

HOST DIET AND PARASITIC HELMINTH INFECTIONS WITHIN NATIVE, ESTABLISHED AND
INTRODUCED FISH OF ALGONQUIN PARK

by

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ABSTRACT

Host diet and parasitic helminth infections within native, established and introduced fish of Algonquin Park

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Freshwater fish biodiversity loss has been attributed to many reasons, including invasive species infectious diseases. I examined 112 invasive Rock Bass (*Ambloplites rupestris*), 59 established Smallmouth Bass (*Micropterus dolomieu*), and 60 native Pumpkinseed sunfish (*Lepomis gibbosus*) from 8 different lakes in Algonquin Park, ON, Canada to evaluate their endohelminth parasites. My results indicate that established and native fish are not only more likely to be infected with trophically-transmitted parasites such as cestodes (tapeworms) and acanthocephalans (thorny-headed worms) than invasive Rock Bass, but they also have a higher infection intensity and greater diversity of endohelminths. There was also a significant difference between the three fish species with respect to non-trophically transmitted larval trematodes (flatworms), which reflect the habitat of fish. Along with host size, I examined fish diet and habitat use to demonstrate how the ecology of different species influences their probability of infection, parasite communities, and possible ease of establishment in novel environments.

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TABLE OF CONTENTS

Author's Declaration	ii
Abstract	iii
Acknowledgements	iv
List of tables	vii
List of figures	viii
Chapter 1 General introduction	1
Contribution of authors.....	1
State of freshwater fishes.....	1
Smallmouth Bass.....	2
Rock Bass.....	5
Pumpkinseeds.....	9
Fishes of Algonquin Park.....	12
The importance of parasitism.....	15
Parasite ecology	21
Invasive species and parasites.....	23
Research aims and hypotheses.....	24
Citations.....	26
Chapter 2 Parasitism of invasive and native species	30
Introduction.....	30
Research objectives and hypotheses.....	35
Materials and Methods.....	37
Field collections.....	37
Dissections and parasite identification.....	41

Data analyses.....	43
Results.....	45
Discussion.....	54
Citations.....	62
Chapter 3 Host habitat and diet in relation to parasitism in Farm Lake.....	65
Introduction.....	65
Research objective and hypotheses.....	71
Materials and Methods.....	72
Parasite and gut content data.....	72
Lipid analysis.....	73
Stable Isotope analysis.....	74
Data analysis.....	75
Results.....	76
Species differences- parasitism.....	76
Species differences- host attributes.....	82
Individual-level patterns.....	91
Discussion.....	102
Species-level parasitism.....	102
Individual-level parasitism.....	108
Significance of findings.....	110
Citations.....	113
Chapter 4 General Discussion.....	117
Future direction.....	121
Citations.....	123

LIST OF TABLES

2.1 Summary of fish caught from each lake.....	38
2.2 Parasites found in Rock Bass and Smallmouth Bass.....	46

LIST OF FIGURES

1.1 Smallmouth Bass ecology and taxonomy.....	3
1.2 Rock Bass ecology and taxonomy.....	7
1.3 Pumpkinseed ecology and taxonomy.....	10
1.4 Generalized cestode life cycle.....	16
1.5 Generalized nematode life cycle.....	18
1.6 Generalized acanthocephalan life cycle.....	20
2.1 Map of Algonquin Park lakes sampled.....	39
2.2 Parasite infection intensity for Rock Bass and Smallmouth Bass.....	47
2.3 Parasite infection intensity by organ for Rock Bass and Smallmouth Bass.....	48
2.4 Parasite richness for Rock Bass and Smallmouth Bass.....	50
2.5 Parasite richness and infection intensity compared to fork length for Rock Bass and Smallmouth Bass.....	51
2.6 Mean fork length of Rock Bass and Smallmouth Bass.....	53
2.7 Life cycle of Bass tapeworm <i>Proteocephalus sp</i>	59
3.1 Infection intensity (excluding metacercariae) for Rock Bass, Smallmouth Bass and Pumpkinseeds from Farm Lake.....	77
3.2 Parasite richness (excluding metacercariae) for Rock Bass, Smallmouth Bass and Pumpkinseeds from Farm Lake.....	78
3.3 Metacercariae infection intensity for Rock Bass, Smallmouth Bass and Pumpkinseeds from Farm Lake.....	80
3.4 Prevalence of cestode infections in Smallmouth Bass, Rock Bass, and Pumpkinseeds from Farm Lake.....	81

3.5 Fork length of Smallmouth Bass, Rock Bass, and Pumpkinseeds from Farm Lake.....	83
3.6 Mean $\delta^{13}\text{C}$ enrichment of Smallmouth Bass, Rock Bass, and Pumpkinseeds from Farm Lake.....	85
3.7 Mean $\delta^{15}\text{N}$ enrichment of Smallmouth Bass, Rock Bass, and Pumpkinseeds from Farm Lake.....	86
3.8 Mean weight percentage of total fatty acids measured from Smallmouth Bass, Rock Bass, and Pumpkinseeds from Farm Lake.....	88
3.9 Occurrence of diet contents in Smallmouth Bass, Rock Bass, and Pumpkinseeds from Farm Lake.....	90
3.10 Omega-3 fatty acids and infection intensity score in Smallmouth Bass, Rock Bass, and Pumpkinseeds from Farm Lake.....	92
3.11 Individual fish measurements of omega-3 fatty acids (w%) in relation to parasite richness in Smallmouth Bass, Rock Bass, and Pumpkinseeds from Farm Lake.....	93
3.12 Individual fish measurements of $\delta^{15}\text{N}$ in relation to parasite richness in Smallmouth Bass, Rock Bass, and Pumpkinseeds from Farm Lake.....	94
3.13 Individual fish measurements of diet in relation to parasite richness in Smallmouth Bass, Rock Bass, and Pumpkinseeds from Farm Lake.....	95
3.14 Infection status and mean ^{15}N isotope values for all Pumpkinseed sunfish, Rock Bass and Smallmouth Bass from Farm Lake	96
3.15 Presence of cestodes and mean ^{15}N isotope values for Rock Bass, Smallmouth Bass and Pumpkinseeds from Farm Lake.....	97

3.16 Fork length and infection intensity score for Smallmouth Bass, Rock Bass and Pumpkinseeds from Farm Lake.....	98
3.17 Palmitoleic acid (w%) and metacercariae infection intensity for Smallmouth Bass, Rock Bass, and Pumpkinseeds from Farm Lake.	99
3.18 Chemical isotope values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in Smallmouth Bass, Rock Bass and Pumpkinseeds from Farm Lake.....	101
3.19 Generalized trematode lifecycle.....	107

CHAPTER 1 GENERAL INTRODUCTION

Contribution of authors

F. Zahlan and J. Koprivnikar conceived of and designed the research questions contained within this thesis and any resulting manuscripts. F. Zahlan performed all data collection (field sampling, dissections, parasite identification, and host attributes) while J. Koprivnikar supervised the project and was responsible for all necessary permits and funding. F. Zahlan and J Koprivnikar performed the data analysis. F. Zahlan wrote the manuscripts with J. Koprivnikar assisting in preparation.

State of freshwater fishes

Freshwater ecosystems are perhaps some of the most threatened areas on Earth, where declines in biodiversity are far greater than in most terrestrial ecosystems (Sala et al. 2000). Approximately 40% of the global fish diversity (Lundberg et al. 2000; Dudgeon et al. 2006), and roughly a third of the global vertebrate diversity, inhabits these waters (Dudgeon et al. 2006). To put this into perspective, freshwater habitats contain approximately 0.01% of the total water on Earth (Gleick, 1996). In North America, extinction rates for unionid mussels, crayfishes, fishes and amphibians are expected to be 4% per decade. Fish species declines have been attributed pollution and habitat loss (Vitousek et al. 1997), invasions and deliberate introductions of non-native species (Mandrak and Crossman, 2003; Koehn, 2004; Gozlan et al. 2005), and infectious diseases including those caused by bacteria, viruses, and parasites (Riley et al. 2008; Johnson and Paull, 2011). Fish loss from parasitism is important not only because of effects on single

populations and species, but cascading effects on the surrounding ecosystem that may follow (Lafferty, 2008). Furthermore, invasive fish may introduce parasites, or be freed from their infections, in novel ranges (Gordon, 1982; Lafferty, 2008). The combination of invasive species and parasitism can therefore create difficult to predict ecological impacts.

This study focuses on 3 fish species with different ecologies, and that are either native, invasive but relatively well-established, or recently invasive, to Algonquin Park, Ontario, in order to elucidate the possible ecological and physiological factors affecting parasitism, and therefore the spread of disease, in these fish. Chapter 1 introduces the three fish species and study area, and highlights the relevance of parasite ecology, especially of invasive species. Chapter 2 evaluates trophically-transmitted parasites between established and invasive Bass in the Park. Chapter 3 looks at three species of fish at different trophic levels in order to determine how host diet and habitat might drive parasitism. Last, Chapter 4 (General Discussion) revisits the overarching theme of whether or not parasites influence the establishment of invasive species.

Smallmouth Bass

Smallmouth Bass (*Micropterus dolomieu*) are one of the world's most widely distributed freshwater fishes. Although the fish is native to east-central North America, its introduction to surrounding water bodies for the purpose of sport fishing began in the late 1800s (Brown et al. 2009). Smallmouth Bass are now one of the world's most highly prized freshwater sports fish and major economic and recreational importance (Scott and Crossman, 1973; Lasenby and Kerr, 2000).

Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Class: Actinopterygii
Order: Perciformes
Suborder: Percoidei
Family: Centrarchidae
Genus: *Micropterus*
Species: *dolomieu*

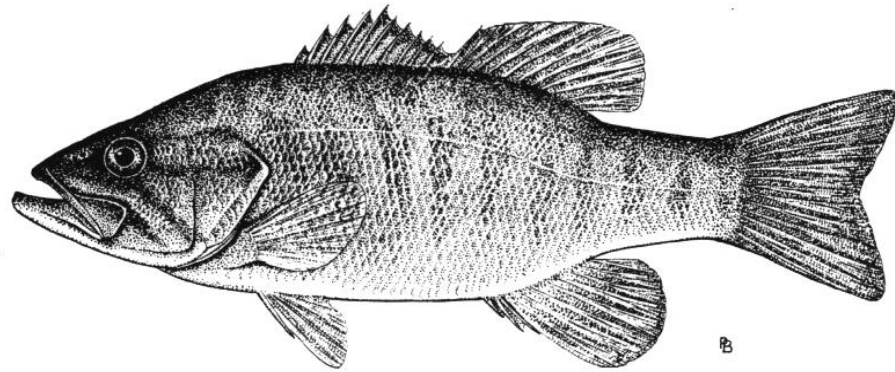


Figure 1.1 Physical appearance of Smallmouth Bass and their taxonomy.
(<http://www.snre.umich.edu/~pwebb/LesChen/species.html>).

When French mineralogist Dieudonne de Dolomieu first characterized the species, his specimen had a damaged fin, resulting in the genus name *Micropterus*, which means "small fin" (Brown et al. 2009). Like all sunfish, Smallmouth Bass have two joined dorsal fins (Brown et al. 2009), where the anterior rays are spiny and posterior soft (Scott and Crossman, 1973). Unlike most other sunfish which are flattened laterally in shape, or compressed in form, Smallmouth Bass are a streamlined, or fusiform, species, with a larger tail for acceleration (Brown et al. 2009) to support its ambush type feeding behaviour and voracious appetite (see Fig. 1.1).

Smallmouth Bass are expected to live no more than 15 years in Canada (Scott and Crossman, 1973). The fish generally reach a full adult size between 25-50 cm, or 0.5-1.6 kg. However the Canadian record, which was caught in Ontario, weighed 4.46 kg (Scott and Crossman, 1973).

Smallmouth Bass have a large and diverse appetite that changes through their development. The first food source all Smallmouth Bass utilize is their yolk sac, which sustains the larval fish until they reach approximately 1 cm (Scott and Crossman, 1973). During this important developmental time, the fish also rely heavily on small zooplankton. As their yolk sac is depleted, they begin to target larger prey items such as chironomids and copepods. Easton and Orth (1992) noted these Bass began to include Ephemeroptera (mayflies) in their diet when they reached approximately 15 cm, and had fully formed fins and a large mouth.

As the fish grow, they tend to rely more on insects than zooplankton. Crayfish and fish fry become a key diet component of Smallmouth Bass >5 cm, and by 10-15 cm these prey items make up the majority of the diet (Moyle, 2002). At the end of their first year, the

aggressive nature of these fish is quite apparent as they consume large fish, crayfish and frogs (Scott and Crossman, 1973).

Bass flourish best in mesotrophic lakes (those with intermediate levels of productivity) that have high visibility, Rocky shores and an average depth >9 m (Edwards et al. 1983). The fish are associated with the littoral (near shore) drop-off and pelagic (open water) zones of lakes, as this is where spawning and rearing occur (Pflug 1981). When they are not spawning, Bass prefer structural habitat such as boulders, Rock fissures and crevices, logs and fallen trees. However, their habitat changes through the season and their lifetimes (Keating, 1970).

Upon introduction to new water bodies, these voracious predators strongly compete with native fish species. Their high fecundity, juvenile use of cover, early onset of piscivory (Brown et al. 2009), parental care (Scott and Crossman, 1973) and low overlap with other predators (Gard, 2004) all favour their survival and establishment in lakes, rivers, estuaries, reservoirs, ponds and creeks. The fish were purposefully introduced into Algonquin Park in the late 1800s, where they immediately established in many of the Park's waters (Mandrak and Crossman, 2003). Smallmouth Bass are so adaptable and resilient that they have been successfully established in Africa, Europe, almost the entire United States and much of Canada (Scott and Crossman, 1973).

Rock Bass

The following description is based on that of Scott and Crossman (1973). Rock Bass (*Ambloplites rupestris*) are a freshwater centrarchid fish widely distributed over eastern and central North America. *Ambloplites* means "blunt armature," and *rupestris* is a

reference to their habitat preference of being "among rocks." Rock Bass are laterally compressed, but to a lesser extent than most other sunfishes (see Fig. 1.2), and have six spines in their anal fin, which distinguishes them from other sunfishes that typically have only three spines. They have a large and bright red eye, for which many anglers call them "goggle-eyed." Their scales are olive to golden brown in colour, and the ventral surface silvery-white. Each scale below the lateral line towards the ventral side has a black spot.

Rock Bass tend to maintain high population densities in lakes, rivers and streams. They inhabit many lakes and water bodies from Manitoba to the east coast, and southwards towards Georgia. They have been introduced to many regions in both North America and Europe as well.

Spawning occurs in the late spring and early summer, when males dig shallow nests approximately 60 cm in diameter with their tails. They have been known to use a wide range of substrates, such as gravel, clay and weeded areas for nesting. Males aggressively attempt to hold females and defend their nests from predators and other males. Offspring growth is rapid and young-of-the-year fish reach between 20-51 mm. Females lay anywhere from 3,000-11,000 eggs on average. Adult Rock Bass span from 15-25 cm and live 10-12 years.

Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Class: Actinopterygii
Order: Perciformes
Suborder: Percoidei
Family: Centrarchidae
Genus: *Ambloplites*
Species: *rupestris*

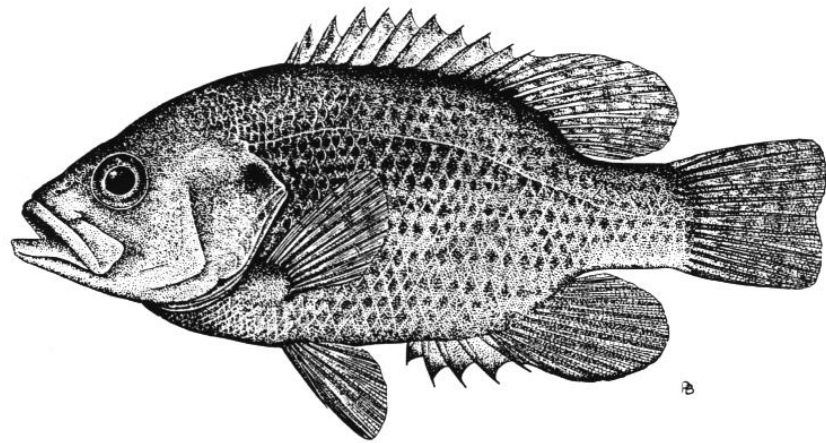


Figure 1.2 Physical appearance of Rock Bass and their taxonomy (<http://www.snre.umich.edu/~pwebb/LesChen/species.html>).

Rock Bass generally inhabit rocky areas in shallow water lakes. Adults are gregarious and can be found in congregations with other sunfishes such as Smallmouth Bass and Pumpkinseed. Areas with vegetation, woody debris, boulders and other cover are also home to Rock Bass. Rock Bass are more associated with the littoral zone than Smallmouth Bass.

Rock Bass are voracious predators of small fish and insects. Keast and Webb (1966) recorded prey items in the stomach contents of various sized Rock Bass from Lake Opinicon in Ontario and found fish below 70 mm strictly eat invertebrates of the classes Chironomida, Ephemeroptera, Odonata, Cladocera, Amphipoda, Isopoda, Copepoda and Hydracarina. Fish greater than 76 mm had a similar diet that also included Trichoptera, fish fry, and crayfish. They found fish greater than 120 mm had diets that consisted almost entirely of crayfish and Anisoptera. The diet of Rock Bass, and other fish, however, depends largely on what is available in their individual environments. Rock Bass are prey items too, especially for large Smallmouth Bass, Northern Pike, Muskellunge and large Walleye.

The introduction of Rock Bass into water bodies can have severe impacts on native fish populations (Mandrak and Crossman, 2003). Rock bass have been rampantly spreading in Algonquin Park since the early 1980s, and now maintain high populations in most of the Park's lakes because the high fecundity and rapid growth of fry permit them to easily establish and have an advantage over native species (Mandrak and Crossman, 2003). Since their habitat overlaps with other fish, such as Smallmouth Bass and brook trout, these voracious eaters no doubt compete for and reduce food availability to other species.

Pumpkinseed

The following description is based on that of Scott and Crossman (1973).

Pumpkinseed (*Lepomis gibbosus*) is a small sunfish capable of living in almost every type of freshwater environment, such as lakes, ponds, creeks and rivers. *Lepomis* means "scaled operculum," which are a key characteristic of this species. *Gibbosus*, meaning like a full moon, refers to the laterally compressed, deep body and disc like shape of the fish (see Fig. 1.3).

The fish has a tawny base, but has random and wavy lines of interconnecting blue and green scales along the lateral sides. The ventral surface is bronze to red/orange and the sides are overspread with spots of orange, red, blue and green. There are often some vertical stripes as well, making the Pumpkinseed one of the most brightly coloured and attractive freshwater fishes of North America. The fish reach an average length of 178-229 mm, but in small highly productive lakes with large populations, growth is often stunted to 102-127 mm. Their lifespan is generally does not exceed 8-10 years.

Pumpkinseeds are one of the most widely distributed fishes in Ontario and are common across most of Canada. Though it is native only to waters of eastern North America, it has been widely introduced in most of central North America and Europe as well.

Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Class: Actinopterygii
Order: Perciformes
Suborder: Percoidei
Family: Centrarchidae
Genus: *Lepomis*
Species: *Gibbosus*

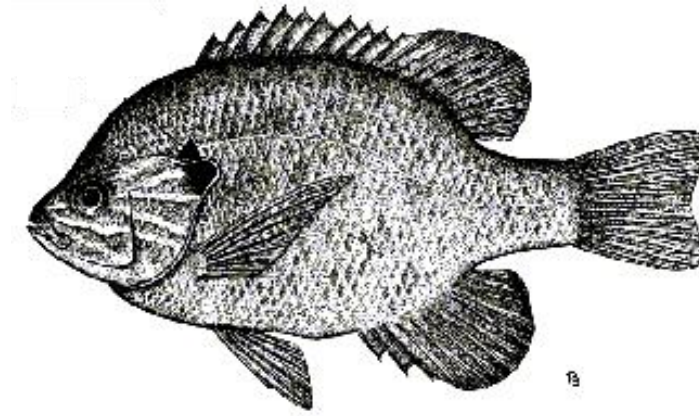


Figure 1.3 Physical appearance of Pumpkinseeds and their taxonomy (<http://www.snre.umich.edu/~pwebb/LesChen/species.html>).

The fish is well known to biologists for its mating behaviour. Nests are shallow and range from 10-40 cm in diameter. Like Smallmouth Bass, Pumpkinseeds use their fins to clear nests near shore free of debris. The males must defend these nests from other males attempting to take over, and after fertilization the males guard the eggs until the fry are hatched. The average adult female is expected to lay 600-2,923 eggs, however this number is likely to increase with fish size. Fry growth is moderate, as young-of-the-year are expected to reach 24-89 mm by age 1.

Pumpkinseeds prefer clear water with submerged vegetation and brush as cover. They are gregarious and often congregate in large numbers near the surface and shore, exposed to the sun. They are primarily associated with the littoral zone, more so than Rock and Smallmouth Bass. Their populations can be large in lakes, often composing the largest portion of an entire given fish community by numbers in small warm bodied waters.

Diet of Pumpkinseed from Welch Lake (New Brunswick) was recorded by Reid (1930) and included dragonfly nymphs, ants, larval salamanders, amphipods, copepods, mayfly nymphs, midge larvae, roundworms, snails, water boatman and other insect larvae. Their food is mostly composed of insects, and secondarily other invertebrates. However, larger Pumpkinseed are known to prey on small fishes and at times, this vertebrate diet can contribute as much as insects to the overall food intake. Pumpkinseed will feed at the surface, within the water column, and the bottom.

Due to their large populations, Pumpkinseed themselves are likely often prey to Basses, Walleye, Perch, Pike and Musky. Recreational and young anglers also target them, as they inhabit areas close to shore that are easily accessible.

Fishes of Algonquin Park

Algonquin Park is famous amongst Canadian anglers for its rich trout populations (Mandrak and Crossman, 2003). The park also has a rich history of fish research dating back to the 1930s, when W.J.K. "Bill" Harkness of the University of Toronto established the park's famous Harkness Research Lab (Mandrak and Crossman, 2003). This lab is also famous for having the longest ongoing study of Lake Trout, or any other fish, in the world, which has been occurring since 1936 (Martin, 1970; Matuszek et al. 1990; Mandrak and Crossman, 2003).

The park has isolated populations of fish when considering their unique means of assembly that were formed owing to glacier movement during the last ice age and also due to the natural variation in altitude throughout the park (Mandrak and Crossman, 2003). Glaciation of the Laurentide Ice Sheet 10,000 years ago led to establishment of cold-water fishes into the park, and also created many compartmental water bodies (Mandrak and Crossman, 2003).

During this time, the cold waters that flowed out of the Park from the thawing glacier could only be used by cold-water species, such as Brook Trout, for colonization (Mandrak and Crossman, 2003). Warm water fishes, such as Smallmouth Bass and Northern Pike, could not establish during glaciation due to low water temperatures (Mandrak and Crossman, 2003). When the water was finally warm enough for other species to withstand, there was a diminished volume of water flowing from the glacier and obstacles such as waterfalls causing natural barriers to these fish (Mandrak and Crossman, 2003).

In the last century, humans have had a major impact on the diversity of fish fauna in the park, where species such as the American eel have been extirpated due to the construction of dams blocking their migratory pathways (Martin and Fry, 1973; Mandrak and Crossman, 2003). Perhaps more importantly, many species have been introduced to the park, purposefully and accidentally, which have had major impacts on the park's natural ecology (Matuszek et al. 1990; Mandrak and Crossman, 2003; Jacques, 2005). Accidental introductions most often happen from bait bucket release by careless fishermen (Ludwig and Leitch, 1996; Mandrak and Crossman, 2003).

However, the purposeful introduction of Smallmouth Bass in the late 1800s was carried out as a way to promote diversity of species for Algonquin as a "game reserve" (Mandrak and Crossman, 2003). Its introduction has had grave consequences, especially for locally adapted Brook Trout populations, which have become seriously reduced since Smallmouth Bass were introduced (Mandrak and Crossman, 2003). Smallmouth Bass have never been native to the park, but by 1901 many populations were extremely well established (Mandrak and Crossman, 2003).

More recently, the rapid establishment of invasive Rock Bass, Rainbow Smelt and Northern Pike has been occurring in the park since the 1980s and is causing degradation to native fish, such as Lake Trout and Brook Trout (Mandrak and Crossman, 2003). There are few fish communities within Algonquin Park that are undisturbed because they have locally adapted to a habitat without large warm water predatory fish such as Bass and Pike and therefore lack any predator avoidance behaviour (Mandrak and Crossman, 2003). Northern pike pose the most serious threat to the natural trout populations of Algonquin waters, but have not yet invaded important trout lakes (Mandrak and Crossman, 2003;

Trumpickas et al. 2011). However, their presence in the Opeongo River system has resulted in declines in unwary small fish and minnow species (Mandrak and Crossman, 2003).

Rainbow smelt have likely been introduced to the park in the 1980s by anglers hoping to increase prey for trout (Mandrak and Crossman, 2003). Ironically, these fish are a prime example of the negative ecological consequences of invasive species, as they prey heavily on eggs and young of the year trout and also pose competition for other resources (Mandrak and Crossman, 2003).

The introduction of Rock Bass in Algonquin Park has had major implications for native fish populations (Vander Zanden et al. 1999; Mandrak and Crossman, 2003). Rock Bass have highly voracious appetites for small fish, therefore reducing the number of prey sustaining the Park's native fish populations (Scott and Crossman, 1973). The impact of their introduction has also fallen heavily upon young lake trout emerging in the spring from shallow rocky spawning beds (Mandrak and Crossman, 2003). This shallow water is used by the trout during the winter, but these naïve fish lack predator avoidance behaviour and are often preyed upon in high quantities by Rock Bass, seriously reducing their population (Mandrak and Crossman, 2003). Rock Bass are likely native to the northwest Amable du Fond and southeastern Petawawa drainage of the Park (Mandrak and Crossman, 2003). Their recent and rapid invasion, most likely due to bait bucket release, in most of the park's waters has been occurring since the 1980s (Mandrak and Crossman, 2003).

The importance of parasitism

Parasitism is a form of symbiosis between two species. In this relationship, the parasite infects a host species and depends on it as both a habitat and source of energy and nutrients. Macroparasites such as helminths (worms) tend to have a complex life cycle that includes a free-living stage and multiple hosts (Roy and May, 1978). The parasitic stage is obligatory for the parasite, and harmful to the host. However, both internal and external parasites can have impacts as minimal as irritation or as severe as mortality in their hosts (Roy and May, 1978).

Helminths can be free-living but also include parasitic worms, and encompass the monogeneans, trematodes, and cestodes within the Phylum Platyhelminths, as well as members of the Phylum Nematoda and Phylum Acanthocephala (Parker et al. 2015). Intermediate hosts are used by these helminths for growth, development, and transmission to the next host, but not for sexual maturation or reproduction, which occurs in the final host (Parker et al. 2015).

Adult cestodes, also known as tapeworms, are dorsal-ventrally flattened and consist of multiple segments called proglottids (Castro, 1996). The head of a tapeworm is known as the scolex and includes suckers for host attachment (Castro, 1996). A neck typically follows the scolex and a chain of proglottids, called strobila, which becomes longer as the tapeworm grows (Castro, 1996).

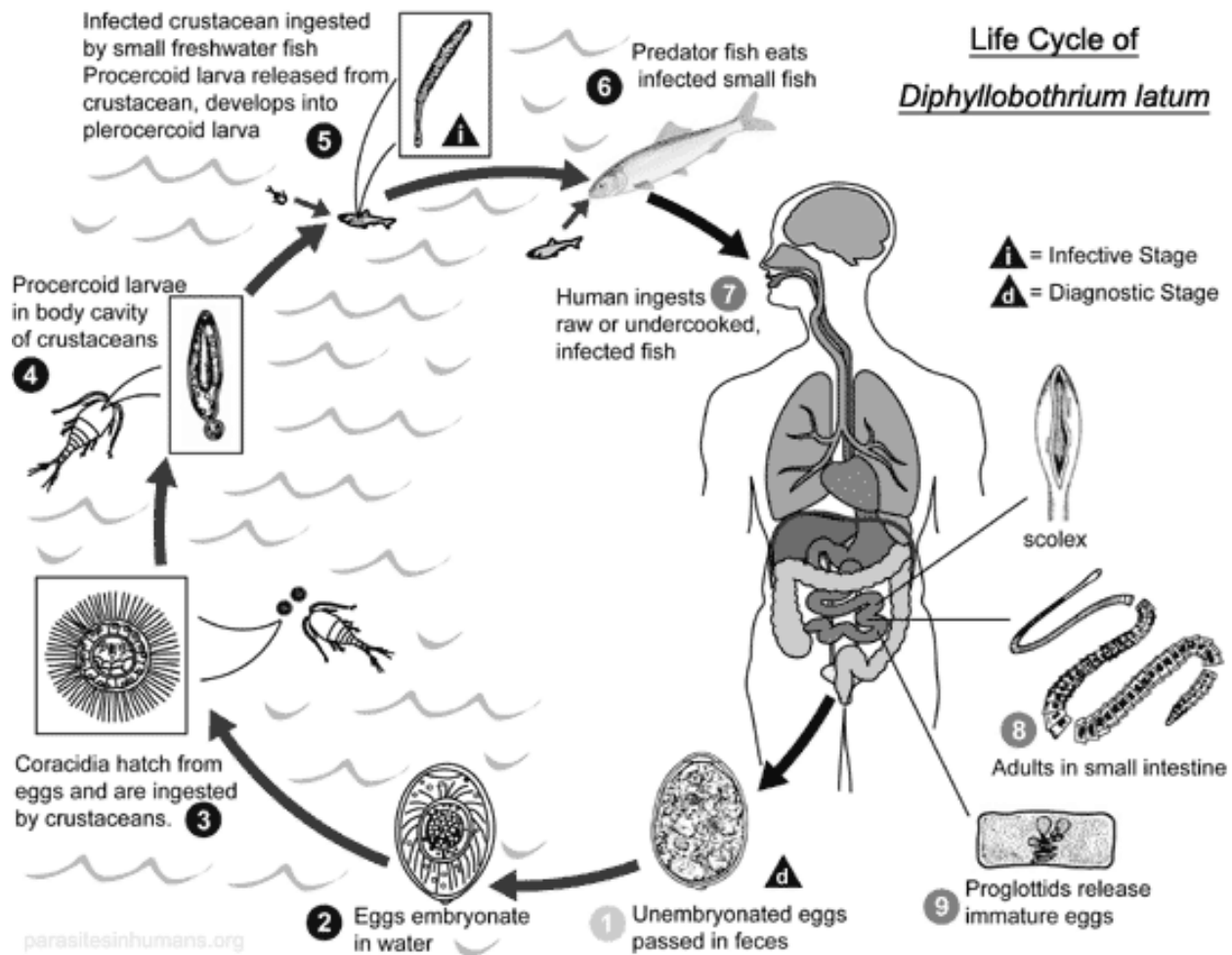


Figure 1.4 General cestode lifecycle
(<http://www.parasesinhumans.org/diphyllobothrium-latum-fish-tapeworm.html>).

Cestodes have a life cycle that typically begins when a mature worm releases gravid proglottids into the gut of its final host, which can be released into an aquatic environment (see Fig. 1.4). Upon entering the water, the proglottids lyse and the eggs are released to become food items of potential hosts, such as small copepods, which become the first intermediate host. The tapeworm develops into an infective larval plerocercoid in this host, before being consumed by a fish second intermediate host, where the larval plerocercoid burrows through the gut into the body cavity to infect and damage multiple tissues. Finally, when the small fish is consumed by the final host, the tapeworms can complete their lifecycle, becoming adults with gravid proglottids.

In contrast to cestodes, nematodes are cylindrical in shape which has resulted in their common name, roundworms (Castro, 1996). Their developmental stages include eggs, several larval forms where the cuticle is molted, and a mature adult phase capable of reproduction (see Fig. 5). Like cestodes, the life cycle for fish-infecting species begins when eggs are released into an aquatic environment by their final host. The resulting larvae of these eggs are prey items for various invertebrates such as zooplankton, which when consumed by a larger fish, become infective. The life cycle is completed when the nematodes reach their final vertebrate host and become mature (see Fig. 1.5).

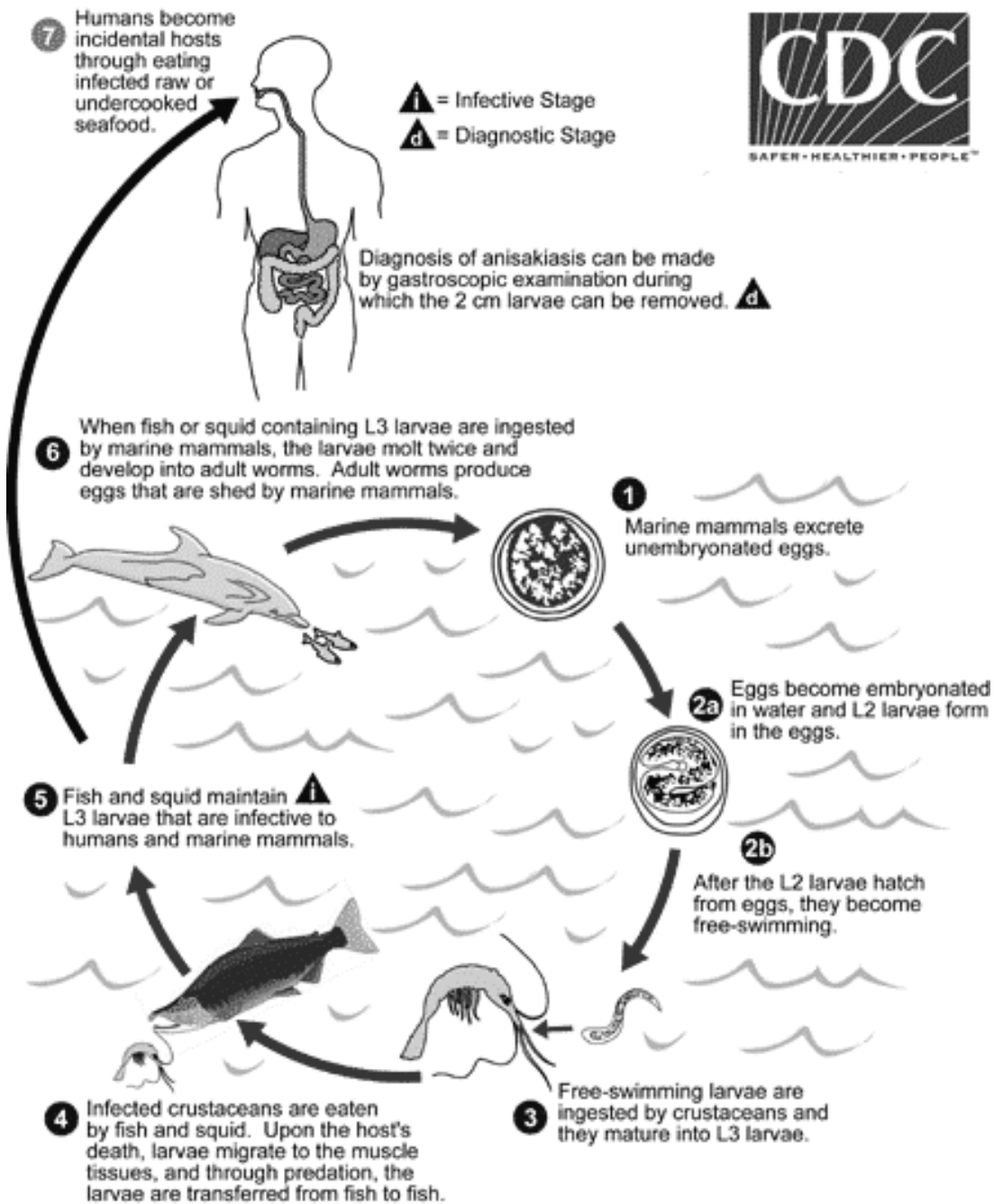


Figure 1.5 General nematode lifecycle
 (<http://www.cdc.gov/parasites/anisakiasis/biology.html>).

Acanthocephalan parasites are also known as thorny-headed worms due to the prominent proboscis at the anterior end of their cylindrical body (Near, 2002). The worms are gonochoristic (unisexual) and reproduce in their final host after transmission from an intermediate arthropod host (Near, 2002). Acanthocephalans in their early life stages fully penetrate the intestinal wall and can enter the body cavity of their intermediate hosts in order to develop (Nickol, 1985). Adults, however, remain in the intestinal lumen of their final vertebrate host where reproduction occurs (Near, 2002) (see Fig. 1.6).

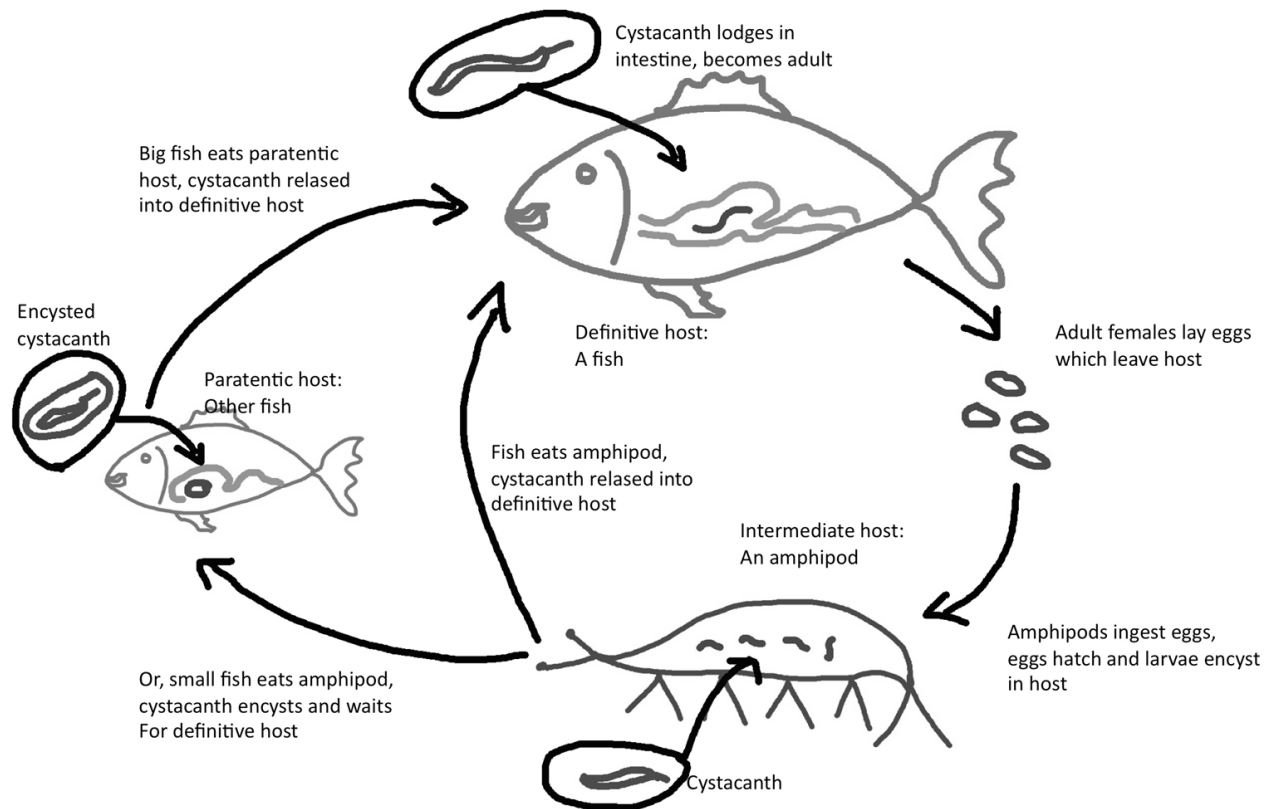


Figure 1.6 General acanthocephalan life cycle
 (<https://increasingdisorder.wordpress.com/2011/12/07/acantho-what/>).

Parasite Ecology

Studying parasite ecology can yield valuable information that can lead to a more sophisticated understanding of energy and nutrient transfer, as well as predator-prey relationships (Lafferty, 2008). The free-living infectious stages of most parasites, such as cercariae in trematodes or eggs of helminths, are traditionally excluded from most food web studies, as their contributions are puzzling and difficult to measure even though they are abundant (Lafferty, 2008). For example, Lafferty (2006) found 505 predator-prey links in the Carpinteria Salt Marsh in California, and an additional 1535 additional parasite-based links. Memmot et al. (2000) found that by adding parasitoid-host information to a food web already containing traditional predator-prey interactions among insects, plants and predators, there was a significant decrease to food web connectivity. This is likely because many parasites are highly specific to their hosts, whereas predators typically have multiple prey items (Lafferty and Kuris, 2002).

This suggests that the topology of food webs excluding parasite information may be largely miscalculated (Lafferty, 2008). The ability to understand animal interactions is incredibly important given current environmental perturbations. For example, one of the many major concerns regarding climate change includes how parasite dynamics and host-parasite interactions will be affected (Elliot et al. 2002). Invasive species are also of concern and can have a variety of effects on novel ecosystems. For example, invasive zebra mussels have become one of the most abundant freshwater animals in the Great Lakes since 1988 (Strayer, 2008) and such bivalves can readily consume free-living parasites, substantially reducing the infection rate in down-stream hosts (Thieltges et al. 2008, 2009). However, these same zebra mussels can also act as intermediate hosts to parasites, which

may serve to amplify their population (Molloy et al. 1997; Minguéz et al. 2011). Many abiotic factors affected by anthropogenic activities are known to influence the transmission of parasites in aquatic ecosystems (Thieltges et al. 2008). Some of these factors include temperature, salinity, pH, hardness, UV-radiation and pollutants (Thieltges et al. 2008).

As they can cause serious diseases, identifying the factors that control parasite transmission can help reveal the importance of changing environments. For instance, climate change will have a significant effect on animal thermal habitats (Cline et al. 2014), and is likely to modify trophic interactions, phenology, habitat distribution and growth. Hellman et al. (2008) believe there at least 5 consequences of climate change for invasive species, including "(1) altered transport and introduction mechanisms, (2) establishment of new invasive species, (3) altered impact of existing invasive species, (4) altered distribution of existing invasive species, and (5) altered effectiveness of control strategies."

Not all parasites are equal in their ecological roles and influence on community structure. Poulin (1999) suggests three mechanisms in which parasites can influence community structure. First, if different hosts have higher levels of susceptibility to infection, then parasites may suppress these populations. If this parasite were to be eliminated or shift its range, its host population could increase in abundance at the expense of other species. Second, a parasite may negatively affect the role of the host at a community level by causing physiological damage. Third, a parasite may positively affect the success of host survival by altering its phenotype in a positive way.

Many predators in aquatic ecosystems, including insect larvae and copepods, prey on parasites (particularly free-living infectious stages); these may serve as a major source of energy and nutrients and contribute to food web energy flow (Johnson et al. 2010).

However, parasites are typically consumed when an infected host is eaten by a predator (Johnson et al. 2010). Predators therefore have a suite of parasites that can very much reflect their dietary preferences (Johnson et al. 2010). In fact, the diet of hosts is likely the most important factor dictating the presence of trophically-transmitted parasites. Differences among host species are therefore important to understand, as parasite infracommunities can yield information regarding trophic interactions (Johnson et al. 2010).

Invasive species and parasites

Understanding why invasive species are able to succeed in novel environments is an obstacle in invasion ecology (Sanchez et al. 2012). The mechanisms behind the success of invasives is of dire importance, considering that invasive species are currently a major driving factor behind species extinction (Poos et al. 2010). Though there has been intensive research in this field in the past 20 years (Kolar and Lodge, 2001), exactly which combinations of biotic and abiotic factors contribute to successful invasions is unknown (Sanchez et al. 2012).

The *Enemy release hypothesis* (Keane and Crawley, 2002; Torchin et al. 2002; Torchin et al. 2003; Colautti et al. 2004) is perhaps the most accepted one in invasive ecology, and states that invasives will experience less regulation by natural enemies such as predators in habitats where they are introduced. However, parasites are clearly an important class of natural enemy, and invasive species that are not easily infected may be able to more easily establish.

Recently, there has been growing evidence suggesting that competition between invasive and native species can be heavily influenced by parasites (Mitchell and Power, 2003; Torchin et al. 2003; Sanchez, 2012). In a study comparing cestode burdens in native and invasive brine shrimp, the invasive demonstrated largely reduced diversity, intensity and prevalence of infection compared to the native species (Georgiev et al. 2007; Sanchez et al. 2012). However, there are mechanisms in which invasive species may act as susceptible and naïve hosts to new parasites too (see Chapter 2).

It has been suggested that the difference in infections is due to host-parasite coevolutionary history (Sanchez et al. 2012). Host species which have never been exposed to a given parasite may be immune to infection if the parasite is unable to overcome host defences. Since the invasive brine shrimp mentioned above has only been present in the study area for 40 years, there may not have been enough time for a potential host shift for cestode infection (Sanchez et al. 2012).

Research aims and hypotheses

Freshwater fish communities are very sensitive, and species declines have been attributed to many factors including invasions and deliberate introductions of non-native species (Mandrak and Crossman, 2003; Koehn, 2004), as well as the spread of disease (Riley et al. 2008; Johnson and Paull, 2011). In Algonquin Park, there has been a rich history of fish introductions, which have already had serious consequences for native species (Mandrak and Crossman, 2003). By evaluating the parasite communities of select fish hosts, it may be possible to determine if disease dynamics are influencing the

establishment of invasive species in Algonquin Park. Parasites, along with other measures, can also be used to identify components of fish diet.

I hypothesized that invasive fish and smaller fish would have a reduced parasite burden because of the known positive relationship between parasitism and host size for many species, and also in accordance with the enemy release hypothesis (Torchin et al. 2002; Poulin, 2007) (Chapter 2). I also expected that larger fish with high trophic positions would have a more diverse suite of trophically transmitted parasites, which will reflect their diet more than their habitat (Chapter 3). In addition, I predicted that non-trophically transmitted parasites should be related to the habitat preferences of fish and not their diet.

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CHAPTER 2: PARASITISM OF INVASIVE AND ESTABLISHED FISH SPECIES

Introduction

The loss of freshwater species today is primarily driven by anthropogenic activities, including habitat loss, invasion by non-native species, climate change, eutrophication, and overharvesting of top-predators (Vitousek et al. 1997; Ricciardi et al. 1999; Jackson et al. 2001; Dudgeon et al. 2006). The invasion and introduction of non-native species has been of key interest to biologists and ecologists (Bauer, 2013). Since the 1800s, and especially with growth in transoceanic trading (Mills et al. 1993), there has been a long history of invasive species in North American freshwater bodies, including the St. Lawrence River and the Great Lakes. Major sources of invasive species include the aquarium and ornamental fish trades (Padilla and Williams, 2008), untreated ballast water and irresponsible bait management by anglers (Egan et al. 2015). Significant correlations between ship traffic and introduction of species has been recorded especially in the Great Lakes (Ricciardi, 2006). Since the construction of the Erie Canal System and St. Lawrence Seaway, more than 182 invasive species have been discovered (Ricciardi, 2006). However, the problems of introduced and invasive species are not limited to such areas of shipping traffic. The introduction of fishes into Algonquin Park, Ontario, is one such example.

Smallmouth Bass (*Micropterus dolomieu*) are one of the world's most widely distributed freshwater fishes (Scott and Crossman, 1973). The purposeful introduction of Smallmouth Bass into Algonquin Park in the late 1800s was carried out as a way to promote a diversity of species to serve as a "game reserve" (Mandrak and Crossman, 2003). By 1901, they were extremely well established in in the majority of Algonquin's major

water systems. Their high fecundity, juvenile use of cover, early onset of piscivory in juveniles (Brown et al. 2009), parental care (Scott and Crossman, 1973), and low overlap with other predators (Gard, 2004) all favour their survival and establishment in lakes, rivers, estuaries, reservoirs, ponds and creeks.

Smallmouth Bass have a large and diverse appetite that changes through their development (Scott and Crossman, 1973). During this ontogenetic shift they are likely to consume many invertebrates, such as copepods and mayflies (Scott and Crossman, 1973; Easton and Orth, 1992). Notably, many of these prey items can serve as routes of transmission for trophically-transmitted parasites. At the end of their first year, these fish climb the food chain to become tertiary and quaternary consumers, consuming larger prey such as other fish, crayfish and frogs (Scott and Crossman, 1973). The presence of such top-predators in ecosystems is crucial for regulating prey populations, thus sustaining the populations of mesopredators and lower trophic levels in general (Bascompte et al. 2005). Their presence is thought to increase the biodiversity and stability of ecosystems (Bascompte et al. 2005).

Rock Bass (*Ambloplites rupestris*) are a freshwater centrarchid fish widely distributed over eastern and central North America (Scott and Crossman, 1973). Their recent and rapid invasion in Algonquin Park, most likely due to bait bucket release or illegal purposeful introduction, has had major implications for native fish populations (Mandrak and Crossman, 2003). Rock Bass are voracious predators of small fish, therefore reducing the number of prey sustaining the native fish populations (Mandrak and Crossman, 2003), and a detailed diet of Rock Bass through their development was recorded by Keast and Webb (1966) – see Chapter 1 (General Introduction) for more information.

Rock Bass are smaller than Smallmouth Bass and also have much smaller mouths, suggesting less intake of large prey items such as other fish compared to Smallmouth Bass. In fact, Keast and Webb (1966) found fish greater than 120 mm had diets that consisted almost entirely of crayfish and Anisoptera (dragonflies). As such, Rock Bass are mesopredators in most of Algonquin's lakes and rivers. In lakes lacking larger fish species, Rock Bass might be top predators, but are still not ecologically identical in their influence as true tertiary consumers (Prugh et al. 2009).

Top predators control mesopredator populations. In many lakes, the population of Rock Bass may be limited by the presence of Smallmouth Bass, especially as the two species overlap in their habitat (Scott and Crossman, 1973; Mandrak and Crossman, 2003). Mesopredator release occurs when top predators are removed from ecosystems, often due to anthropogenic disturbances, and this can result in mesopredator increases (Prugh et al. 2009). Trophic cascades caused by mesopredator release from predation have been recorded in fish, birds, sea turtles, marsupials, rodents, lizards, scallops, rabbits, and ungulates (Brashares et al. 2010). However, there are many other ecological implications related to natural enemies that may occur when species are introduced or invade into new habitats.

A parasite infracommunity is the entire diversity of parasite species living inside or on a single host, regardless of whether or not those parasites interact with one another (Bush et al. 1997). The composition of any given infracommunity is reliant on host exposure and individual susceptibility to parasites in a particular environment (Bauer, 2013). Exposure itself depends on many factors, such as interaction with intermediate

hosts for parasites with complex life cycles, the life history of the parasite, and environmental influences (Poulin, 2007).

Trophic transmission occurs when a parasite is ingested by a host in which it is able to establish, and is a fundamental aspect for many parasite life cycles that utilize multiple hosts for different phases (Lafferty and Kuris, 2002; Poulin, 2011). This requirement is prominent for many helminth parasites that require one or more intermediate hosts to complete their life cycle (Poulin, 2007). As detailed in Chapter 1 (General Introduction), this occurs in all cestodes and acanthocephalans, many nematodes, and the vast majority of trematodes (Parker et al. 2015). For these helminths, trophic transmission (consumption of infected hosts) is necessary (Poulin, 2007). However, some parasites are able to infect their hosts through a free swimming infectious stage depending on the phase of the life cycle (Poulin, 2007). Hosts likely encounter many parasites that are not represented by their infracommunities, which is likely a result of their immunocompetence as well as parasite host specificity (Poulin, 2007).

A component parasite community refers to the total number of parasites that occupy an individual host population rather than those found in individual hosts (Bush et al. 1997). The component communities of two different host species may overlap depending on a variety of ecological factors (Bauer, 2013). Infracommunities are usually composed of a random subset of the total parasites found in their corresponding component communities (Norton, 2004; Poulin, 2007). Biological factors, such as body size, age (Poulin and Valtonen, 2001) and diet (Johnson et al. 2004) have been found to influence infracommunity composition. Ecological factors such as habitat have also been found to be strong drivers of parasite community composition (Poulin and Fitzgerald,

1989). Because so many helminth parasites depend on trophic transmission (Lafferty, 1999; Johnson, 2004; Bertrand et al. 2008), diet and trophic position have been found to be strong drivers structuring host parasite communities (Johnson et al. 2004). For instance, the diversity of trophically-transmitted parasites increases with prey diversity in fish (Valtonen et al. 2010) and saltmarsh animals including birds and mammals (Rossiter and Sukhdeo, 2011).

There is strong justification to believe that invasive species can alter the composition of parasite infracommunities (Kelly et al. 2009a, 2009b), and it has been well observed that introduced and invasive species tend to have a reduced parasite burden in their novel range (Bauer 1991; Torchin et al. 2003). It is unclear if their release from parasite stress significantly assists with their propagation in new environments. Although evidence does support this possible driver of invasive success (Torchin et al. 2001), every ecosystem and population is unique and should be examined individually before drawing broad conclusions (Colatti et al. 2004). In theory, it is possible for invasive species to interfere with the natural integrity of ecosystems by having an effect on parasite transmission to native or established species. For example, the process of parasite *spillover* describes an event in which an invasive species bears parasites capable of infecting hosts in a novel range (Kelly et al. 2009a, 2009b). However, spillover is more likely to assist in the transmission of parasites with simple life cycles. Parasites with complex life cycles relying on specific intermediate and final hosts are unlikely to successfully establish in new regions (Torchin et al. 2003).

Parasite *spillback* occurs when an introduced species serves as a naïve host to the new parasites in its novel range (Kelly et al. 2009a, 2009b). The effects of the mechanism

are two-fold: first it inflates the parasite population; and second, this increases native host risk of exposure (Kelly et al. 2009a, 2009b). In contrast, if the invasive species is susceptible to infection, but not beneficial for parasite survival, the native host(s) may experience a release from their native parasites (Paterson et al. 2013). In this way, naïve invasive species may act as a net sink for infections, thereby reducing the burden on native hosts, which is a process known as *dilution* (Kelly et al. 2009a, 2009b).

The ways in which an invasive species reacts to parasites in a novel environment are complicated and difficult to understand and predict. The overall low diversity and intensity of infections in invasive species may result in decreased trophic transmission of parasites to their intermediate and final hosts. Predators consuming these invasive species are therefore likely to experience increased fitness (Bordes and Morand, 2009; Bulté et al. 2012). Similarly, if invasive species prey on intermediate hosts of native parasites and can resist infection, there can be a reduction in parasite burden as well (Pulkinen et al. 2012). In addition, invasive species that cause declines of native species, such as by predation or competition, can also indirectly cause reductions in native parasite populations (Johnson et al. 2009; Bauer, 2013). In contrast, invasive species can also act to increase the populations of native species, therefore increasing the abundance and transmission of native parasites between native species (Kelly et al. 2009a, 2009b). For example, the initial introduction of zebra mussels into the Great Lakes resulted in large increases to amphipod populations (Mercer et al. 1999), which are a common host for many trophically-transmitted parasites.

Research objectives and hypotheses

Given the alterations of fish communities in Algonquin Park, and the potential role for parasites to facilitate the establishment of introduced species, the aims of this study was to: 1) Determine if there are any differences in infection with trophically-transmitted helminth parasites between invasive Rock Bass and the longer-established invasive Smallmouth Bass in Algonquin Park; and 2) Examine factors that might be drivers any differences, focusing on host diet and size given the transmission pathway for trophically-transmitted helminths.

It is thought spillover is relatively rare as trophically-transmitted parasites require multiple specific hosts, and is less likely to occur as the phylogenetic distance between hosts increases (Torchin et al. 2003). Spillback and high susceptibility of Rock Bass to infection is certainly possible, but the large amount of literature documenting invasive species report reduced parasite burdens (Bauer, 1991; Torchin et al. 2003). Because Smallmouth Bass are a large species able to consume a broad range of prey items, including other large fish, they may acquire more trophically-transmitted parasites than Rock Bass. Notably, the total length of fish has been positively correlated with high parasite diversity and intensity (Poulin, 2007; Ravi and Yahaya, 2015) as larger fish provide more habitat. Larger fish are generally older as well, suggesting they have had more time to accumulate parasites.

My hypotheses therefore were as follows:

- 1) Smallmouth Bass will have a higher diversity and intensity of infection with trophically-transmitted helminths than Rock Bass.
- 2) Fork length of fish will be positively related with infection intensity and diversity and infection both between and within species.

Materials and Methods

Field collections

Fish were collected from 8 different lakes in Algonquin Park between 2 July and 30 July 2015 (see Table 1), with 112 invasive Rock Bass and 60 Smallmouth Bass sampled in total (see Table 1). Of these 8 lakes, 3 were part of the Opeongo River system, and 5 part of the Lake of Two Rivers system (Table 2.1, Fig. 1).

Table 2.1 Number of Rock Bass, Smallmouth Bass and Pumpkinseed caught from 8 different lakes, and their coordinates, in Algonquin Park, Ontario.

Lake	Rock Bass	Smallmouth	Pumpkinseed	Total Fish	Latitude	Longitude
Sasajewun	15	0	15	30	45.59315	-78.52426
Pog	15	0	17	32	45.65284	-78.09234
Opeongo	0	7	17	24	45.65201	-78.12117
Whitefish	18	15	0	33	45.49829	-78.36759
Rock	15	15	0	30	45.57385	-78.45466
Galeairy	18	15	0	33	45.66689	-78.37096
Farm	15	6	17	38	45.53989	-78.41771
Crotch	15	2	0	17	45.48981	-78.33382
total	112	60	66	237	-	-

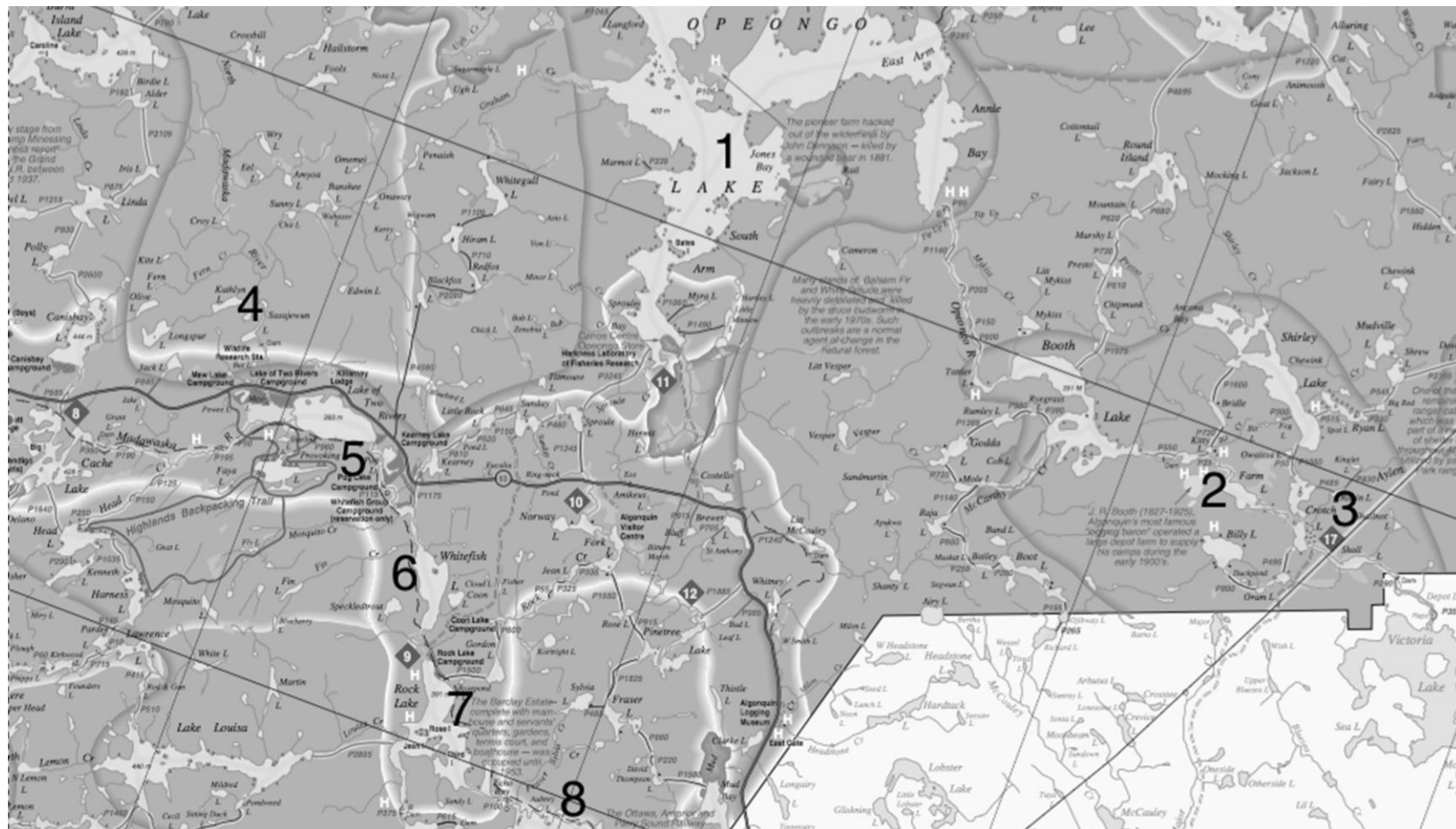


Figure 2.1 Map of lakes sampled from in July 2015 in Algonquin Park, ON (1=Opeongo, 2= Farm, 3= Crotch, 4=Sasajewun, 5=Pog, 6= Whitefish, 7=Rock, 8=Galeairy)
http://algonquinpark.on.ca/virtual/canoe_routes_map/index.php.

Lakes were chosen based on accessibility, and probable occurrence of both Rock Bass and Smallmouth Bass based on previously-documented presence given the records summarized by Mandrak and Crossman (2003). Consultation with park staff and the MNR conservation branch in Algonquin was also carried out prior to sampling. Each of these lakes was sampled several times at various locations until the target sample size of at least 15 fish of each species was achieved. Sampling locations within the lake were chosen to reflect the known preferred habitats of invasive Rock Bass and Smallmouth Bass, including Rocky and weedy habitats near high current areas.

Sampling was conducted via rod and hook during the hours of 08:00-21:00. Most fish were caught using small spinner baits (e.g. Blue Fox Super Vibrax #2), small crank or jerk baits (Live Target Trout Fry), and handcrafted fly lures (tungsten steel and feather). No live bait was used, as it is not permitted in Algonquin Park. Angling was done both from shore and from canoes. Upon capture, fish were identified as Rock Bass or Smallmouth Bass in the field, placed in a portable live-well cooler, and brought back to the lower laboratory at the Wildlife Research Station within Algonquin Park. Fish were euthanized by pithing, and one skinless dorsal muscle plug (~1 g) was removed from either side of the dorsal fin (2 samples per fish) for stable isotope and fatty acid analysis (see Chapter 3). These tissue samples were removed with a scalpel and dissection scissors, placed in small microcentrifuge tubes and frozen at -20°C. Fish were placed individually in sealable plastic bags and frozen at -20°C until they were brought back to Ryerson University on 31 July 2015. Tissue samples were then transferred to a -80°C freezer, and the fish were transferred to another -20°C freezer.

Animal use complied with standards of the Canadian Council of Animal Care and occurred with an approved protocol by the local animal care committee. Collection permits were issued by the Ministry of Natural Resources.

Dissections and parasite identification

Fish were thawed at Ryerson University for necropsy. Each fish was first examined for external parasites on the skin, fins, operculum and eyes with a dissecting microscope. Fork length of the fish was measured before making a lateral incision on the left ventral side of the fish. Another two longitudinal incisions were made running just posterior to the operculum and anterior of the anal fin. The skin and flesh of the fish were then removed to reveal the internal organs. The flesh was first examined for metacercariae infections (cysts of trematodes), then organs including the stomach, liver, spleen, digestive tract and gonads were examined for trophically-transmitted helminth infections. The digestive tract was examined by removing it from the fish and placing it into a dissecting tray, making one consistent cut along the entire length to reveal the interior contents. Prey items were recorded from both the stomach and digestive tract. The sex of each fish was also recorded during these dissections.

I focused on trophically transmitted helminths in 4 major groups: Phylum Platyhelminthes – juvenile and adult tapeworms in the Class Cestoda along with adult trematodes in the Class Trematoda; adult nematodes in the Phylum Nematoda, and adult thorny-headed worms in the Phylum Acanthocephala. These are most likely to reflect host diet and habitat use but I also examined fish for trematode metacercariae (cysts acquired via penetration, i.e. not trophically-transmitted). Upon finding a helminth, except for

metacercariae which were recorded but left in the flesh, the parasite was removed and preserved in vials containing 70% ethanol. For the purposes of identification, it was then necessary to prepare slide mounts of representative specimens.

Cestodes and acanthocephalans were removed from 70% ethanol storage and prepared in the following way based on well-established methods (e.g. Schell 1970; Schmidt, 1986):

1. Fixation in a mixture of alcohol, formalin and acetic acid (AFA) for 48 hours (acanthocephalan parasites were first pricked several times with a pin to allow for diffusion)
2. Immersed in 70% ethanol for 2 hours
3. Stained in acetocarmine for 3 hours
4. Washed with 70% ethanol for 20 minutes
5. Immersed in 70% acid ethanol (1 mL 1N HCl/10 ml 70% ethanol)
6. Immersed in 70% ethanol for 10 minutes
7. Immersed in 95% ethanol for 20 minutes
8. Immersed in toluene 20 minutes (2 washes as a fixative)

After preparation, cestodes and acanthocephalans were fixed onto slides and cover slips were affixed using Canada Balsam as a permanent mounting medium. For large cestodes, it was sometimes necessary to only prepare and mount the anterior portion of the worm containing the critical features for identification. Nematodes were not prepared for permanent mounting. Rather, they were immersed in 70% ethanol containing several drops of 5% glycerol for clearing. They were temporarily examined as wet mounts, then placed back in 70% ethanol for storage. To identify helminths, I used standard keys for each major group (e.g. Schell 1970; Schmidt, 1986) to the lowest level of taxonomic classification possible. I also relied on published host-parasite checklists (e.g. Margolis and Arthur, 1979) to narrow down the possibilities. When multiple worms were present in a single location within an individual fish, such as the presence of adult cestodes in the small

intestine, we assumed that these represented multiple infections by the same species.

Owing to the high intensity of infection in some fish, it was not possible to remove, prepare, and identify each individual helminth.

Data Analyses

The presence of an infection with a trophically-transmitted helminth was categorized for each fish by either a 0 (no infection) or a 1 (infection), and was also coded separately for the presence of trematode metacercariae. Intensity of infection with any such helminth was categorized on a scale of 0-5 (0=no infection, 1= 1-2 individual helminths, 2= 3-5, 3=5-10, 4=10-15, 5=15+ individual helminths). Mean infracommunity (parasite richness within a given fish) was also determined by assuming infection in different organs by helminths in the same class represented different species given the highly-specific site selection of most parasites, along with counting the number of different classes or phyla; this represents standard methodology (Bush et al., 1997). For instance, a fish with 1 acanthocephalan in the intestine, 1 cestode in the liver, and 1 cestode in the stomach was considered to have a richness score of 3. This approach is likely to have underestimated helminth richness given that multiple species of cestode and acanthocephalan can occupy certain organs such as the small intestine; however, we decided on a conservative approach. Host sex was categorized as 0-3 (0=unknown, 1=male, 2=female). Fork length was included as a continuous measure. The sampling sites (lakes) were each given a category (1-8) and host gut contents were broken down into 5 categories (0=none, 1=invertebrate, 2=vertebrate, 3=both invertebrate and vertebrate, 4= plant

matter). No fish were observed with different combinations of gut contents in this study beyond those listed above.

I first determined whether there was a difference between Smallmouth Bass and Rock Bass in terms of their infection status (infected or not) using a Generalized Linear Mixed Model (GLMM) with a binomial distribution and logit link function for this dependent variable. Host species was used as a categorical fixed effect, and I included site (lake i.d. as category), sex (category), fork length (continuous) as random effects. To investigate species differences in infection with trematode metacercariae, I also used a GLMM in the same way, substituting the presence/absence of these cysts as the dependent variable. I calculated the infection intensity score by summing those for each of the 6 major fish body regions examined (liver, body cavity, gonads, spleen, digestive tract, and stomach) and then did a \log_{10} transformation to meet the assumptions of a normal distribution. This measure was then analyzed with a Linear Mixed Model (LMM) using an identity link function. The same fixed and random effects were used as for infection status. A species difference in parasite richness (\log_{10} -transformed; linear with identity link) was also examined with a LMM with the same fixed and random effects as above.

To see if Smallmouth Bass and Rock Bass differed in their fork length, I used a LMM as well, but now used \log_{10} -transformed fork length as the dependent measure rather than as a random effect. Because fork length could be a major driver of parasite infection, I then used a LMM to look at the relationship between fork length (fixed effect) and intensity score, using site, sex, and host species as random effects. This was also done for parasite richness as the dependent variable. Finally, I examined host species differences in gut contents after removing fish from dataset who didn't have anything in their guts – this left

112 fish in the analysis. Although there were 4 original diet categories, there were only 2 fish in category 3, and 5 fish in category 4, thus I removed these, leaving 105 fish and a binary outcome for diet (either category 1 or 2). I then again used a GLMM with host species as the fixed effect (predictor), with site and fork length as random effects. All analyses were carried out with SPSS 23.1.

Results

A total of 59 Smallmouth and 110 Rock Bass were caught from 8 different lakes. Cestodes were the most common parasite found in these fish (Table 2). There was a tendency for Smallmouth Bass to be infected with a trophically-transmitted helminth compared to Rock Bass ($F_{1,120} = 3.652$, $P = 0.058$). Rock Bass had a mean trophically-transmitted helminth infection intensity of $3.12 (\pm 3.93 \text{ S.D.})$, which was significantly lower ($F_{1,100} = 7.929$, $P = 0.006$) than Smallmouth Bass, which had a score of $4.80 (\pm 4.44 \text{ S.D.})$ (Fig. 2.1). Infections were found in the stomach, digestive tract, spleen, gonads and body cavity (Fig. 2.2).

Table 2.2 Parasites found in Rock Bass (RB) and Smallmouth Bass (SMB) in Algonquin Park, July 2015.

Parasite	Classification	Fish
<i>Neoechinorhynchus cylindratus</i>	Acanthocephala	RB
<i>Leptorhynchoides thecatus</i>	Acanthocephala	SMB
<i>Proteocephalus ambloplitis</i>	Cestoda	SMB, RB
<i>Bothriocephalus sp.</i>	Cestoda	SMB, RB
<i>Trianophorus sp.</i>	Cestoda	SMB, RB
<i>Rhabdochona sp.</i>	Nematoda	SMB, RB

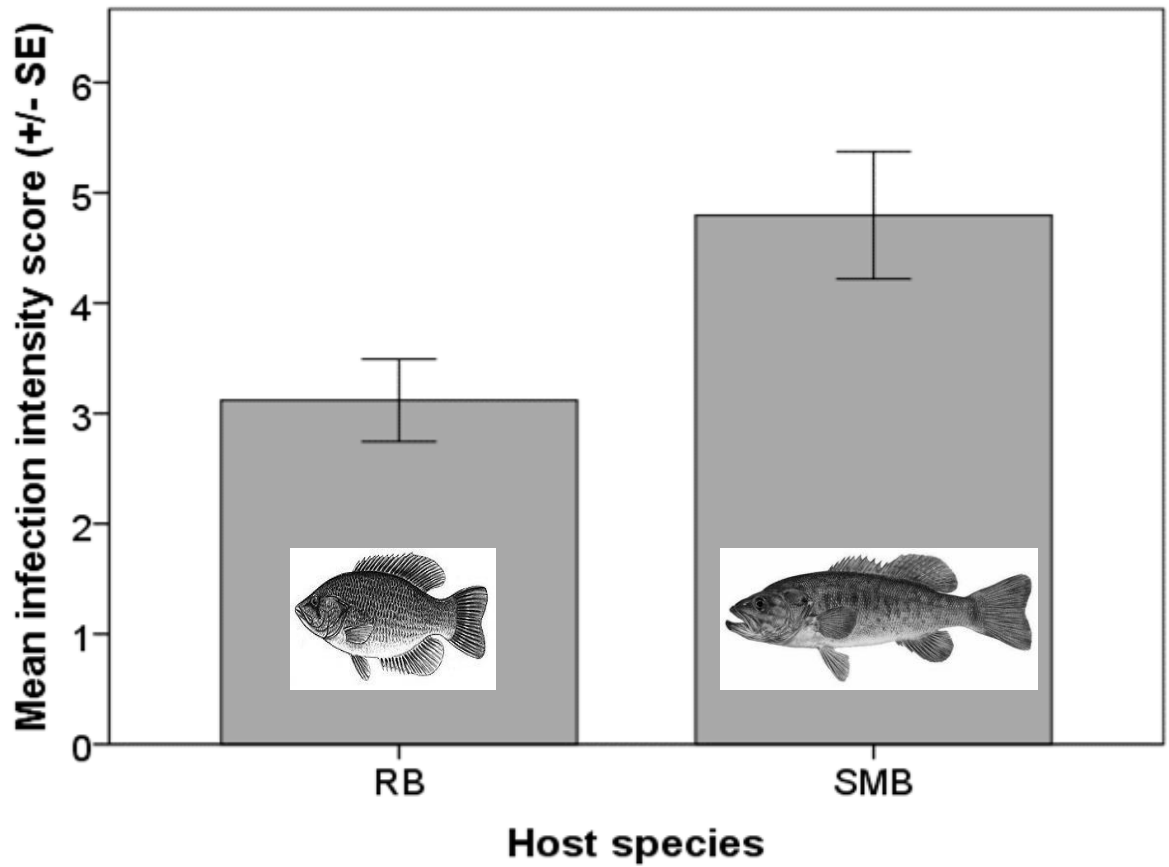


Figure 2.3 Mean macroparasite infection intensity score (\pm S.E.) for Rock Bass (RB) and Smallmouth Bass (SMB) caught in Algonquin Park, July 2015.

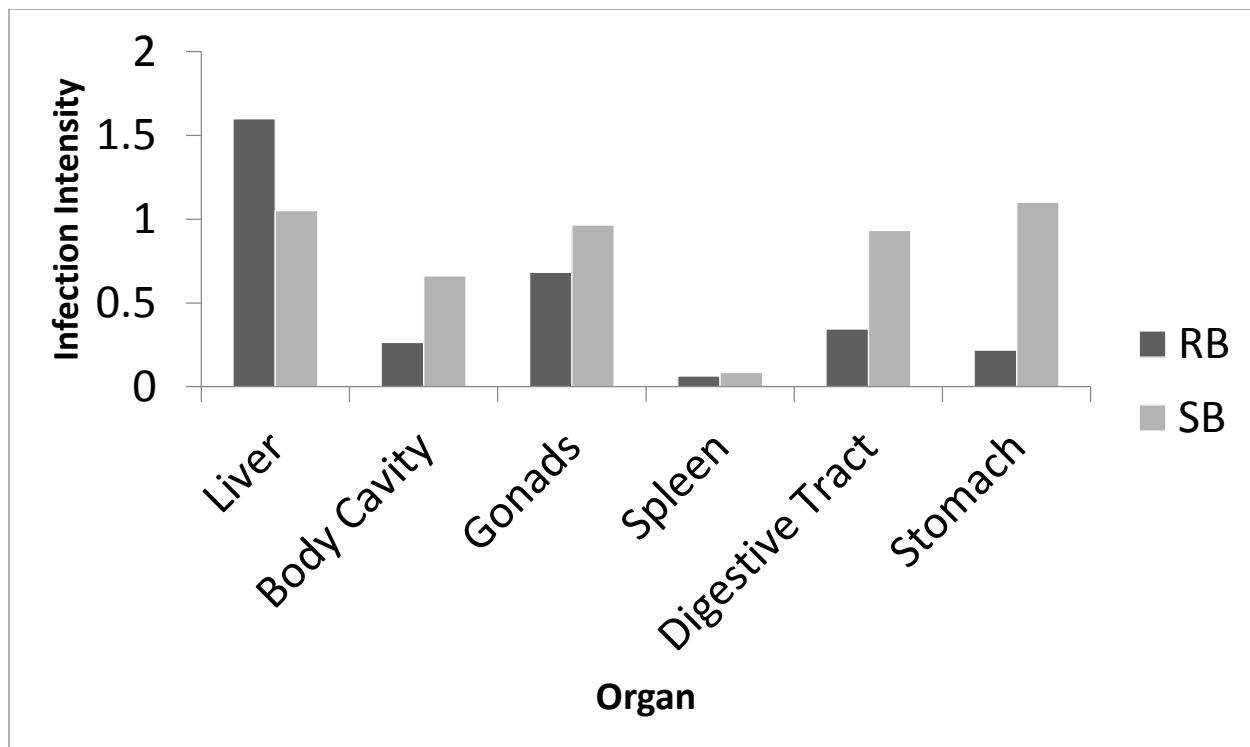


Figure 2.4 Mean macroparasite infection intensity by major organ in Rock Bass (RB) and Smallmouth Bass (SB).

Smallmouth Bass had a mean parasite richness of 1.81 (\pm 0.221 S.E.), which was significantly higher than Rock Bass ($F_{1,101} = 5.906$, $P = 0.017$), where the mean was 1.26 (\pm 0.158 S.E.) (2.3).

Macroparasite intensity was positively correlated with fork length in both Smallmouth and Rock Bass ($F_{1,91} = 5.481$, $P < 0.001$), as was parasite species richness ($F_{1,90} = 1.933$, $P = 0.023$) (Fig. 2.4).

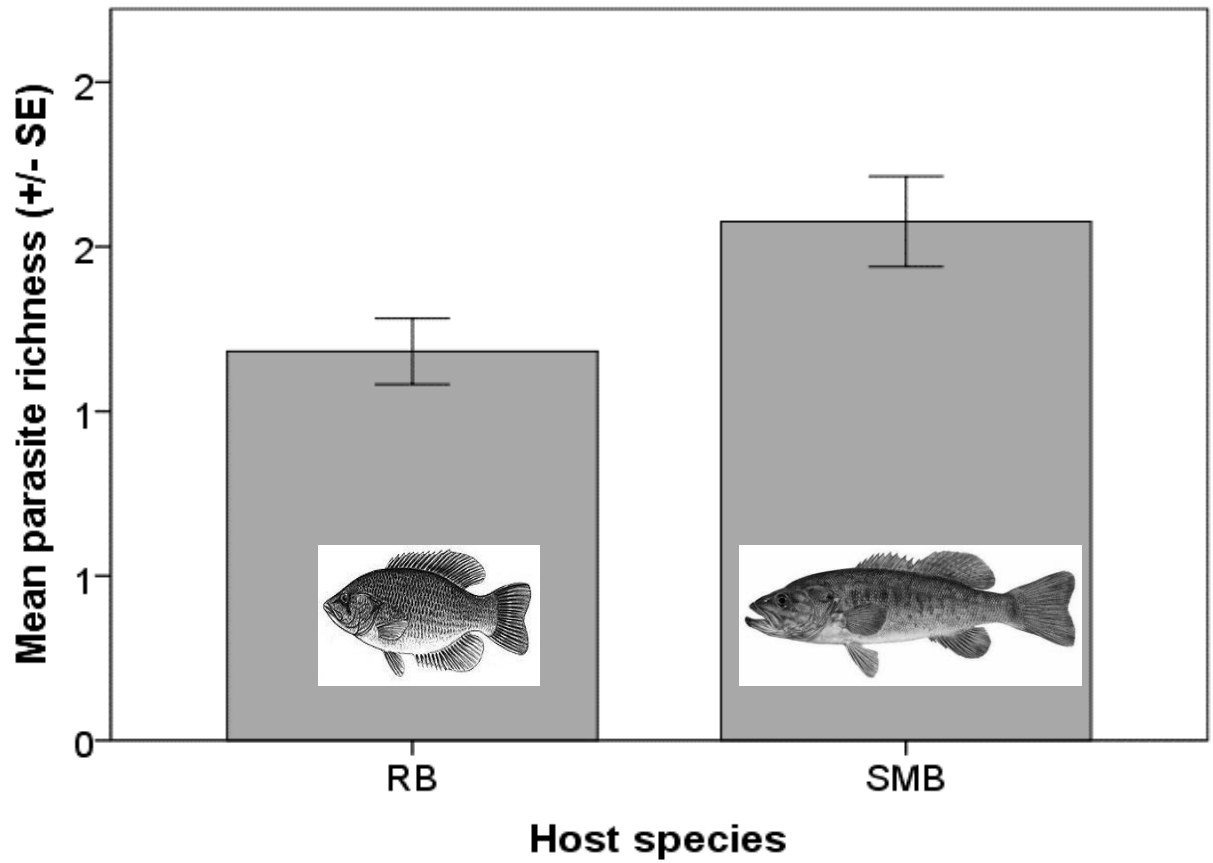


Figure 2.5 Mean macroparasite species richness (\pm S.E.) for Rock Bass (RB) and Smallmouth (SMB) Bass.

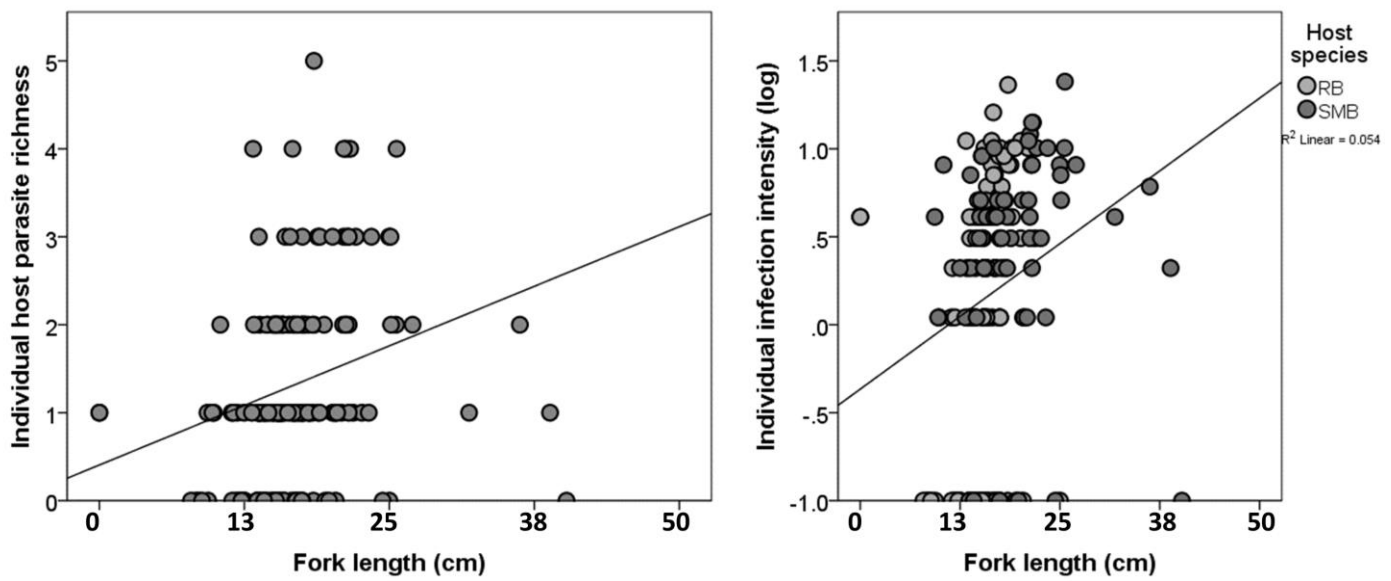


Figure 2.6 Macroparasite intensity scores and parasite species richness compared to the fork length of Rock Bass (RB) and Smallmouth Bass (SMB).

Related to this, mean fork length was significantly greater in Smallmouth Bass compared to Rock Bass ($F_{1,117} = 18.938$, $P < 0.001$) (see Fig. 2.5). In contrast, there was no difference between Smallmouth Bass and Rock Bass with respect to infection with trematode metacercariae that are not trophically-transmitted ($F_{1,118} = 1.479$, $P = 0.226$). There was also no broad difference between the two host species regarding their diet, based on the presence of either invertebrates or vertebrates in their gut contents ($F_{1,103} = 0.473$, $P = 0.493$).

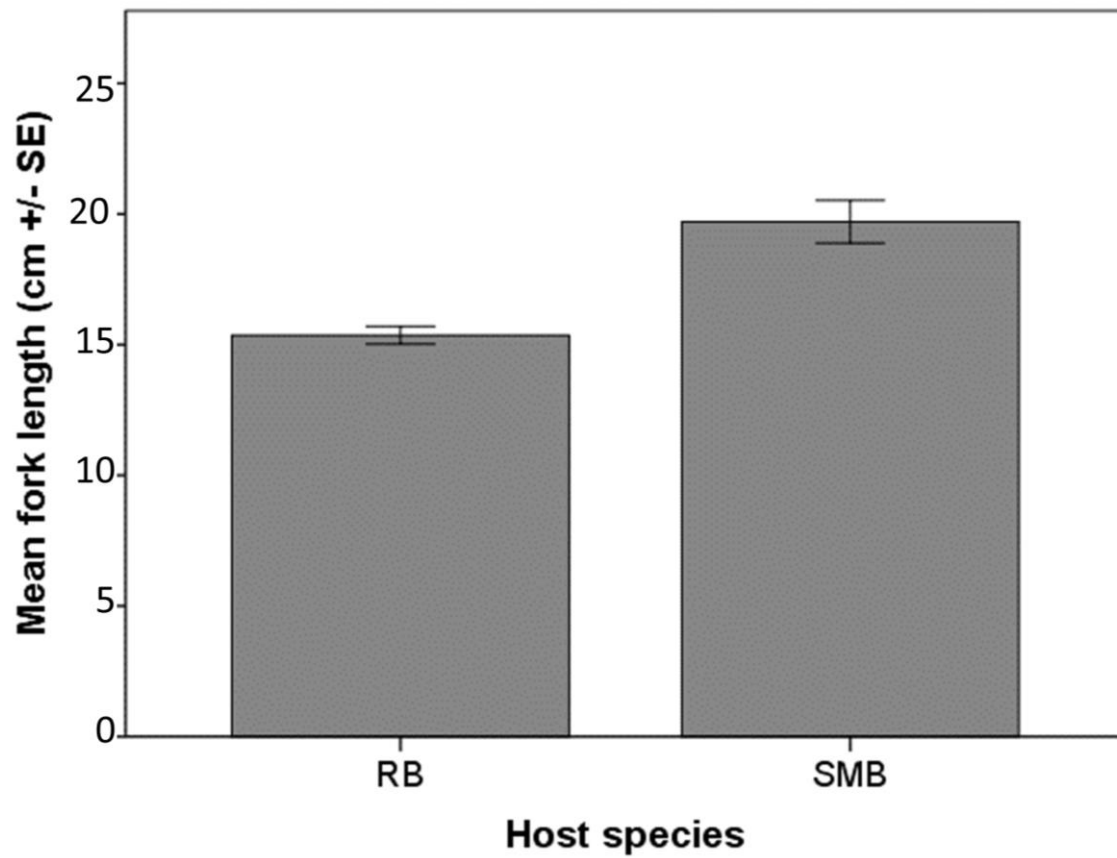


Figure 2.7 Mean fork length (\pm S.E.) of 112 Rock Bass (RB) and 59 Smallmouth Bass (SMB).

Discussion

My survey of fish and their parasites generated findings mostly consistent with my hypotheses. The longer-established host species (Smallmouth Bass) was more likely to be infected with trophically-transmitted helminths such as cestodes and acanthocephalans compared to Rock Bass, which have more recently invaded various lakes in Algonquin Park. In addition, Smallmouth Bass harboured a greater richness of these helminths. Differences in the parasite component infracommunities between Smallmouth and Rock Bass could be a reflection of their general ecology, including their habitat and dietary preferences, or driven by the unique interactions of invasive species and local parasites.

Although there was no significant difference in the number of invertebrate and vertebrate prey items found in the gut contents of the two species, the common presence of adult tapeworms (e.g. *Proteocephalus ambloplitis*) in Smallmouth Bass suggests a more fish-based diet given the necessary ingestion of a smaller fish containing larval cestodes which may not have been accurately reflected in their gut contents (Rybczynski, 2008). Although Smallmouth Bass are associated with the pelagic zone more so than Rock Bass (Pflug 1981), which prefer littoral areas (Scott and Crossman, 1973), the similarity in gut contents supports they may have largely overlapping habitat, or seek out common areas where invertebrate prey are widely available. The presence of tapeworm plerocercoid infections in both Smallmouth Bass and Rock Bass further suggests a preference of both fish species for shallow, open water habitats, where the intermediate copepod hosts exist (See Chapter 1 for overview of helminth life cycles). At the least, this may suggest Smallmouth Bass are moving from the pelagic to the littoral zone for feeding, where they would ingest these copepods.

The likelihood for overlapping habitat of Smallmouth and Rock Bass is furthermore supported by the lack of a significant difference in their trematode metacercariae infection intensities. Before metacercariae encyst in a second intermediate host such as a fish, they are cercariae (a free-swimming infectious stage) that emerge from the aquatic mollusks, particularly snails, which are utilized as first intermediate hosts (Olsen, 1986). These are therefore not trophically-transmitted and can be used to indicate similarities in habitat, rather than diet. Given that both Smallmouth Bass and Rock Bass had similar infection intensities of metacercariae, this suggests they spend a considerable amount of time in littoral zones where the mollusks are found, as well as the emergent cercariae that penetrate into hosts to form cysts.

If host diet and habitat use are similar, then it is possible that the relative lack of infection by trophically-transmitted helminths in Rock Bass reflects their recent introduction into Algonquin Park lakes compared to Smallmouth Bass. The stark difference in infection status, infection intensity and parasite richness might correspond to the *enemy-release hypothesis* (see Introduction), which suggests that invasive species will have a reduced parasite burden in a novel environment that will contribute to their establishment and success (Keane and Crawley, 2002; Torchin et al. 2003). Indeed, many studies have demonstrated this phenomenon (Bauer and Hoffman, 1976; Bauer, 1991; Torchin et al. 2003). For example, Kvach and Stepien (2008) found that invasive gobies in the Great Lakes have a reduced parasite load compared to those in their native habitats. Although it is very difficult for invasive species to become established, those that do tend to reach high population densities (Carlton et al. 1990; Buttermore et al. 1994), such as Rock Bass in Algonquin Park. However, it cannot be said with any certainty that this release from the

intensity and diversity of infections contributes to the overall establishment of Rock Bass in Algonquin Park. Ecological, physical and life history traits could be responsible for this pattern, and this phenomenon should be examined on a case-by-case basis (Colautti et al. 2004).

My results are inconsistent with parasite spillover (see Introduction), which occurs when invasive species bring their invasive parasites into a novel environment (Torchin et al. 2002; Gendron et al. 2012). This mechanism is less common for trophically-transmitted parasites that require one or more intermediate host, as these hosts are less likely to occur with increasing distance from the native environment (Torchin et al. 2002; Gendron et al. 2012). Since Rock Bass have been rampantly spreading in the park since 1990s, any spillover would have likely been observed by now, or occurs so slowly that it has not yet manifested itself. This result matches the prediction that parasite introduction is unlikely for parasites with complex life cycles (Bulté et al. 2012).

Parasite spillback (see Chapter 1), which occurs when invasive species amplify the parasite population in their new range (Kelly et al. 2009a, 2009b), is also unlikely as my results indicate that Rock Bass were not a particularly naïve or susceptible host to infection in Algonquin Park. However, the high overall infection in the livers of Rock Bass could indicate that Rock Bass are susceptible to helminths that use this one particular organ. The infections in the liver were identified as larval cestodes, but I was unable to taxonomically identify them with my chosen methods. Any evidence of dilution, which occurs when invasive species act as a net sink for infection (Kelly et al. 2009a, 2009b), would be impossible to identify without precise historical records of parasitism in Smallmouth and

Rock Bass from each of the 8 sampled lakes. Since Smallmouth Bass had a higher diversity and intensity of infections, this is not a likely mechanism.

As predicted, fork length was positively correlated with both infection intensity and parasite richness across both fish species. Although diet analysis did not indicate any differences between Rock and Smallmouth Bass, Smallmouth Bass were significantly larger than Rock Bass, so it may be assumed larger fish are consuming larger prey items and acquiring a different parasite fauna that could be driving differences between the two species. Based on this assumption, it may be said that the ability to consume larger prey contributes to the diversity of infection, as only adult tapeworms (e.g. *Proteocephalus sp.*, *Trianophorus sp.*) may be acquired from eating vertebrate prey (Olsen, 1986). It can also be assumed that larger species eat more food in general, therefore increasing their overall exposure to all parasites. The total length of fish has been positively correlated with high parasite diversity and intensity in other studies (Ravi and Yahaya, 2015).

Larger animals have often found to be more heavily parasitized (Saad-Fares and Combes, 1992) and this may occur for various reasons. Firstly, larger hosts offer more habitat for parasites. For instance, Harrison's rule states that parasites of large-bodied host species tend to be bigger than those of small-bodied species (Harrison, 1915), and this is also likely true in terms of overall infection. Since Bass length is also a proxy for age, this reflects how parasites are accumulated over time. Simply put, the longer an animal lives, the more likely it is to acquire parasites (Saad-Fares and Combes, 1992), and this is another likely explanation for why size is positively correlated with both diversity and intensity in my study.

The surprising lack of diversity of macroparasite species, and the overall absence of adult trematodes, can perhaps be explained by several reasons. First, parasite communities

can vary greatly over time and space (Calvete et al. 2004; Vidal-Martinez and Poulin, 2003). Second, it may be possible that one of the intermediate hosts for trematodes, typically mollusks, was not present at these particular sites in the park; however, I did not actively survey such intermediate hosts as part of this study. Lastly, while the presence of metacercariae, the encysted larval form of trematodes, in fish muscle indicates the presence of this type of parasite, trematodes are known to infect a variety of final hosts and it may be possible the species observed here does not utilize larger fish as a final host. Since many fish were infected with metacercariae, and there were plentiful observations of Smallmouth Bass consuming smaller fish such as Rock Bass, fish-infecting adult trematodes would likely have been identified if they were present in the park.

My findings of greater infections in the established, and highly sought-after sportfish, Smallmouth Bass have various implications. High intensities of Bass tapeworm larvae can cause significant host damage, including host castration during larval migration to the reproductive organs (Bangham, 1941; McCormick and Stokes, 1982), which has obvious reproductive consequences. The Bass tapeworm *Proteocephalus ambloplitis* was the most commonly identified cestode in both Bass species (see Fig. 2.6). The species has been recorded to be prevalent in the Park as early as 1941 and is a very well documented in fish species across North America (Bangham, 1925, 1933, 1939, 1941). The multiple migrations and potential for high infection intensity can result in considerable damage to the visceral organs and tissues, which often appear as one single adhered mass (Durborow, 1988).

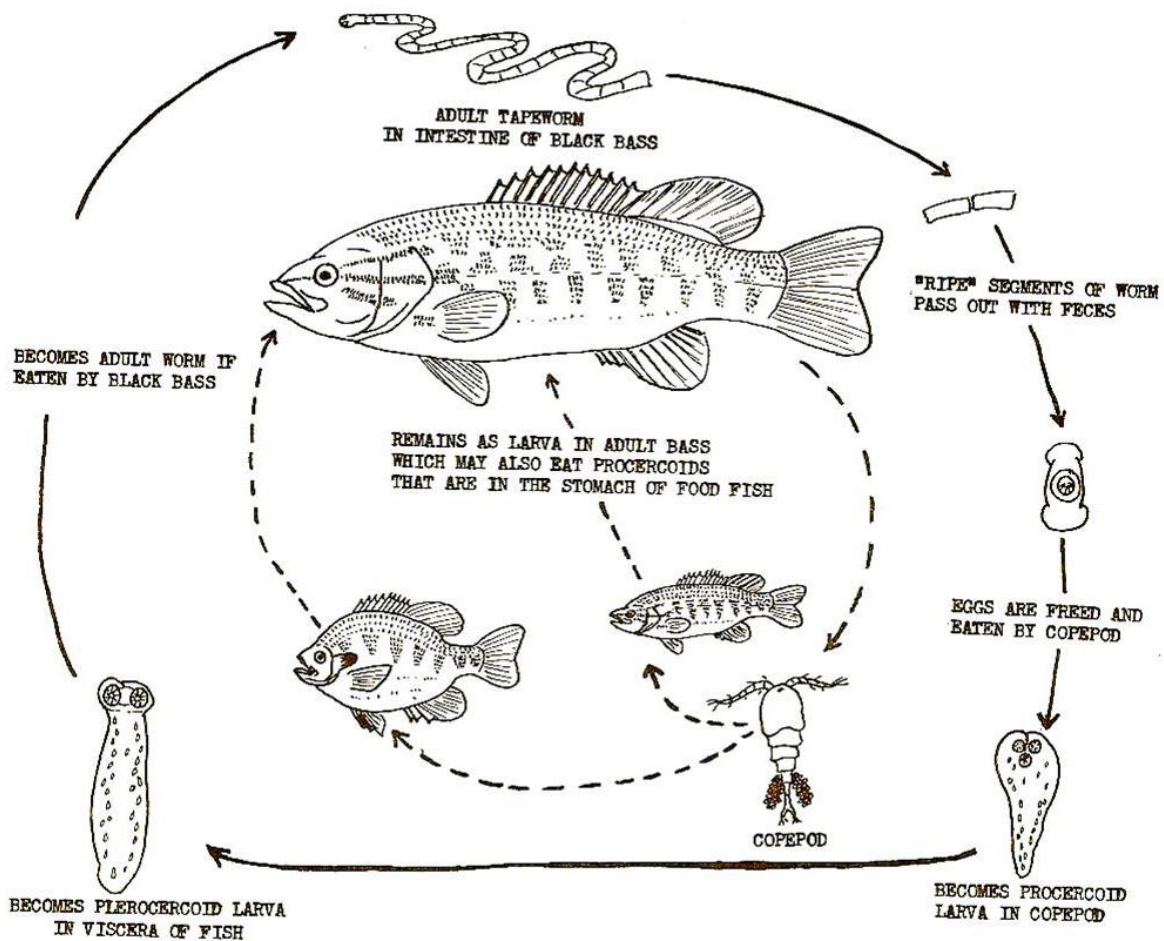


Figure 2.7 Life cycle of Bass tapeworm *Proteocephalus* sp.
(<https://fishpathogens.net/image/proteocephaluslifecycle2jpg>).

The acanthocephalan parasites found, such as *Leptorhynchoides thecatus* and *Neoechinorhynchus cylindratus*, are also known to cause damage to the alimentary canal and intestinal walls (Venard and Warfel, 1953). Damage from their penetration could leave the fish vulnerable to secondary infections (Venard and Warfel, 1953; Reyda et al. 2012). Adel-Meguid et al. (1995) found that *N. cylindratus* can evoke goblet cell hyperplasia in green sunfish, a host response presumed to create a protective barrier of antibodies to reduce further attachment.

The introduction of Rock Bass to Algonquin Park may have resulted in considerable but difficult to understand consequences to the parasite dynamics of Algonquin Park fishes. It is generally recognized that parasite surveys such as this one must be understood as a snapshot in time that cannot account for seasonal and temporal prevalence and dynamics (Vidal-Martinez and Poulin, 2003; Calvete et al. 2004). The random and non-random patterns of community assembly tend not to be repeatable in the same environment (Vidal-Martinez and Poulin, 2003; Calvete et al. 2004). It has even been suggested that this non-systematic means of assembly may be a restricted characteristic to helminth parasites (Holmes 1990). However, it is hard to ignore the overall complexity of a given ecosystem and various ecological, physical and biological interactions as another explanation.

It is suggested that those wishing to pursue similar research utilize entomological taxonomic keys for the more accurate identification of stomach contents. It is also recommended that future researchers utilize polymerase chain reaction (PCR) for the absolute identification of parasite species via DNA barcoding methods. However, searching for intermediate hosts in the sample areas would too provide a better idea of what parasites may be present. Improving the sample size by harvesting the same number of fish

from all lakes would also be an asset. Lastly, by assembling historical records into a meta-analysis, it may be possible to accurately observe how the parasite communities in Algonquin Park have changed with disturbances such as invasive species. Such work will help to better understand the driving forces behind parasite infections in freshwater lakes.

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CHAPTER 3: HOST HABITAT AND DIET IN RELATION TO PARASITISM IN FARM LAKE

Introduction

An ecosystem is composed of many communities of interacting organisms and their surrounding physical environment. Understanding exactly how all these organisms are connected is extremely difficult; however, studying food web structure can be a reliable and effective way to understand the trophic network (Woodward and Hildrew, 2002) and flow of organic matter in a community (Rosi-Marshall and Wallace, 2002). It is critical to understand how species interact with one another to predict how changes in community composition may affect ecosystems. By utilizing both indirect and direct forms of diet analysis, food web structure can be carefully constructed for better resource management (Rybczynski, 2008). However, food webs traditionally only include free-living species and largely exclude parasites despite their large effects on individuals, populations, and communities (Marcogliese and Cone, 1997; Lafferty et al. 2006; Dunne et al. 2013).

Most free-living organisms are hosts for at least one parasitic species (Bauer, 2013). The high prevalence of parasites and complexity of their life cycles make them integral parts of ecosystems (Bauer, 2013). Parasites can be utilized reliably as indicators of overall ecosystem health as well as the ecological effects of disturbance, such as invasive species, as they are highly susceptible to extinction (Marcogliese, 2005; Bauer, 2013). Parasites can be transmitted in many ways. Trophic transmission is common in many macroparasites and involves multiple hosts to complete the life cycle. Transmission occurs when an infected intermediate host harbouring an infective parasite is consumed by a suitable final host in which the adult stage can establish, and is a fundamental adaptation for many

parasite life cycles (Lafferty and Kuris, 2002; Poulin, 2011). This interaction is prominent in aquatic ecosystems, where it occurs in all cestodes and acanthocephalans, many nematodes, and almost all trematodes (Parker et al. 2015). Typically, parasites in their larval stage are specific to one or only a few intermediate hosts (Johnson, 2004).

Given the particular nature of transmission for most parasites, as well as host specificity, the parasite community of an animal can be used to infer ingestion of specific prey items within a given final host or the parasite could not be present (Johnson, 2004). As such, host trophic position can also be inferred from their parasite composition (Johnson, 2004), and the diversity of trophically-transmitted parasites is documented to increase with prey diversity (Valtonen et al. 2012; Rossiter and Sukhdeo, 2011). Parasites may also reveal the precise identification of prey items too small or easily digested to be inferred from stomach contents (Kitchella and Windella, 1968; Hyslop, 1980; Budge et al. 2002). For example, Locke et al. (2013) did not find any copepods, ostracods or oligochaetes in the stomachs of 99 examined Pumpkinseed sunfish (*Lepomis gibbosus*), yet identified parasite species in these fish which utilize these prey items as obligatory intermediate hosts. Locke et al. (2013) also found that parasites indicated a diet consisting almost exclusively of amphipods in many fish; however, alternate methods (gut content analysis and stable isotope analysis – discussed below) of diet estimation indicated very little amphipod consumption. Along with host diet, other factors also influence the parasite fauna within individual hosts. For example, fish of the same species but of different sizes are known to vary in their parasite communities (Poulin, 2000; Poulin and Valtonen, 2001).

Gut content analysis (GCA) has traditionally been a well-used identifier of animal diet (Rybczynski, 2008). In the past, GCA has helped shed light on diet characterizations

such as prey resource overlap (Creque and Czesny, 2012) and ontogenetic diet shifts (Roswell et al. 2013). Although GCA provides detailed information on diet as prey items can be identified with relatively high taxonomic resolution, it does often not elucidate long-term diet (Rybczynski 2008). Variability in prey items can occur due to short-term diet irregularities or unique digestion time of prey items (Macdonald et al. 1982). Empty stomachs do not provide any data (Locke et al. 2013), which suggests that a higher sample size may be needed. Even ambient conditions, such as temperature, have been known to influence stomach contents (Legler et al. 2010). GCA is also incredibly onerous in terms of laboratory work, and identification requires a high level of taxonomic precision, which can be especially difficult when evaluating insectivorous animals such as fishes (Rybczynski, 2008). For such reasons, it may be more appropriate to utilize GCA to identify diet in conjunction with one or more indirect methods, which can include fatty acid analysis, stable isotope analysis, and parasite transmission data.

Chemical isotope composition within tissue is a reflection of the organic compounds that have accumulated into the bodies of consumers (Chen et al. 2012). The measurements of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (the isotopic ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$, respectfully) are particularly useful for insight into the habitat and diet of animals. The values determined by analysis incorporate the modification of these isotopes during assimilation by one or more consumers (Post, 2002). Isotopic enrichment occurs in differing magnitudes in entire organisms, organs, and individual tissues (Pinnegar and Polunin, 1999; Miller, 2006); however, Chen et al. (2012) suggest muscle tissue is the most consistent. Studies by DeNiro and Epstein (1978, 1981) demonstrated that isotope fractionation differs more interspecifically (between species) than intraspecifically (within a species) for fish fed the

same diet. Although a range of fractionation rates have been recorded (Post 2002; McCutchan et al. 2003), organisms tend to become enriched with ~ 3.4 ppt of ^{15}N compared to their prey (DeNiro and Epstein, 1981; Cabana and Rasmussen, 1994; Rybczynski et al. 2008).

The use of $\delta^{15}\text{N}$ signatures for identifying the trophic position of consumers can neglect naturally occurring environmental variation. By adjusting for the baseline $\delta^{15}\text{N}$ of a given environment, it is possible to more accurately determine an organism's trophic position. Given that there is high temporal variation of primary producers (e.g. periphyton), primary consumers (e.g. mollusks) are typically used to calculate the baseline (Cabana and Rasmussen 1996). Primary consumers are also more useful due to their longer lifespan and body size (Cabana and Rasmussen, 1996). SIA of $\delta^{13}\text{C}$ can be a valuable tool for assessing food web components such as diet and habitat, rather than trophic position. Since the $\delta^{13}\text{C}$ values in consumers are similar to those of their prey (Fry and Sherr, 1984, Wada et al. 1993), the values are conserved up the food chain, but vary at the base (Vander Zanden and Rasmussen, 1999). For example, in freshwater lakes, phytoplankton have been found to have greater carbon fractionation during carbon fixation than benthic algae (Hecky and Hesslein, 1995). Furthermore, the most profundal organisms have more negative $\delta^{13}\text{C}$ signatures, which is likely due fixation of respired CO_2 (Rau, 1980). Carbon therefore can reflect the source of food.

Inferring diet by comparing the isotopic ratios in tissue of consumers compared to their potential prey items is not straightforward. Diversity of prey, combination of prey items, and prey that were not sampled can produce overlapping and hard to interpret compositions in their predators (Caut et al. 2008; Locke et al. 2013). Isotopic fractionation

and turnover rates also vary with the growth rate, size and age of the consumer (Herzka, 2005; Caut et al. 2008; Paradis et al. 2008). Despite this, stable isotope analysis (SIA) is advantageous to GCA in that it provides temporally integrated data information (Rybczynski et al. 2008; Chen et al. 2012). It has become increasingly used to calculate trophic positions in aquatic ecosystems and is therefore a valuable tool for describing food web interactions (Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1996). $\delta^{15}\text{N}$ is especially useful for establishing trophic connections in food webs, but does not provide direct information on prey items (Rybczynski et al. 2008).

Fatty acid analysis (FAA) has been used as a biological identifier for animal diets in aquatic ecosystems for quite some time (Ackman and Eaton, 1966; Sargent et al. 1987; Graeve et al. 1994). Fatty acids are a component of a larger group of lipids, and are plentiful in organisms in all environments (Budge et al. 2002). They can have a variable chemical appearance, but in fishes, generally contain 14-24 carbon atoms with different degrees of saturation (Budge et al. 2002). Due to the energy demands of altering these fatty acids, they are often directly assimilated into animal tissue with very little modification, and are therefore appropriate diet biomarkers (Budge et al. 2002).

FAA can be used to identify patterns in prey and subsequently to trace food webs and diets of predators (Budge et al. 2002). In the past they have been used to study the diet of fish and copepods (Sargent et al. 1989), to indicate the presence of fish and other prey in diets of carnivores (Colby et al. 1993), and the quantity of plant matter in carnivore diet (Iverson et al. 2001). In order to better understand the diet of aquatic predators, the fatty acid compositions of their prey items must be better distinguished (Budge et al. 2002). Though fatty acids are typically stored in consumer tissues for 4-12 weeks (Kirsh et al.

1998; Budge et al. 2011), changes in the diet of a fish can reflect in its fatty acid composition in as little as three weeks (Kirsh et al. 1998), suggesting FAA is an effective indirect method for temporal diet identification. Fatty acids have also been shown to be altered by the reproductive status of fish (Sargent et al. 1989; Sargent et al. 1995), as well as increasing body size within a species (Iverson et al. 1997).

Polyunsaturated fatty acids (PUFAs), particularly from the n-6 and n-3 families, are required by animals for many essential functions, such as maintaining cell membranes, but most complex organisms are unable to biosynthesize them (Glencross, 2009).

Phytoplankton are typically the major source of these essential n-3 and n-6 PUFAs in aquatic ecosystems (Ahlgren et al. 1990; Arts, 2009; Brett et al. 2009, Taipale et al. 2012). Eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6) have been regarded as the most important PUFAs for physiological processes (Ahlgren et al. 1990; Arts et al. 2009).

Individual fatty acids are found in varying concentrations in unique prey items. For example, α -linolenic acid (18:3n-3), palmitoleic acid (16:1n-7), and EPA are highly associated with diatoms, detritus and bacteria, which typically correspond to the benthic zone (Bell et al. 1994; Napolitano, 1999; Arts et al. 2009; Kelly and Scheibling, 2012). Stearadonic acid is associated with Cryptophyceae, which are pelagic algae (Arts et al. 2009; Taipale, 2013). Consumers with high concentrations of DHA and ARA are likely to have assimilated non-diatom flagellates and their zooplankton consumers, which is as reflection of a more pelagic-based diet (Napolitano, 1999; Arts et al. 2009; Kelly and Scheibling, 2012). It can said that the primary producers of a community are therefore

strong predictors of the food web dynamics and availability of fatty acids to consumers (Arts et al. 2009; Galloway and Winder 2015; Strandberg et al. 2015).

Research objectives and hypotheses

As discussed above, parasites are an excellent indirect method for predicting consumption of specific prey items by their hosts (Johnson, 2004). Like SIA and FAA, parasites can predict diet over a long temporal period (Johnson, 2004), and can provide more than just a snap shot in time, like GCA does. Parasites have been used as indicators of diet in the past in combination with SIA and have been strongly correlated with both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Johnson et al. 2004; Bertrand et al. 2011). Since any given method of diet identification is influenced by the local environment, both parasites and isotopes can be used to discriminate the spatial origins of fish (Bertrand et al. 2008, 2011). The aim of this research was to examine the possible ecological drivers of parasitism in three fish species (Smallmouth Bass, Rock Bass, and Pumpkinseed) within a single lake, using a comprehensive approach to focus on the role of host diet and habitat use. Because diet, habitat, and trophic position of fish have been examined as potential predictors of trophically-transmitted parasites via GCA, SIA and FAA, I chose to concentrate on helminths with such life cycles. My research employed all four of the above-mentioned techniques, and no other study has ever before evaluated host diet in this way to the best of our knowledge.

My hypotheses were as follows:

- 1) Fish species occupying higher trophic levels (e.g. Smallmouth Bass) will have greater parasite species richness.
- 2) Fish with a more diverse diet will have a higher parasite species richness.
- 3) Host habitat preference will not be correlated with infections by trophically-transmitted helminths.

Materials and methods

Parasite and gut content data

For detailed information on field methods and data collection (fish dissections and parasite identification), see Chapter 2. A total of 12 Pumpkinseeds, 6 Smallmouth Bass and 13 Rock Bass were removed from Farm Lake (45.539899, -78.417712) via rod and hook and fish traps baited with dog food. Farm Lake was evaluated as it was the only lake where all three fish species were sampled (Table 2.1). Two tissues samples (~1 g) were taken in the field from either side of the dorsal fin of each of these fish for FAA and SIA. These fish were then frozen and brought back to Ryerson University for examination of trophically-transmitted helminths of the Class Cestoda and Phyla Acanthocephala and Nematoda. Non-trophically transmitted trematode metacercariae were also observed for infection intensity. The fish were also measured in their fork length and examined for their sex. During dissections, the stomach and intestines of each fish were removed, opened laterally, and the contents examined under a dissecting microscope. Prey items in the stomach and the intestines were identified as invertebrate, vertebrate, invertebrate and vertebrate, or plant matter.

Lipid Analysis

The tissues of 12 Pumpkinseeds, 6 Smallmouth Bass and 13 Rock Bass caught from Farm Lake were analyzed with respect to their lipid compositions. Prior to this, samples were kept frozen at -80°C. The samples were prepared for fatty acid extraction by placement in a freeze dryer for 72 hours at -50°C and 0.0100 mbar. The samples were weighed before and after freeze-drying to determine their moisture content. Extraction was completed by grinding each sample in liquid nitrogen. Four mL of CHCl₃:MeOH (2:1), 800 µL of 0.88% KCl, and 15 µg of a previously prepared solution of free fatty acid (23:0, Nu-Chek Prep) were added to individual samples. The samples were sealed in glass vials, vortexed, and centrifuged at 4°C and 2000 rpm for 5 minutes. The lower phase, consisting of chloroform:methanol, was removed and placed in a new vial. Three mL of CHCl₃:MeOH was added to the remaining tissue. The samples were then centrifuged as before, and the lower phases of each sample were combined and stored again at -80°C.

Samples were then evaporated of chloroform:methanol with a nitrogen air stream. The samples were combined with 1 mL hexanes and 2 mL H₂SO₄ in MeOH. Nitrogen gas was added to the samples and they were sealed before placement on a heat block at 90°C for 1.5 hours. The samples were cooled and 1.5 mL of ultra-pure water (Milli-Q) and 4 mL hexanes were added. Samples were vortexed and centrifuged at 4°C and 2000 rpm for 3 minutes. The upper phase, containing hexanes and fatty acid methyl esters, was transferred to a new vial. Four mL of hexanes were added to the remaining lower phase, then vortexed and centrifuged as before. The upper phases were combined then evaporated with N₂ gas before 300 µL of hexanes were added to the samples, which then were transferred to 1.5 mL amber vials suitable for gas chromatography. Another aliquot of 300 µL of hexanes

were combined in the amber vials before the samples were placed under a nitrogen gas stream and evaporated. Finally, a final volume of 500 μL of hexanes was added, and the samples were stored at -80°C . A total of 3 extractions were performed.

A Shimadzu 2010+ gas chromatograph was used to identify the fatty acid methyl esters. The samples were injected using a 1:100 split ratio on a Supelco SP-2560 column (100 m x 0.25 mm ID x 0.20 μm film). The chromatograph oven was programmed to operate for 5 minutes at 140°C , and then increase to 240°C at a rate of $4^{\circ}\text{C min}^{-1}$ where the temperature was held for 12 minutes. The injector was set to 250°C with helium as the carrier gas flowing at 1.2 mL min^{-1} . The FID detector was set to 250°C and ran for 42 minutes. Identification of standards was conducted using a GLC68F standard (Nu-Chek Prep Inc.).

Stable Isotope Analysis

Fish tissue and algae samples (to determine baseline C and N in primary producers from Farm Lake) were freeze dried for 72 hours at -50°C and 0.0100 mbar. The dehydrated tissue was crushed and the fatty acids were extracted (see above). The recovered tissue was sent to the Earth and Environmental Sciences department at the University of Waterloo for stable isotope analysis. The analysis of solid materials for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope measurements was determined through combustion conversion of sample material to gas through a 4010 Elemental Analyzer (Costech Instruments, Italy) coupled to a Delta Plus XL (Thermo-Finnigan, Germany) continuous flow isotope ratio mass spectrometer (CFIRMS).

Data analysis

Because I was primarily interested in the role of host ecology in driving differences among fish species in terms of their infection with trophically-transmitted helminths, I focused on species-level differences in my analyses and removed individual fish that had empty guts (i.e. no diet data using this measure). To look for differences among species in terms of their infection intensity score with respect to trophically-transmitted helminths (see Chapter 2 for categories), I used a Generalized Linear Mixed Model (GLMM) with a normal distribution and identity link function after a \log_{10} transformation of this measure. Species category was used as the fixed effect and host sex and fork length were entered as random effects. I used a GLMM to examine species differences in parasite species richness in a similar way, and also did this for intensity of infection with trematode cysts (acquired through penetration, not consumption). To determine whether diet, habitat use, and other host attributes differed among fish species and could be driving any differences in parasitism, I then used separate GLMMs with the following dependent variables to see if these differed among species: fork length, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, total lipids, and diet (see categories above). Because cestodes were the most commonly-found helminth, I also examined species differences in the presence of this parasite using a binomial distribution and logit link function. Fork length and sex were entered as random effects for these analyses, with the exception of the model where fork length was the dependent.

Along with these species-level analyses, I conducted select individual-level analysis to more closely look at the effect of diet and habitat use on parasitism (trophically-transmitted parasite richness and intensity score, respectively as dependent variables) in each fish (infected and uninfected individuals). Here I also used GLMMs but added host

species as a random effect along with sex and fork length. Fixed effects included: diet, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, palmitoleic acid, stearidonic acid, and omega fatty acids. I conducted an additional analysis for the presence of cestodes as done above, and another with host infection status (yes/no); both used a binomial distribution with logit link function. For these individual-level analyses that began with multiple fixed effects, insignificant predictors were dropped and the models re-run until only significant fixed effects were retained. Posthoc tests (pairwise comparisons) were always used to further examine significant (or marginally insignificant) fixed effects in all analyses. All analyses were conducted with SPSS 23.0.

Results

Species differences – parasitism

There was a significant difference among species for intensity of infection by trophically-transmitted helminths ($F_{2,28} = 10.168$, $P < 0.001$) as Rock Bass had a lower intensity compared to Smallmouth Bass ($P = 0.003$) and Pumpkinseed ($P < 0.001$), but there was no difference between Smallmouth Bass and Pumpkinseed ($P = 0.900$)(see Fig. 3.1). A similar pattern was seen for parasite species richness (excluding metacercariae), with an overall difference among the three host species ($F_{2,28} = 4.839$, $P = 0.016$). Once again, Rock Bass had lower richness compared to Smallmouth Bass ($P = 0.013$), and also less species than Pumpkinseed ($P = 0.039$), but there was no difference between Smallmouth Bass and Pumpkinseed ($P = 0.411$)(see Fig. 3.2).

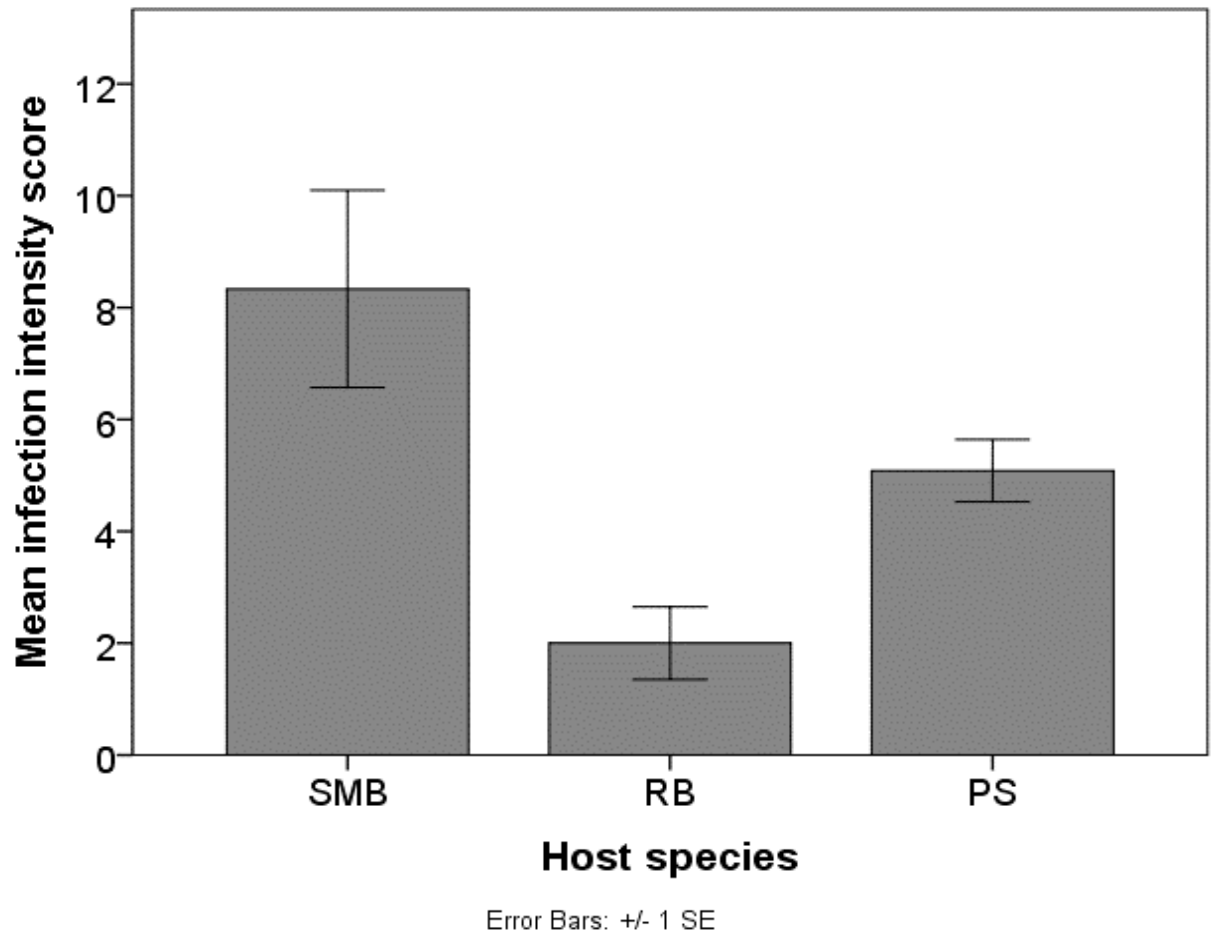


Figure 3.1 Mean infection intensity (+ S.E.) (excluding metacercariae) of Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseeds (PS) caught in Farm Lake.

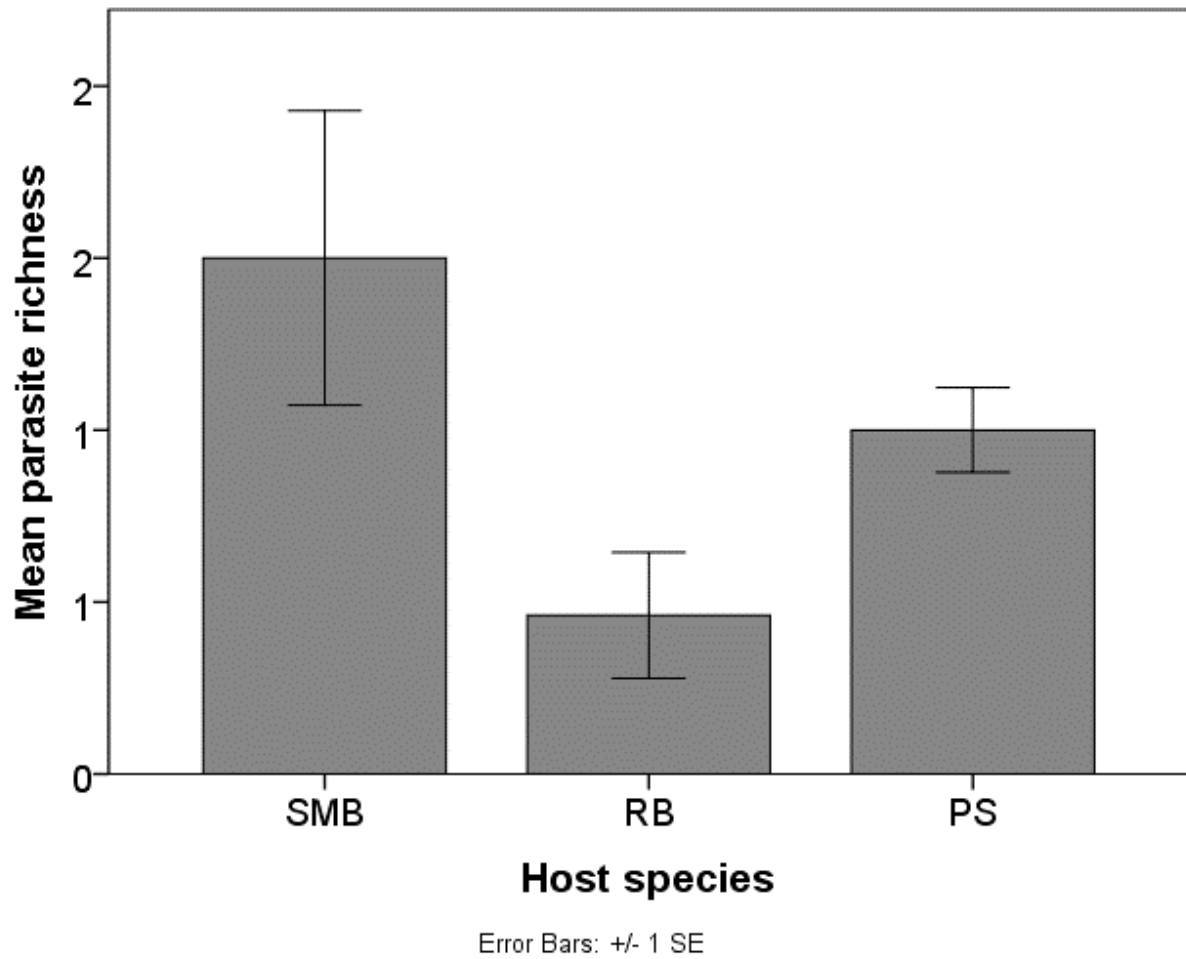


Figure 3.2 Mean macroparasite richness (+ S.E.) (excluding metacercariae) of Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseeds (PS) caught in Farm Lake.

The pattern for trematode metacercariae (not trophically-transmitted) differed. While there was a marginally insignificant difference among species ($F_{2,28} = 2.897$, $P = 0.072$), Pumpkinseed had the highest mean metacercariae infection score, which showed a tendency to be greater than that in Smallmouth Bass ($P = 0.063$) and Rock Bass ($P = 0.082$), although it was similar between the two Bass species ($P = 0.682$)(see Fig. 3.3).

The prevalence of cestodes differed among species ($F_{2,28} = 3.823$, $P = 0.034$). Cestodes were most common in Pumpkinseed and Smallmouth Bass, but specific between-species posthoc comparisons were not significant (see Fig. 3.4).

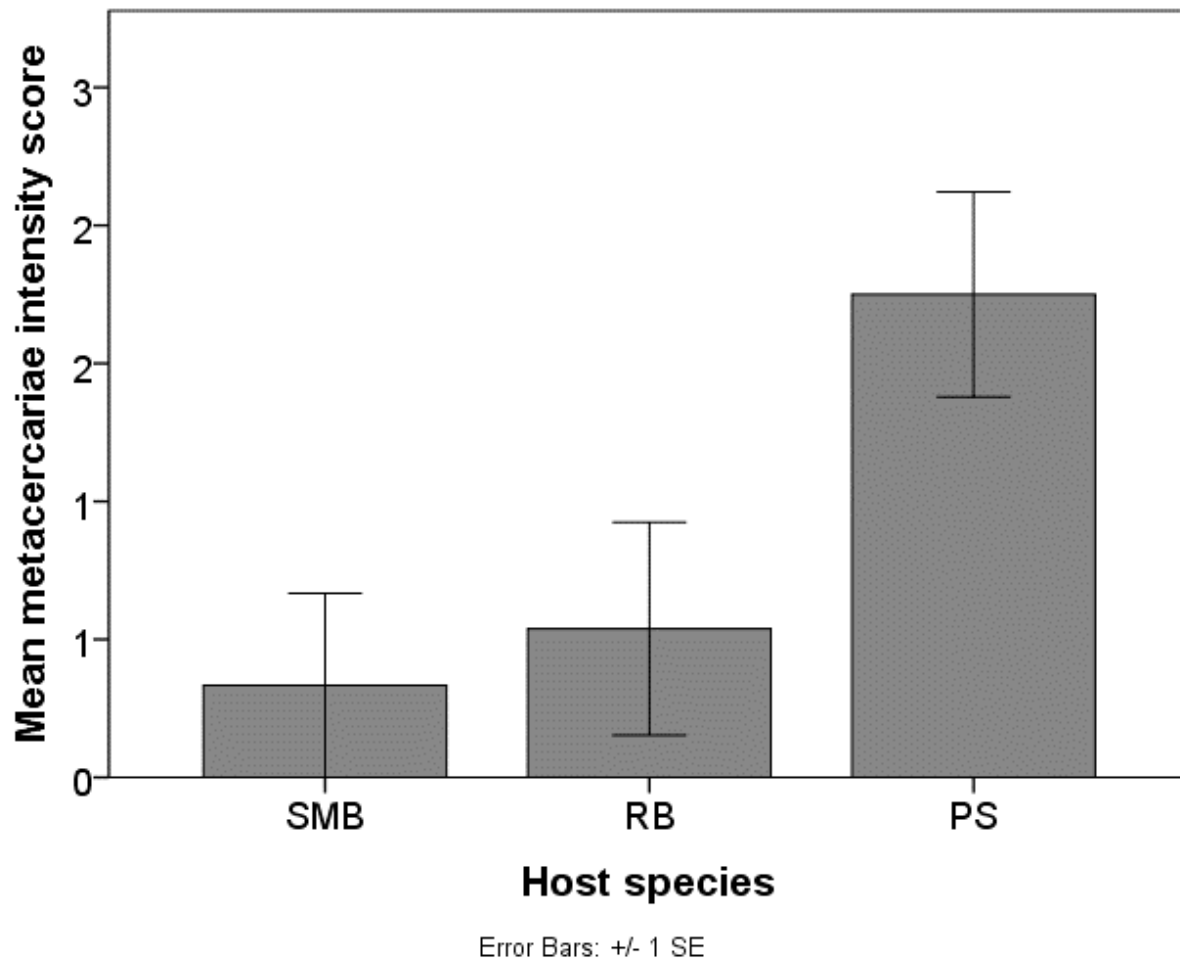


Figure 3.3 Mean metacercariae intensities (\pm S.E.) of Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseed (PS) sunfish caught in Farm Lake.

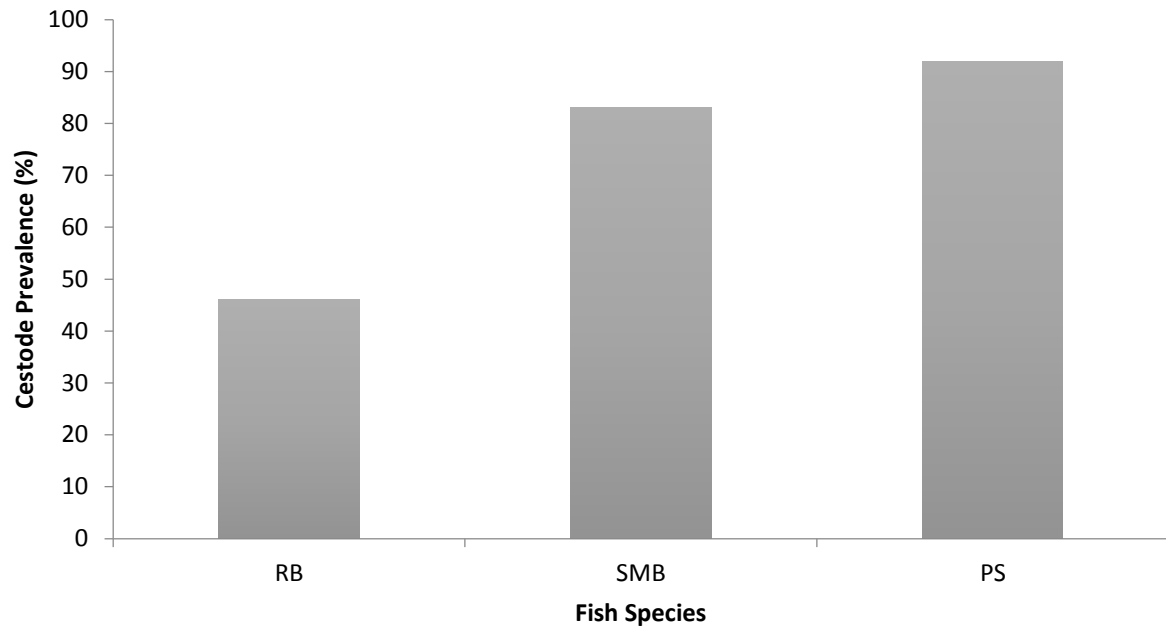


Figure 3.4 Prevalence of cestode infections in Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseed (PS) sunfish caught in Farm Lake, July 2015.

Species differences – host attributes

There was a significant difference among hosts with respect to their fork length ($F_{2,28} = 17.591$, $P < 0.001$). Pumpkinseed were the smallest species, and were significantly shorter than both Rock Bass ($P = 0.004$) and Smallmouth Bass ($P < 0.001$), and Smallmouth Bass were also longer than Rock Bass ($P = 0.002$)(see Fig. 3.5).

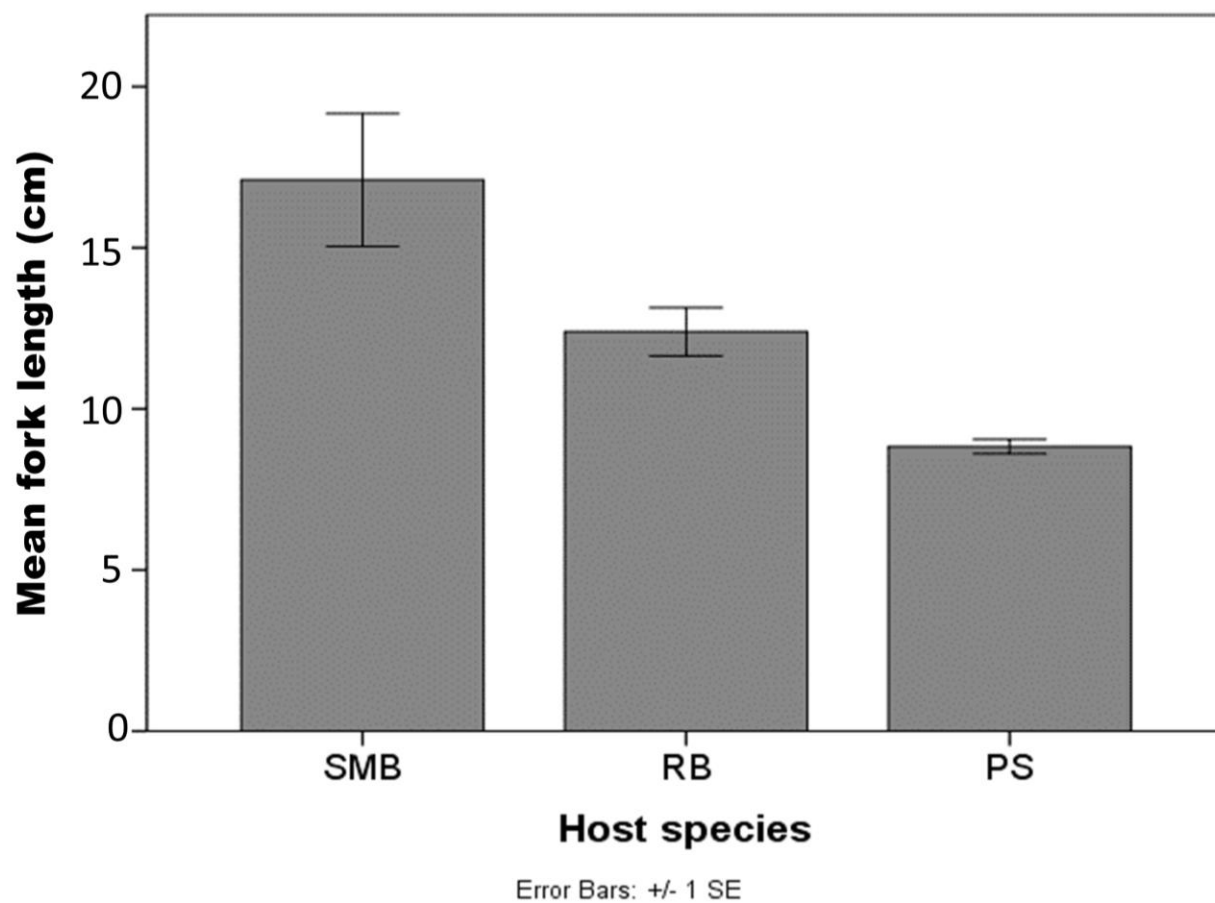


Figure 3.5 Mean fork length (+ S.E.) of Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseed (PS) sunfish caught in Farm Lake.

The stable isotope analysis revealed a tendency for a difference among species in terms of $\delta^{13}\text{C}$ enrichment ($F_{2,28} = 3.193$, $P = 0.056$). The values for Smallmouth Bass were significantly more negative than that of Pumpkinseeds ($P = 0.018$), and there was a tendency for a difference between the two Bass species ($P = 0.073$), but there was no difference between Rock Bass and Pumpkinseed ($P = 0.402$) (see Fig. 3.6). In contrast, there was a highly significant difference in $\delta^{15}\text{N}$ enrichment among species ($F_{2,28} = 17.639$, $P < 0.001$). $\delta^{15}\text{N}$ enrichment was highest in Rock Bass, and significantly differed from that in both Smallmouth Bass ($P < 0.001$) and Pumpkinseeds ($P = 0.003$), but there was only a tendency for a difference between Smallmouth Bass and Pumpkinseed ($P = 0.067$)(Fig. 3.7).

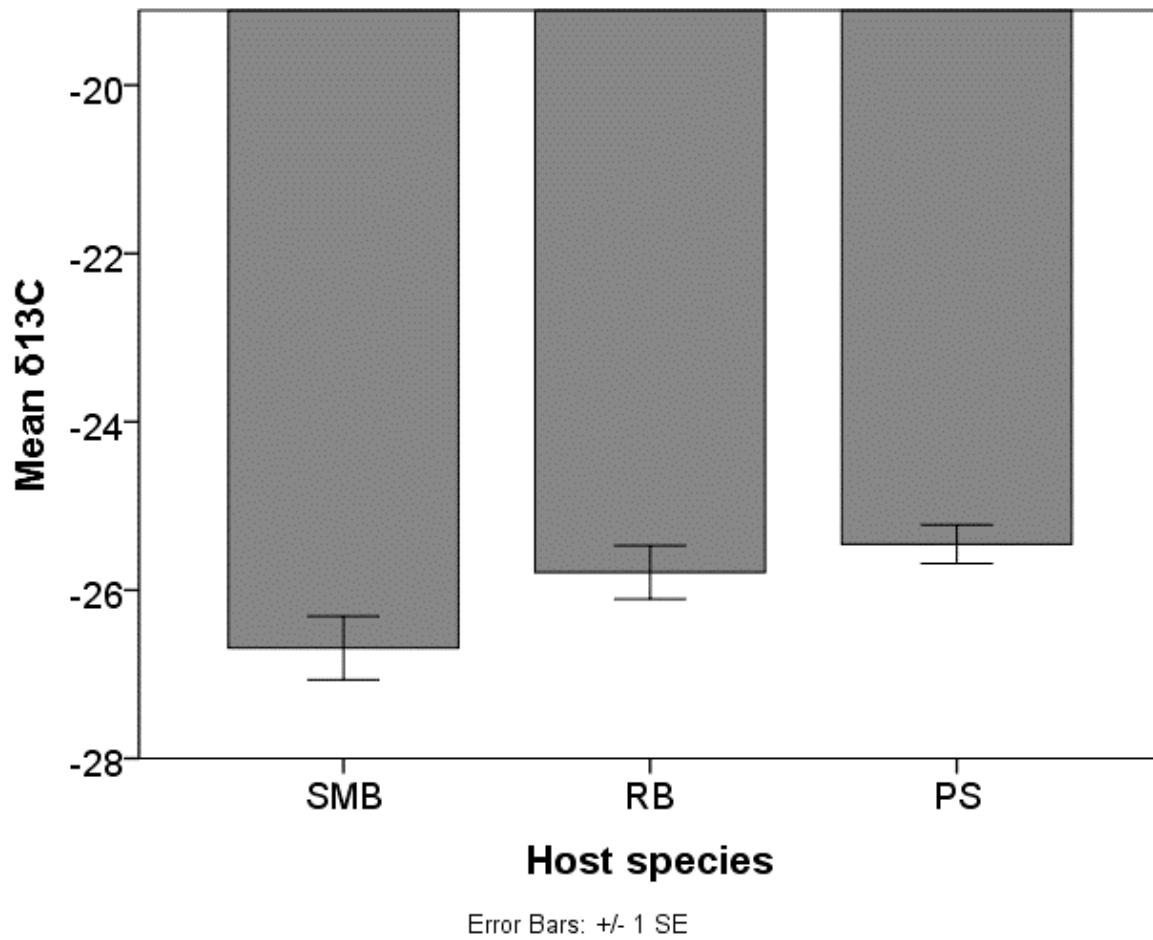


Figure 3.6 Mean $\delta^{13}\text{C}$ enrichment (+ S.E.) for Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseed (PS) sunfish caught in Farm Lake.

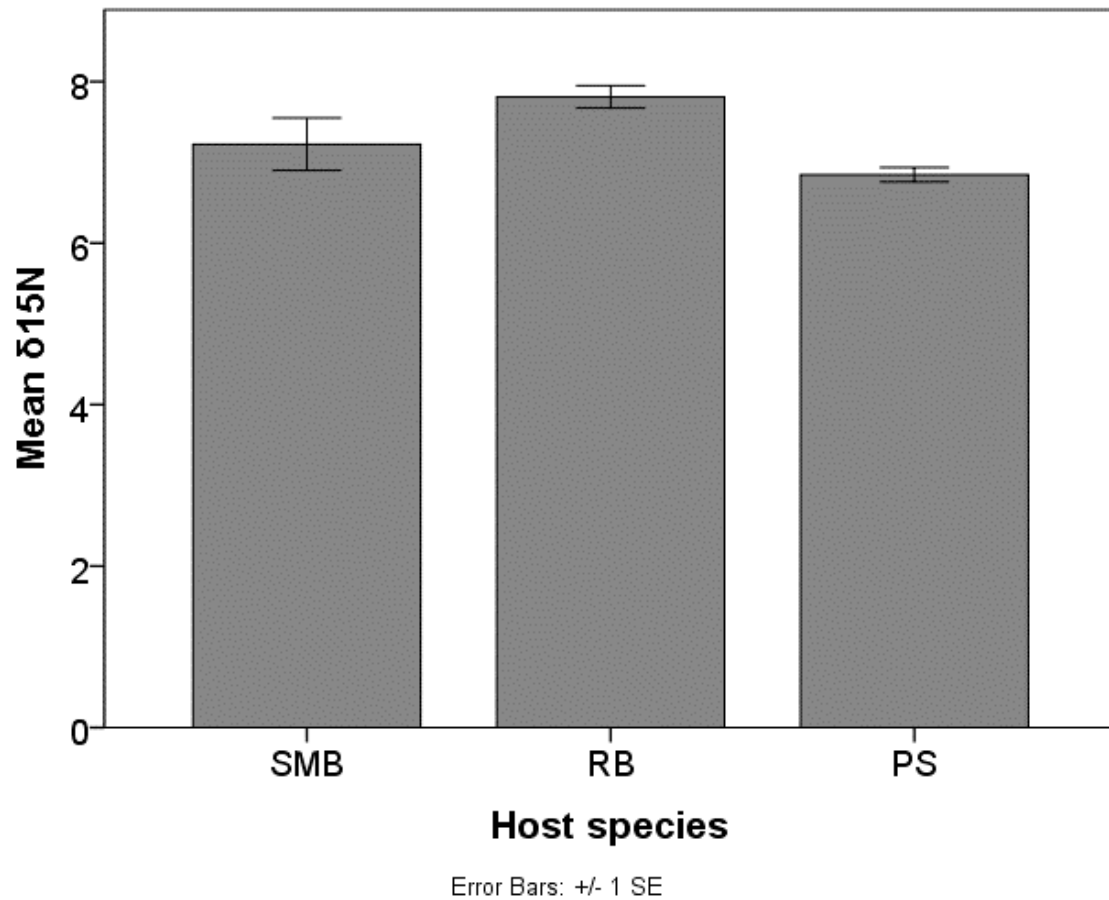


Figure 3.7 Mean $\delta^{15}\text{N}$ enrichment (+ S.E.) for Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseed (PS) sunfish caught in Farm Lake.

There was also a difference among species for the mean weight percentage of total fatty acids ($F_{2,28} = 3.928$, $P = 0.031$). Total lipid content in Rock Bass was significantly lower than that of Pumpkinseeds ($P = 0.009$), which were the species with the highest percentage (see Fig. 3.8). There was no difference between Smallmouth Bass and Rock Bass ($P = 0.214$), or between Smallmouth Bass and Pumpkinseed ($P = 0.333$).

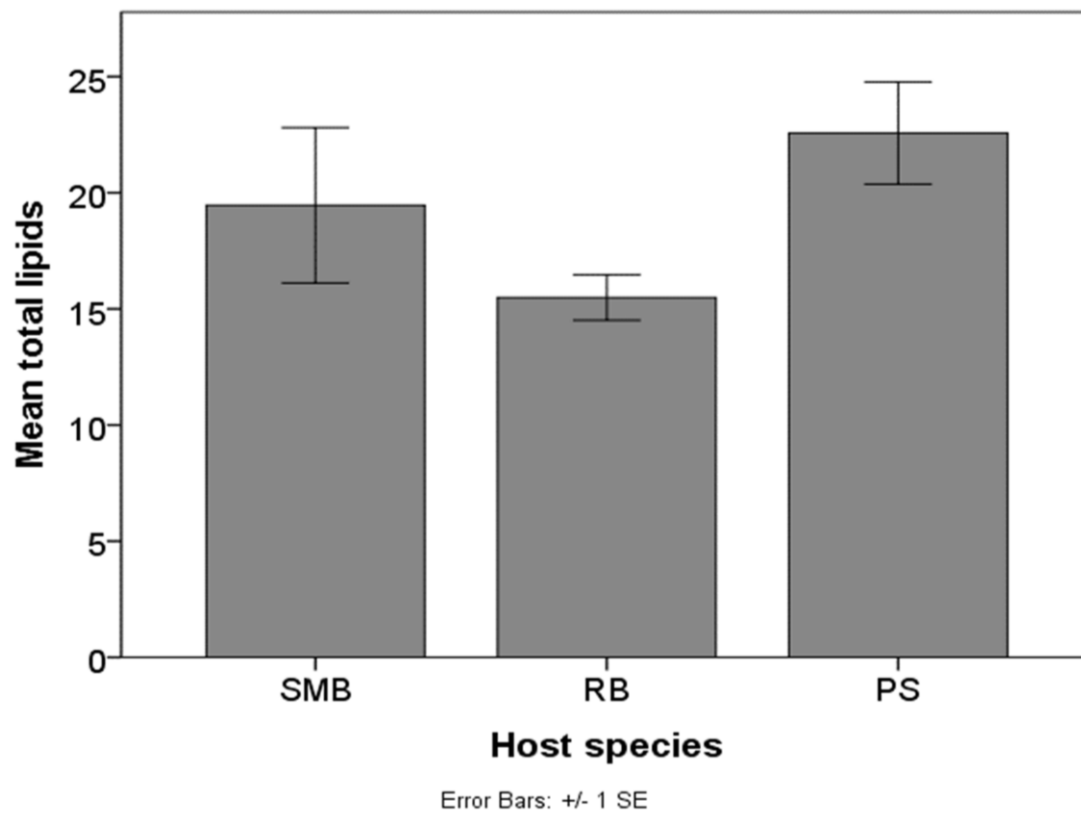


Figure 3.8 Mean weight percentage (+ S.E.) of total fatty acids measured from Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseed (PS) sunfish caught in Farm Lake.

Although the three species showed different patterns with respect to their gut contents, diet based on this measure did not differ among them ($F_{6,22} = 0.695$, $P = 0.657$). Rock Bass had a large occurrence of invertebrates, Pumpkinseeds had a strictly invertebrate diet, and Smallmouth Bass were found to have a mixture of both invertebrates and vertebrates within individual fish (see Fig. 3.9).

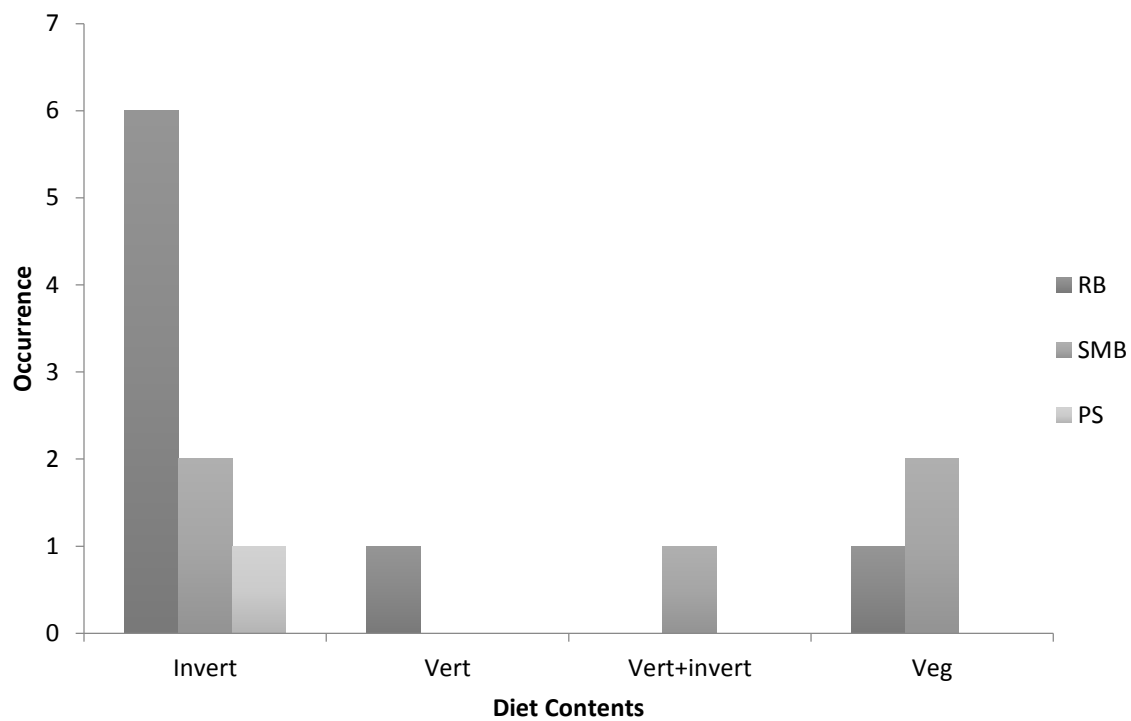


Figure 3.9 Occurrence of diet contents (+ S.E.) in Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseed (PS) sunfish caught in Farm Lake.

Individual-level patterns

The best overall model for individual infection intensity score as related to trophically-transmitted helminths was marginally insignificant ($F_{1,29} = 3.386$, $P = 0.076$) and only retained the percentage of omega-3 fatty acids as a predictor as there was a tendency for infection intensity to decrease with greater levels of these lipids (see Fig. 3.10). The overall model for richness of these parasites was significant ($F_{5,25} = 5.721$, $P = 0.001$) and included diet ($P = 0.007$), $\delta^{15}\text{N}$ ($P = 0.01$), and omega-3 fatty acids ($P = 0.023$). Higher values of both $\delta^{15}\text{N}$ and omega-3 fatty acids were negatively associated with parasite richness, but individual fish with a mixture of invertebrate and vertebrate prey in their gut contents had the greatest number of parasite species (see Fig. 3.11). There was a marginally insignificant difference ($F_{1,29} = 3.914$, $P = 0.057$) in the mean percentage of $\delta^{15}\text{N}$ enrichment between fish either infected or not infected with any trophically-transmitted helminth as this was slightly higher for infected individuals (see Fig. 3.14). Fish infected with any cestode were significantly more likely to have a higher mean percentage of $\delta^{15}\text{N}$ enrichment than those fish without these helminths ($F_{1,29} = 6.292$, $P = 0.018$) (Fig. 13). Individual fork length was somewhat positively correlated with infection intensity score for all fish ($F_{1,29} = 3.436$, $P = 0.074$) (see Fig. 3.16), but showed no relationship with parasite species richness ($F_{1,29} = 2.247$, $P = 0.145$). With respect to helminths not acquired through trophic transmission, palmitoleic acid was strongly positively correlated with the intensity of metacercariae infection ($F_{1,29} = 9.012$, $P = 0.005$) (see Fig. 3.17).

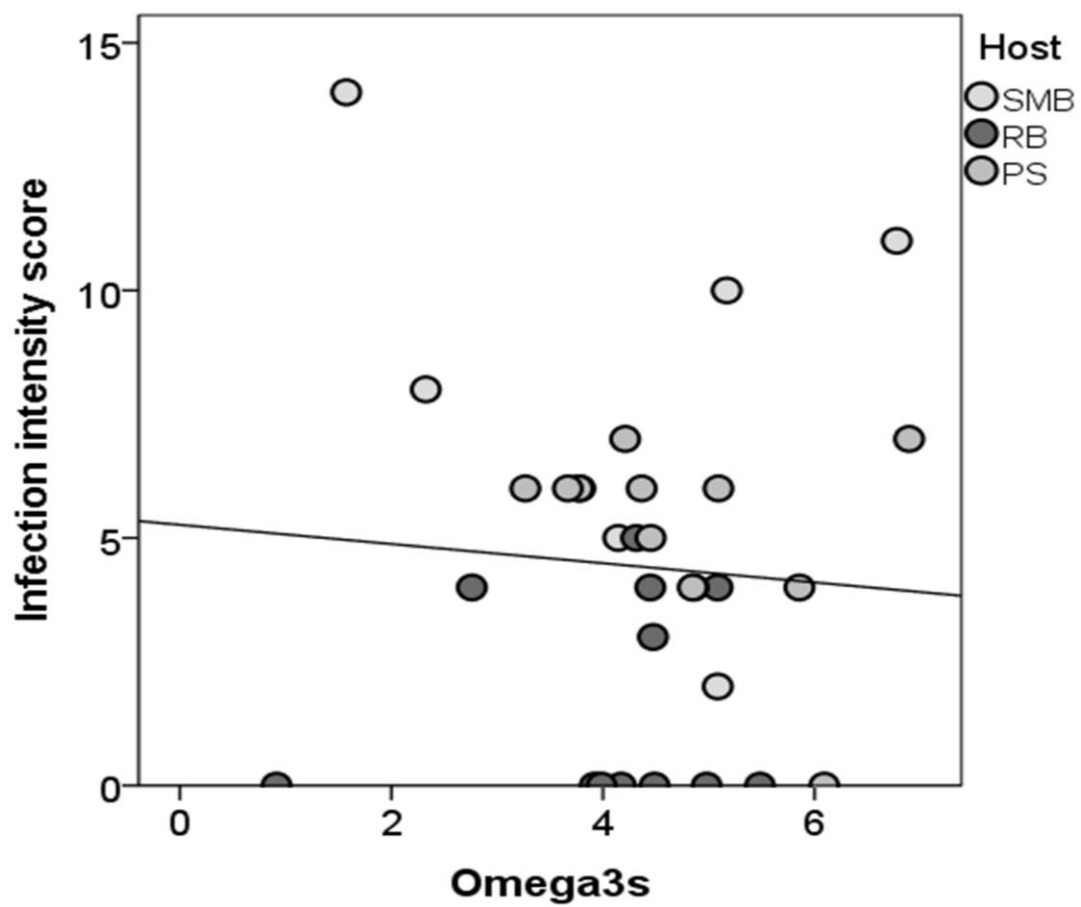


Figure 3.10 Omega-3 fatty acids (w%) and infection intensity score in Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseed (PS) sunfish caught in Farm Lake.

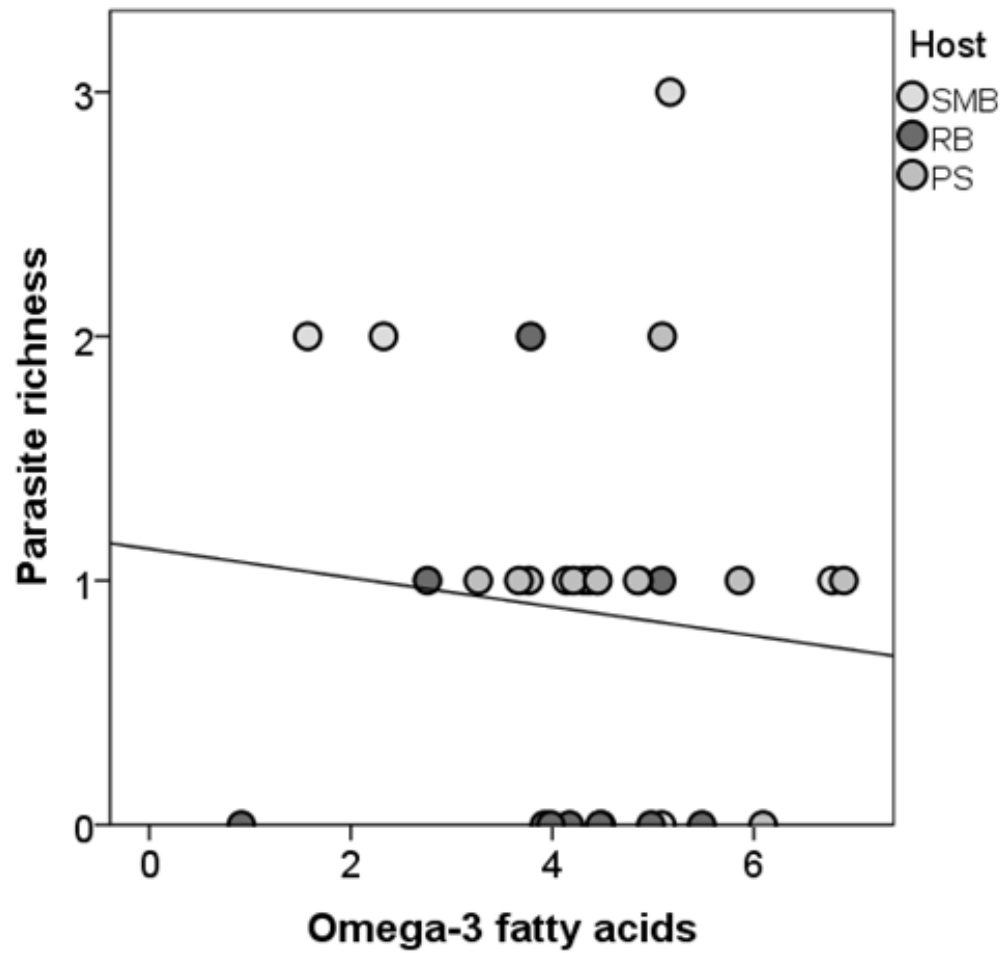
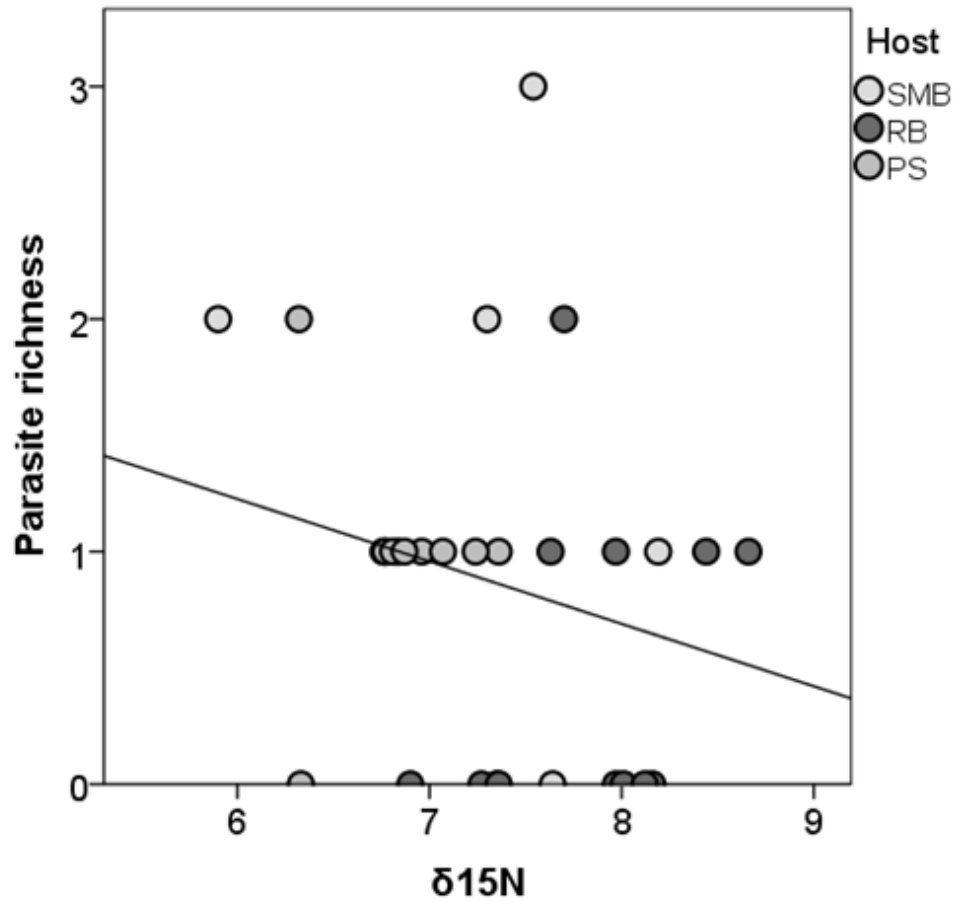


Figure 3.11 Individual fish measurements of omega-3 fatty acids (w%) in relation to parasite richness in Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseed (PS) sunfish caught in Farm Lake.



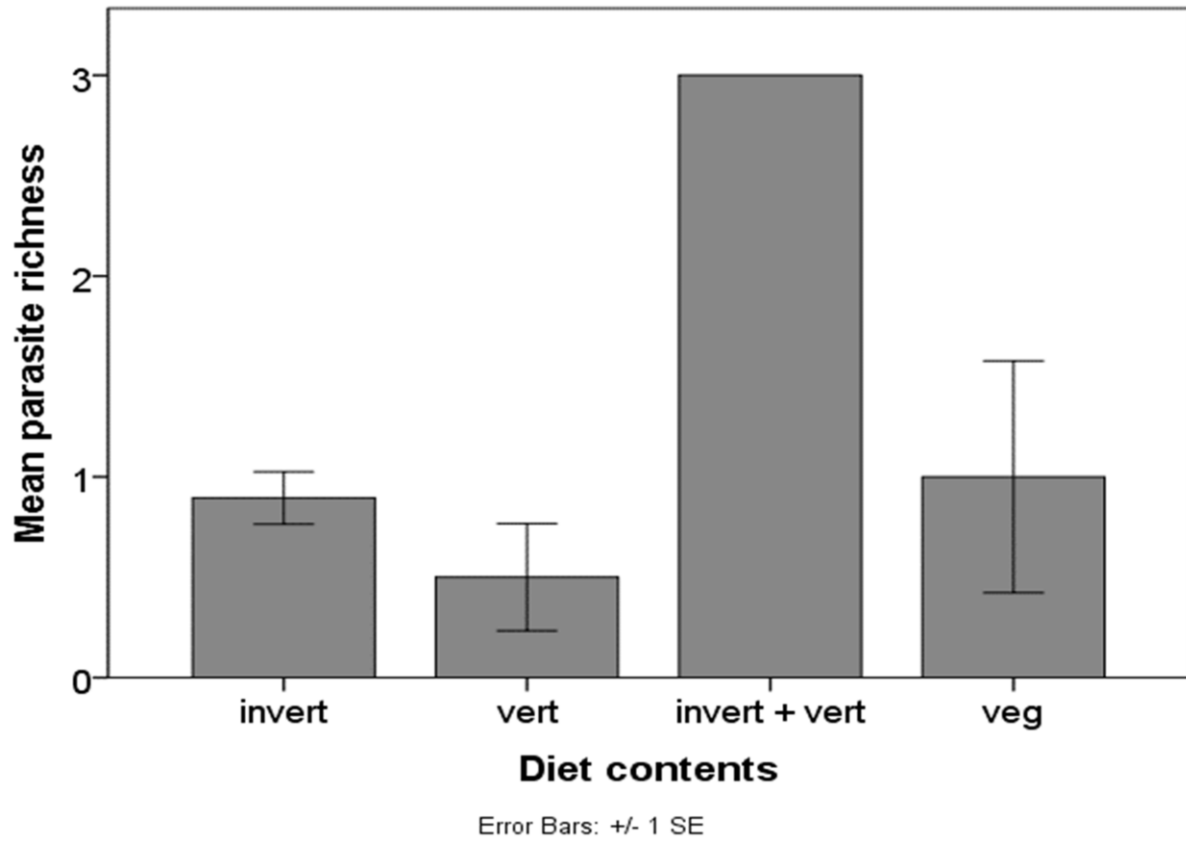


Figure 3.13 Individual fish measurements of diet in relation to parasite richness in Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseed (PS) sunfish caught in Farm Lake.

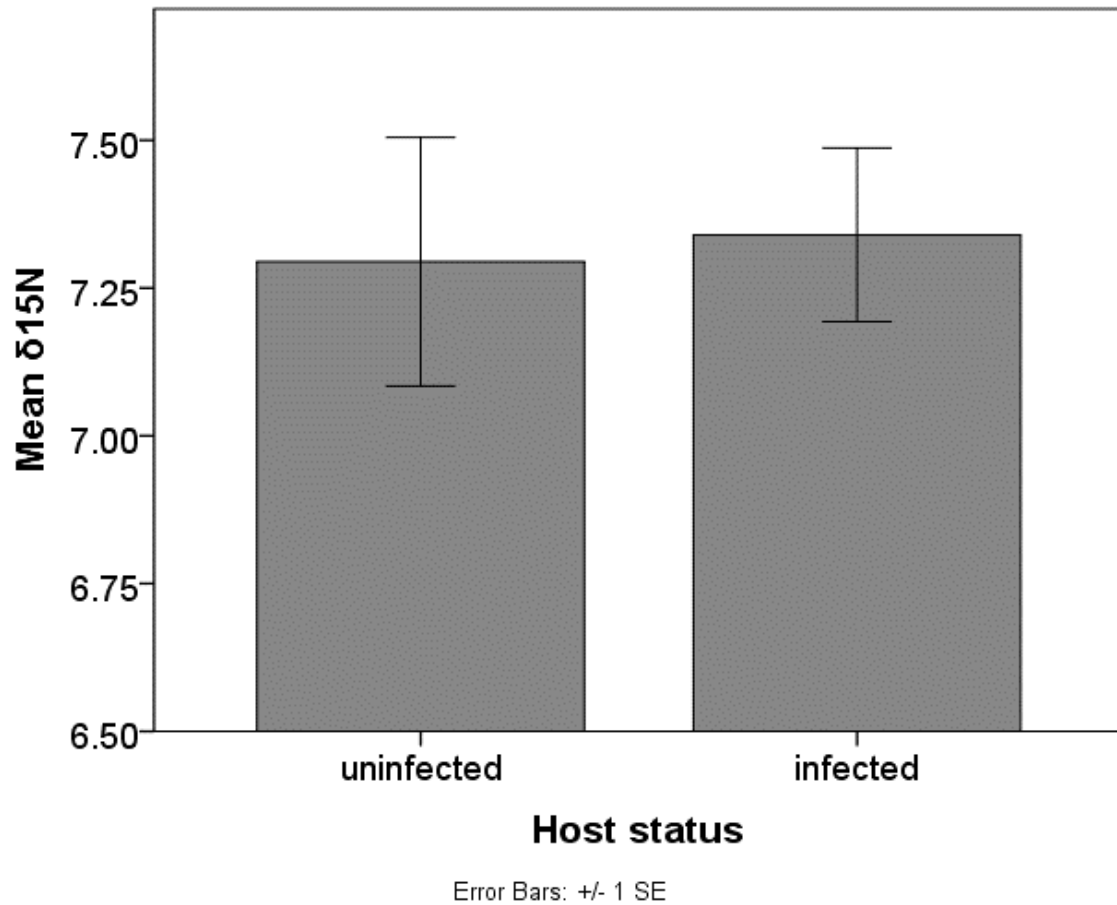


Figure 3.14 Infection status and mean ^{15}N isotope values (+ S.E.) for all Pumpkinseed sunfish, Rock Bass and Smallmouth Bass caught in Farm Lake.

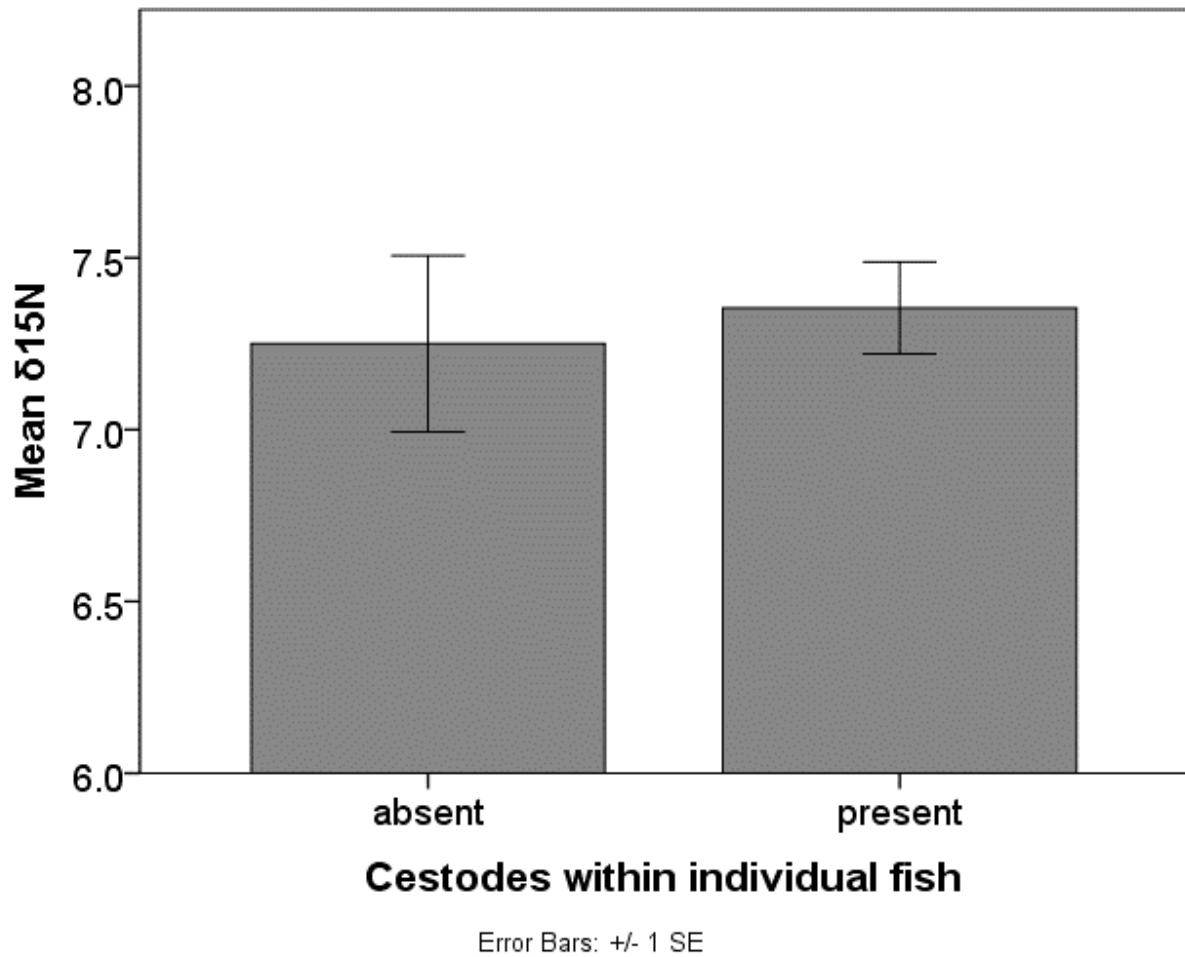
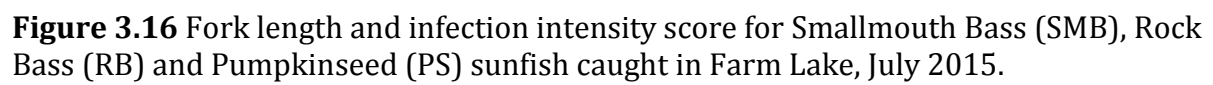


Figure 3.15 Presence of cestodes and mean $\delta^{15}\text{N}$ isotope values (+ S.E.) for all Pumpkinseed sunfish, Rock Bass and Smallmouth Bass caught in Farm Lake.



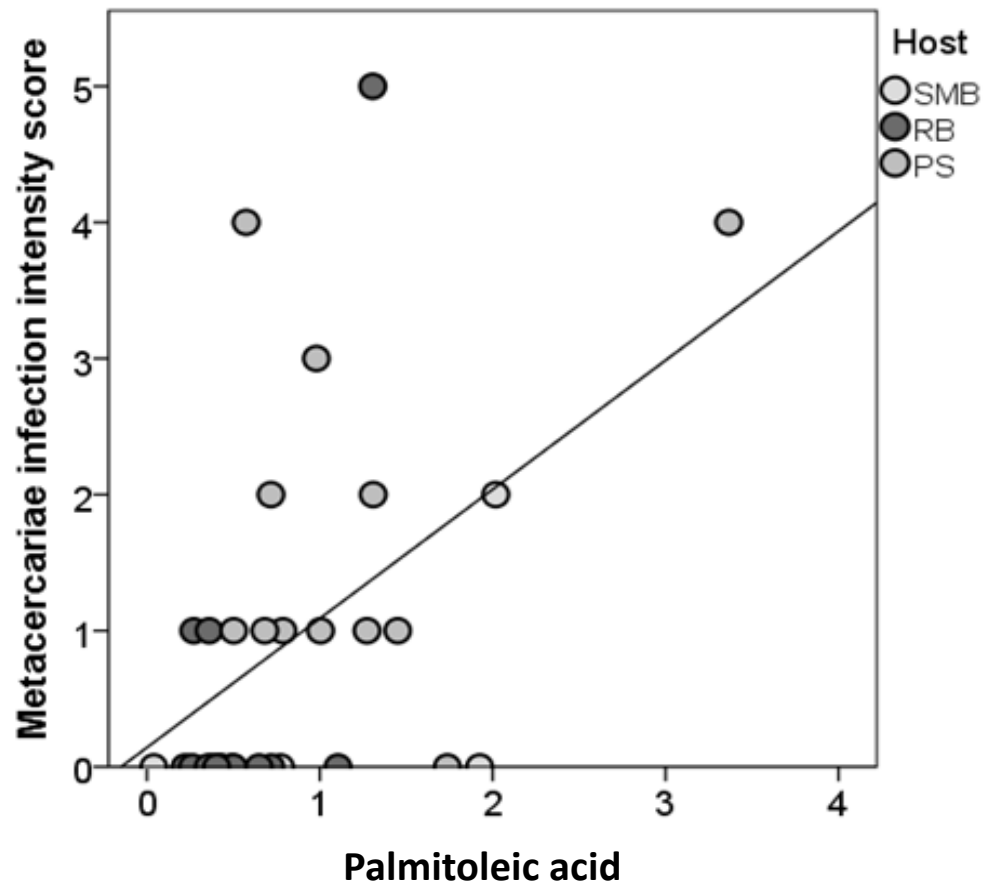


Figure 3.17 Palmitoleic acid (w%) and metacercariae infection intensity for Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseed (PS) sunfish caught in Farm Lake.

When plotting the unique combination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for individual fish, it can be seen that distinct species-specific patterns emerge such that Pumpkinseed and Rock Bass generally have lower $\delta^{13}\text{C}$ scores compared to Smallmouth Bass, but the two Bass species have higher $\delta^{15}\text{N}$ values relative to Pumpkinseed (see Fig. 3.18).

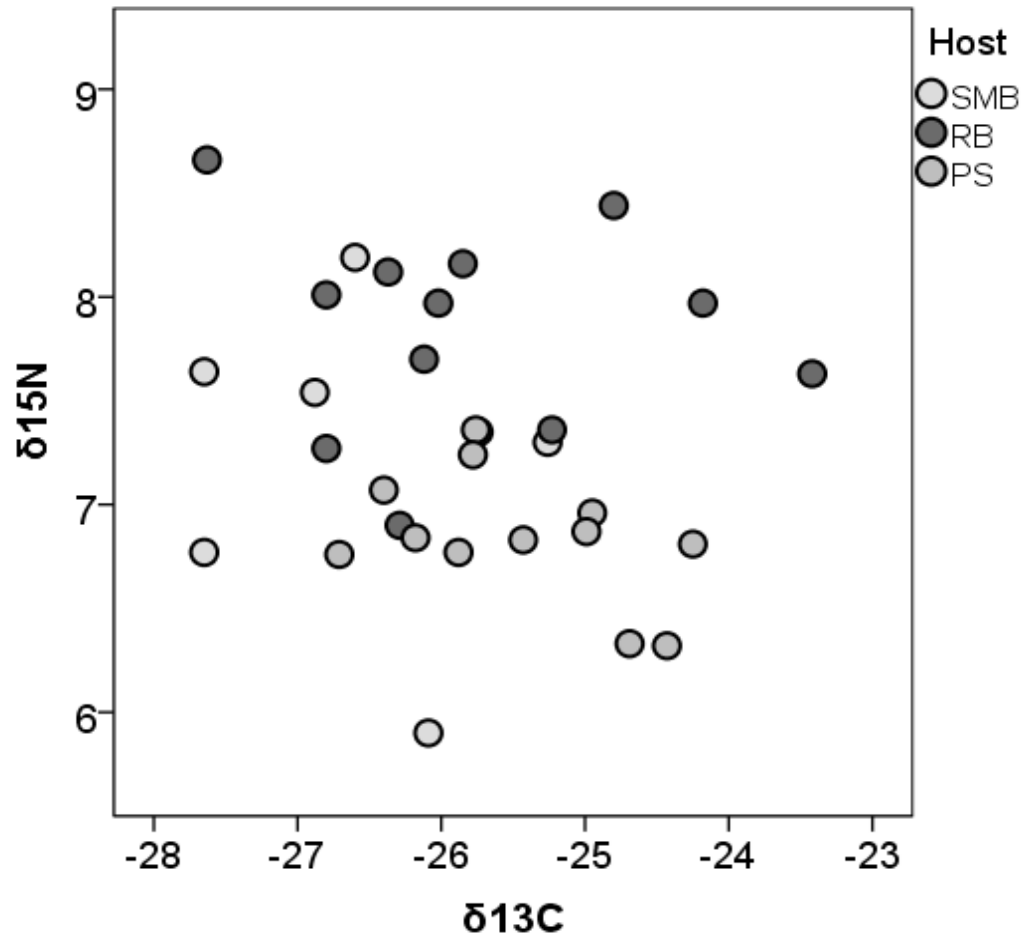


Figure 3.18 Chemical isotope values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in Smallmouth Bass (SMB), Rock Bass (RB) and Pumpkinseed (PS) sunfish caught in Farm Lake.

Discussion

Species-level parasitism

Host species differences were somewhat consistent with my hypotheses, with a few exceptions. I predicted that fish occupying higher trophic levels would have greater parasite species richness and intensity of infection (Ravi and Yahaya, 2015). This was observed for Smallmouth Bass compared to Pumpkinseed sunfish, but not for Rock Bass even though they clearly ate many vertebrates and secondary-consumers based on their $\delta^{15}\text{N}$ values, even more so than Smallmouth Bass. In fact, Rock Bass had the lowest richness of trophically-transmitted helminths. However, $\delta^{15}\text{N}$ cannot be used alone as an absolute measure of trophic position, which is why gut content analysis (GCA) and fatty acid profiles were also conducted. Stomach contents from the fish of Farm Lake indicate that Rock Bass rely heavily on invertebrates, but were the only species that were recorded with vertebrate prey, even though this was only in one fish. In contrast, Smallmouth Bass were the only species where individual fish had a mixture of invertebrate and vertebrate prey in their guts. The fact that many fish had empty stomachs confirms the disadvantages of GCA and supports the concept that identified prey items cannot be used alone to assess the overall diet (Rybcyncyski, 2008).

Species differences in parasitism related to host size (fork length) were expected as larger predators can consume a wider variety of prey potentially hosting parasites, and some adult cestodes (the most commonly-found helminth) can only be acquired by eating smaller fish harbouring larval stages. Trophic position of predators can also be partly interpreted by body size as large animals are more capable of consuming secondary

consumers, although there was no difference in gut content among the three species here. Indeed, my results indicate that Smallmouth Bass had a significantly longer fork length than Rock Bass and Pumpkinseed, and this species had the highest parasite richness. However, Rock Bass were larger than Pumpkinseed sunfish and yet their infection intensity, and richness, of trophically-transmitted parasites was the lowest of all three species. Interestingly, Rock Bass had the lowest content of total lipids by weight, a measure of overall condition (Arts et al. 2009) even though they were the least infected. It was expected that parasite infections would be related as low lipid levels are known to compromise immune systems in fishes (Shul'man, 1974; Gurr, 1983). However, lipid quality, rather than quantity, should also be evaluated in order to better observe fish condition (Arts, 2009).

My observations for the lowest infections of trophically-transmitted helminths in Rock Bass cannot seemingly be explained by their diet and/or trophic position, or their size, but I did also expect that the “enemy release hypothesis” (Keane and Crawley, 2002; Torchin et al. 2002; Torchin et al. 2003) might be observed and help to explain differential patterns of parasitism among the three species given my results in Chapter 2. Since Pumpkinseed and Smallmouth Bass are established species in Algonquin Park compared to the more recent establishment of Rock Bass in many lakes, it may be expected that both invasiveness and trophic position are strong predictors of parasitism in freshwater fishes. Cestodes were most prevalent in Pumpkinseed, and found the least in Rock Bass even though some can only be required by preying upon secondary consumers, which Rock Bass clearly do in Farm Lake. This could be explained by the reliance on zooplankton as prey by Pumpkinseed, and these serve as the primary route of infection for most cestodes, but

many invertebrates were found in the guts of the Rock Bass. However, this lack of cestodes in Rock Bass also supports the enemy release hypothesis, particularly because the similarity in cestode prevalence in Smallmouth Bass and Pumpkinseed is not supported by overlaps in diet in Farm Lake based on their $\delta^{15}\text{N}$ values (strong tendency for difference). In the past, Rock Bass less than 70 mm have been recorded as having a heavily invertebrate based diet, with fish greater than this length consuming a mixture of invertebrates and fish fry (Keast and Webb, 1966). Simply put, Rock Bass are not acquiring cestode infections even though their diet based on GCA and stable isotope analysis suggested that they should be consuming infected prey.

Host habitat use might also play a role in explaining patterns of parasitism. Of the three species examined in Farm Lake in this study, Pumpkinseed are the fish most known to be associated with the littoral zone (Scott and Crossman, 1973), and this has also indicated by their individual values of $\delta^{13}\text{C}$, where more negative values indicate greater use of pelagic habitat (France, 1995). Although Pumpkinseed are the most littoral species, they do overlap in habitat with Rock Bass as illustrated by the relatively low $\delta^{13}\text{C}$ values of both species compared to Smallmouth Bass. Based on this, Smallmouth Bass here seem to be the most pelagic species, but there is individual-level overlap in habitat ($\delta^{13}\text{C}$) and trophic position ($\delta^{15}\text{N}$) with Rock Bass, more so than with Pumpkinseed. As such, Rock Bass overlap in their habitat use with both of the species that had higher parasite infection intensity and richness, suggesting ample opportunity to become infected. That they are not also further supports the enemy-release hypothesis for invasive species (Keane and Crawley, 2002; Torchin et al. 2002; Torchin et al. 2003).

While my focus was on trophically-transmitted helminths, examining infection with parasites acquired through other means can help to clarify the possible influences of host diet and habitat. The results for infection intensity of non-trophically transmitted trematode metacercariae indicate that Pumpkinseed were the most infected species, which is a much different pattern than that observed with trophically transmitted helminths. This is very likely a reflection of fish habitat use, as free-living cercariae that penetrate into fish to form metacercariae are released from their molluscan intermediate hosts near shore (Wisenden, 2012) (see Fig. 3.19). Interestingly, Pumpkinseed and Rock Bass differ significantly in their metacercariae infection intensity even though similarity in their $\delta^{13}\text{C}$ values indicate that they both use littoral areas where infected hosts shedding the infectious stage would be found. That Smallmouth Bass differ in their habitat use likely explains their difference from Pumpkinseeds, but not why Rock Bass have such low infections with metacercariae as well as trophically-transmitted helminths.

Similar to my results for Chapter 2, the reduced parasite burden in Rock Bass, as predicted by the enemy release hypothesis, might be at play because it seems the status of fish as invasive or not is the strongest predictor of infection. This suggests that perhaps physiological and immunological drivers may be more responsible compared to ecological factors, although the mechanism(s) releasing invasive species from parasitism are still not fully elucidated (Keane and Crawley, 2002; Torchin et al. 2003). This relationship could also be driven by native parasites not having yet adapted to invasive species, as parasites may not as easily establish in new potential host species even if there is moderate exposure (Combes, 2001). Successful infections are often thought to be a result of both encounter and susceptibility (Combes, 2001), therefore suggesting that ecological drivers, such as diet

and habitat, and the potentially low susceptibility of invasive species, may play equal components in infection.

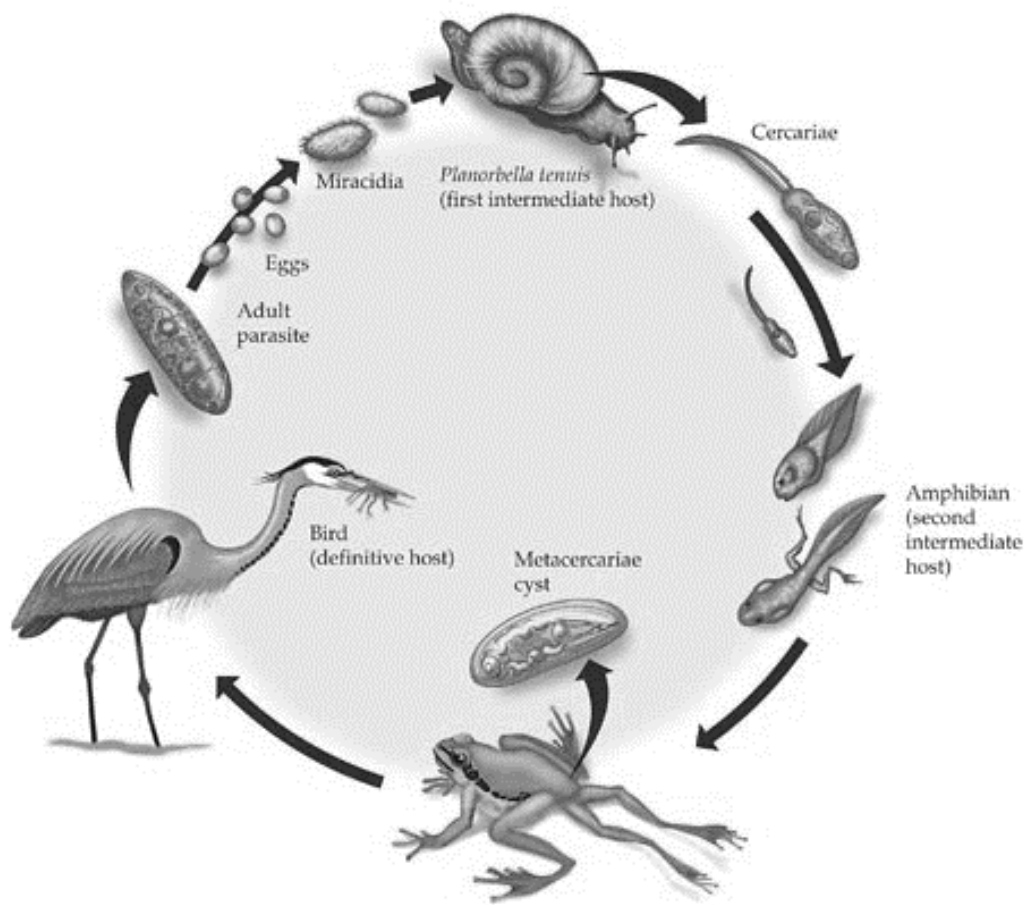


Figure 3.19 Generalized trematode life cycle (<http://www.aquaticparasites.org/background.html>).

Individual-level parasitism

While my focus was on species-level differences in parasitism in this study, the select individual-level analyses yielded some interesting results that might help to explain interspecific patterns and lay the foundation for future directions. The level of omega-3 fatty acids demonstrated a marginally insignificant negative correlation with infection intensity score for trophically-transmitted helminths. Since omega-3 fatty acids can be used as a proxy for piscivory among the 3 fish species (Arts, 2009), this tendency indicates that more piscivorous fish (e.g. Smallmouth and Rock Bass) are less infected than those that primarily eat zooplankton (e.g. Pumpkinseeds). These results suggest that a vertebrate-based diet limits host exposure to parasites, whereas fish that are consuming multiple invertebrate are at higher risk. Because most cestodes and all acanthocephalan parasites utilize zooplankton as intermediate hosts (Parker et al. 2015), a diet relying mostly on vertebrates could therefore limit the diversity and intensity of trophically-transmitted parasites that fish may accumulate. Indeed, there was a significant negative relationship using omega-3 fatty acids as a predictor of parasite richness. Furthermore, this is reinforced by the fact that the fish species (Smallmouth Bass) consuming both invertebrates and vertebrates based on GCA had the highest parasite richness. This might then also help to explain the low richness and intensity found in Rock Bass at the species level given that their $\delta^{15}\text{N}$ scores indicate a diet rich with secondary consumers (i.e. highly piscivorous).

However, there was a strong tendency for infected fish to have a higher trophic level as interpreted by their $\delta^{15}\text{N}$ values even though Rock Bass had the highest $\delta^{15}\text{N}$ values and

also the lowest parasitism (diversity and intensity). Fish at higher trophic levels might simply consume more food given that trophic position and body size are often positively related (Jennings et al. 2001) therefore putting them at higher exposure via greater overall exposure to infected prey. This suggests $\delta^{15}\text{N}$ may indeed be worthy predictor of infection for non-invasive species given that invasive status is potentially an overpowering predictor of infection. Individual values of $\delta^{15}\text{N}$ were also significantly positively correlated with the presence of cestodes within individual fish. Since tapeworms can be acquired both by consumption of invertebrate hosts (larval cestodes) and vertebrate hosts (adult cestodes), fish consuming both diet types are therefore more likely to be infected. That $\delta^{15}\text{N}$ was also negatively correlated with parasite richness might also relate to a greater chance of acquiring many cestodes and acanthocephalan through an invertebrate-dominated diet compared to high piscivory. However, these results for $\delta^{15}\text{N}$, and that for omega-3 fatty acids, are also likely to be strongly driven by the low parasite richness and intensity within Rock Bass.

Once again, looking at infections with helminths that are not trophically-transmitted yielded interesting information. Palmitoleic acid had a very significant positive correlation with the intensity of infection with trematode metacercariae, which is largely explainable by both the large sample size of Pumpkinseeds and their preference for a very littoral habitat where infectious cercariae are prevalent. Palmitoleic acid is produced by diatoms (Kates and Volcani, 1966; Smirnov, 2013), which are a main food source for zooplankton - a common prey item for Pumpkinseed (Reid, 1930; Scott and Crossman, 1973).

Significance of findings and future directions

The aim of this study was to identify the trophic position, diet and, habitat of invasive and non-invasive species that are known to differ in their ecology, and to determine what factors may be the best predictors of parasitism using different methods. There was a general inconsistency regarding the 4 methods used to identify fish diet and trophic position, which probably reflects biological, ecological and methodological factors. These inconsistencies may also reflect the small sample size of fish, few fish species, and the fact only one lake was evaluated.

Parasite diversity was rather low in the three fishes examined from Farm Lake, with cestodes being the most common. Since tapeworms are acquired from eating copepods, it was expected they would have been strongly predicted by invertebrate presence in the GCA. When examining Pumpkinseed from Algonquin Park, Locke et al. (2013) also found cestodes to be prevalent, yet were unable to relate them to stomach contents as well. This is likely due to copepods being too small or easily digested to be identified (Macdonald et al., 1982), but could also be representative of a seasonal or ontogenetic diet shift, where larval cestodes persist for months prior to sampling. Those wishing to conduct similar studies may want to consider revising methods to include a higher specificity in GCA options, such as volume v. abundance of prey, wet v. dry mass, and stomach fullness (Locke et al. 2013). Searching for potential prey items could also assist in SIA, where the isotopic signatures of prey items could be determined from the contents of sampled fish. This study should also be expanded to include more fish species, as the overarching relationship

between parasitism, host diet, and establishment of invasive species may become more apparent.

Another issue encountered with GCA was the lack of stomach contents in many of the fish sampled. For example, only one Pumpkinseed was found to have any stomach contents at all, and the low sample size of Smallmouth Bass may be unrepresentative of what was expected to be a more piscivorous diet. However, no difference in omega-3 fatty acids, which are a proxy for a piscivorous diet, were observed among the host species. FAA showed a high degree of similarity among the three examined species, except for the presence of palmitoleic acid, which is more likely be a reflection of a littoral habitat rather than trophic position or diet.

SIA was also difficult to interpret in Farm Lake. The fact that Rock Bass had the highest trophic level was certainly not expected. The lack of adult cestodes, vertebrates in the GCA, and low omega-3 fatty acids, suggest that there may be other unknown driving factors behind their high $\delta^{15}\text{N}$ values, although release from parasitism may also play a role. Related to this, it was expected that fish species with a broad diet and high trophic level would have higher parasite richness. The results demonstrated this to be partly true, where species consuming both invertebrate and vertebrate prey had a higher parasite richness. I also expected that host habitat would not correspond to trophically transmitted helminth infections, which was found to be true. Habitat was, however, clearly a driving factor for non-trophically transmitted metacercariae infections. There was no attempt to meticulously characterize the diet of each fish with all four mechanisms, but the low degree

of correlation among many of these factors suggests a higher sample size of both fish and lakes may be required.

The results suggest there are trophically-based factors that drive parasitism in Algonquin Park fish. In particular, diet and habitat were demonstrated to be significantly related to parasitism and therefore the ecological perturbations in the Park could have major impacts for the disease dynamics of Algonquin fish. The release from parasites seen in Rock Bass and other invasive species may be strongly influencing their ability to establish and succeed over native species. Future studies should focus on revealing exactly how ecological, physiological or immunological factors are resulting in enemy release. Such work is critical for the development and implementation of freshwater fish conservation strategies, which may help to improve the diversity and health of entire ecosystems.

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CHAPTER 4 GENERAL DISCUSSION AND SIGNIFICANCE

The primary objectives of this thesis were twofold: 1) compare parasitism by trophically-transmitted helminths in invasive Rock Bass and established Smallmouth Bass in Algonquin Park; and 2) perform a detailed examination regarding the role of host diet and habitat use on infection by trophically-transmitted helminths in three host fish species in one lake in Algonquin Park.

In chapter 2, I evaluated the differences between Rock Bass and Smallmouth Bass in their trophically-transmitted helminth communities, focusing on cestodes (tapeworms), acanthocephalans (thorny-headed worms), nematodes, and trematodes (flukes). I hypothesized that invasive Rock Bass would experience reduced parasitism in the relatively novel Algonquin lakes in which they have recently been reported, and therefore experience a lower diversity and intensity of infection as predicted by the “enemy release hypothesis” (Keane and Crawley, 2002; Torchin et al. 2002; Torchin et al. 2003). I also expected that independent of the species, individual fish size (fork length) would be positively correlated with parasite richness and intensity of infection.

This evaluation was completed by sampling 112 Rock Bass and 60 Smallmouth Bass from 8 different lakes in the park. These fish were dissected for their parasite composition, which were identified after staining creating microscope slides, as well as their gut contents to examine whether species differences in diet might also play a role. My hypotheses were supported by my findings. Not only did Smallmouth Bass harbour more trophically-transmitted parasites, but they also had a higher diversity of these. However,

there was no difference in infection by trematode metacercariae, which are not acquired by trophic transmission, and there was no difference in gut content between the two host species with respect to the presence of invertebrates or vertebrates. Fork length was positively correlated with parasite intensity and richness between and within species, and Smallmouth Bass were on average significantly larger than Rock Bass.

My results support the enemy release hypothesis (Keane and Crawley, 2002; Torchin et al. 2002; Torchin et al. 2003), which suggests that invasive species lose their native parasites but do not acquire local ones in their new habitats; however, it cannot be said with any certainty whether or not this contributes to the success of Rock Bass in Algonquin Park, especially because they are typically smaller than Smallmouth Bass given the importance of fork length as a predictor. More detailed examinations of host diet and habitat use in relation to parasitism are also needed for these two species. It is unlikely that parasite spillover (introduction of exotic parasites by the invasive hosts) occurred, as trophically-transmitted parasites are often specific in their use of intermediate hosts (Torchin et al. 2002). Since Rock Bass did not appear to be a naïve host to novel infections, spillback (use of invasive host by local parasites) is another mechanism which did not likely occur.

In chapter 3, I evaluated the diet, habitat use, and trophic position of 13 Rock Bass, 12 Pumpkinseed, and 6 Smallmouth Bass from Farm Lake in relation to host parasite communities, again focusing on trophically-transmitted helminths. I used 3 measures to evaluate the influence of host diet on parasitism: gut content analysis (GCA), stable isotope analysis (SIA), and fatty acid analysis (FAA). This represents the first time that these 3

measures have been simultaneously used in such an effort to examine parasitism to the best of our knowledge. It was expected that fish with broader diets, and at higher trophic levels, would have a greater diversity and intensity of trophically-transmitted helminth infections. I also anticipated that habitat would be related only to non-trophically-transmitted infections, such as trematode metacercariae, based on the location of intermediate hosts such as aquatic gastropods.

My predictions were largely supported by the results, where it was determined that both diet and habitat could affect parasitism. However, the 3 methods of diet identification yielded results that were difficult to interpret as they sometimes contradicted one another in terms of their ability to predict parasitism. The lack of association among these methods, and their differential relationships with parasitism, is likely a reflection of methodological and biological factors (Locke et al. 2013). The different temporal scales of diet assimilation between the 3 methods, as well as parasite acquisition and persistence, may be one potential source of disparity (Locke et al. 2013).

Nonetheless, I found that Smallmouth Bass and Pumpkinseed sunfish had a higher diversity, and intensity, of trophically-transmitted helminths compared to Rock Bass, and this was related to certain aspects of diet even though there was no difference among species as evaluated by GCA. Based on the average $\delta^{15}\text{N}$ value, Rock Bass were found to be at a higher trophic position than Smallmouth Bass in Farm Lake, which contradicts the literature (Scott and Crossman, 1973; Mandrak and Crossman, 2003). Because of this, both $\delta^{15}\text{N}$ and level of omega-3 fatty acids (a proxy for piscivory) were negatively related to parasitism – contrary to expectations. These results may have been skewed by a small

sample size of Smallmouth Bass compared to Rock Bass and Pumpkinseeds, and the short fork length of Smallmouth Bass compared to their potential growth capacity. Some Smallmouth Bass caught in other lakes as part of Chapter 2 exceeded 46 cm in length, whereas the longest Smallmouth Bass caught in Farm Lake was only 21.6 cm. Larger Smallmouth Bass would likely hold a different trophic position and diet than shorter fish. Interestingly, parasitism was highest in individual fish that had a mix of invertebrates and vertebrates in their guts (only seen for Smallmouth Bass) rather than only vertebrates. However, the low infections in Rock Bass overall likely heavily influence any relationships with host diet.

My results generally indicated that invasive Rock Bass often overlapped in their prey and habitat with Smallmouth Bass and Pumpkinseeds, respectively, further supporting a possible release from natural enemies such as parasites. Release from parasites in invasive species can have profound consequences for their ability to establish and should be further examined to see if their diet and habitat use plays a role, or if factors related to their susceptibility are more important. These consequences could be most severe in freshwater ecosystems, where species loss has been attributed to introductions of non-native species (Mandrak and Crossman, 2003; Koehn, 2004), as well as the spread of disease (Riley et al. 2008; Johnson and Paull, 2011).

The overlap in diet and habitat of Rock Bass with native Pumpkinseeds and established Smallmouth Bass also suggests their introduction could have effects on these populations outside of parasitism. However, any changes to the diets of these native species as a consequence of the introduction of Rock Bass could ultimately result in changes to their parasite communities. For example, if an invasive species such as Rock

Bass creates competition that causes a shift from piscivory in Smallmouth Bass to a more zooplankton-based diet, this could result in an increased prevalence and abundance of *Proteocephalus ambloplitis*, the most common tapeworm found in all three species. Larval stages of this cestode are acquired by consuming infected zooplankton such as crustaceans, and can cause significant pathology (Gilliland and Muzzall, 2004). The health of fish heavily infected with this parasite was likely compromised in my samples, with visible damage to the reproductive organs and adhesion of visceral organs with thick connective tissue seen in several Smallmouth and Rock Bass.

Future directions

Since parasite surveys such as this one are generally considered to be a snapshot in time (Bauer, 2013), it has been suggested that yearly examinations be carried out in order to better assess how disease dynamics in local fish populations may change in relation to perturbations such as introduced species. A meta-analysis using carefully assembled historical records could also help reveal patterns. It would be especially interesting to observe changes in these records dating to when Rock Bass were becoming rapidly established in Algonquin Park, as well as continue to monitor parasitism in fish populations.

Through studies such as this one, it is possible to gain a better understanding of how parasitism in freshwater fishes is related to their ecology, which will allow for more accurate predictions regarding the possible impacts of ecosystem perturbations. The dynamics of fish parasitism in freshwater environments may help shed light on drivers of community structure in aquatic populations (Rowley et al. 2013). Some species that may be

impacted by parasitism, either directly or indirectly, may be considered keystone species (Whiles et al. 2006) and changes in their abundance carry consequences for the ecosystem. In order to avoid negative ecosystem effects, the early detection of, and rapid response to, emerging parasites is critical (Rowley et al. 2013). Thus, further studies and greater knowledge of freshwater parasites may help prevent ecosystem collapses in the future.

Identifying predictable patterns of parasite infection resulting from environmental changes may also help to identify conservation priorities and act as a basis for preventative management. The International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species was created to act as a species-based conservation prioritization tool. While the list is regarded as the most authoritative list of globally threatened species (Lamoureaux et al. 2003), its use as a tool for assessing the biodiversity impact of specific disturbances has been criticized due to the general criteria used to classify species extinction risks (Ackakaya et al. 2006), and the lack of consideration for cascading community disassembly (Zavaleta et al. 2009). Although no fish species in Algonquin Park are currently on the Red List, my research has the potential to impact future risk criteria by identifying any vulnerabilities of fish species from parasitism due to trophic position and habitat type in relation to specific biodiversity alterations, such as that posed by invasive species, or other perturbations that affect host ecology.

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