anthony, D.D. 1987. Helminth parasites of burbot (*Lota lota*) from Lake Nipissing, Ontario. Program and Abstracts of the 62nd Annual Meeting of American Society of Parasitologists, 2–5 August 1987, Lincoln, Nebraska, 42–43.

Applegate, R.L. and J.W. Mullan. 1967. Food of young largemouth bass, *Micropterus salmoides*, in a new and old reservoir. Transactions of the American Fisheries Society, 96: 74-77.

Applegate, V.C. 1950. Natural history of the sea lamprey (*Petromyzon marinus*) in Michigan. U.S. Fish and Wildlife Service Special Science Report. No. 55, 237 p.

Arai, H.P. 1989. Acanthocephala, Part III. *In* L. Margolis and Z. Kabata, editors. Guide to the Parasites of Fishes of Canada. Fisheries and Oceans, Ottawa, Canada. 95 p.

Arthur, J.R., L. Margolis, and H.P. Arai. 1976. Parasites of fishes of Aishihik and Stevens Lakes, Yukon Territory, and potential consequences of their interlake transfer through a proposed water diversion for hydroelectrical purposes. Journal of the Fisheries Research Board of Canada, 33: 2489–2499.

Bailey, R.M. and G.R. Smith. 1981. Origin and geography of the fish fauna of the Laurentian Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences, 38: 1539–1561.

Baker, J.C. and J.L. Crites. 1976. Parasites of channel catfish, *Ictalurus punctatus* Rafinesque, from the island region of western Lake Erie. Proceedings of the Helminthological Society of Washington, 43: 37-39.

Baldwin, R.E. and C.P. Goater. 2003. Circulation of parasites among fishes from lakes in the Caribou Mountains, Alberta, Canada. The Journal of Parasitology, 89(2): 215-225.

Bangham R.V. 1926. Parasites other than cestodes in black bass of Ohio. The Ohio Journal of Science, 26: 117-127.

Bangham, R.V. 1939. Parasites of Centrarchidae from Southern Florida. Transactions of the American Fisheries Society, 68: 263-268.

Bangham, R.V. 1955. Studies on fish parasites of Lake Huron and Manitoulin Island. American Midland Naturalist, 53: 184-194.

Bangham, R.V. and J.R. Adams. 1954. A survey of the parasites of freshwater fishes from the mainland of British Columbia. Journal of the Fisheries Research Board of Canada, 11: 673-708.

Bangham, R.V. and C.E. Venard. 1942. Studies on parasites of Reelfoot Lake fish. Distribution studies and check-list of parasites. Journal of the Tennessee Academy of Science, 17: 22-38.

Banks, S.M. and D.C. Ashley. 2000. Observations on the internal helminth parasite fauna of largemouth bass, *Micropterus salmoides*, from Smithville Reservoir, Missouri. Journal of Freshwater Ecology, 15(3): 299-306.

Barger, M.A. and G.W. Esch. 2001. Downstream changes in the composition of the parasite community of fishes in an Appalachian stream. The Journal of Parasitology, 87: 250-255.

Barger, M.A. and B.B. Nickol. 1998. Structure of *Leptorhynchoides thecatus* and *Pomphorhynchus bulbocolli* (Acanthocephala) eggs in habitat partitioning and transmission. Journal of Parasitology, 84(3): 534-537.

Bates, D. and M. Maechler. 2010. Lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-33. http://CRAN.R-project.org/package=lme4.

Bauer, O.N. 1987. Guide for identification of parasites of the freshwater Fish Fauna of the USSR, No. 3. Zoological Institute, Academy of Sciences of the USSR, Leningrad.

Becker, D.A. 1978. Pre- and post-impoundment ichthyoparasite succession in a new Arkansas reservoir. Arkansas Water Resources Research Center, University of Arkansas, Fayetteville, Publication No. 54, 85 p.

Becker, D.A., R.G. Heard, and P.D. Holmes. 1966. A pre-impoundment survey of the helminth and copepod parasites of *Micropterus* spp. of Beaver Reservoir in Northwest Arkansas. Transactions of the American Fisheries Society, 95: 23-34.

Begge, A.M., R. Poulin, and E.T. Valtonen. 2003. Fish population size, and not density, as the determining factor of parasite infection: a case study. Parasitology, 128: 305–313.

Behnke, J.M., J.W. Lewis, S.N. Mohd Zain, and F.S. Gilbert. 1999. Helminth infections in *Apodemus sylvaticus* in southern England: interactive effects of host-age, sex and year on prevalence and abundance of infections. Journal of Helminthology, 73: 31-44.

Berger, W.H. and F.L. Parker. 1970. Diversity of planktonic foraminifera in deep sea sediments. Science, 168: 1345–1347.

Bernatchez, L., F. Colombani, and J.J. Dodson. 1991. Phylogenetic relationship among the subfamily Coregoninae as revealed by mitochondrial DNR restriction analysis. Journal of Fish Biology, 39 (Supplement A): 283–290.

Bierbower S.M. and T.C. Sparkes. 2007. Parasite-related pairing success in an intermediate host, *Caecidotea intermedius* (Isopoda): effects of male behavior and reproductive physiology. Journal of Parasitology, 93: 445-449.

Bierman, V.J., J. Kaur, J.V. DePinto, T.J. Feist, and D.W. Dilks. 2005. Modeling the role of zebra mussels in the proliferation of blue-green algae in Saginaw Bay, Lake Huron. Journal of Great Lakes Research, 31(1): 32–55.

Black, G.A. 1983*a*. Origin, distribution, and postglacial dispersal of a swimbladder nematode, *Cystidicola stigmatura*. Canadian Journal of Fisheries and Aquatic Sciences, 40: 1244–1253.

Black, G.A. 1983*b*. Taxonomy of a swimbladder nematode, *Cystidicola stigmatura* (Leidy), and evidence of its decline in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences, 40: 643–647.

Black, G.A. 1984. Swimbladder lesions associated with mature *Cystidicola stigmatura* (Nematoda). The Journal of Parasitology, 70: 441–443.

Black, G.A. and M.W. Lankester. 1980. Migration and development of swimbladder nematodes, *Cystidicola* spp. (Habronematoidea), in their definitive hosts. Canadian Journal of Zoology, 58: 1997–2005.

Black, G.A. and M.W. Lankester. 1981. The transmission, life span, and population biology of *Cystidicola cristivomeri* White, 1941 (Nematoda: Habronematoidea) in char, *Salvelinus* spp. Canadian Journal of Zoology, 59(3): 498-509.

Black, G.A. and M.W. Lankester. 1984. Distribution and biology of swimbladder nematodes *Cystidicola* spp. (Habronematoidea), in charr, *Salvelinus* spp. Pages 395-411 *in* L. Johnson and B. Burns, editors. Biology of the Arctic Charr. Proceedings of the International Symposium on Arctic Charr. University of Manitoba Press, Winnipeg, Canada.

Bolker, B.M., M.E. Brooks, C.J. Clark, S.W. Geange, J.R. Poulsen, M.H.H. Stevens, and J.S.S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology and Evolution, 24: 127-135.

Brinker, H. 2007. Evidence for negative impact of plerocercoid infection of *Triaenophorus nodulosus* on *Perca fluviatilis* L. stock in Upper Lake Constance, a water body undergoing rapid reoligotrophication. Journal of Fish Biology, 71: 129-147.

Brown, R., M. Ebener, and T. Gorenflo. 1999. Great Lakes commercial fisheries: historical overview and prognosis for the future. Pages 307–354 *in* W.W. Taylor and C.P. Ferreri, editors. Great Lakes fisheries policy and management. Michigan State University Press, East Lansing, Michigan.

Bullock, W.L. 1963. Intestinal histology of some salmonids with particular reference to the histopathology of acanthocephalan infection. Journal of Morphology, 112: 23-44.

Burnham, K.P. and D.R. Anderson. 2002. Model selection and inference: a practical information-theoretic approach, 2nd edition. Springer-Verlag, New York.

Buron, D.I. and B.B. Nickol. 1994. Histopathological Effect of the Acanthocephalan *Leptorhynchoides thecatus* in the ceca of the Green Sunfish, *Lepomis cyanellus*. Transactions of the American Microscopical Society, 113: 161-168.

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

Camp, J.W., L.M. Blaney, and D.K. Barnes. 1999. Helminths of the round goby, *Neogobius melanostomus* (Perciformes: Gobiidae), from southern Lake Michigan, Indiana. Journal of the Helminthological Society of Washington, 66: 70-72.

Carlander, K.D. 1977. Handbook of Freshwater Fishery Biology. Vol. 2. Iowa State University Press, Ames, Iowa, USA.

Cheetham, A.H. and J.E. Hazel. 1969. Binary (presence-absence) similarity coefficients. Journal of Paleontology, 43: 1130-1136.

Choudhury, A. and T.A. Dick. 1998. Patterns and determinants of helminth communities in the Acipenseridae (Actinopterygii: Chondrostei), with special reference to the lake sturgeon, *Acipenser fulvescens*. Canadian Journal of Zoology, 76: 330-349.

Choudhury, A. and T.A. Dick. 2000. Richness and diversity of helminth communities in tropical freshwater fishes: Empirical evidence. Journal of Biogeography, 27: 935–956.

Choudhury, A., T.L. Hoffnagle, and R.A. Cole. 2004. Parasites of native and nonnative fishes of the Little Colorado River, Grand Canyon, Arizona. Journal of Parasitology, 90: 1042-1053.

Chubb, J.C. 1970. The parasite fauna of British freshwater fish. Pages 119-144 *in* A.E.R. Taylor and R. Muller, editors. Aspects of Fish Parasitology. Blackwell Scientific Publications, Oxford, U.K.

Clady, M.D. 1974. Food habits of yellow perch, smallmouth bass and largemouth bass in two unproductive lakes in Northern Michigan. American Midland Naturalist, 91: 453- 459.

Cleland, C.E. 1982. The inland shore fishery of the northern Great Lakes: its development and importance in prehistory. Society for American Archaeology, 47: 761–784.

Clers, S. 1993. Modeling the impact of disease-induced mortality on the population size of wild salmonids. Fisheries Research, 17: 237-248.

Cloutman, D.G. 1975. Parasite community structure of largemouth bass, warmouth, and bluegill in Lake Fort Smith, Arkansas. Transactions of the American Fisheries Society, 104: 277–283.

Collins, J.J. and A.O. Dechtiar. 1974. Parasite fauna of kokanee salmon (*O. nerka*) introduced into Lake Huron. Journal of the Fisheries Research Board of Canada, 31: 1818-1821.

Combes, C. 1996. Parasites, biodiversity and ecosystem stability. Biodiversity and Conservation, 5: 953-962.

Cornet, S., N. Franceschi, A. Bauer, T. Rigaud, and Y. Moret. 2009. Immune depression induced by acanthocephalan parasites in their intermediate crustacean host: consequences for the risk of super-infection and links with host behavioural manipulation. International Journal for Parasitology, 39(2): 221-229.

Curtis, M.A. 1988. Determinants in the formation of parasite communities in coregonids. Finnish Fisheries Research, 9: 303-312.

Dechtiar, A.O. 1972. Parasites of fish from Lake of the Woods, Ontario. Journal of the Fisheries Research Board of Canada, 29: 275-283.

Dechtiar, A.O. and W.J. Christie. 1988. Survey of the parasite fauna of Lake Ontario fishes, 1961-1971. Pages 66-95 *in* S.J. Nepszy, editor. Parasites of fishes in the Canadian waters of the Great Lakes. Great Lakes Fishery Commission Technical Report No. 51.

Dechtiar, A.O. and A.H. Lawrie. 1988. Survey of the parasite fauna of Lake Superior fishes, 1969-1975. Pages 1-18 *in* S.J. Nepszy, editor. Parasites of fishes in the Canadian waters of the Great Lakes. Great Lakes Fishery Commission Technical Report No. 51.

Dechtiar, A.O., J.J. Collins, and J.A. Reckahn. 1988. Survey of the parasite fauna of Lake Huron fishes, 1961-1971. Pages 19-48 *in* S.J. Nepszy, editor. Parasites of fishes in the Canadian waters of the Great Lakes. Great Lakes Fishery Commission Technical Report No. 51.

Deutsch, W.G. 1977. Fish parasites from the Susquehanna River in Pennsylvania, with new host records. Proceedings of the Pennsylvania Academy of Science, 51: 122-124.

Dextrase, A.J. 1987. The biology of *Cystidicola farionis* Fischer 1798 (Nematoda: Cystidicolidae) in salmonid fishes. Master of Science thesis, Lakehead University, Thunder Bay, Ontario, Canada.

Dezfuli, B.S., S. Arrighi, C. Domeneghini, and G. Bosi. 2000. Immunohistochemical detection of neuromodulators in the intestine of *Salmo trutta* Linnaeus naturally infected with *Cyathocephalus truncatus* Pallas (Cestoda). Journal of Fish Diseases, 23: 265–273.

Dezfuli, B.S., L. Giari, S. De Biaggi, and R. Poulin. 2001. Associations and interactions among intestinal helminths of the brown trout, *Salmo trutta*, in northern Italy. Journal of Helminthology, 75: 1–6.

Dezfuli, B.S., F. Pironi, L. Giari, C. Domeneghini, and G. Bosi. 2002. Effect of *Pomphorhynchus laevis* (Acanthocephala) on putative neuromodulators in the intestine of naturally infected *Salmo trutta.* Diseases of Aquatic Organisms, 51: 27-35.

Dobson, A.P. 1986. Inequalities in the individual reproductive success of parasites. Parasitology, 92: 675-682.

Dogiel, V.A. 1966. General Parasitology. Oliver and Boyd, Edinburgh and London, 516 p. (English translation by Z. Kabata).

Durborow, R.M., W.A. Rogers, and P.H. Klesius. 1988. Interaction of bass tapeworm, *Proteocephalus ambloplitis*, and *Neoechinorhynchus* sp. (Acanthocephala) in largemouth bass, *Micropterus salmoides*. The Journal of Parasitology, 74: 1056-1059.

Dzeikonska-Rynko, J., J. Rokicki, and Z. Jablonowski. 2003. The activity of selected hydrolases in excretion-secretion products and extracts from larvae and mature specimens of *Cystidicola farionis*. Oceanological and Hydrobiological Studies, 32(4): 117-129.

Ebener, M.P. 1997. Recovery of lake whitefish populations in the Great Lakes: a story of successful management and just plain luck. Fisheries, 22(7): 18-20.

Ebener, M.P. and F.A. Copes. 1985. Population Statistics, Yield Estimates, and Management Considerations for Two Lake Whitefish Stocks in Lake Michigan. North American Journal of Fisheries Management, 5(3b): 435-448.

Ebener, M.P., T.O. Brenden, G.M. Wright, M.L. Jones, and M. Faisal. 2010. Spatial and temporal distributions of lake whitefish spawning stocks in northern lakes Michigan and Huron, 2003-2008. Journal of Great Lakes Research, 36 (Supplement 1): 38-51.

Ebener, M.P., R.E. Kinnunen, L.C. Mohr, P.J. Schneeberger, J.A. Hoyle, and P. Peeters. 2008. Management of commercial fisheries for lake whitefish in the Laurentian Great Lakes of North America. Pages 99-143 *in* M.G. Schechter, W.W. Taylor, N.J. Leonard, editors. International governance of fisheries ecosystems: learning from the past, finding solutions for the future. American Fisheries Society Symposium, Bethesda, Maryland.

El-Matbouli, M., T. Fischer-Scherl, and R.W. Hoffman. 1992. Present knowledge on the life cycle, taxonomy, pathology, and therapy of some *Myxosporea* spp. important for freshwater fish. Annual Review of Fish Diseases, 2: 367-402.

Esch, G.W. 1971. Impact of ecological succession on the parasitic fauna in centrarchids from oligotrophic and eutrophic ecosystems. American Midland Naturalist, 86: 160–168.

Esch, G.W. 1994. Population biology of the diplostomatid trematode *Uvulifer ambloplitis*. Pages 321-335 *in* M.E. Scott and G. Smith, editors. Parasitic and infectious diseases: Epidemiology and Ecology. Academic Press, San Diego.

Esch, G.W. and W.J. Huffins. 1973. Histopathology Associated with Endoparasitic in Bass. The Journal of Parasitology, 59: 306-313.

Eure, H. 1976. Seasonal abundance of *Neoechinorhynchus cylindratus* taken from largemouth bass (*Micropterus salmoides*) in a heated reservoir. Parasitology, 73: 355-370.

Ezenwaji, H.M.G. and P.C.O. Ilozumba. 1992. Helmintho-fauna of four West African small *Clarias* species (Osteichthyes: *Claridae*) from Nigeria. Journal of African Zoology, 106: 391-400.

Faisal, M. and J.G. Hnath. 2005. Fish health and disease issues in the Laurentian Great Lakes. Pages 331–349 *in* R.C. Cipriano, I.S. Shchelkunov, and M. Faisal, editors. Health and Diseases of Aquatic Organisms: Bilateral perspectives. Michigan State University, East Lansing, MI.

Fellis, K.J. and G.W. Esch. 2004. Natural history of the sea lamprey (*Petromyzon marinus*) in Michigan sch. Community Structure and Seasonal Dynamics of Helminth Parasites in *Lepomis cyanellus* and *L. Macrochirus* from Charlie's Pond, North Carolina: Host Size and Species as Determinants of Community Structure. Journal of Parasitology, 90: 41-49.

Fischer, S.A. and W.E. Kelso. 1990. Parasite fauna development in juvenile bluegills and largemouth bass. Transactions of the American Fisheries Society, 119: 877–884.

Fleischer, G.W. 1992. Status of coregonine fishes in the Laurentian Great Lakes. Pages 247–259 *in* T.N. Todd and M. Luczysnki, editors. Biology and management of coregonid fishes, Proceedings of the 4th international symposium on the biology and management of coregonid fishes. Quebec City, Quebec, Canada, August 19–23, 1990. Polskie Archiwum Hydrobiologii 39.

Forstie, M.D. and H.L. Holloway Jr. 1984. Parasites of fish from the James and Sheyenne Rivers, Jamestown Reservoir Complex, and Lake Ashtabula in North Dakota. Prairie Naturalist, 16: 11-20.

Frank R., M. Holdrinet, H.E. Braun, D.P. Dodge, and G.E. Sprangler. 1978. Residues of organochlorine insecticides and polychlorinated biphenyls in fish from Lakes Huron and Superior, Canada-1968-76. Pesticides Monitoring Journal, 12(2): 60-68.

Gash, R. and S.L. Gash. 1973. Helminth fauna of Centrarchidae from two strip-mine lakes and a stream in Crawford County, Kansas. Transactions of the Kansas Academy of Science, 75: 236-244.

Genten, F., E. Terwinghe, and A. Danguy. 2009. Atlas of Fish Histology. Brussels: Université Libre de Bruxelles.

Giæver, A., A. Klemetsen, and O. Halvorsen. 1991. Infection of *Cystidicola farionis* Fischer (Nematoda: Spiruroidea) in the swimbladder of Arctic charr, *Salvelinus alpinus* (L.), from Takvatn, North Norway. Nordic Journal of Freshwater Research, 66: 63-71.

Gillilland III, M.G. and P.M. Muzzall. 2004. Microhabitat analysis of bass tapeworm, *Proteocephalus ambloplitis* (Eucestoda: Proteocephalidae), in smallmouth bass, *Micropterus dolomieu*, and largemouth bass, *Micropterus salmoides*, from Gull Lake, Michigan, U.S.A. Comparative Parasitology, 71: 221-225.

Goater, C.P., R.E. Baldwin, and G.J. Scrimgeour. 2005. Physico-chemical determinants of helminth component community structure in whitefish (*Coregonus clupeaformes*) from adjacent lakes in Northern Alberta, Canada. Parasitology, 131(5): 713-722.

Gollock, M.J., C.R. Kennedy, E.S. Quabius, and J.A. Brown. 2004. The effect of parasitism of European eels with the nematode, *Anguillicola crassus* on the impact of netting and aerial exposure. Aquaculture, 233: 45-54.

Gulland, F.M.D. 1995. Impact of infectious diseases on wild animals. Pages 20-51 *in* B.T. Grenfell and A.P. Dobson, editors. Ecology of Infectious Diseases in Natural Populations. Cambridge University Press, Cambridge.

Gupta, A.K., A. Niyogi, M.L. Naik, and S.M. Agarwal. 1984. Population dynamics of endohelminths of *Channa punctatus* at Raipur, India. Japanese Journal of Parasitology, 33: 105-118.

Hart, J.L. 1931. The growth of the whitefish *Coregonus clupeaformis* (Mitchill) with a note on the parasites. Contributions to Canadian Biology and Fisheries (New Series), 20(4): 429-444.

Hartvigsen, R. and C.R. Kennedy. 1993. Patterns in the composition and richness of helminth communities in brown trout *Salmo trutta* in a group of reservoirs. Journal of Fish Biology, 43: 603-615.

Haukisalmi, V., H. Henttonen, and F. Tenora. 1988. Population dynamics of common and rare helminths in cyclic vole populations. Journal of Animal Ecology, 57: 807-825.

Hazen, T.C. and G.W. Esch. 2006. Observations on the ecology of *Clinostomum marginatum* in largemouth bass (*Micropterus salmoides*). Journal of Fish Biology, 12: 411-420.

Heidinger, A. 2000. Helminth transmission in marine pollution studies. Advances in Parasitology, 35: 86-144.

Higgins, S.N., R.E. Hecky, and S.J. Guildford. 2005. Modeling the growth, biomass, and tissue phosphorus concentration of *Cladophora glomerata* in eastern Lake Erie: model description and field testing. Journal of Great Lakes Research, 31: 439-455.

Hoffman, G. 1999. Parasites of North American Freshwater Fishes, 2nd Edition. Cornell University Press, Ithaca, New York. 539 p.

Holmes, J.C. and P.W. Price. 1986. Communities of parasites. Pages 187-213 *in* D.J. Anderson and J. Kikkawa, editors. Community ecology: Pattern and process. Blackwell Scientific Publications, Oxford, U.K.

Howard, C.N. and J.V. Aliff. 1980. Metazoan parasites of fishes from Piedmont and coastal plain Georgia. Georgia Journal of Science, 8: 173-179.

Hoyle, J.A. 2005. Status of lake whitefish (*Coregonus clupeaformis*) in Lake Ontario and the response to the disappearance of *Diporeia* spp. Pages 47-66 *in* L.C. Mohr and T.F. Nalepa, editors. Proceedings of a workshop on the dynamics of lake whitefish (*Coregonus clupeaformis*) and the amphipod *Diporeia* spp. in the Great Lakes. Great Lakes Fishery Commission Technical Report 66, Ann Arbor, Michigan.

Hoyle, J.A., J.M. Casselman, R. Dermott, and T. Schaner. 1999. Changes in Lake Whitefish (*Coregonus clupeaformis*) stocks in eastern Lake Ontario following *Dreissena* mussel invasion. Great Lakes Research Review, 4: 5-10.

Hubbs, C. 1964. Interactions between a bisexual fish species and its gynogenetic sexual parasite. Bulletin of the Texas Memorial Museum, 8: 1-72.

Hubert, W.A. and M.C. Warner. 1975. Note on the occurrence of *Leuceruthrus micropteri* (Trematoda, Azygiidae) in bass, *Micropterus* spp., from the Tennessee River. Journal of Wildlife Diseases, 11(1): 38-39.

Hudson, P.J. and A.P. Dobson. 1997. Transmission dynamics and host-parasite interactions of *Trichostrongylus tenuis* in red grouse (*Lagopus lagopus scoticus*). Journal of Parasitology, 83: 194-202.

Hudson, P. and J. Greenman. 1998. Competition mediated by parasites: biological and theoretical progress. Trends in Ecology and Evolution, 13: 387-390.

Hùeg, J.T. 1995. The biology and life cycle of the Rhizocephala (Cirripedia). Journal of the Marine Biological Association of the United Kingdom, 75: 517- 550.

Hugghins, E. 1959. Parasites of fishes in South Dakota. South Dakota, Department of Game, Fish and Parks, Agricultural Experiment Station, Bulletin 484, p. 73.

Huh, C., D. Thomas, P. Udomkusonsri, and E.J. Noga. 2005. Epidemic trichodinosis associated with severe epidermal hyperplasia in largemouth bass, *Micropterus salmoides*, from North Carolina, USA. Journal of Wildlife Diseases, 41(3): 647-653.

Hunter, G.W. III and R.V. Bangham. 1933. Studies on the fish parasites of Lake Erie. New Cestoda and Nematoda. Journal of Parasitology, 19: 304-311.

Ingham, R.E. and N.O. Dronen Jr. 1980. Endohelminth parasites from largemouth bass, *Micropterus salmoides*, in Belton and Livingston Reservoirs, Central Texas. Proceedings of the Helminthological Society of Washington, 47: 140-142.

Ingham, R.E. and N.O. Dronen Jr. 1982. Some effects of seasonality on helminthic infection in largemouth bass, *Micropterus salmoides* (Lacepede) (Centrarchidae). Southwestern Naturalist, 27: 223-225.

Janovy, J. Jr., and G.W. Kutish. 1988. A model of encounters between host and parasite populations. Journal of Theoretical Biology, 134(3): 391-401.

Janovy, J. Jr, R.E. Clopton, and T.J. Percival. 1992. The roles of ecological and evolutionary influences in providing structure to parasite species assemblages. Journal of Parasitology, 78(4): 630-640.

Johnson, D.E. 1998. Applied Multivariate Methods for Data Analysts. Duxbury, Pacific Grove, CA.

Johnson, P.T.J., K.B. Lunde, E.G. Ritchie, and A.E. Launer. 1999. The effect of trematode infection on amphibian limb development and survivorship. Science, 284: 802-804.

Joy, J.E. and E. Madan. 1989. Pathology of black bass hepatic tissue infected with larvae of the tapeworm *Proteocephalus ambloplitis.* Journal of Fish Biology, 35: 111-118.

Karen, D.J., R. Draughn, M.H. Fulton, and P. Ross. 1998. Bone strength and acetylcholinesterase inhibition as endpoints in chlorpyrifos toxicity to *Fundulus heteroclitus*. Pesticide Biochemistry and Physiology, 60: 167-175.

Karvonen, A. and E.T. Valtonen. 2004. Helminth assemblages of whitefish (*Coregonus lavaretus*) in interconnected lakes: Similarity as a function of species specific parasites and geographical separation. Journal of Parasitology, 90: 471-476.

Kennedy, C.R. 1978. An analysis of the metazoan parasitocoenoses of brown trout *Salmo trutta* from British Lakes. Journal of Fish Biology, 13: 255-263.

Kennedy, C.R. 1990. Helminth communities in freshwater fish: Structured communities or stochastic assemblages? Pages 131-156 *in* G.W. Esch, A.O. Bush, and J.M. Aho, editors. Parasite communities: Patterns and processes. Chapman and Hall, New York.

Kennedy, C.R. 1993. The dynamics of intestinal helminth communities in eels *Anguilla anguilla* in a small stream: longterm changes in richness and structure. Parasitology, 107: 71-78.

Kennedy, C.R. 2007. The pathogenic helminth parasites of eels. Journal of Fish Diseases, 30(6): 319-334.

Kennedy, C.R. 2009. The ecology of parasites of freshwater fishes: the search for patterns. Parasitology, 136(12): 1653-1662.

Kennedy, C.R. and A.O. Bush. 1994. The relationship between pattern and scale in parasite communities: A stranger in a strange land. Parasitology, 109: 187-196.

Kennedy, C.R. and R.A. Hartvigsen. 2000. Richness and diversity of intestinal metazoan communities in brown trout, *Salmo trutta,* compared to those of eels, *Anguilla anguilla,* in their European heartlands. Parasitology, 121: 55-64.

Kennedy, C.R., D. Dicave, F. Berrilli, and P. Orecchia. 1997. Composition and structure of helminth communities in eels *Anguilla anguilla* from Italian coastal lagoons. Journal of Helminthology, 71: 35-40.

Kenyon, F. and D. Knox. 2002. The proteinases of *Psoroptes ovis*, the sheep scab mite: their diversity and substrate specificity. Veterinary Parasitology, 105: 317-325.

Kirk, R.S. 2003. The impact of *Anguillicola crassus* on European eels. Fisheries Management and Ecology, 10(6): 385-394.

Knopf, K. and M. Mahnke. 2004. Differences in susceptibility of the European eel (*Anguilla anguilla*) and the Japanese eel (*Anguilla japonica*) to the swim-bladder nematode *Anguillicola crassus*. Parasitology, 129: 491-496.

Knudsen, R., P. Amundsen, and A. Klemetsen. 2002. Parasite-induced host mortality: indirect evidence from a long-term study. Environmental Biology of Fishes, 64: 257-265.

Knudsen, R. and A. Klemetsen. 1994. Infections of *Diphyllobothrium dendriticum, D. ditremum* (Cestoda), and *Cystidicola farionis* (Nematoda) in a north Norwegian population of Arctic charr (*Salvelinus alpinus)* during winter. Canadian Journal of Zoology, 72: 1822-1930.

Knudsen, R., M.A. Curtis, and R. Kristoffersen. 2004. Aggregation of Helminths: the role of feeding behavior of fish hosts. Journal of Parasitology, 90: 1-7.

Ko, R.C. and R.C. Anderson. 1969. A revision of the genus *Cystidicola* Fischer, 1798 (Nematoda: Spiruroidea) of swimbladder of fishes. Journal of the Fisheries Research Board of Canada, 26: 849-864.

Kratzer, J.F., W.W. Taylor, and M. Turner. 2007. Changes in fecundity and egg lipid content of lake whitefish (*Coregonus clupeaformis*) in the upper Laurentian Great Lakes between 1986-87 and 2003-05. Journal of Great Lakes Research, 33: 922-929.

Lafferty, K.D. and A.K. Morris. 1996. Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. Ecology, 77: 1390-1397.

Landry, R.C. and W.E. Kelso. 2000. Physicochemical influences on parasites of age-0 largemouth bass in the Atchafalaya River Basin. Louisiana Journal of Freshwater Ecology, 14(4): 519-534.

Langley, R. and J.S. Fairley. 1982. Seasonal variations in infestations of parasites in a wood mouse *Apodemus sylvaticus* population in the west of Ireland. Journal of Zoology, 198: 249-261.

Lankester, M.W. and J.D. Smith. 1980. Host specificity and distribution of the swim-bladder nematodes, *Cystidicola farionis* Fischer, 1798 and *C. cristivomeri* White, 1941 (Habronematoidea), in salmonid fishes of Ontario. Canadian Journal of Zoology, 58: 1298-1305.

Lawler, G.H. 1970. Parasites of coregonid fishes. Pages 279-303 *in* C.C. Lindsey and C.S. Woods, editors. Biology of coregonid fishes. University of Manitoba Press, Winnipeg, Canada.

Leadabrand, C.C. and B.B. Nickol. 1993. Establishment, survival, site selection and development of *Leptorhynchoides thecatus* in largemouth bass, *Micropterus salmoides*. Parasitology, 106 (Pt. 5): 495-501.

Lefebvre, F., P. Contournet, and A.J. Crivelli. 2002. The health state of the eel swimbladder as a measure of parasite pressure by *Anguillicola crassus*. Parasitology, 124: 457- 463.

Lengy, J. and L. Fishelson. 1972. On an immature *Didymozoid* larva (Trematoda: Didymozoidae) in the muscles and swimbladder of *Anthias squamipinnis* (Anthiidae) from the Gulf of Aqaba. Journal of Parasitology, 58: 879-881.

León, P.P., G.L. Gagnon, V.G. Prieto, L.R. Mendivil, and S.A. Alvarez. 2000. Digenean fauna of amphibians from Central Mexico. Nearctic and Neotropical influences. Comparative Parasitology, 67: 92-106.

Leong, T.S. and J.C. Holmes. 1981. Communities of metazoan parasites in open water fishes of Cold Lake, Alberta. Journal of Fish Biology, 18: 693-713.

Lile, N.K. 1998. Alimentary tract helminths of four pleuronectid flatfish in relation to host phylogeny and ecology. Journal of Fish Biology, 53: 945-953.

Lowe, P.O., P. Ffolliott, and J.G. Goodwin. 1977. Nematode parasites in largemouth bass, Presa de Novillo Reservoir, Sonora, Mexico. The Southwestern Naturalist, 22(4): 537-538.

Marcogliese, D.J. and D.K. Cone. 1991. Importance of lake characteristics in structuring parasite communities of salmonids from insular Newfoundland. Canadian Journal of Zoology, 69: 2962-2967.

Marcogliese, D.J. and D.K. Cone. 1997. Parasite communities as indicators of ecosystem stress. Parasitology, 39: 227-232.

Margolis, L., G.W. Esch, J.C. Holmes, A.M. Kuris, and G.A. Schad. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). Journal of Parasitology, 68: 131-133.

McCormick, J.H. and G.N. Stokes. 1982. Intraovarian invasion of smallmouth bass oocytes by *Proteocephalus ambloplitis* (Cestoda). Journal of Parasitology, 68: 973-975.

McDonough, J.M. and L.N. Gleason. 1981. Histopathology in the rainbow darter, *Etheostoma caeruleum*, resulting from infections with the Acanthocephalans, *Pomphorhynchus bulbocolli* and *Acanthocephalus dirus*. The Journal of Parasitology, 67: 403-409.

McFarland, L.H., K.N. Mouritsen, and R. Poulin. 2003. From first to second and back to first intermediate host: The unusual transmission route of *Curtuteria australis* (Digenea: Echinostomatidae). The Journal of Parasitology, 89: 625-628.

McLain, A.L. 1951. Diseases and parasites of the sea lamprey, *Petromyzon marinus,* in the Lake Huron Basin. Transactions of the American Fisheries Society, 81: 94-100.

McNickle, G.G., M.D. Rennie, and W.G. Sprules. 2006. Changes in benthic invertebrate communities of South Bay, Lake Huron following invasion by zebra mussels (*Dreissena polymorpha*), and potential effects on lake whitefish (*Coregonus clupeaformis*) diet and growth. Journal of Great Lakes Research, 32: 180-193.

Meguid, M.A. and H.E. Eure. 1996. Pathobiology associated with the spiruroid nematodes *Camallanus oxycephalus* and *Spinitectus carolini* in the intestine of green sunfish, *Lepomis cyanellus*. Journal of Parasitology, 82(1): 118-23.

Messick, G.A., R.M. Overstreet, T.F. Nalepa, and S. Tyler. 2004. Prevalence of parasites in amphipods *Diporeia* spp. from Lakes Michigan and Huron, USA. Diseases of Aquatic Organisms, 59: 159-170.

Meyer, M.C. 1954. (Reprinted 1962). The larger animal parasites of the fresh-water fishes of Maine. State of Maine, Department of Inland Fish and Game, Fisheries Research Management Division, Bulletin Number 1, 88 p.

Mikaelian, I., Y. de Lafontaine, C. Menard, P. Tellier, J. Harshbarger, and D. Martineau. 1998. Neoplastic and nonneoplastic hepatic changes in lake whitefish (*Coregonus clupeaformis*) from the St. Lawrence River, Quebec, Canada. Environmental Health Perspectives, 106: 179-83.

Mikaelian, I., Y. de Lafontaine, J.C. Harshbarger, L.L. Lee, and D. Martineau. 2002. Health of lake whitefish (*Coregonus clupeaformis*) with elevated tissue levels of environmental contaminants. Environmental Toxicology and Chemistry, 21: 532-541.

Mills, E.L., J.M. Casselman, R. Dermott, J.D. Fitzsimons, G. Gal, K.T. Holeck, J.A. Hoyle, O.E. Johannsson, B.F. Lantry, J.C. Makarewicz, E.S. Millard, I.F. Munawar, M. Munawar, R. O'Gorman, R.W. Owens, L.G. Rudstram, T. Schaner, and T.J. Stewart. 2005. A synthesis of ecological and fish-community changes in Lake Ontario, 1970-2000. Great Lakes Fishery Commission Technical Report 67, Ann Arbor, Michigan.

Minchella, D.J. and M.E. Scott. 1991. Parasitism: a cryptic determinant of animal community structure. Trends in Ecology and Evolution, 6: 250-254.

Miscampbell, A.E., M.W. Lankester, and M.L. Adamson. 2004. Molecular and morphological variation within swimbladder nematodes, *Cystidicola* spp. Canadian Journal of Fisheries and Aquatic Sciences, 61: 1143-1152.

Mohr, L.C. and M.P. Ebener. 2005. Status of lake whitefish (*Coregonus clupeaformis*) in Lake Huron. Pages 105-125 *in* L.C. Mohr and T.F. Nalepa, editors. Proceedings of a workshop on the dynamics of lake whitefish (*Coregonus clupeaformis*) and the amphipod *Diporeia* spp. in the Great Lakes. Great Lakes Fisheries Commission Technical Report 66, Great Lakes Fishery Commission, Ann Arbor, Michigan.

Molnar, K., S. Marton, E. Eszterbauer, and C. Szekely. 2006. Comparative morphological and molecular studies on *Myxobolus* spp. infecting chub from the River Danube, Hungary, and description of *M. muellericus* sp. n. Source Diseases of Aquatic Organisms, 73(1): 49-61.

Montgomery, S.S.J. and W.I. Montgomery. 1989. Spatial and temporal variation in the infracommunity structure of helminths of *Apodemus sylvaticus* (Rodentia: Muridae). Parasitology, 98: 145-150.

Moravec, F. 1978. The development of the nematode Philometra obturans (Prenant, 1886) in the intermediate host. Folia Parasitologica, 25: 303-315.

Moravec, F. 1980. Biology of *Cucullanus truttae* (Nematoda) in a trout stream. Folia Parasitologica, 27: 217-226.

Mouillot, D., M. George-Nascimento, and R. Poulin. 2005. Richness, structure and functioning in metazoan parasite communities. Oikos, 109: 447-460.

Moyle, P.B. and J.J. Cech Jr. 2000. Fishes: An Introduction to Ichthyology, 4th Edition. Prentice-Hall.

Mudry, D.R. and P.J. McCarty. 1976. Metazoan parasites of Arctic char (*Salvelinus alpinus*) from the north slope of Canada and Alaska. Journal of the Fisheries Research Board of Canada, 33: 271-275.

Muzzall, P.M. 2007. Parasites of juvenile brook trout (*Salvelinus fontinalis*) from Hunt Creek, Michigan. Journal of Parasitology, 93: 313-317.

Muzzall, P.M. and C.A. Bowen II. 2000. Helminths in an intensively stocked population of lake trout, *Salvelinus namaycush*, from Lake Huron. Journal of Parasitology, 86: 639-642.

Muzzall, P.M. and C.A. Bowen II. 2002. Parasites of the slimy sculpin, *Cottus cognatus* Richardson, 1836, from Lake Huron, U.S.A. Comparative Parasitology, 69: 196-201.

Muzzall, P.M. and M.G. Gillilland. 2004. Occurrence of acanthocephalans in largemouth bass and smallmouth bass (Centrarchidae) from Gull Lake, Michigan. Journal of Parasitology, 90: 663-664.

Muzzall, P.M. and C.R. Peebles. 1986. Helminths of pink salmon, *Oncorhynchus gorbuscha*, from five tributaries of Lake Superior and Lake Huron. Canadian Journal of Zoology, 64: 508-511.

Muzzall, P.M. and C.R. Peebles. 1988. Helminths of rainbow smelt, *Osmerus mordax*, from five localities in Lake Huron and Lake Michigan, with emphasis on *Diplostomum spathaceum*. Proceedings of the Helminthological Society of Washington, 55: 281-285.

Muzzall, P.M., B.T. Eggold, and R.J. Fahey. 2006. Helminths of pond-reared walleye from Wisconsin. Journal of Parasitology, 92(2): 408-410.

Muzzall, P.M., M.G. Gillilland III, C.A. Bowen II, N.R. Coady, and C.R. Peebles. 2003. Parasites of burbot, *Lota lota*, from Lake Huron, Michigan, U.S.A., with a checklist of the North American parasites of burbot. Comparative Parasitology, 70: 182-195.

Nachev, M. and B. Sures. 2009. The endohelminth fauna of barbel (*Barbus barbus*) correlates with water quality of the Danube River in Bulgaria. Parasitology, 136(5): 545-552.

Nalepa, T.F., D.L. Fanslow, and G.A. Lang. 2009*b*. Transformation of the offshore benthic community in Lake Michigan: recent shift from the native amphipod *Diporeia* spp. to the invasive mussel *Dreissena rostriformus bugensis*. Freshwater Biology, 54: 466-479.

Nalepa, T.F., D.L. Fanslow, and G.A. Messick. 2005*a*. Characteristics and potential causes of declining *Diporeia* spp. populations in southern Lake Michigan and Saginaw Bay, Lake Huron. Proceedings of a workshop on the dynamics of lake whitefish (*Coregonus clupeaformis*) and the amphipod *Diporeia* spp. in the Great Lakes. Great Lakes Fishery Commission Technical Report 66.

Nalepa, T.F., S.A. Pothoven, and D.L. Fanslow. 2009*a*. Recent changes in benthic macroinvertebrate populations in Lake Huron and impact on the diet of lake whitefish (*Coregonus clupeaformis*). Aquatic Ecosystem Health and Management, 12: 2-10.

Nalepa, T.F., D.W. Schloesser, S.A. Pothoven, and S.J. Lozano. 1998. Declines in benthic macroinvertebrate populations in southern Lake Michigan, 1980-1993. Canadian Journal of Fisheries and Aquatic Science, 55: 2402-2413.

Nalepa, T.F., D.L. Fanslow, S.A. Pothoven, A.J. Foley III, and G.A. Lang. 2007. Long-term trends in benthic macroinvertebrate populations in Lake Huron over the past four decades. Journal of Great Lakes Research, 33: 421-436.

Nalepa, T.F., L.C. Mohr, B.A. Henderson, C.P. Madenjian, and P.J. Schneeberger. 2005*b*. Lake whitefish and *Diporeia* spp. in the Great Lakes: an overview. Pages 3-20 *in* L.C. Mohr and T.F. Nalepa, editors. Proceedings of a Workshop on the Dynamics of Lake Whitefish (*Coregonus clupeaformis*) and the Amphipod *Diporeia* spp. in the Great Lakes. Great Lakes Fishery Commission Technical Report 66, Ann Arbor, Michigan. 27 February 2002.

Neiland, K.A. 1952. A new species of *Proteocephalus* Weinland, 1858 (Cestoda), with notes on its life history. Journal of Parasitology, 38: 540-545.

Noga, E.J., R.A. Bullis, and G.C. Miller. 1990. Epidemic oral ulceration in largemouth bass (*Micropterus salmoides*) associated with the leech *Myzobdella lugubris.* Journal of Wildlife Diseases, 26: 132-134.

Oehlert, G.W. 1992. A note on the delta method. American Statistician, 46: 27-29.

Oksanen, J., F.G. Blanchet, R. Kindt, P. Legendre, R.B. O'Hara, G.L. Simpson, P. Solymos, M.H.H. Stevens, and H. Wagner. 2010. vegan: Community Ecology Package. R package version 1.17-3. http://CRAN.R-project.org/package=vegan

Olson, P.D. and B.B. Nickol. 1996. Comparison of *Leptorhynchoides thecatus* (Acanthocephala) recruitment into green sunfish and largemouth bass populations. Journal of Parasitology, 82: 702-706.

Olson, M.H. and B.P. Young. 2003. Patterns of diet and growth in co-occurring populations of largemouth bass and smallmouth bass. Transactions of the American Fisheries Society, 1207-1213.

O'Sullivan, H.M., C.M. Smal, and J.S. Fairley. 1984. A study of parasitic infestations in populations of small rodents (*Apodemus sylvaticus* and *Clethrionomys glareolus*) on Ross Island, Killarney. Journal of Life Sciences of the Royal Dublin Society, 5: 29-42.

Page, L.M. and B.M. Burr. 1991. Peterson field guide to freshwater fishes. Houghton Mifflin Company, New York. 432 p.

Pearse, A.S. 1924. The parasites of lake fishes. Transactions of the Wisconsin Academy of Sciences, Arts, and Letters, 21: 161-194.

Petersson, A.Ê. 1971. The effect of lake regulation on populations of cestodan parasites of Swedish whitefish *Coregonus*. Oikos, 22: 74-83.

Petrochenko, V.I. 1956. Acanthocephala of domestic and wild animals. Moscow: Izadatel'stvo Akademii Nauk SSSR, Vol. 1, 435 p. (in Russian: English translation, Israel Program for Scientific translations, Ltd., Jerusalem, 1971, 465p.)

Poole, B.C. 1985. Fish-parasite population dynamics in seven small boreal lakes of central Canada. Master of Science thesis, University of Manitoba, Winnipeg, Canada.

Poppe, T.T., T.A. M.O., and L. Iversen. 1992. Disseminated hexamitosis in sea-caged Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms, 14: 91-97.

Pothoven, S.A. 2005. Changes in lake whitefish diet in Lake Michigan, 1998-2001. Pages 127-140 *in* L.C. Mohr and T.F. Nalepa, editors. Proceedings of a workshop on the dynamics of lake whitefish (*Coregonus clupeaformis*) and the amphipod *Diporeia* spp. in the Great Lakes. Great Lakes Fishery Commission Technical Report 66, Ann Arbor, Michigan.

Pothoven, S.A. and C.P. Madenjian. 2008. Changes in consumption by alewives and lake whitefish after dreissenid mussel invasions in lakes Michigan and Huron. North American Journal of Fisheries Management, 28: 308-320.

Pothoven, S.A., T.F. Nalepa, P.J. Schneeberger, and S.B. Brandt. 2001. Changes in diet and body of lake whitefish in southern Lake Michigan associated with changes in benthos. North American Journal of Fisheries Management, 21: 876-883.

Poulin, R. 1997. Species richness of parasite assemblages: Evolution and patterns. Annual Review of Ecology and Systematics, 28: 341-358.

Poulin, R. 1999. The functional importance of parasites in animal communities: many roles at many levels. International Journal of Parasitology, 29: 903-914.

Price, P.W. and K.M. Clancy. 1983. Patterns in number of helminth parasite species in freshwater fishes. Journal of Parasitology, 69: 449-454.

Prophet, E., B. Mills, and J. Arrington. 1992. Laboratory methods in histotechnology. Armed Forces Institute of Pathology, Washington, D.C.

R Development Core Team. 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org.

Rennie, M.D., W.G. Sprules, and T.B. Johnson. 2009. Factors affecting the growth and condition of lake whitefish (*Coregonus clupeaformis*). Canadian Journal of Fisheries and Aquatic Sciences, 66: 2096-2108.

Ricklefs, R.E. 1993. The economy of nature: A textbook in basic ecology, 3rd Edition. W.H. Freeman and Company, New York, New York, 576 p.

Roberts, L. and J. Janovy. 2008. Foundations of Parasitology, 8th Edition. McGraw-Hill Publishing Company, New York.

Rohde, K. and M. Heap. 1998. Latitudinal gradients in species and community richness and in community structure of metazoan endo- and ectoparasites of marine teleost fish. International Journal of Parasitology, 28: 461-478.

Rynko, J.D., J. Rokicki, and Z. Jablonowski. 2003. The activity of selected hydrolases in excretion secretion products and extracts from larvae and mature specimens of *Cystidicola farionis*. Oceanological and Hydrobiological Studies, 32: 117-129.

Salgado-Maldonado, G. and C.R. Kennedy. 1997. Richness and similarity of helminth communities in the tropical cichlid fish *Cichlasoma urophthalmus* from the Yucatan Peninsula, Mexico. Parasitology, 114: 581-590.

Schmidt, G.D. 1986. CRC Handbook of Tapeworm Identification. CRC Press, Boca Raton, Florida. 675 p.

Scott, M.E. 1988. The impact of infection and disease on animal populations: Implications for conservation biology. Conservation Biology, 2: 40-56.

Selgeby, J.H. and M.H. Hoff. 1996. Seasonal bathymetric distributions of 16 fishes in Lake Superior, 1958-75. United States Department of the Interior, National Biological Service, Biological Science Report 7.

Shannon, C.E. 1948. A mathematical theory of communication. Bell System Technical Journal, 27: 379-423.

Simberloff, D. and J. Moore. 1997. Community ecology of parasites and free-living animals. Pages 174-197 *in* D.H. Clayton and J. Moore, editors. Host-parasite evolution: General principles and avian models. Oxford University Press, Oxford, U.K.

Simpson, E.H. 1949. Measurement of diversity. Nature, 163: 163-688.

Smith, J.D. and M.W. Lankester. 1979. Development of swimbladder nematodes (*Cystidicola* spp.) in their intermediate hosts. Canadian Journal of Zoology, 57: 1736-1744.

Smith, B. and J.B. Wilson. 1996. A consumer's guide to evenness indices. Oikos, 76: 70-82.

Snoj, N., V. Jencic, J. Berglez, and M. Vidmar. 1986. Cystidiculosis in two-year-old brown trout in nursery streams. Veterinarstvo, 23: 263-268.

Sogandares-Bernal, F. 1955. Some helminth parasites of fresh and brackish water fishes from Louisiana and Panama. Journal of Parasitology, 41: 587-594.

Sotelo, J. and H. Del Brutto. 2002. Review of Neurocysticercosis. American Association of Neurological Surgeons, 12: 61-69.

Spangler, G.R. and C.C. Collins. 1980. Response of lake whitefish (*Coregonus clupeaformis*) to the control of sea lamprey (*Petromyzon marinus*) in Lake Huron. Canadian Journal of Fisheries and Aquatic Sciences, 37: 2039-2046.

Spangler, G.R. and J.H. Peters. 1995. Fisheries of Lake Huron: an opportunity for stewardship. Pages 103-123 *in* M. Munawar, T. Edsall, and J. Leach, editors. The Lake Huron ecosystem: ecology, fisheries and management. Ecovision World Monograph Series, SPB Academic Publishing, Amsterdam, The Netherlands.

Spangler, G.R., D.S. Robson, and H.A. Regier. 1980. Estimates of lamprey-induced mortality in whitefish, *Coregonus clupeaformis*. Canadian Journal of Fisheries and Aquatic Sciences, 37: 2146-2150.

Sparks, A.K. 1951. Some helminth parasites of the largemouth bass in Texas. Transactions of the American Microscopical Society, 70: 351-358.

Sparkes, T.C., K.A. Weil, D.T. Renwick, and J.A. Talkington. 2006. Development-related effects of an acanthocephalan parasite on pairing success of its intermediate host. Animal Behavior, 71: 439-448.

Sparkes, T.C., V.M. Wright, D.T. Renwick, K.A. Weil, J.A. Talkington, and M. Milhalyov. 2004. Intra-specific host sharing in the manipulative parasite *Acanthocephalus dirus*: does conflict occur over host-modification? Parasitology, 129: 335-340.

Steinauer, M.L. and W.F. Font. 2003. Seasonal dynamics of the helminths of bluegill (*Lepomis macrochirus*) in a subtropical region. Journal of Parasitology, 89: 324-328.

Steinauer, M.L., J.E. Parham, and B.B. Nickol. 2006. Geographic analysis of host use, development, and habitat use of an acanthocephalan species, *Leptorhynchoides thecatus*. Journal of Parasitology, 92(3): 464-472.

Steinstrasser, W. 1936. Acanthocephalen als Forellen-parasiten. Zeitschrift der Fischerei, 34: 174-212.

Stewart, D.B. and L.M.J. Bernier. 1999. Common Parasites, Diseases and Injuries of Freshwater Fishes in the Northwest. Arctic Biological Consultants for the Canada Department of Fisheries and Oceans, Central and Arctic Region, Winnipeg. 41 p.

Stott, W., T.N. Todd, and L. Kallemeyn. 2004. Genetic variability among lake whitefish from Isle Royale and the upper Great Lakes. Pages 51-60 *in* O. Heikinheimo, P. Amundsen, H. Auvinen, D. Brodaly, R. Eshenroder, A. Huusko, K. Mills, R. Muller, T. Todd, and I. Winfield, editors. Biology and management of coregonid fishes. Proceedings of the 8th international symposium on the biology and management of coregonid fishes, Rovaniemi, Finland, 26-29 August 2002. Annales Zoologici Fennici 41, Helsinki, Finland.

Stratoudakis, Y., A. Barbosa, and I. Meneses. 2000. Infection of sardine eggs by the protistan endoparasite *Ichthyodinium chabelardi* off Portugal. Journal of Fish Biology, 57: 476-482.

Stromberg, P.C. and J.L. Crites. 1975. Population biology of *Camallanus oxycephalus* Ward and Magath, 1916 (Nematoda: Camallanidae) in white bass in Western Lake Erie. Journal of Parasitology, 81: 123-132.

Szalai, A.J. and T.A. Dick. 1990. *Proteocephalus ambloplitis* and *Contracaecum* sp. from largemouth bass (*Micropterus salmoides*) stocked into a boundary reservoir, Saskatchewan. Journal of Parasitology, 76: 598-601.

Szekely, C., K. Molnar, and O.Z. Racz. 2005. Radiodiagnostic method for studying the dynamics of *Anguillicola crassus* (Nematoda: Dracunculoidea) infection and pathological status of the swimbladder in Lake Balaton eels. Diseases of Aquatic Organisms, 64: 53-61.

Taraschewski, H. 2000. Host-parasite interactions in Acanthocephala: a morphological approach. Advances in Parasitology, 46: 1-179.

Tedla, S. and C.H. Fernando. 1969. Observations on the seasonal changes of the parasite fauna of yellow perch (*Perca flavescens*) from the Bay of Quinte, Lake Ontario. Journal of the Fisheries Research Board of Canada, 26: 833-843.

Timi, J.T. and R. Poulin. 2003. Parasite community structure within and across host populations of a marine pelagic fish: how repeatable is it? International Journal for Parasitology, 33(12): 1353-1362.

Tompkins, D.M. and M. Begon. 1999. Parasites can regulate wildlife populations. Parasitology Today, 15: 311-313.

Uznanski, R.L. and B.B. Nickol. 1976. Structure and function of the fibrillar coat of *Leptorhynchoides thecatus* eggs. Journal of Parasitology, 62(4): 569-573.

Valtonen, E.T. and T. Valtonen. 1978. *Cystidicola farionis* as a swimbladder parasite of the whitefish in the Bothnian Bay. Journal of Fish Biology, 13(5): 557-561.

Wagner, T., M.L. Jones, M.P. Ebener, M.T. Arts, T.O. Brenden, D.C. Honeyfield, G.M. Wright, and M. Faisal. 2010. Spatial and temporal dynamics of lake whitefish (*Coregonus clupeaformis*) health indicators: linking individual-based indicators to a management-relevant endpoint. Journal of Great Lakes Research, 36 (Supplement 1): 121-134.

Ward, H.B. 1894. Some notes on the biological relations of fish parasites of the Great Lakes. Proceedings of the Nebraska Academy of Sciences, 4: 8-11.

Wardle, R.A. 1932*a*. The Cestoda of Canadian fishes. I. The Pacific Coast region. Contributions to Canadian Biology and Fisheries, 7: 221-243.

Wardle, R.A. 1932*b*. The Cestoda of Canadian fishes. II. The Hudson Bay drainage system. Contributions to Canadian Biology and Fisheries, 7: 377-403.

Warren, M.L. Jr., and J.L. Wilson. 1978. Parasitic nematode *Contracaecum* in two subspecies of largemouth bass. The Progressive Fish-Culturist, 40(4): 137.

Watson, R.A. and T.A. Dick. 1979. Metazoan parasites of whitefish, *Coregonus clupeaformis* (Mitchill) and cisco, *C. artedii* LeSueur from Southern Indian lake, Manitoba. Journal of Fish Biology, 15: 579-587.

Watson, R.A. and T.A. Dick. 1980. Metazoan parasites of pike, *Esox lucius* Linnaeus, from Southern Indian Lake, Manitoba, Canada. Journal of Fish Biology, 17: 255-261.

Willers, W.B., R.R. Dubielzig, and L. Miller. 1991. Histopathology of the swimbladder of the cisco due to the presence of the nematode *Cystidicola farionis* Fisher. Journal of Aquatic Animal Health, 3: 130–133.

Wilsey, B.J., D.R. Chalcraft, C.M. Bowles, and M.R. Willig. 2005. Relationships among indices suggest that richness is an incomplete surrogate for grassland biodiversity. Ecology, 86: 1178-1184.

Wisniewski, W.L. 1958. Characterization of the parasitofauna of an eutrophic lake. Acta Parasitologica Polonica, 6: 1-64.

Yamaguti, S. 1971. Synopsis of digenetic trematodes of vertebrates. Vols. 1-2, Keigaku Publishing Company, Tokyo, Japan. 1074 p.

Zander, C.D. 2007. Parasite diversity of sticklebacks from the Baltic Sea. Parasitology Research, 100: 287-297.

Zander, C.D., L.W. Reimer, and K. Barz. 1999. Parasite communities of the Salzhaff (Northwest Mecklenburg, Baltic Sea) I. Structure and dynamics of communities of littoral fish, especially small-sized fish. Parasitology Research, 85: 356–372.

Zółtowska, K., E. Lopieńska, J. Rokicki, and M. Dmitryjuk. 2001. The enzymes of carbohydrates metabolism from *Cystidicola farionis* (Cystidicolidae). Wiadomosci parazytologiczne, 47(3): 311-315.