EFFECTS OF HOST COMMUNITY STRUCTURE ON PARASITE TRANSMISSION AND DISEASE RISK

SANGWOOK AHN
Bachelor of Science, University of Calgary, 2017

A thesis submitted
in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

in

BIOLOGICAL SCIENCES

Department of Biological Sciences
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

EFFECTS OF HOST COMMUNITY STRUCTURE ON PARASITE TRANSMISSION
AND DISEASE RISK

SANGWOOK AHN

Date of Defence: 27 November, 2019

Dr. Cameron Goater Thesis Supervisor	Professor	Ph.D.
Dr. Joseph Rasmussen Thesis Examination Committee Member	Professor	Ph.D.
Dr. Shaun Dergousoff Thesis Examination Committee Member	Research Scientist	Ph.D.
Dr. Tony Russel Chair, Thesis Examination Committee	Associate Professor	Ph.D.

DEDICATION

To my mother and brother, who have continued to support whatever endeavors I find myself in, and to my father, who's desire for knowledge continues to motivate me.

ABSTRACT

This thesis aims to determine how parasite transmission responds to temporal, spatial, or ecological factors such as host biodiversity. For this study, I used a fathead minnow/trematode parasite study system and incorporated both field and experimental components. Long-term data on the parasite population sizes in fathead minnows from two lakes in Alberta indicate that parasite population sizes are highly variable. Simultaneous infections by multiple parasite species in fathead minnows is the norm, with closely related parasite species exhibiting similar long-term trends in population dynamics. Increases in host biodiversity is thought to decrease parasite transmission, however, my experiments indicate that biodiversity is not directly causing a decrease in transmission of the trematode *Ornithodiplostomum ptychocheilus* into fathead minnows. Instead, I suggest that the mechanism behind the decline in transmission is the species-specific modulation of transmission pathways as host biodiversity increases.

ACKNOWLEDGEMENTS

University of Lethbridge, who have been enthusiastic about supporting my research ethically and efficiently.

Finally, I would like to thank my family for supporting my decision to move to Lethbridge. Even if they didn't understand why I chose to move to Lethbridge to look at worms inside fish, it is only with their love that I am confident in having pursued my passion.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

1.1 *General background*

Of all animal species on the Tree of Life, almost 50% of them are parasites for at least one stage of their lifecycle (Windsor 1998; Poulin & Morand 2000). In terms of biodiversity, parasites comprise key components of ecosystem structure (Lafferty et al. 2008) and can play large functional roles within ecosystems (Lafferty & Kimo 1996, Morris 1996; Frainer et al. 2018). However, research into the functional role of parasites within ecosystems is not proportional to their ubiquity in nature (Windsor 1998). Research focusing on parasites that directly affect human health, such as malaria, are consistently pursued, yet the role of parasites on wildlife health is an emerging field, and researchers are still attempting to define the sheer diversity of parasite species (Dobson et al. 2005). As researchers continue to struggle to understand fundamental characteristics of parasite biology, research into their role in ecosystems is still at its infancy (Wood & Johnson 2015). Recently, recognition of the ecological role of parasites in natural systems is becoming more mainstream, particularly with respect to how parasites can be an integral link in energy transfer between trophic levels. Specifically in aquatic environments, free-livings stages of parasites are known to constitute a large proportion of overall biomass (Preston et al. 2013) and can subsequently play important roles as prey species (Lafferty et al. 2008). Furthermore, parasites can facilitate energy transfer in food webs (Lafferty et al. 2008; Johnson et al. 2010), as infected hosts are often more prone to predation.

A key example of the role of parasites in food web dynamics has been reported for char fish ingesting orthopterans (crickets and grasshoppers) infected with a parasitic nematomorph (Sato et al. 2011, 2012). Crickets and grasshoppers are typically not

consumed by aquatic predators simply because the bridge between aquatic and terrestrial habitats is insurmountable. However, orthopterans infected with larval nematomorphs display suicidal behaviour by jumping into bodies of water, providing an available resource of food for char. The role of parasites in facilitating this interaction was so dramatic that in one study, 60% of the food ingested by char was shown to be comprised by infected orthopterans (Sato et al. 2011). Another key consideration of parasites is their ability to regulate host populations. In a seminal paper by Hudson et al. (1998) parasites were recognized as regulatory factors for red grouse populations. Thus, the evidence continues to mount to indicate the importance of parasites in shaping our environments.

However, parasites are not solely agents of change and are as much affected by their environment as the hosts they inhabit. Unfortunately, much research involving parasites is host-centric and focuses on the effects of problem parasites on host individuals, especially on humans and domestic animals. In that respect, there is a lack of understanding of how parasites themselves can be influenced by both biotic and abiotic factors. To address these shortcomings, researchers have suggested the use of a community-ecology framework to understand complex host-parasite-environment interactions (Johnson et al. 2015). By studying infectious disease with ecologically relevant questions, researchers have identified how parasite risk is impacted by some of the worlds' most contemporary environmental issues such as habitat loss and climate change (Brownstein et al. 2005; Pongsiri et al. 2009; Medlock & Leach 2015). As our environments continue to undergo dramatic anthropogenic changes, a community ecology framework for studying parasites can provide information on what drives variation in parasite burdens and biomass in individual hosts, within host

populations, and within geographic regions for targeted control strategies (Johnson et al. 2015).

For a more complete understanding of how parasites respond to their natural environments, long-term studies are needed to provide key information on what factors can influence rates of parasite transmission. Intrinsic (host sex, intraspecific competition between parasites, etc.) and extrinsic (temperature, nutrient availability, etc.) factors determine the rate of parasite transmission. Based on these factors, parasite populations often demonstrate long-term dynamics that are highly variable. Understanding the underlying causes of this variation in parasite population dynamics is often a central goal for parasite ecologists and epidemiologists. However, the current literature suggests that parasite population dynamics are context dependent, and are not necessarily a consequence of the same extrinsic or intrinsic pressures at different sites and in different years. For example, a 31-year study of larval stages of the cestode *Ligula intestinalis* in roach populations (Kennedy et al. 2001) concluded that parasite populations were inherently unstable and that the multiple epizootic cycles observed in the *Ligula*-roach system were not spatially or temporally consistent. *L. intestinalis* population dynamics appeared to consistently cycle throughout the study period, however, the authors concluded that the epizootics were independent of each other and reflected stochastic habitat changes, and not regulatory factors that were dependent on parasite burden (Kennedy et al. 2001). Therefore, even the dynamics of a single parasite population in a single host population can exhibit context dependent and highly stochastic variation over the long-term.

In contrast, other parasite populations demonstrate seemingly stable population sizes from year to year with remarkably little variation (Kennedy 1987; Valtonen et al.

3

2004). For example, acanthocephalan populations in eels were temporally consistent in populations of eels collected from a large, stable, anthropogenically-controlled river in Ireland (Kennedy & Moriarty 2002). However, in this case, the observed pattern of stability was attributed to the stability of the local and regional habitat (Kennedy 2009). In a review, Kennedy (2009) used examples such as these to conclude that parasite populations are fundamentally unstable and unregulated. The instability of parasite populations was further emphasized from an observation by Kennedy and Burrough (1977) that showed that the colonization of a lake with larval *Tylodelphus clavata* in the eyes of perch was associated with the stochastic visitation to the lake by a single pair of breeding grebes that are final hosts to the parasite. In that respect, the composition of host species communities has been implicated as being a key determinant of variation in rates of parasite transmission.

Evidence from recent empirical studies have indicated the significance of local host species biodiversity in determining the notoriously high variation in rates of parasite transmission. This approach is particularly relevant as biodiversity of organisms is declining at unprecedented rates, particularly through anthropogenic influences such as climate change and habitat loss (Vitousek et al. 1997; Thomas et al. 2004; Ceballos et al. 2015). From a parasite and disease perspective, one consequence of biodiversity loss is described by the dilution effect hypothesis (DEH). The DEH predicts that parasite transmission is negatively associated with host species richness (Keesing et al. 2006). The results from contemporary field studies indicate that host biodiversity can act to dilute risk of transmission of important human and wildlife diseases (Ostfeld & Keesing 2000; Allan et al. 2009; Suzán et al. 2009; Gendron & Marcogliese 2017). Therefore, understanding how rates of disease transmission change relative to ongoing losses of biodiversity is a vital

4

component of preserving public and environmental health as emerging diseases are contributing to global environmental crises (Jones et al. 2008). However, evidence in support of the DEH remains contentious with no definitive consensus on the general applicability of the DEH across a range of host-parasite interactions (Wood et al. 2014; Halsey 2019).

Much of the contentious nature of the DEH arises from the sheer diversity of parasites and their highly variable life cycles. First, much of the literature regarding the dilution effect focuses on vector-transmitted diseases that have different life-history strategies relative to direct, or complex-life cycle species. Furthermore, vectored systems are often intractable to experimentation (Johnson & Thieltges 2010) resulting in a lack of mechanistic insight on the biological processes that might underlie the DEH. To address these shortcomings, researchers have begun using aquatic trematode systems to experimentally test the DEH (Thieltges et al. 2008; Raffel et al. 2010; Johnson et al. 2013). The results of experimental tests indicate that the rate of transmission of the trematodes *Ribeiroia ondatrae* and *Echinostoma trivolvis* from its snail intermediate hosts to its amphibian host is, in part, determined by host species richness (Raffel et al. 2010; Johnson et al. 2013). These results are consistent with the DEH because rates of transmission were shown to decline with increases in host (larval amphibians) diversity. In both of these cases, supportive evidence for the DEH is especially important because these trematodes are associated with host pathology and mortality that can contribute to the dramatic decline in global amphibian populations (Stuart et al. 2004).

Within the scope of declining animal populations, the loss of other freshwater animals such as fishes (Moyle & Leidy 1992; Ricciardi & Rasmussen 1999) are equally

alarming. Recently, host survey approaches have suggested that the dilution effect can be observed in fish parasites through the introduction of a novel fish species into fish communities (Paterson et al. 2011; Gendron & Marcogliese 2017). These field studies provide the first evidence that the DEH can be an important factor in determining risk of infection of hosts within fish communities. Unfortunately, no experimental tests of the DEH have been performed with fish/parasite interactions, meaning that the effect of biodiversity losses on disease and parasite transmission into fish remains unclear. Ideally, the resolution of this knowledge gap requires carefully-selected model systems that can incorporate field surveys to assess natural patterns of infection and experimental manipulation of both fish species diversity and exposure.

Similar to the parasites used in amphibian studies (Johnson et al. 2013; Wojdak et al. 2014) fish are commonly infected with the encysted stages (known as metacercariae) of larval trematodes. Trematodes are parasitic flatworms that have complex life cycles. The completion of their life cycle requires transmission of infective larval stages into multiple obligate hosts, the first of which is almost always a snail. Fathead minnows (*Pimephales promelas*) are known to harbour the metacercariae of at least 6 species of trematode parasites (Sandland *et al.* 2001; Wisenden *et al.* 2012) and previous studies in my supervisor's lab have shown that the fathead/trematode model system is highly amenable to both field studies (Sandland et al. 2001) and experimental manipulation in laboratory and mesocosm settings (Matisz & Goater 2010; Stumbo et al. 2012). Furthermore, fathead minnows have been consistently sampled from sites in southern Alberta since 2005. This prior research effort has the potential to provide a picture of long-term, spatio-temporal changes in metacercarial population sizes. The availability of extensive parasite census

data in this region, together with the demonstrated amenability of the fathead/trematode system to experimental manipulation provides the basis for my tests of how parasites generally, and trematodes specifically, respond to environmental change.

1.2 Thesis objectives

The aim of Chapter 2 is to characterize spatio-temporal patterns of larval parasite infection in two populations of fathead minnows in Southern Alberta. This approach is made possible by previous sampling efforts by students in the Goater lab that started in 2005 at two sites (McQuillan Reservoir and Goldspring Park Pond). In my study, I continued field surveys of parasites in fathead minnows at these same sites between 2016-2019. By combining these recent and past sampling efforts, I could assess long-term (15 years) changes in the structure of the parasite communities in fatheads. More specifically, I could assess spatio-temporal changes in the population sizes of the common and frequent species of parasite over this interval. Characterizing spatio-temporal variation in the presence and number of parasites in individual minnows, and in populations of minnows, will provide two key pieces of information. First, fathead minnows are known to be infected with multiple different species of parasite, especially trematodes. Long-term data sets are rare, particularly those with complete parasite surveys. Therefore, this chapter uses this long-term data to test whether variation in component populations (Bush et al. 1997) of parasite communities are predictable through time. Second, this chapter lays the foundation for subsequent experimental lab work (e.g. Chapter 3) by providing a benchmark of natural parasite community structure and parasite population sizes in fathead minnows from southern Alberta.

Chapter 3 provides the first test of the Dilution Effect Hypothesis in a fish/parasite model system. Here, I ask whether fish host species richness can dilute parasite risk into a focal host, the fathead minnow. Using a combination of laboratory experiments involving controlled exposures and outdoor mesocosm experiments, I test if diverse fish species assemblages reduce rates of transmission of the trematode *Ornithodiplostomum ptychocheilus* into fathead minnows. Consistent with the DEH, I predict that as fish host species richness increases, parasite transmission into fathead minnows will decrease. My experimental test of DEH will provide two key pieces of information. First, by testing the DEH in a novel host-species system, this chapter will clarify the general applicability of the DEH in predicting parasite transmission in natural systems. Secondly, by using multiple host diversity treatments along with host density treatments, I can isolate the effects of increasing fish host biodiversity independent of fish density, providing a higher-resolution understanding of the factors that influence parasite transmission in aquatic systems.

1.3 Literature cited

Allan, B. F., B. R. Langerhans, W. A. Ryberg, W. J. Landesman, and N. W. Griffin. 2009. Ecological correlates of risk and incidence of West Nile virus in the United States. Oecologia 158:699–708.

Brownstein, J. S., T. R. Holford, and D. Fish. 2005. Effect of climate change on lyme disease risk in North America. EcoHealth 2:38–46.

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

Ceballos, G., P. R. Ehrlich, A. D. Barnosky, A. García, R. M. Pringle, and T. M. Palmer. 2015. Accelerated modern human – induced species losses : Entering the sixth mass extinction. Science Advances 1:9–13.

Dobson, A., K. D. Lafferty, A. M. Kuris, R. F. Hechinger, and W. Jetz. 2005. Homage to Linnaeus: How many parasites? How many hosts? Proceedings of the National Academy of Sciences of the United States of America 47:109–117.

Frainer, A., B. G. McKie, P. A. Amundsen, R. Knudsen, and K. D. Lafferty. 2018. Parasitism and the biodiversity-functioning relationship. Trends in Ecology and Evolution 33:260–268.

Gendron, A. D., and D. J. Marcogliese. 2017. Enigmatic decline of a common fish parasite (*Diplostomum* spp.) in the St. Lawrence River: Evidence for a dilution effect induced by the invasive round goby. International Journal for Parasitology: Parasites and Wildlife 6:402–411.

Halsey, S. 2019. Defuse the dilution effect debate. Nature Ecology and Evolution 3:145–146.

Hudson, P. J., A. P. Dobson, and D. Newborn. 1998. Prevention of population cycles by parasite removal. Science 282:2256–2258.

Johnson, P. T. J., A. Dobson, K. D. Lafferty, D. J. Marcogliese, J. Memmott, S. A. Orlofske, R. Poulin, and D. W. Thieltges. 2010. When parasites become prey: Ecological and epidemiological significance of eating parasites. Trends in Ecology and Evolution 25:362–371.

Johnson, P. T. J., D. L. Preston, J. T. Hoverman, and K. L. D. Richgels. 2013. Biodiversity decreases disease through predictable changes in host community competence. Nature 494:230–233.

Johnson, P. T. J., J. C. De Roode, and A. Fenton. 2015. Why infectious disease research needs community ecology. Science 349.

Johnson, P. T. J., and D. W. Thieltges. 2010. Diversity, decoys and the dilution effect: How ecological communities affect disease risk. The Journal of Experimental Biology 213:961–970.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature 451:990–993.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485–498.

Kennedy, C. R. 1987. Long-term stability in the population levels of the eyefluke *Tylodelphys podicipina* (Digenea: Diplostomatidae) in perch. Journal of Fish Biology 31:571–581.

Kennedy, C. R. 2009. The ecology of parasites of freshwater fishes: The search for patterns. Parasitology 136:1653–1662.

Kennedy, C. R., and R. Burrough. 1977. The population biology of two species of eyefluke, *Diplostomum gasterostei* and *Tylodelphys clavata*. Journal of Fish Biology 11:619–633.

Kennedy, C. R., and C. Moriarty. 2002. Long-term stability in the richness and structure of helminth communities in eels, *Anguilla anguilla*, in Lough Derg, River Shannon, Ireland. Journal of Helminthology 76:315–322.

Kennedy, C. R., P. C. Shears, and J. A. Shears. 2001. Long-term dynamics of *Ligula intestinalis* and roach *Rutilus rutilus*: A study of three epizootic cycles over thirty-one years. Parasitology 123:257–269.

Lafferty, K. D., S. Allesina, M. Arim, C. J. Briggs, G. De Leo, A. P. Dobson, J. A. Dunne, P. T. J. Johnson, A. M. Kuris, D. J. Marcogliese, N. D. Martinez, J. Memmott, P. A. Marquet, J. P. McLaughlin, E. A. Mordecai, M. Pascual, R. Poulin, and D. W. Thieltges. 2008. Parasites in food webs: The ultimate missing links. Ecology Letters 11:533–546.

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

Matisz, C. E., and C. P. Goater. 2010. Migration, site selection, and development of *Ornithodiplostomum* sp. metacercariae (Digenea: Strigeoidea) in fathead minnows (*Pimephales promelas*). International Journal for Parasitology 40:1489–1496.

Medlock, J. M., and S. A. Leach. 2015. Effect of climate change on vector-borne disease risk in the UK. The Lancet Infectious Diseases 15:721–730.

Moyle, P. B., and R. A. Leidy. 1992. Loss of biodiversity in aquatic ecosystems: Evidence from fish faunas. Pages 127-169 Conservation Biology: The tehory and practive of nature conservation, preservation, and management. Chaptman & Hall, New York.

Ostfeld, R. S., and F. Keesing. 2000. Biology biodiversity and disease risk : The case of Lyme disease. Conservation Biology 14:722–728.

Paterson, R. A., C. R. Townsend, R. Poulin, and D. M. Tompkins. 2011. Introduced brown trout alter native acanthocephalan infections in native fish. Journal of Animal Ecology 80:990–998.

Pongsiri, M. J., J. Roman, V. O. Ezenwa, T. L. Goldberg, H. S. Koren, S. C. Newbold, R. S. Ostfeld, S. K. Pattanayak, and D. J. Salkeld. 2009. Biodiversity loss affects global disease ecology. BioScience 59:945–954.

Poulin, R., and S. Morand. 2000. The diversity of parasites. The Quarterly Review of Biology 75:277–293.

Preston, D. L., S. A. Orlofske, J. P. Lambden, and P. T. J. Johnson. 2013. Biomass and productivity of trematode parasites in pond ecosystems. Journal of Animal Ecology 82:509–517.

Raffel, T. R., J. T. Hoverman, N. T. Halstead, P. J. Michel, and J. R. Rohr. 2010. Parasitism in a community context: Trait-mediated interactions with competition and predation. Ecology 91:1900–1907.

Ricciardi, A., and J. B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. Conservation Biology 13:1220–1222.

Sandland, G. J., C. P. Goater, and A. J. Danylchuk. 2001. Population dynamics of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows (*Pimephales promelas*) from four northern-Alberta lakes. The Journal of Parasitology 87:744–748.

Sato, T., T. Egusa, K. Fukushima, T. Oda, N. Ohte, N. Tokuchi, K. Watanabe, M. Kanaiwa, I. Murakami, and K. D. Lafferty. 2012. Nematomorph parasites indirectly alter the food web and ecosystem function of streams through behavioural manipulation of their cricket hosts. Ecology Letters 15:786–793.

Sato, T., K. Watanabe, M. Kanaiwa, Y. Niizuma, Y. Harada, and K. D. Lafferty. 2011. Nematomorph parasites drive energy flow through a riparian ecosystem. Ecology 92:201–207.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Stumbo, A. D., C. T. James, C. P. Goater, and B. D. Wisenden. 2012. Shoaling as an antiparasite defence in minnows (*Pimephales promelas*) exposed to trematode cercariae. Journal of Animal Ecology 81:1319–1326.

Suzán, G., E. Marcé, J. T. Giermakowski, J. N. Mills, G. Ceballos, R. S. Ostfeld, B. Armién, J. M. Pascale, and T. L. Yates. 2009. Experimental evidence for reduced rodent diversity causing increased hantavirus prevalence. PLoS ONE 4. Page numbers? See Wojdak

Thieltges, D. W., M. D. Bordalo, A. Caballero Hernández, K. Prinz, and K. T. Jensen. 2008. Ambient fauna impairs parasite transmission in a marine parasite-host system. Parasitology 135:1111–1116.

Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. C. Collingham, B. F. N. Erasmus, M. Ferreira De Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A. S. van Jaarsveld, G. F. Midgley, L. Miles, M. A. Ortega-Huerta, A. T. Peterson, and O. L. Phillips. 2004. Extinction risk from climate change. Nature

427:145–148.

Valtonen, T. E., E. Helle, and R. Poulin. 2004. Stability of *Corynosoma* populations with fluctuating population densities of the seal definitive host. Parasitology 129:635–642.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth's ecosystems. Science 277. Need page number

Windsor, D. A. 1998. Most of the species on Earth are parasites. International Journal for Parasitology 28:1939–1941.

Wisenden, B. D., J. Y. Martinez-Marquez, E. S. Gracia, and D. C. McEwen. 2012. High intensity and prevalence of two species of trematode metacercariae in the fathead minnow (*Pimephales promelas*) with no compromise of minnow anti-predator competence. The Journal of Parasitology 98:722–727.

Wojdak, J. M., R. M. Edman, J. A. Wyderko, S. A. Zemmer, and L. K. Belden. 2014. Host density and competency determine the effects of host diversity on trematode parasite infection. PLoS ONE 9:14–16.

Wood, C. L., and P. T. J. Johnson. 2015. A world without parasites: Exploring the hidden ecology of infection. Frontiers in Ecology and the Environment 13:425–434.

Wood, C. L., K. D. Lafferty, G. Deleo, H. S. Young, J. Peter, A. M. Kuris, C. L. Wood, K. D. Lafferty, G. Deleo, H. S. Young, P. J. Hudson, and A. M. Kūris. 2014. Does biodiversity protect humans against infectious disease? Ecology 95:817–832.

CHAPTER 2: SPATIO-TEMPORAL VARIATION IN LARVAL TREMATODE POPULATION SIZES IN FATHEAD MINNOWS IN TWO ALBERTA PONDS OVER 15 YEARS

2.1 Abstract

Current hypotheses suggest that natural parasite populations are unregulated over the long-term due to the variability in intrinsic (e.g. host immunity) and extrinsic (e.g. temperature) factors. In this study, I used parasite census data from fathead minnows collected from two lakes in southern Alberta over 15 years to evaluate spatio-temporal variation in parasite prevalences and mean intensities. Twelve parasite species infected these minnows, 5 of which were larval trematodes. Specifically, two *Ornithodiplostomum* (*O. ptychocheilus* and *Ornithodiplostomum* sp.) species were dominant and their mean population sizes varied by up to two orders of magnitude relative to both site and year. Interestingly, the two *Ornithodiplostomum* species had similar population crashes at one site, suggesting that closely-related species are under similar ecological constraints over the long-term. The prevalence and mean intensities of the other trematode species were also highly variable, with no consistent year or site effect. This study also identified an emerging disease caused by a myxozoan that has become established in fathead minnow populations in southern Alberta in 2017. My results highlight the highly context-dependent nature of parasite populations and suggest that parasite populations are inherently unstable and are affected by environmental processes such as changes in host visitation/availability.

2.2 Introduction

Long-term monitoring of animal populations and communities is an essential component of modern ecology. For parasite populations, long-term studies can provide key information regarding changes in rates of transmission from year to year (Kennedy 1987, Heins and Ecke 2012) and rates of colonization of new habitats and hosts (Gendron and Marcogliese 2017). Environmental factors underlying changes in parasite risk within host populations can be identified when long-term survey data is matched with parallel data on habitat characteristics (Heins et al. 2010b). For problem parasites of humans and domestic animals, this information provides the foundation for the design of parasite control strategies (Ouedraogo et al. 2018). However, long-term studies involving parasites and disease of wild animals are rare (Bajer et al. 2014), resulting in a knowledge gap regarding the magnitude, direction, and consistency of parasite transmission rates in natural environments. High spatio-temporal variation of parasite transmission rates in wild animal populations is common (Kennedy 2009, Thieltges et al. 2009, Budischak et al. 2016, Young and MacColl 2017), but the relative importance of intrinsic (host sex, host immunity, intraspecific competition between parasites) or extrinsic (temperature, nutrient availability, humidity/moisture) factors on predicting changes in parasite transmission over time remains vague (Bajer et al. 2014).

High variation in rates of parasite transmission is a consequence of the combination of intrinsic and extrinsic factors. Although the results from a handful of monitoring studies indicate that some parasite population sizes are spatially and temporally stable, Kennedy (2009) concluded that, at least for parasites in aquatic systems, any observed stability in parasite population sizes was not regulatory. Instead, population stability in some systems

could be attributed to habitat stability, especially in anthropogenically-altered habitats (e.g. canalization, impoundments, dams) (Kennedy 2009). In contrast, fluctuations in parasite populations sizes, especially in hosts sampled from relatively pristine aquatic habitats, is often notoriously high (Sandland et al. 2001, Goater et al. 2005, Shaw et al. 2010, Wisenden et al. 2012). One extreme example is the colonization of perch in an English lake by larvae of the trematode *Tylodelphus clavate*, which was associated with the visitation to the lake by a single pair of piscivorous bird that are final hosts (Kennedy and Burrough 1977). Similarly, the results of a 31-year study of larva population sizes of the cestode *Ligula intestinalis* in roach populations (Kennedy et al. 2001) showed that parasite populations were inherently unstable and that any seemingly predictable epizootic cycles were deceptively complex, independent of each other, and reflected environmental changes and not regulatory factors (Kennedy et al. 2001). These long-term studies contribute to the understanding of the causes of temporal epidemiological patterns in natural and anthropogenically-altered habitats.

Most long-term epidemiological studies focus on the population dynamics of one parasite species (Kennedy et al. 2001, Heins et al. 2010b), overlooking the common phenomenon that hosts are co-infected with multiple species. Therefore, many long-term studies searching for patterns that are repeatable over space and time do not consider if parasites within the same host species demonstrate similar population dynamics. This missing link is important, as understanding how multiple parasites within the same host respond to similar environmental factors can help clarify which factors drive rates of parasite transmission. Parasite of freshwater fish provide particularly powerful models to

evaluate variation in transmission due to their ease of collection and high parasitism rates (Kennedy 2009, Young and MacColl 2017).

Ongoing long-term surveys of parasite population sizes in fathead minnows (*Pimephales promelas*) in southern Alberta provides an opportunity to evaluate spatio-temporal patterns of parasite populations of several trematode species of parasite and to assess potential underlying factors. Sandland et al. (2001) describe the dominance of trematode species in fathead minnows, particularly the two congeners, *Ornithodiplostomum ptychocheilus* and *Ornithodiplostomum* sp.. Metacercariae of *O. ptychocheilus* infect the brain of minnows and have been demonstrated to show log-scale site- and year-differences in population sizes (Sandland et al. 2001). However, fathead minnows are always co-infected with several other common trematodes (Sandland 1999, Schleppe and Goater 2004, Stumbo et al. 2012, Wisenden et al. 2012). Long-term changes in the population sizes of *O. ptychocheilus* larvae in minnows are unknown, as are the population sizes of co-occurring larvae of other species.

The purpose of this study is to characterize annual changes in the population sizes of five species of trematode metacercariae in two populations of fathead minnows in southern Alberta over a 15-yr period. The two sites were selected for monitoring based on their similar sizes, depths, and water chemistry. In addition, because both sites are similarly anthropogenically-altered to provide recreational trout-fishing and camping opportunities. The nature of the two contrasting sites allows a test of Kennedy's prediction that parasite populations are intrinsically unstable (Kennedy 2009). Understanding the factors that influence parasite population sizes over the long-term will provide key data that can be used to determine temporal and spatial variation in parasite risk.

16

2.3 Materials and Methods

2.3.1 Study sites

Fathead minnows were collected from two sites in southern Alberta: Goldspring Park Pond (49°05'41.5"N 111°59'28.2"W) and McQuillan Reservoir (49°38'51.7"N 112°27'30.4"W) (Fig. 1). These collections are part of an ongoing survey of fish and their parasites from numerous sites within the region (Goater, unpublished data). We selected these two sites for intensive sampling for their ease of accessibility and because the two sites were representative of other sites in the region where fathead minnows dominate the fish community. Furthermore, the parasite communities were similar to other sites located in southern Alberta where the two species of *Ornithodiplostomum* dominated (Goater, unpublished data).

Goldspring Park Pond is an oxbow that has been physically separated from the adjacent Milk River by an earthen dam and is approximately 100 km southeast of Lethbridge. The riparian area of the pond has been converted into a small campground which is surrounded by non-irrigated crop fields and grassland habitat. The water level in this pond is maintained by a weir that is operated by Park personnel. McQuillan Reservoir is an off-stream impoundment that is part of the St. Mary's irrigation system. This reservoir is approximately 30 km east of Lethbridge. The water levels are maintained using dams and irrigation canals that feed the reservoir. Similar to Goldspring Park Pond, McQuillan Reservoir is adjacent to a recreational campsite and is surrounded by crop fields and a golf course. Both ponds are stocked annually with rainbow trout by the Alberta Conservation Association to support a recreational fishery.

17

Environmental data for the two sites were collected for spring and summer conditions by the Alberta Conservation Association in 2011 and 2016. The two study sites are broadly similar in their physical and chemical characteristics (Table 2.1). In general, McQuillan Reservoir is larger and shallower than Goldspring Pond. The former is also slightly more productive, based upon its consistently higher phosphorous and chlorophyll-α concentrations in spring and summer (Table 2.1).

2.3.2 Host collection

Fathead minnows have been collected once each year from the two sites since 2005. In most years, collections occurred to facilitate experimental studies in the Goater laboratory that required a source of minnows that were heavily infected with both species of *Ornithodiplostomum* (eg. James et al. 2008, Stumbo et al. 2012b). For those studies, infected fish were required, but enumeration of trematode metacercariae in individual hosts was unnecessary. Thus, there was no formal monitoring program until a crash in *Ornithodiplostomum* species was observed in McQuillan Reservoir in 2016. This crash prompted a decision to conduct consistent annual surveys at the two sites. This sequence of anecdotal observations explains the intermittent enumeration that occurred at the two sites between 2005-2015.

Minnows collected for parasite enumeration were sampled in 2005 and 2008 and then annually between 2016-2019. All collections were restricted to 2-yr old adult, sexually-mature males during their breeding seasons between early May and late June each year. Male minnows were considered sexually mature if they possessed tubercles on the snout, a secondary sexual characteristic exclusive to sexually mature males (Danylchuk and Tonn 2001). Minnows were collected using un-baited Gee traps placed haphazardly around

18

the perimeter of the two ponds using procedures for minnow collection that are detailed in Sandland et al. (2001). Up to 10 traps were set 1-2 meters from the shore, at approximately 50cm in depth for 4-8 hours. If mature males were found in more than one trap, individuals were haphazardly selected from each trap. Sample sizes of minnows varied between years (n = 24-60) but were consistent between the two sites.

2.3.3 Host and parasite characteristics

Fathead minnows were necropsied within 48 hours of collection following standard laboratory protocols with the aim to fully-census each parasite population in each minnow (Sandland et al. 2001). In the laboratory, fish were euthanized by concussion, followed by cerebral dislocation. Following the death of the host, the skin and musculature was evaluated for the encysted stages of trematode metacercariae. Next, the brain and viscera were removed, and the tissue was pressed between two glass slides and then observed under a dissecting microscope. This procedure enabled the identification and enumeration of trematode metacercariae within each tissue. The intact eyes of each fish were also dissected for the larval stages of the trematode, *Diplostomum* sp. If no *Diplostomum* sp. were observed in the eyes of the first 5 fish that were dissected at each site, I assumed the species was absent in that sample and did not dissect the eyes of additional fish.

Fathead minnows from both sites were infected with myxozoans. Various stages were observed in the brains, gill filaments, and viscera. When large pseudocysts were observed, they were teased out, placed on a microscope slide, and observed under a compound microscope. Since myxozoan spores reproduce asexually within their site-specific host tissues, enumeration was not possible. Starting in 2017, I observed white, irregular, swellings in the spleens of samples of minnows from both sites. Follow-up and

19

ongoing studies have indicated that this is a new species of myxozoan that is now common in all individuals within all sites that have been sampled in the region (Tilley et al., unpublished observations). For this and other species of myxozoans in samples of fatheads, infection in individual hosts was characterized as presence or absence.

Fork length of each individual fish was measured to the nearest 0.01mm using digital calipers and defined as the length between the tip of the snout and split in the caudal fin. Wet weight was calculated to the nearest 0.01g using a digital scale.

2.3.4 Data analyses

A total of 386 minnows was collected from the two sites between 2005-2019. For formal analyses, only the 276 minnows from the annual collections that occurred between 2016-2019 were used to evaluate year-to-year changes in parasite intensities and prevalances. I used generalized linear models (GLMs) to analyze for annual and between-pond differences in mean metacercariae intensity and prevalence (Bush et al. 1997; Young and MacColl 2017). The mean intensities and prevalence of five trematode species were tested with models that included year (continuous factor) and site (categorical factor), as well as their interaction, with fork length as a covariate:

$$y \sim Year + Site + Year \times Site + Fork\ Length$$

where *y* stands for mean intensity or prevalence of the five trematode species. The GLMs for mean intensities were modelled using either a Poisson or a quasi-Poisson error distribution (Ver Hoef and Boveng 2007) with a log link function, dependent on the residuals by species. GLMs testing prevalence was modelled using a binomial error distribution with a logit link function. Following the methods described in Young and

MacColl (2017), a deletion approach was used in which full models were initially conducted, with non-significant factors being removed in sequential models. *P*-values were adjusted using a Bonferroni correction for multiple comparisons.

2.4 Results

2.4.1 Parasite assemblages in fathead minnows

Fathead minnows collected from the two sites were infected with 12 species of parasite, 5 of which were larval trematodes (Table 2.2). *Ornithodiplostomum ptychocheilus* metacercariae were found encysted in the brain of fathead minnows, while the congener, *Ornithodiplostomum* sp. was found encysted in the liver along with encysted *Posthodiplostomum minimum* metacercariae*. Crassiphialia bulboglossa* was identified by melanisation visible on the host epidermis, a consequence of their encystment within the musculature. Larvae of *Diplostomum* sp. were unencysted within the lens of eye. The overall parasite assemblages were dominated by larval stages of *Ornithodiplostomum ptychocheilus* and *Ornithodiplostomum* sp., each of which was present in greater than 85% of the 386 minnows examined. The prevalences of the other trematodes tended to be lower or their occurrence was pond-specific. For the larval trematodes, overall patterns of intensity were strongly bimodal. Whereas the two species of *Ornithodiplostomum* often exceeded intensities of >100 larvae/host, mean intensities in the other 3 trematodes rarely exceeded 5 larvae/host (Table 2.2).

The prevalence of larvae of the nematode *Philometra* sp. was moderately high (21.7%), particularly in minnows sampled from McQuillan Reservoir (Table 2.2). Of the 137 fish that had their nostrils evaluated for adult stages of the monogenean *Dactylogyrus*

olfactorius, 27% were infected. Larvae of the cestode *Ligula intestinalis* and the acanthocephalan *Pomphorhynchu* sp. occurred sporadically, never exceeding 10% in either pond. Of the 3 myxozoans recovered, only *Myxobolous* sp. 1 was common (Table 2.2). This species was not observed in fathead minnows until 2017, with a dramatic increase in prevalence approaching 100% in adult males from both sites in 2018 and 2019.

2.4.2 Variation in larval trematode intensities and prevalences

The prevalence of *O. ptychocheilus* remained consistently high throughout all sampling years for both sites, only dropping slightly below 100% in 2016 at McQuillan Reservoir (Figure 2.1). The prevalence of *O. ptychocheilus* was not affected by year, site, or their interaction (Table 2.3). However, GLM analyses indicated that year, site, and their interaction had highly significant effects on mean *O. ptychocheilus* intensity (Table 2.3). For samples from Goldspring, mean intensities were roughly consistent at 100-150 metacercariae/host between 2016-2017 and these values were similar to those observed in the 2005 and 2008 samples (Fig. 2.1). However, mean intensity at this site approximately doubled in 2018 to over 300 metacercariae/host, after which there was a sharp decline towards the pre-2019 mean intensities. Annual variation in mean *O. ptychocheilus* intensities in McQuillan Reservoir differed markedly from those in Goldspring Pond (Fig. 2.1). Whereas mean intensities in 2005 and 2008 in McQuillan Reservoir were consistently high and similar to those in Goldspring Pond, there was a precipitous decline that started in the 2016 samples. This decline coincided with the one year that prevalence was not 100% (Fig. 2.1). Following the 2016 decrease in McQuillan Reservoir, mean intensity remained approximately unchanged at < 20 metacercariae/host.

Ornithodiplostomum sp. had similarly high prevalence at both sites and was the second most common parasite species found in fathead minnows (Table 2.2). Unlike *O. ptychocheilus*, the prevalence of *Ornithodiplostomum* sp. was affected by year (Table 2.3), with prevalence increasing after the sharp declines observed in both ponds (Fig. 2.2; 2017 for Goldspring Park Pond, 2016 for McQuillan Reservoir). Like *O. ptychocheilus*, the intensity of *Ornithodiplostomum* sp. was similar between the two sites in 2005/2008, yet between 2016-2019, mean intensities were erratic (Fig. 2.2) resulting in significant differences between the two sites (Table 2.3). Minnows in Goldspring Park Pond had an exponential increase in mean intensity between 2016-2019 with an approximate 8x increased in metacercariae intensity relative to the 2005/2008 intensities (Fig. 2.2). Minnows in McQuillan Reservoir did not show this dramatic increase but instead had a consistently low intensity between 1-6 metacercariae/host between 2016-2019 that has shown no sign of recovery (Fig. 2.2). Due to the dramatic year-to-year differences in Goldspring Park Pond, both year and the interaction between year and site were highly significant (Table 2.3).

The prevalence of *P. minimum* was relatively stable and remained low, never surpassing 40% at either site (Fig. 2.3). Due to the stability observed at both sites as well as throughout 2016-2019, *P. minimum* prevalence was not predicted by site, year, or their interaction (Table 2.3). However, mean *P. minimum* intensities were highly erratic between years in both ponds. Initially, minnows from Goldspring Park Pond in 2005 had the highest intensity at approximately 11 metacercariae/host, followed by a decline in 2008 (Fig. 2.3) that continued to a collapse in 2017 in which both prevalence and intensity was zero. Following the collapse, there was a partial recovery back to a mean intensity of 3

metacercariae/host in 2019. McQuillan Reservoir displayed stable intensities of *P. minimum* around 1-2 metacercariae/host throughout all collection years. However, like Goldspring Park Pond, a collapse in the parasite population occurred in 2016, with a recovery in mean intensity to 1-2 metacercariae/host (Fig. 2.3). Between 2016-2019, variation in mean intensity of *P. minimum* was only attributed to changes in year with neither site nor it's interaction with year being significant (Table 2.3).

Variation in prevalence of *C. bulboglossa* was attributed to both site and year as well as their interaction (Table 2.3). *Crassiphiala bulboglossa* metacercariae were not present in Goldspring Park Pond until 2018, following which both prevalence and intensities remained low. Metacercercariae of this species were observed in McQuillan Reservoir since 2005, after which patterns of prevalence and intensity were highly erratic (Fig. 2.4).

Diplostomum spp. was rare in Goldspring Pond and intensities were low (< 2 larvae/host (Table 2.2) but it was consistently found in fish from McQuillan Reservoir. (Fig. 5). Mean larval *Diplostomum* sp. intensities peaked at 16 metacercariae/host in 2017 but spatial and annual changes in mean intensity were not significant (Table 2.3).

Fork length was inconsistent in predicting prevalence or intensity of trematode metacercariae (Table 2.3). Fork length was positively associated with the intensities of the two *Ornithodiplostomum* species and this association was not significant for any other trematode.

2.5 Discussion

My results showed that mean metacercariae intensities in fathead minnows were highly spatially and temporally variable. Furthermore, the parasites that were most common and abundant in fathead minnows (the two species of *Ornithodiplostomum*), demonstrated dramatic changes in mean intensity between samples, sometimes exceeding two orders of magnitude relative to both year and site. Less common and abundant trematodes such as *P. minimum*, *Diplostomum* sp., and *C. bulboglossa* did not exhibit as dramatic changes in prevalence or population size. These results are consistent with those from other studies where prevalence and intensity of metacercariae are highly variable in individual freshwater fish and also between samples of fish (Kennedy et al. 2001, Heins et al. 2010a).

This high level of observed variation in mean metacercariae intensities could be attributed to annual, spatial, and species-specific factors. In general, year was the most common factor explaining the observed variation in metacercariae intensity and prevalence, being a significant factor in 6 out of the 10 models (based on the reduction method, only 6 models incorporated year). Furthermore, the changes in year-to-year intensities and prevalences were highly variable among parasite species. In general, these results support the hypothesis that parasite populations are unregulated over the long-term (Kennedy 2009). For example, the prevalence of *O. ptychocheilus* was near 100% for the duration of the study and only slightly decreased when intensity collapsed in 2016 at McQuillan Reservoir. Furthermore, even with continuously low intensities in McQuillan Reservoir following the collapse, prevalence rapidly recovered to 100%. This pattern suggests that well-established parasites may continue to exhibit consistent prevalence over long time-scales, whereas mean intensity fluctuates markedly. Prevalence and intensities of O*rnithodiplostomum* sp. demonstrated a similar pattern. In this case, high prevalence was

maintained throughout the study period, despite the collapse in mean intensity to near zero in 2016 at McQuillan Reservoir.

The structure of the overall data set meant that site differences in mean intensity or prevalence could only be assessed for four of the five species – *O. ptychocheilus*, *Ornithodiplostomum* sp., *P. minimum*, and *C. bulboglossa*. In these cases, the differences between sites explained by far, most of the variation. The strong site differences for the *Ornithodiplostomum* species can likely be attributed to site-specific differences following the collapses in their respective metacercariae populations. This contention is supported by the significant Site × Year interactions for both species. In the case of Goldspring Park Pond, there was a significant and exponential recovery in metacercariae populations following the collapse of both *Ornithodiplostomum* spp. in 2017. However, for McQuillan Reservoir, there was no recovery and the population sizes have remained low since the crash. Similar observations have been made by other researchers who have reported on long-term spatial-temporal changes in larval helminth populations. For example, epizootics of larval cestodes (*Schistocephalus solidus*) that parasitize stickleback were independent between two lakes that were 140 air km apart (Heins et al. 2011). Likewise, the difference in intensity between lakes was almost an order of magnitude. This pattern of variation between lakes is similar to the extreme variation I observed for both *Ornithodiplostomum* species between McQuillan Reservoir and Goldspring Park Pond which are approximately 80 air km apart.

Between-site differences in mean intensities were not significant for the three other less common trematodes, suggesting that spatial effects manifest differently relative to species. Interestingly, temporal patterns of the two *Ornithodiplostomum* species appeared

to parallel each other within sites, indicating that closely related species (both *Ornithodiplostomum* sp. use the same hosts at all developmental and adult stages) may be under similar intrinsic and extrinsic constraints – and subsequently exhibit similar patterns of transmission within individual sites. Taken together, my results indicate that variation in metacercariae intensities in minnows is year, site, and species-specific. The context-dependent nature of variation in mean metacercariae intensities among samples of hosts has been well documented in other fish/trematode interactions (Sandland et al. 2001, Thieltges et al. 2009, Shaw et al. 2010).

The current study was sparked by anecdotal observations regarding the marked decline in intensity of the two species of *Ornithodiplostomum* that occurred at McQuillan Reservoir in 2016. The relatively high and consistent intensities of *Ornithodiplostomum* in this lake, and its close proximity to our Parasite Ecology Laboratory at the University of Lethbridge has provided a convenient source of metacercariae to support more than 20 years of experimental work (e.g. Sandland and Goater 2001, Shirakashi and Goater 2002, James et al. 2008, Stumbo et al. 2012). Indeed, had the current study been completed in approximately 2015, a contrasting conclusion of population-level stability would have been warranted for both species of *Ornithodiplostomum*, in both lakes. Furthermore, the introduction of *C. bulboglossa* into Goldspring Park Pond would not have been recognized unless the survey was continued consistently through 2018. The introduction of a new parasite provides researchers with the opportunity to track how a newly introduced parasite might exhibit population dynamics relative to a site that has an established population, (e.g. McQuillan Reservoir has had *C. bulboglossa* in the lake since surveys started in 2005).

27

These results emphasize the importance of long-term monitoring of spatio-temporal patterns of infection in free-ranging hosts (Kennedy 2009).

Similar to the results of this current study, the high spatio-temporal variation of aquatic parasite populations have been attributed to the environmental context such as local site influences (Heins and Ecke 2012) or the availability of final and intermediate hosts (Kennedy et al. 2001). For example, Kennedy and Burrough (1977) observed that the colonization of a parasite population into fish in a lake in the United Kingdom, and their rate of transmission, was related to the visitation and water-use by waterfowl. We did not assess visitation rates of great blue herons, *Ardea herodias,* the final host of the two most common parasites (*Ornithodiplostomum* species) but observed them consistently each year at both sites. Furthermore, the two sites are relatively close geographically, and are similar in both water chemistry and human recreation use. Therefore, other factors are likely to be causing the extreme spatio-temporal variation that I observed in each of the metacercariae populations. One anecdotal observation made during the survey was the rapid introduction in 2016, followed by the apparent decline, of an invasive snail (*Radix auricularia*) into McQuillan Reservoir. This introduction coincided approximately with the collapse in the population sizes of both *Ornithodiplostomum* species in the reservoir. The addition of snail hosts into naïve snail communities has been demonstrated to alter parasite transmission rates of aquatic trematodes (Johnson et al. 2009). Experimental tests under laboratory conditions are required to assess the role of introduced *Radix* on overall patterns of transmission. One intriguing possibility is that the introduction of a susceptible host led to a decline in the transmission of *Ornithodiplostomum* sp. miracidia into *Physa* snails via dilution. Another is that interspecific interactions between *Physa* and *Radix* led to a decline

in population densities of the former, ultimately leading to a decline in transmission rates (Johnson and Thieltges 2010).

The purpose of this study was to investigate the long-term changes in metacercariae population sizes. My results support the contention that trematode parasite population sizes are highly variable over time and space. Throughout the 15-year study, there were dramatic changes in metacercariae population sizes in fathead minnows. Specifically, for the two most common parasites (the two *Ornithodiplostomum* species), changes in mean metacercariae intensity over the study was an order of magnitude higher in some years relative to others. Furthermore, the less common parasite species, even if not consistent, appeared to show year-to-year variation, especially in prevalence. The spatial effects of variation on parasite intensity were only noticeable for the two *Ornithodiplostomum* species which were established in both sites since the start of the study, but not for the other trematodes. Moreover, even though there were site differences in the intensities of *Ornithodiplostomum* species over time, the two closely-related species exhibited parallel changes in population sizes over time. Although multiple intrinsic and extrinsic factors likely contributed to this extreme variation, my results support the idea that closely-related species may be under similar constraints, and subsequently exhibit similar population dynamics. My results suggest that trematode metacercariae exhibit unstable population patterns over the long-term, as suggested earlier by Kennedy (2009), and emphasizes the context-dependent nature of parasite population dynamics.

2.6 Literature cited

Bajer, A., R. Welc-Faleciak, M. Bednarska, M. Alsarraf, J. Behnke-Borowczyk, E. Siński, and J. M. Behnke. 2014. Long-term spatiotemporal stability and dynamic changes in the haemoparasite community of bank voles (*Myodes glareolus*) in NE Poland. Microbial Ecology 68:196–211.

Budischak, S. A., E. P. Hoberg, A. Abrams, A. E. Jolles, and V. O. Ezenwa. 2016. Experimental insight into the process of parasite community assembly. Journal of Animal Ecology 85:1222–1233.

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

Danylchuk, A. J., and W. M. Tonn. 2001. Effects of social structure on reproductive activity in male fathead minnows (*Pimephales promelas*). Behavioral Ecology 12:482–489.

Gendron, A. D., and D. J. Marcogliese. 2017. Enigmatic decline of a common fish parasite (*Diplostomum* spp.) in the St. Lawrence River: Evidence for a dilution effect induced by the invasive round goby. International Journal for Parasitology: Parasites and Wildlife:1–10.

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

Heins, D. C., J. A. Baker, and D. M. Green. 2011. Processes influencing the duration and decline of epizootics in *Schistocephalus solidus*. Journal of Parasitology 97:371–376.

Heins, D. C., J. A. Baker, M. A. Toups, and E. L. Birden. 2010a. Evolutionary significance of fecundity reduction in threespine stickleback infected by the diphyllobothriidean cestode *Schistocephalus solidus*. Biological Journal of the Linnean Society 100:835–846.

Heins, D. C., E. L. Birden, and J. A. Baker. 2010b. Host mortality and variability in epizootics of *Schistocephalus solidus* infecting the threespine stickleback, *Gasterosteus aculeatus*. Parasitology 137:1681–1686.

Heins, D. C., and J. K. Ecke. 2012. The rise and fall of an epizootic of the diphyllobothriidean cestode *Schistocephalus pungitii* infecting the ninespine stickleback. Journal of Parasitology 98:1–5.

Ver Hoef, J. M., and P. L. Boveng. 2007. Quasi-poisson vs. negative binomial regression: How should we model overdispersed count data? Ecology 88:2766-2772.

James, C. T., K. J. Noyes, A. D. Stumbo, B. D. Wisenden, and C. P. Goater. 2008. Cost of exposure to trematode cercariae and learned recognition and avoidance of parasitism risk by fathead minnows Pimephales promelas. Journal of Fish Biology 73:2238–2248.

Johnson, P. T. J., P. J. Lund, R. B. Hartson, and T. P. Yoshino. 2009. Community diversity reduces *Schistosoma mansoni* transmission, host pathology and human infection risk. Proceedings. Biological sciences / The Royal Society 276:1657–1663.

Johnson, P. T. J., D. L. Preston, J. T. Hoverman, and K. L. D. Richgels. 2013. Biodiversity decreases disease through predictable changes in host community competence. Nature 494:230–233.

Johnson, P. T. J., and D. W. Thieltges. 2010. Diversity, decoys and the dilution effect: How ecological communities affect disease risk. The Journal of Experimental Biology 213:961–970.

Kennedy, C. R. 1987. Long-term stability in the population levels of the eyefluke *Tylodelphys podicipina* (Digenea: Diplostomatidae) in perch. Journal of Fish Biology 31:571–581.

Kennedy, C. R. 2009. The ecology of parasites of freshwater fishes: The search for patterns. Parasitology 136:1653–1662.

Kennedy, C. R., and R. Burrough. 1977. The population biology of two species of eyefluke, *Diplostomum gasterostei* and *Tylodelphys clavata*, in perch. Journal of Fish Biology 11:619–633.

Kennedy, C. R., P. C. Shears, and J. A. Shears. 2001. Long-term dynamics of *Ligula intestinalis* and roach *Rutilus rutilus*: A study of three epizootic cycles over thirty-one years. Parasitology 123:257–269.

Ouedraogo, B., Y. Inoue, A. Kambiré, K. Sallah, S. Dieng, R. Tine, T. Rouamba, V. Herbreteau, Y. Sawadogo, L. S. L. W. Ouedraogo, P. Yaka, E. K. Ouedraogo, J. C. Dufour, and J. Gaudart. 2018. Spatio-temporal dynamic of malaria in Ouagadougou, Burkina Faso, 2011-2015. Malaria Journal 17:1–12.

Pila, E. A., H. Li, J. R. Hambrook, X. Wu, and P. C. Hanington. 2017. Schistosomiasis from a snail's perspective: Advances in snail immunity. Trends in Parasitology 33:845–857.

Sandland, G. J. 1999. Interactions between a brain-encysting trematode and its intermediate host, the fathead minnow. University of Lethbridge.

Sandland, G. J., and C. P. Goater. 2001. Parasite-induced variation in host morphology: brain-encysting trematodes in fathead minnows. Journal of Parasitology 87:267–272.

Sandland, G. J., C. P. Goater, and A. J. Danylchuk. 2001. Population dynamics of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows (*Pimephales promelas*) from four Northern-Alberta lakes. Journal of Parasitology 87:744–748.

Schleppe, J. L., and C. P. Goater. 2004. Comparative life histories of two diplostomid trematodes, *Ornithodiplostomum ptychocheilus* and *Posthodiplostomum minimum*. Journal of Parasitology 90:1387-1390.

Shaw, J. C., R. F. Hechinger, K. D. Lafferty, and A. M. Kuris. 2010. Ecology of the brain trematode *Euhaplorchis californiensis* and its host, the California killifish (*Fundulus*

parvipinnis). Journal of Parasitology 96:482–490.

Shirakashi, S., and C. P. Goater. 2002. Intensity-dependent alteration of minnow (*Pimephales promelas*) behavior by a brain-encysting trematode. Journal of Parasitology 88:1071–1074.

Stumbo, A. D., C. T. James, C. P. Goater, and B. D. Wisenden. 2012. Shoaling as an antiparasite defence in minnows (*Pimephales promelas*) exposed to trematode cercariae. Journal of Animal Ecology 81:1319–1326.

Thieltges, D. W., B. L. Fredensborg, and R. Poulin. 2009. Geographical variation in metacercarial infection levels in marine invertebrate hosts: Parasite species character versus local factors. Marine Biology 156:983–990.

Wisenden, B. D., J. Y. Martinez-Marquez, E. S. Gracia, and D. C. McEwen. 2012. High intensity and prevalence of two species of trematode metacercariae in the fathead minnow (*Pimephales promelas*) with no compromise of minnow anti-predator competence. Journal of Parasitology 98:722–727.

Young, R. E., and A. D. C. MacColl. 2017. Spatial and temporal variation in macroparasite communities of three-spined stickleback. Parasitology 144:436–449.

Table 2.1. Physical and chemical characteristics of Goldspring Park Pond and McQuillan Reservoir. Water chemistry data were collected May 31, 2011 (spring) whereas temperature and pH data were collected June 2-7, 2016. Summer water temperature, pH, and water chemistry data were collected July 12, 2011. Data collected and provided by the Alberta Conservation Association.

Site	Area (ha)	Mean Depth (m)	Season (date)	Surface temperature (°C)	Surface pH	Surface DO (mg/L)	TP (mg/L)	TKN (mg/L)	Chl$_\alpha$ (µg/L)	Turbidity (NTU)
Goldspring park pond	6.4	3.1	Spring	15.9	9.1	10.5	0.02	0.9	NA	4.1
			Summer	21.2	8.6	10.4	0.02	0.9	2.2	1.8
McQuillan Reservoir	9.3	2.5	Spring	19.1	9.3	8.7	0.08	1.9	NA	4.9
			Summer	20.3	8	6.9	0.05	1.9	9.4	5.6

Table 2.2. Parasite assemblages and summary infection characteristics for samples of adult fathead minnows collected from two ponds in southern Alberta, Canada between 2005-2019. Not all collected minnows were evaluated for *Dactylogyrus olfactorius* or *Diplostomum* spp. Sample sizes are indicated in parentheses. The presence of Myxozoans was not evaluated until 2017 (sample size in parentheses). Asterisk (*) indicate unconfirmed site of host tissue.

Parasite	Host tissue	Goldspring Park Pond (n=197)		McQuillan Reservoir (n=189)	
		Mean intensity ± s.d.	Prevalence (%)	Mean intensity ± s.d.	Prevalence (%)
Trematoda					
Ornithodiplostomum ptychocheilus	Brain	202.1 ± 105.4	100	65.2 ± 76.9	97.9
Ornithodiplostomum sp.	Viscera	56.0 ± 78.4	93.4	18.9 ± 28.5	77.2
Posthodiplostomum minimum	Viscera	2.8 ± 6.0	21.3	1.1 ± 0.4	15.3
Crassiphiala bulboglossa	Musculature		10.7		46.5
Diplostomum sp.	Lens	1.2 ± 0.4 (n = 108)	12.0	7.6 ± 7.5	85.2
Monogenea					
Dactylogyrus olfactorius	Nostril	2.4 ± 1.4 (n = 74)	29.7	2.1 ± 1.2 (n = 63)	23.9
Nematoda					
Philometra sp.	Intestine	1.3 ± 0.5	6.6	1.6 ± 0.7	21.7
Cestoda					
Ligula intestinalis	Body cavity	1 ± 0	0.5	1 ± 0	7.9
Acanthocepha					
Pomphorhynchu spp.	Intestine	0	0	1 ± 0	2.6
Myxozoa					
Myxobolus hendricksoni	Brain	-	0.90 (n = 111)	-	5.4 (n = 112)
Myxobolus spp. 1	Spleen and head*	-	58.6 (n = 111)	-	68.5 (n = 112)
Myxobolus spp. 2	Visceral mesentery*	-	7.2 (n = 111)	-	8.9 (n = 112)

Table 2.3. Summary of associations between the intensity and prevalence of parasite species by site, year, site*year interaction, and fork length of minnow using GLM analysis. Sample sizes are denoted in the parentheses. A reduction method was used in which non-significant factors were removed from subsequent models to increase model fit, denoted by a dash (-). Analysis for *Diplostomum* spp. did not test site differences (and subsequently no site × year interaction was tested) since prevalence in Goldspring Park Pond was under 10% to avoid zero inflation, as such, these cells are denoted as blanks.

	Site		Year		Site * Year		Fork Length	
	Estimate ± S.E.	P	Estimate ± S.E.	P	Estimate ± S.E.	P	Estimate ± S.E.	P
O. ptychocheilus intensity (n = 382)	627.2 ± 33.7	*	0.31 ± 0.0055	*	-0.31 ± 0.017	*	0.030 ± 0.0013	*
O. ptychocheilus prevalence	-	-	-	-	-	-	-	-
Ornithodiplostomum spp. intensity (n = 330)	2574 ± 121.1	*	1.38 ± 0.019	*	-1.00 ± 0.053	*	0.050 ± 0.0029	*
Ornithodiplostomum spp. prevalence	-	-	1.29 ± 0.023	*	-	-	0.14 ± 0.03	*
P. minimum intensity (n = 71)	-	-	0.19 ± 0.071	*	-	-	-	-
P. minimum prevalence	-	-	-	-	-	-	-	-
C. bulboglossa intensity (n = 108)	-	-	-	-	-	-	-	-
C. bulboglossa prevalence	1900.81 ± 768.8	*	1.20 ± 0.34	*	-0.94 ± 0.38	*	-	-
Diplostomum spp. intensity (n = 161)			-				-	-
Diplostomum spp. prevalence			1.29 ± 0.23	*			0.15 ± 0.030	*

Note: (*) indicates significance relative to the Bonferroni correction

Figure 2.1. Year-to-year variation in intensity and prevalence of *Ornithodiplostomum ptychocheilus* metacercariae in the brain of fathead minnows in Goldspring Park Pond and McQuillan Reservoir. Bars indicate mean intensity (± s.e.) denoted by the left axis, and the line indicates prevalence denoted by the right axis. Dashed lines indicate non-continuous years.
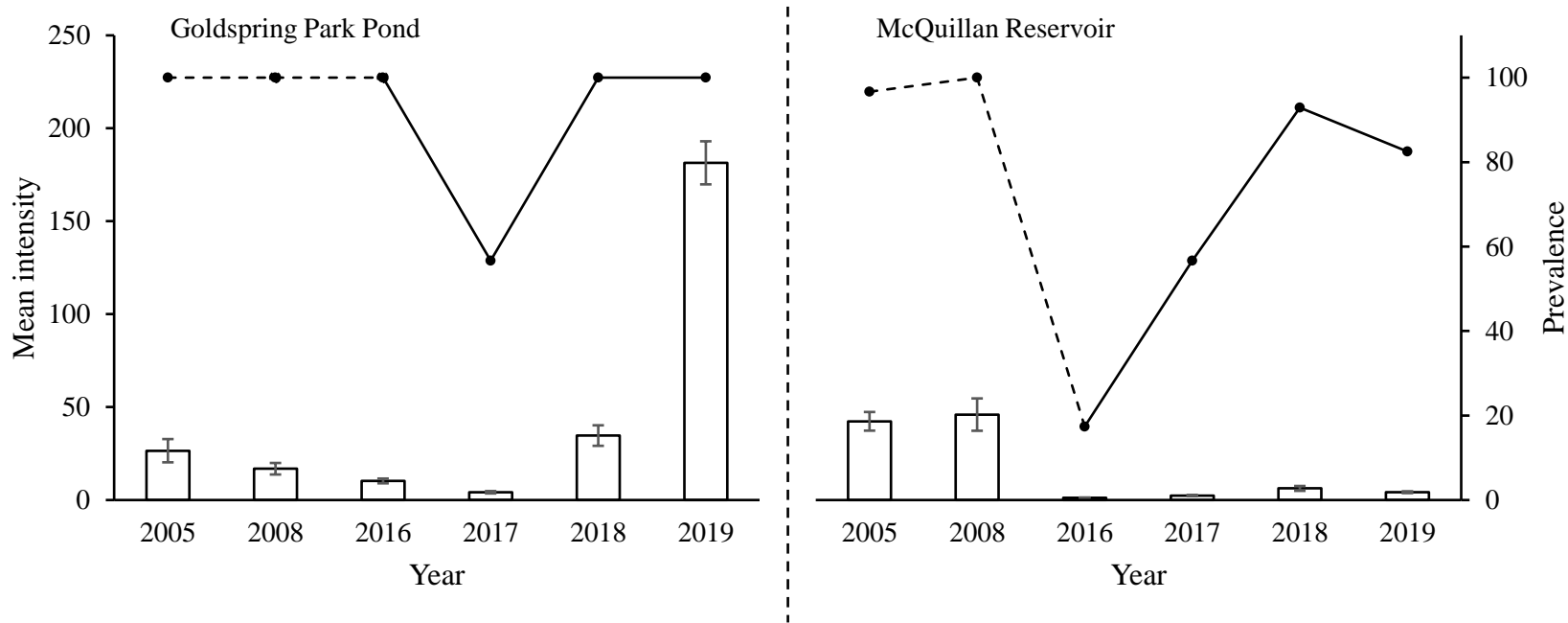
Figure 2.2. Year-to-year variation in intensity and prevalence of *Ornithodiplostomum* spp. metacercariae in the viscera of fathead minnows in Goldspring Park Pond and McQuillan Reservoir. Bars indicate mean intensity (± s.e.) denoted by the left axis, and the line indicates prevalence denoted by the right axis. Dashed lines indicate non-continuous years.
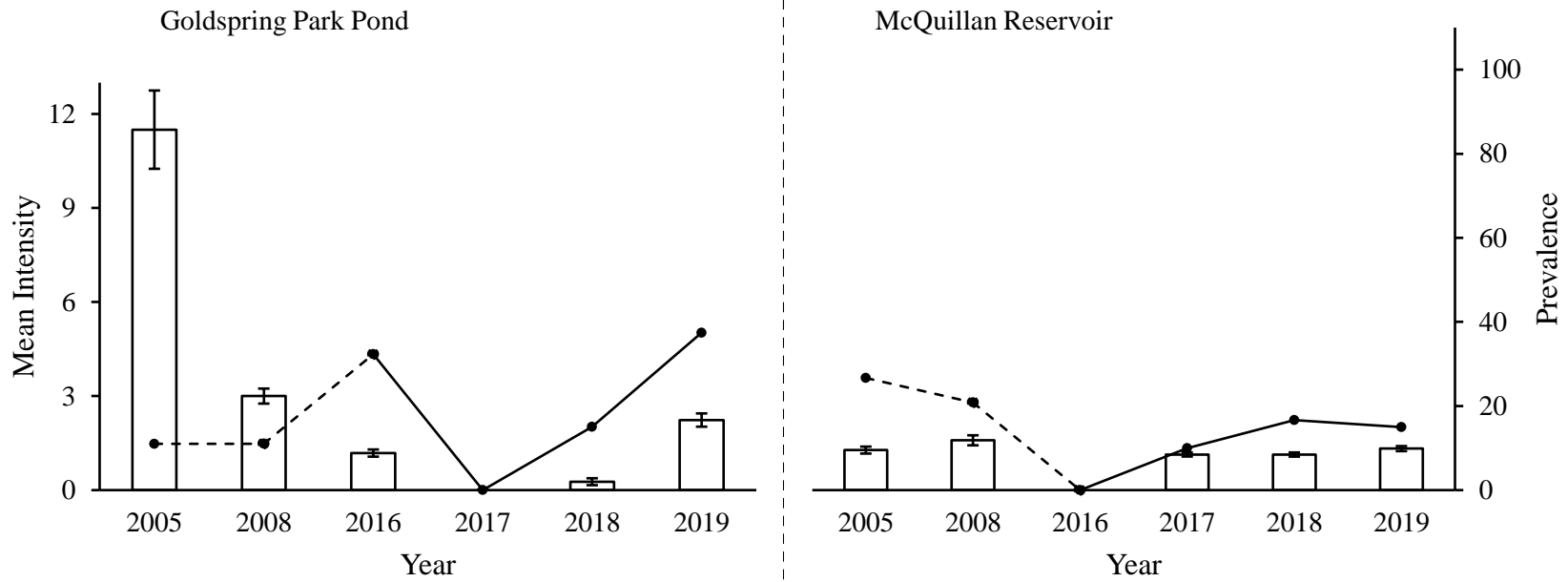
Figure 2.3. Year-to-year variation in intensity and prevalence of *Posthodiplostomum minimum* metacercariae in the viscera of fathead minnows in Goldspring Park Pond and McQuillan Reservoir. Bars indicate mean intensity (± s.e.) denoted by the left axis, and the line indicates prevalence denoted by the right axis. Dashed lines indicate non-continuous years.
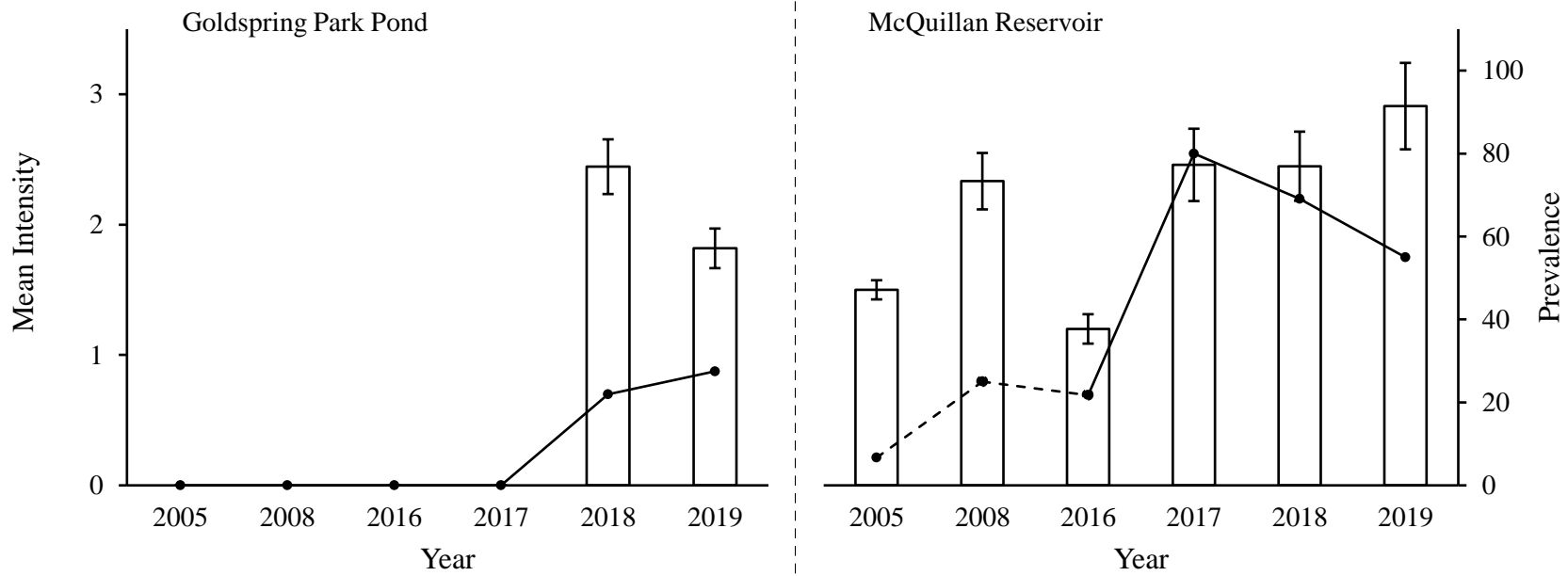
Figure 2.4. Year-to-year variation in intensity and prevalence of *Crassiphiala bulboglossa* metacercariae in the musculature of fathead minnows in Goldspring Park Pond and McQuillan Reservoir. Bars indicate mean intensity (± s.e.) denoted by the left axis, and the line indicates prevalence denoted by the right axis. Dashed lines indicate non-continuous years.
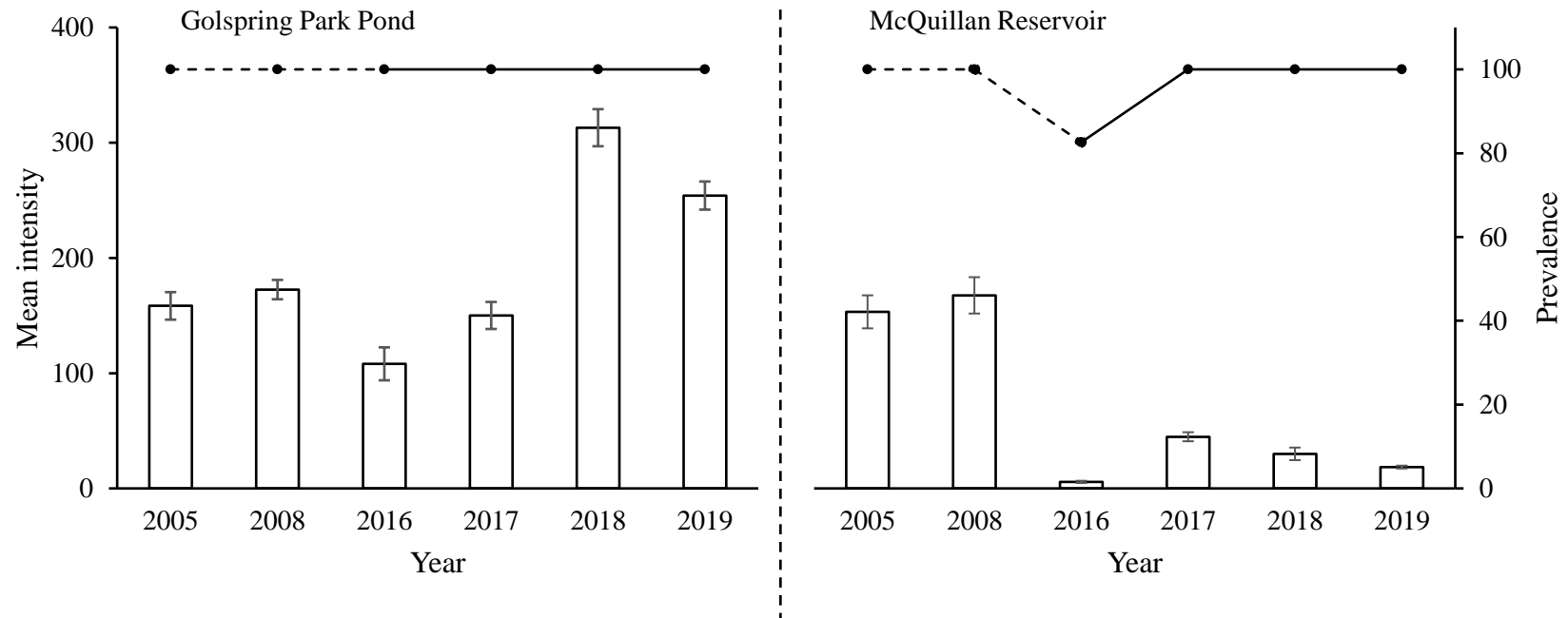
Figure 2.5. Year-to-year variation in intensity and prevalence of *Diplostomum* spp. metacercariae in the lens of fathead minnows in Goldspring Park Pond and McQuillan Reservoir. Bars indicate mean intensity (± s.e.) denoted by the left axis, and the line indicates prevalence denoted by the right axis. Dashed lines indicate non-continuous years.
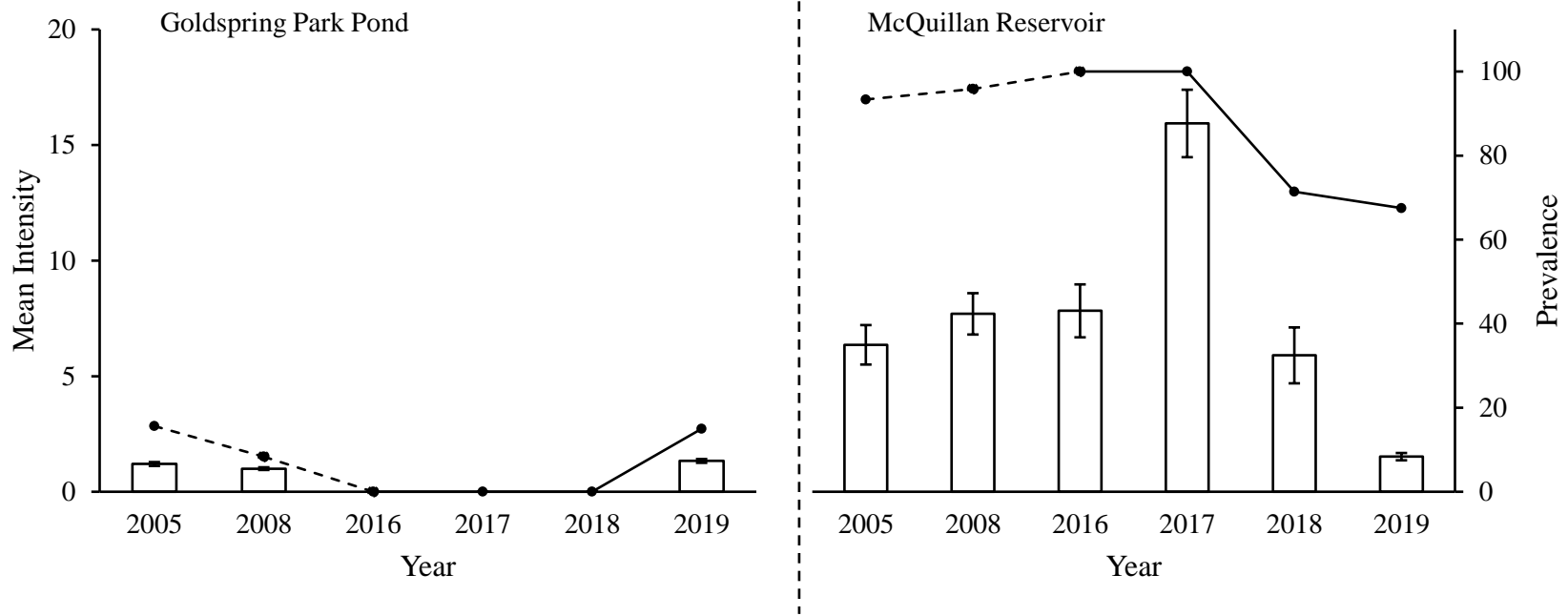
CHAPTER 3: EXPERIMENTAL TESTS OF THE HOST BIODIVERSTY-DISEASE RELATIOSHIP IN A FISH/TREMATODE SYSTEM

3.1 Abstract

Ecologists have proposed that host community structure can mediate rates of parasite transmission. An extension of this idea is that rates of transmission decline within diverse host communities via a dilution effect. But there is limited support for the dilution effect hypothesis because few systems are amenable to manipulation of both host diversity and rates of exposure. I tested for a dilution effect by exposing fathead minnows to larvae of the trematode *Ornithodiplostomum ptychocheilus* in outdoor mesocosms and in indoor aquaria. The containers held minnows alone or with 1-3 other species of fish, each of which varied in their susceptibility to *O. ptychocheilus* larvae. Trematode larvae were added to containers and minnows were assessed for metacercariae intensity 10 days later. When minnows co-occurred with emerald shiners in mesocosms and in aquaria, there was a 52% and 21% decrease, respectively, in metacercariae intensity in minnows. Co-occurrence with brook sticklebacks and longnose dace had no effect on intensity in minnows relative to monospecies treatments. These results indicate that the significant dilution effect that I observed was independent of host diversity, but strongly affected by host identity. Due to the incompatibility of emerald shiners to *O. ptychocheilus* larvae, the reduction in transmission rates is best explained by shiner-induced changes in minnow behaviour, micro-habitat selection, or activity that reduce encounter rates between parasite larvae and minnows. My results that highlight the importance of host identity over host diversity within multi-host communities is particularly relevant to our understanding of transmission processes in host communities that contain introduced species.

3.2 Introduction

One consequence of biodiversity loss is described by the dilution effect hypothesis (DEH) (Keesing *et al.* 2006). This hypothesis predicts a negative relationship between host biodiversity and disease risk. The hypothesis proposes that the decrease in disease risk results from alterations to the relative densities of host species in a community, particularly those that are susceptible to infective stages of a parasite (Ostfeld & Keesing 2000; Johnson & Thieltges 2010). The results of studies completed over the last decade indicate that host biodiversity can act to dilute risk of transmission of infective stages of *Borrelia burgdorferi*¸ which causes Lyme disease (Ostfeld & Keesing 2000), and other human and wildlife pathogens (Allan *et al.* 2009; Suzán *et al.* 2009). Understanding how rates of pathogen transmission change relative to ongoing losses of biodiversity on the one hand, and ongoing gains in biodiversity due to host introductions on the other, is a vital component of public health and conservation biology as emerging diseases are contributing to global health and environmental crises (Jones *et al.* 2008).

Currently, much of the evidence for the DEH is anecdotal, and underlying mechanisms are still hotly debated (Wood *et al.* 2017). First, much of the literature regarding the dilution effect focuses on vector-transmitted pathogens which are often intractable to experimentation (Johnson & Thieltges 2010), with neither host nor the vector being amendable to manipulation. Furthermore, emulating a natural environment to experimentally test host-vector interactions either require abundant resources or are logistically impossible, making biologically relevant interpretations difficult. The shortcomings of experimental opportunities for vector-disease systems results in correlative studies that lack the mechanistic insight on what biological processes underlie

the DEH. Therefore, the ability to experimentally test the dilution effect concerns the applicability of the dilution effect hypothesis as a disease management tool.

To address the shortcomings of correlative studies, researchers have begun using aquatic trematode systems to test the DEH in both mesocosm and laboratory settings (Thieltges *et al.* 2008; Raffel *et al.* 2010; Johnson *et al.* 2013). Experimental tests by Johnson et al. (2013) have indicated that the rate of transmission of the trematode *Ribeiroia ondatrae* from its snail intermediate hosts to its amphibian host is, in part, determined by host species richness. Tests by Wojdak et al. (2014) involving the trematode *Echinostoma trivolvis* suggest that rates of cercariae transmission from snails into it's amphibian host are decreased as alternative host species were added to experimental containers. These results are consistent with the DEH because the rate of transmission was decreased with an increase in host (larval amphibian) diversity. In both of these cases, supportive evidence for the DEH is especially important because these trematodes are associated with host pathology (altered limb number and morphology, and impaired kidney function, respectively) and mortality that can contribute to the dramatic decline in global amphibian populations (Stuart *et al.* 2004). Within the scope of declining animal populations, the loss of other freshwater animals such as fishes (Moyle & Leidy 1992) are equally alarming. Recently, host survey approaches have suggested that the dilution effect can be observed for fish parasites through the introduction of a novel fish species (Paterson *et al.* 2011; Gendron & Marcogliese 2017). However, no experimental tests of the DEH in fish/trematode systems have been performed and the effect of biodiversity losses on disease and parasite transmission into fish remain unclear.

Recent studies have shown that to understand the mechanism underlying the DEH, it is necessary to differentiate the relative roles of host identity and host species richness in decreasing parasite transmission rates (Johnson *et al.* 2019). Studies indicate that the co-occurrence of specific host species in a community was a stronger predictor of disease risk than host species richness alone (Johnson *et al.* 2008, 2019). Johnson et al. (2008) suggested that immunological differences between host species provided a possible mechanism for a decrease in parasite transmission in cases where certain host species acted as 'immunological sponges' to cercariae, ultimately decreasing the total number of parasites successfully establishing within a host community. However, the mechanism responsible for the reduction in physical encounter rates within a single host is speculative, resulting in a disconnect between the consequences of the DEH and the ecological context. Furthermore, experimental tests of the DEH have been performed on the generalist parasites such as *Ribeiroia ondatrae* (Johnson *et al.* 2008, 2013, 2016) or echinostomes (Raffel *et al.* 2010), but experimental tests of the DEH involving host specialist parasites are absent. Host-specific parasites are common, and the constraints of their transmission contrast those of vectors or generalist parasites, especially when it comes to their sensitivity to host biodiversity changes (Johnson & Thieltges 2010).

Complicating the understanding of the DEH are other ecological considerations that can independently and concurrently alter parasite transmission rates along with host diversity. Host density has often been the focus of predicting parasite transmission, rates (McCallum *et al.* 2001), and host density can be of particular interest if it influences behaviours that are known to decrease parasite risk. For example, shoaling can decrease the rate of parasite transmission into fish (Stumbo *et al.* 2012), whereas fish density can

affect shoaling behaviours (e.g. shoal size (Krause et al. 2005)). The indirect effects of variables such as host density on parasite risk have been recognized in amphibian-echinostome systems with higher intra-host competition delaying development of hosts out of their highly susceptible life-stages in mesocosms (Raffel *et al.* 2010). However, the role of host density is inconsistent, even within the same system, with null results of host density on parasitism rate being reported in aquaria (Raffel *et al.* 2010) and mesocosm (Marino *et al.* 2016).

In this study, I used a hierarchical experimental approach to test the DEH. I used a fish/trematode model system to distinguish the relative roles of host identity and host diversity on the rate of parasite transmission. Larvae (= cercariae) of the trematode *Ornithodiplostomum ptychocheilus* encyst as metacercariae in the brains of fathead minnows (*Pimephales promelas*). Free-swimming *O. ptychocheilus* cercariae penetrate the epidermis of fathead minnows and migrate along the peripheral nervous system to the optic lobes (Matisz *et al.* 2010). Encystment of these larvae is known to interfere with host visual acuity (Shirakashi & Goater 2005) and development of the cranium (Sandland & Goater 2001a). Cercariae are released by its' first intermediate host, the snail *Physa* sp. The larvae are easily enumerated prior to exposure to fish (e.g. Stumbo et al. 2012), providing a powerful study system to test the effects of host diversity on parasite transmission. First, I tested the nature of host specificity by assessing interspecific differences in the susceptibility of 3 host species to *O. ptychocheilus* cercariae. I then used combinations of these same species of hosts to assess the relative effects of host density, host identity, and host diversity on rates of *O. ptychocheilus* transmission in both aquaria and mesocosm settings.

3.3 Materials and Methods

3.3.1 *Source of infected snails*

Following methods described in Seppälä et al. (2007), I used naturally-infected adult snails as a source of infective cercariae for experiments. Adult *Physa gyrina* were collected from Coulee Creek Stormwater Pond, Lethbridge, Alberta (49°66'N, 112°78'W) between 5 July and 31 August, 2018. Results from previous field surveys indicated that this site contained snails that were infected with larval stages of the trematodes *Ornithodiplostomum ptychocheilus* (in the brain) and *Ornithodiplostomum* sp. (in the body cavity). Fathead minnows at this site were heavily infected with the metacercariae of both species (Ahn and Goater, unpublished data).

A total of 294 adult snails was collected haphazardly from the perimeter of Coulee Creek Pond and brought to the laboratory to assess their infection status with larval trematodes. The experimental exposures of fish to cercariae required that I distinguish the two species of *Ornithodiplostomum* that were present in *P. gyrina* at this site. First, the infection status of individual snails was determined by placing each adult snail into glass vials with aquarium grade water for 2-3 hours under direct light (Seppälä *et al.* 2007). Infected snails released large numbers of cercariae daily. A solution of water and approximately 300 cercariae was poured into a 1L container that contained 3 juvenile fathead minnows. After 3 days, exposed minnows were necropsied, and the brain and viscera were placed between two microscope slides to assess for the presence of metacercariae. The presence of immature metacercariae in the optic lobes of the brain confirmed *O. ptychocheilus* (Matisz & Goater 2010), whereas the presence of immature metacercariae in the liver and viscera confirmed *Ornithodiplostomum* sp. (Matisz &

Goater 2010). Of 159 snails collected on 5 July 2018, 19 were infected with *O. ptychocheilus*. Of the 134 *P. gyrina* adults collected on 31 August, 12 were infected with *O. ptychocheilus*. All infected snails were isolated and kept under standard laboratory conditions that included weekly water changes and continual access to fresh Romaine lettuce and commercial fish food (Sandland & Goater 2001b).

3.3.2 *Source of fish hosts*

For the exposure experiments, juveniles of 5 fish species were collected using a seine net in summer 2018 from several locations in southern Alberta. Fathead minnows (*Pimephales promelas*) were collected from a small pond located on the grounds of the University of Lethbridge, Alberta (49°68'N, 112°87'W). Emerald shiners (*Notropis atherinoides*) and longnose dace (*Rhinichthys cataractae*) were collected from the Oldman River along a stretch adjacent to the University (49°68'N, 112°86'W). Samples of brook stickleback (*Culaea inconstans*) were collected from McQuillan Reservoir (49°65'N, 112°46'W). In southern Alberta, fathead minnows are one of the most common and widespread species of cyprinid and are heavily infected with *O. ptychocheilus* metacercariae (Sandland *et al.* 2001). Thus, fathead minnows were considered as the focal host of interest in this study. The other species of fish tend to co-occur with fathead minnows at most sites. Emerald shiners and longnose dace are more commonly found in moving water, while brook stickleback are more common in standing water. Even though all species of fish are sympatric at sites in southern Alberta, brook stickleback are syntopic at all sites.

All field-collected fish were acclimated for at least 24 h in laboratory aquaria at room temperature on a 16L:8D light cycle. While under laboratory conditions, fish were

fed *ad libitum* with a combination of commercial fish food (Tetramin ®) and zooplankton (copepods and *Daphnia*) collected from a local reservoir. Aquaria water was changed every 3 days. For the mesocosm experiments, an additional transfer was performed that involved moving fish from laboratory aquaria to outdoor holding tanks. These fish were given an additional 24 hours acclimation in outdoor holding tanks before transfer to the mesocosms.

A subset of 30 individuals of each species of fish was necropsied immediately after collection to determine natural rates of exposure to *O. ptychocheilus*. Exposure to cercariae starts in mid- to late July and peaks in late summer/early fall (Sandland *et al.* 2001). Thus, the presence of encysted *O. ptychocheilus* metacercariae in the juvenile hosts sampled in 2018 resulted from exposure to cercariae during the previous late-summer/fall.

3.3.3 *Relative host susceptibility to O. ptychocheilus cercariae*

To determine relative host susceptibility, 14 individuals of each species of fish host were exposed to *O. ptychocheilus* cercariae. Size-matched samples of fathead minnow, emerald shiner, and longnose dace were selected haphazardly from the holding tanks and placed into 200mL plastic containers that held aquarium grade water. Brook stickleback were already confirmed to be uninfected with *O. ptychocheilus* metacercariae prior to the experiment and were thereby considered to be non-susceptible (Goater, unpublished data). Ten *O. ptychocheilus*-infected snails were placed in 200mL of water and placed under a light source for 3-4 hours. Once cercariae release was observed, three, 1mL aliquots of the cercariae-filled water was collected and immersed in 70% ethanol (Sandland & Goater 2000). The mean number of dead cercariae was calculated from

counts made under a gridded Petri dish following standard protocol to obtain replicate densities of cercariae/200 mL. The volume corresponding to 50 cercariae was then calculated. Individual fish were exposed to cercariae for 2 h. Cercariae-exposed fish were then transferred into aquariums with aquarium grade water and housed according to species. Standard necropsy procedures (Sandland and Goater 2000) were used to assess metacercariae intensity at 14 days post exposure. Complete maturation of *O. ptychocheilus* metacercariae requires a minimum of 8 weeks (Matisz *et al.* 2010), therefore new infections resulting from experimental exposures were easily distinguished from previous infections within the timeframe of the necropsies. At necropsy, the wet weight (0.1 g) and standard length (mm) of each host was assessed.

3.3.4 *Laboratory experiment*

For the aquaria experiment, fish were transferred to 3L experimental plastic containers (H: 17cm X L: 21cm X W: 14cm) from the holding tanks and provided 24 h acclimation prior to exposure to cercariae. This experiment was completed in the Aquatic Research Facility at the University of Lethbridge. The experiment was set-up with a randomized block design with 5 replicates of each of the following 7 treatments: 2 fathead minnows; 4 fathead minnows; 8 fathead minnows; 2 fathead minnows/2 emerald shiners; 2 fathead minnows/2 longnose dace; 2 fathead minnows/2 brook sticklebacks; 2 fathead minnows/2 emerald shiners/ 2 longnose dace/ 2 brook sticklebacks. Following the 24 h acclimation period, fish in each container were exposed to *O. ptychocheilus* cercaria using methods similar to those described for the relative susceptibility experiment. In this experiment, fish in a container were batch-exposed to 200 cercariae from 10 *O. ptychocheilus*-infected *P. gyrina* on 8 September 2018. The survivors in a container were

necropsied 10 days post-exposure to obtain metacercariae counts. Fish were fed zooplankton (*Daphnia* and copepods) daily and water was changed every 2 days. Live food was used to prevent fouling of the water in the containers and because all species of fish readily consumed these prey items. Food quantity/container was determined on a *per capita* basis by the density of fish in the containers and was estimated as approximately 1mL of concentrated zooplankton per individual/day.

3.3.5 *Outdoor mesocosm experiment*

Sixteen 1200L outdoor mesocosms and 4 outdoor holding tanks were set-up within a fenced area on the University of Lethbridge campus. The methods used to set-up the mesocosms and to batch-expose fathead minnows to *O. ptychocheilus* cercariae followed a standard protocol (Pearson & Goater 2009). In brief, mesocosms were filled with irrigation water and dried *Typha sp*. reeds (800g) from a local wetland on 18 May, 2018. A 500mL inoculation of concentrated phyto- and zooplankton was collected from 4 local ponds and added to each mesocosm on 15 June and 12 July, 2018. Each mesocosm was covered with a mesh lid for the duration of the experiment to prevent the colonization of predatory invertebrates. The mesocosms were left undisturbed until 27 July, 2018 to allow for the reproduction and development of plankton that would provide a food source for the experimental populations of fish.

Field caught fish were transferred from outdoor holding tanks to experimental mesocosms on 27 July 2018 and were acclimated for a minimum of 24 h prior to exposure to *O. ptychocheilus* cercariae. The design included 4 replicates of 4 treatments: 30 fathead minnow, 60 fathead minnow, 30 fathead minnow/30 emerald shiner, 30 fathead minnow/30 longnose dace. Cercarial exposures started on 30 July, 2018 with

50

cercariae from 10 *O. ptychocheilus*-infected *P. gyrina*. A total of 10,000 cercariae was placed into each mesocosm between 1:00-5:00 p.m. each day for 7 consecutive days. Fish necropsies were conducted starting 16 August 2018, at least 10 days from the last day of cercariae exposure.

3.3.6 *Data analyses*

The susceptibility of each fish species to *O. ptychocheilus* cercariae was determined as 'percent recovery', calculated as the mean abundance (Bush *et al.* 1997) of metacercariae relative to the estimated numbers of cercariae used in exposures (n = 50). A 1-Way ANOVA, with species as a fixed factor, was used to evaluate differences in metacercariae intensity between host species.

The effect of fathead minnow density on metacercariae intensity was analyzed with mixed-effect generalized linear models (GLMMs). The mean intensity of *O. ptychocheilus* metacercariae was predicted using a GLMM with host density as a fixed effect, host body condition as a covariate, and experimental container as a random effect. I used a Poisson error distribution with a log link function. To test the effects of host density for the aquaria experiments, I analyzed differences in metacercariae intensities determined from three density treatments (n=2, 4, 8 fish/container). To test the effects of host density for the outdoor mesocosm experiments, I analyzed metacercariae intensities determined from two density treatments (n=30, 60 fish/container). A Poisson distribution was used since metacercariae count data (with no zeros) approximated a normal distribution. The body condition of individual hosts was calculated as the residual of natural log wet weight against natural log standard length (Jakob *et al.* 1996) and was independently calculated for each model.

Similar analyses were performed to test the effects of host species diversity on *O. ptychocheilus* transmission. Mixed-species treatments were compared to their respective single-species treatments at the same total host density for both the aquaria and mesocosm experiments (e.g. 60 fathead minnow treatment vs 30 fathead minnow/30 emerald shiner or 4 fathead minnow treatment vs 2 fathead minnow/2 longnose dace). Models included host diversity as a fixed effect, with fish body condition as a covariate, and experimental aquaria/mesocosm as a random effect. Through the GLMMs, contrasts between mixed- and single-species treatments were performed with a Wald test. Statistical analyses were performed using R (version 3.4.1) and the GLMMs were performed using the lme4 package.

3.4 Results

3.4.1 *General patterns of infection*

The prevalence and mean intensities of *O. ptychocheilus* metacercariae in natural populations of juvenile fish varied by species (Table 3.1). In general, 30-50% of the young-of-the-year fathead minnows and emerald shiners contained 0-2 mature *O. ptychocheilus* metacercariae in their brains, whereas samples of longnose dace and sticklebacks were uninfected.

3.4.2 *Relative host Susceptibility*

The results of the susceptibility trials indicated that fathead minnows were most susceptible to *O. ptychocheilus* cercariae (Table 3.1). Recovery of metacercariae in emerald shiners was approximately 1% and it was zero for longnose dace. The hierarchical nature of host susceptibility was significant (ANOVA, $F_{3, 51} = 68.06$, p <

0.0001) with mean intensity highest in fathead minnows relative to all other host species (Tukey's HSD, p < 0.05). Even though emerald shiners were susceptible to *O. ptychocheilus* while longnose dace were not, the difference in susceptibility between emerald shiners and longnose dace was not significant (Tukey's HSD, p < 0.05).

3.4.3 *Laboratory experiment*

There was a significant effect of host density on rates of cercariae transmission into fathead minnows (Fig. 3.1; GLMM, density: -0.17 ± 0.038, df = 37, p < 0.0001). Overall, there was a 44.2% decrease in transmission between the 2 and 4-density containers and a 31.9% decrease between the 4 and 8-density containers (Fig. 3.1). The 27.4% decline in metacercariae intensity between minnows in the 4-minnow only treatment (n = 15) and minnow/emerald shiner (n = 6) treatment was significant (Fig. 3.1; GLMM, treatment: -0.47 ± 0.22, p < 0.0001). Metacercariae intensities in minnows that co-occurred with dace (n = 10) were not significantly different from those that occurred in the 4-minnow only treatment (Fig. 3.1; GLMM, treatment: -0.060 ± 0.19, p = 0.75). There was no significant difference in metacercariae intensity in minnows in the 8-minnow only treatment (n = 18) compared to minnows in the mixed treatment (n = 5) that contained shiners, dace, and sticklebacks (Fig. 3.1; GLMM, treatment: -0.24 ±0.34, p = 0.47). The 2 fathead minnow/2 brook stickleback treatment was not included in this analysis due to high mortality of fathead minnows in these containers.

3.4.4 *Outdoor mesocosm experiment*

There was no significant effect of host density on rates of cercariae transmission into fathead minnows (Fig. 3.2; GLMM, density: -0. 024 ± 0.11, df = 208, p = 0.82) with

minnows in the 30-density mesocosms (n = 78) containing 38.2 (± 11.7 s.d.) metacercariae and minnows in the 60-density mesocosms (n = 134) containing 37.4 (± 13.1 s.d.) metacercariae. Furthermore, fathead minnows (n = 84) that were sympatric with longnose dace showed no difference in metacercariae intensity compared to fathead minnows in the 60 fathead minnow treatments (Fig. 3.2; GLMM, p = 0.73). However, there was a 50.9% decline in metacercariae intensity in fathead minnows (n = 81) that were sympatric with emerald shiners which had, on average, 18.4 (± 9.0 s.d.) metacercariae (Fig. 3.2). The decline in cercariae transmission into fathead minnows sympatric with emerald shiners was highly significant (GLMM, treatment: -0.75 ± 0.13, p < 0.0001).

3.5 Discussion

Support for the host dilution hypothesis arises when an increase in host diversity within a community is associated with a decrease in parasite transmission. My results provide partial support for the hypothesis. On the one hand, there was a decrease in transmission into fathead minnows when they occurred together with emerald shiners compared to when they were by themselves. This is a robust result because the reduction in transmission was consistent within both mesocosm and laboratory settings. Thus, when host biodiversity increased from one to two species of host through the addition of emerald shiners, there was a marked reduction in transmission when overall host density was kept constant. These results are consistent with previous literature showing that increased host species richness resulted in decreased rates of parasite transmission (Johnson *et al.* 2009, 2013). On the other hand, in all other pairwise host compositions, transmission rates were equivalent to those in monospecific minnow containers at the

same total density. Furthermore, transmission rates were not affected when exposure occurred within high biodiversity containers that contained mixtures of 4 species. Ultimately, my results indicate that transmission reduction only occurred under the conditions that involved co-occurrence with a single species of host. As such, my results emphasize the importance of specific host species compositions as a driver for the dilution effect hypothesis (Johnson *et al.* 2008, 2019).

The results of the laboratory susceptibility assays showed that less than 1% of the 50 cercariae used in exposure trials were recovered as metacercariae in the brains of shiners. Along with the zero susceptibility of *O. ptychocheilus* into longnose dace or brook stickleback, I conclude that *O. ptychocheilus* cercariae are host-specific. The specialization of *O. ptychocheilus* to fathead minnows is particularly important since changes in host biodiversity are thought to have disproportionate effects on host-specific parasites over generalist parasites (Johnson & Thieltges 2010). Previous experiments by Mitchell et al. (2002) on fungal pathogens of plants support the hypothesis that the composition of host community is important for host-specific parasites. However, Mitchell et al. (2002) also conclude that the decrease in parasite transmission was not due to biodiversity, but due to the availability of specific plant hosts that arose from interspecific competition. Their results indicate that the dilution of parasite risk is not necessarily a direct consequence of host biodiversity, but an indirect result of biotic interactions between species. Thieltges et al. (2008) attribute decreases in the transmission of trematode cercariae into marine cockles to the disturbances caused by crabs and shrimps that decreased cockle feeding rates, decreasing the window of opportunity of parasites to infect a cockle. Consequently, any alterations to an

environment that affects the availability of the host may have cascading effects on parasite risk.

One explanation for the observed decline in *O. ptychocheilus* transmission rate into fathead minnows is that the presence of shiners leads to alterations in minnow behaviour that leads to encounter reduction with cercariae. In this study, I did not evaluate differences in minnow behaviours in the presence of conspecific or heterospecific fish. However, my anecdotal observations indicated that minnows formed mixed shoals with emerald shiners but not with longnose dace or stickleback. Furthermore, interspecific shoaling involving cyprinids is associated with changes in swimming behaviours of individual fish (Tang *et al.* 2017), their positioning within a shoal (Allan 1986), and with overall shoal architecture (Pollock *et al.* 2006). Results from previous work in the laboratory have indicated that rates of transmission by *O. ptychocheilus* cercariae into minnows are influenced by minnow behaviours such as host activity (Shirakashi & Goater 2005) and positioning within a conspecific shoal (Stumbo et al., 2012). Therefore, the interspecific interactions between emerald shiners and fathead minnows may cause alterations to minnow microhabitat preferences, shoaling architecture, place/time foraging, or overall activity which may singularly or collectively influence their contact rates with cercariae. Accordingly, the alterations in minnow behaviours described are alterations to social behaviour, indicating that changes in biodiversity may affect social conditions for competent hosts, and ultimately alter parasite risk.

The activity and behaviour of emerald shiners within the water column may influence the swimming behaviours of the cercariae themselves and thereby interfere with the probability that cercariae encounter minnows. Trematode cercariae are millimeter-

scale, short-lived (ca. 12-24 hours), non-feeding, and free-swimming (Goater *et al.* 2014). Following their daily release from snails, they engage in efficient, but temporary, swimming behaviours that are driven by the muscular action of the posterior forked tail coupled with flexure of the anterior region of the body (Combes *et al.* 1994). For fork-tailed cercariae such as *O. ptychocheilus*, these complex behaviours often lead to the aggregation of cercariae at the air-water interface. Since emerald shiners tend to prefer swimming near the surface (Trautman 1981), it is possible that cercariae encountered emerald shiners more frequently which may have indirect consequences on the interaction between cercariae and fathead minnows. Predation of cercariae by emerald shiners is a possibility since they are zooplanktonivorous (Hartman *et al.* 1992) and cercariae predation is a recognized mechanism of decreased parasite transmission in aquatic environments (Kaplan *et al.* 2009). It is also possible that shiners may influence the mobility of cercariae indirectly via the creation of water currents that influence swimming performance. Overall, these results support the contention that interspecific interactions, at either host or parasite scales, can result in reduced probability of contact between a focal host (fathead minnows in our experiment) and its parasites, contributing to patterns of transmission in diverse communities.

My results support the idea that rates of parasite transmission are strongly impacted by the presence of specific host species in a community. Previous work on the cercariae stage of aquatic trematode has indicated that the identity of specific hosts in the environment is an important factor for predicting parasite risk (Johnson *et al.* 2008, 2019). Up to now, the dilution effect hypothesis has not been experimentally tested on communities of fish. Therefore, my results demonstrate that support for the dilution effect

hypothesis can be observed for trematode parasites of fish, consistent with previous field studies of fish parasites (Gendron & Marcogliese 2017). In conclusion, these results indicate that the predictive capacity of the dilution effect is generalizable in aquatic systems but that the ecology of the host and the specific interactions within their environments is a necessary consideration.

Reduction of parasite risk due to increased host density was observed in a stepwise fashion for the in-lab experiments. These results follow model predictions for cercariae/host interactions, with host density having an inverse relationship with rates of cercariae transmission (Anderson & May 1978). These density results further confirm the 'safety in numbers' phenomenon for *per capita* parasite risk; a result consistent with other parasite/host interactions (e.g. Arneberg et al. 1998). Thus, these results are consistent with those demonstrated for other aquatic free-living parasites in which high host densities dilute *per capita* risk (Buck *et al.* 2017). The total proportion of successful cercariae could not be assessed due to mortality observed in the lab experiment. Therefore, no definitive conclusions can be made regarding the ecological consequences of minnow density on parasite transmission into the final host. However, the consistency in metacercariae intensity in fathead minnows within density treatments suggests that cercariae penetration into a fish host is likely random and that the reduction in *per capita* encounter rates occurs as more hosts are available in a container.

An unexpected result from my experiment was the contrasting effects of host density on the transmission of *O. ptychocheilus* between the laboratory and mesocosm experiments. In the mesocosm experiment, there was no decrease in cercariae transmission, even when the numbers of fathead minnows doubled. The contrasting

results are likely due to environmental differences between the two experimental settings. The size of the mesocosms may permit fathead minnows to display behaviours such as shoaling that may reduce exposure to parasites (eg. James et al. 2008, Stumbo et al. 2012). The greater number of fish in mesocosms may also have permitted more opportunities to shoal compared to the low numbers in the aquaria, which is an important consideration with minnows in shoals potentially being able to recognize cercariae more efficiently (Mikheev *et al.* 2013). Ultimately, contrasting results of the effects of host density highlight the highly context-dependent nature of parasite transmission.

This study provides mechanistic insight on how parasite transmission varies within complex host communities. These results support the dilution effect hypothesis by showing decreased parasite transmission with addition of another host. However, results also highlighted that the dilution effect is not solely a consequence of biodiversity *per se* because only specific host community compositions lead to reduced rates of parasite transmission. Thus, understanding the biotic interactions caused by other hosts is an important consideration for how changes to biodiversity will alter parasite transmission. As well, the inconsistent dilution effect caused by changes in density between the in-laboratory experiments and the outdoor mesocosm experiments show that abiotic environment is an important consideration for predicting disease risk. As our environments approach an age of unprecedented changes, a greater understanding of how host ecology affects disease risk will provide the framework for a preventative approach to managing human and wildlife diseases.

3.6 Literature cited

Allan, B. F., B. R. Langerhans, W. A. Ryberg, W. J. Landesman, and N. W. Griffin. 2009. Ecological correlates of risk and incidence of West Nile virus in the United States. Oecologia 158:699–708.

Allan, J. R. 1986. The influence of species composition on behaviour in mixed-species cyprinid shoals. Journal of Fish Biology 29:97–106.

Anderson, R. M., and R. M. May. 1978. Regulation and stability of host-parasite population interactions: I. Regulatory Processes. The Journal of Animal Ecology 47:219–247.

Arneberg, P., A. Skorping, B. Grenfell, and A. F. Read. 1998. Host densities as determinant of abundance in parasite communities. Proceedings of the Royal Society B-Biological Sciences 265:1283–1289.

Buck, J. C., R. F. Hechinger, A. C. Wood, T. E. Stewart, A. M. Kuris, and K. D. Lafferty. 2017. Host density increases parasite recruitment but decreases host risk in a snail–trematode system. Ecology 98:2029–2038.

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

Combes, C., A. Fournier, H. Moné, and A. Théron. 1994. Behaviours in trematode cercariae that enhance parasite transmission: Patterns and processes. Parasitology 109:S3–S13.

Gendron, A. D., and D. J. Marcogliese. 2017. Enigmatic decline of a common fish parasite (*Diplostomum* spp.) in the St. Lawrence River: Evidence for a dilution effect induced by the invasive round goby. International Journal for Parasitology: Parasites and Wildlife 6:402–411.

Goater, T. M., C. P. Goater, and G. W. Esch. 2014. Parasitism: The diversity and ecology of animal parasites, 2nd Edition. Cambridge University Press, Cambridge.

Hartman, K. J., B. Vondracek, D. L. Parrish, and K. M. Muth. 1992. Diets of emerald and spottail shiners and potential interactions with other western Lake Erie planktivorous fishes. Journal of Great Lakes Research 18:43-50.

Jakob, E. M., S. D. Marshall, and G. W. Uetz. 1996. Estimating fitness: A comparison of body condition indices. Oikos 77:61–67.

James, C. T., K. J. Noyes, A. D. Stumbo, B. D. Wisenden, and C. P. Goater. 2008. Cost of exposure to trematode cercariae and learned recognition and avoidance of parasitism risk by fathead minnows *Pimephales promelas*. Journal of Fish Biology 73:2238–2248.

Johnson, P. T. J., D. M. Calhoun, T. Riepe, T. McDevitt-Galles, and J. Koprivnikar. 2019. Community disassembly and disease: realistic-but not randomized-biodiversity

losses enhance parasite transmission. Proceedings. Biological Sciences 286:20190260.

Johnson, P. T. J., R. B. Hartson, D. J. Larson, and D. R. Sutherland. 2008. Diversity and disease: Community structure drives parasite transmission and host fitness. Ecology Letters 11:1017–1026.

Johnson, P. T. J., P. J. Lund, R. B. Hartson, and T. P. Yoshino. 2009. Community diversity reduces *Schistosoma mansoni* transmission, host pathology and human infection risk. Proceedings. Biological sciences / The Royal Society 276:1657–1663.

Johnson, P. T. J., D. L. Preston, J. T. Hoverman, and K. L. D. Richgels. 2013. Biodiversity decreases disease through predictable changes in host community competence. Nature 494:230–233.

Johnson, P. T. J., and D. W. Thieltges. 2010. Diversity, decoys and the dilution effect: How ecological communities affect disease risk. The Journal of Experimental Biology 213:961–970.

Johnson, P. T. J., C. L. Wood, M. B. Joseph, D. L. Preston, S. E. Haas, and Y. P. Springer. 2016. Habitat heterogeneity drives the host-diversity-begets-parasite-diversity relationship: Evidence from experimntal and field studies. Ecology Letters 19:752–761.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature 451:990–993.

Kaplan, A. T., S. Rebhal, K. D. Lafferty, and A. M. Kuris. 2009. Small estuarine fishes feed on large trematode cercariae: Lab and field investigations. Journal of Parasitology 95:477–480.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485–498.

Krause, J., E. Hensor, I. D. Couzin, and R. James. 2005. Modelling density-dependent fish shoal distributions in the laboratory and field. Oikos 110:344–352.

Marino, J. A., M. P. Holland, and E. E. Werner. 2016. Competition and host size mediate larval anuran interactions with trematode parasites. Freshwater Biology 61:621–632.

Matisz, C. E., and C. P. Goater. 2010. Migration, site selection, and development of *Ornithodiplostomum* sp. metacercariae (Digenea: Strigeoidea) in fathead minnows (*Pimephales promelas*). International Journal for Parasitology 40:1489–1496.

Matisz, C. E., C. P. Goater, and D. Bray. 2010. Migration and site selection of *Ornithodiplostomum ptychocheilus* (Trematoda: Digenea) metacercariae in the brain of fathead minnows (*Pimephales promelas*). Parasitology 137:719–731.

McCallum, H. I., N. Barlow, and J. Hone. 2001. How should pathogen transmission be modelled? Trends in Ecology and Evolution 16:295–300.

Mikheev, V. N., A. F. Pasternak, J. Taskinen, and T. E. Valtonen. 2013. Grouping facilitates avoidance of parasites by fish. Parasites and Vectors 6:1–8.

Mitchell, C. E., D. Tilman, and J. V. Groth. 2002. Effects of grassland species diversity, abundance, and composition on foliar fungal disease. Ecology 83:1713–1726.

Morand, S., and R. Poulin. 1998. Density, body mass and parasite species richness of terrestrial mammals. Evolutionary Ecology 12:717–727.

Moyle, P. B., and R. A. Leidy. 1992. Loss of biodiversity in aquatic ecosystems: Evidence from fish faunas. Pages 127-169 Conservation Biology. Chapman & Hall, New York.

Ostfeld, R. S., and F. Keesing. 2000. Biology biodiversity and disease risk : The case of Lyme disease. Conservation Biology 14:722–728.

Paterson, R. A., C. R. Townsend, R. Poulin, and D. M. Tompkins. 2011. Introduced brown trout alter native acanthocephalan infections in native fish. Journal of Animal Ecology 80:990–998.

Pearson, K. J., and C. P. Goater. 2009. Effects of predaceous and nonpredaceous introduced fish on the survival, growth, and antipredation behaviours of long-toed salamanders. Canadian Journal of Zoology 87:948–955.

Pollock, M. S., R. J. Pollock, and D. P. Chivers. 2006. Social context influences the antipredator behaviour of fathead minnows to chemical alarm cues. Ethology 112:801–806.

Raffel, T. R., J. T. Hoverman, N. T. Halstead, P. J. Michel, and J. R. Rohr. 2010. Parasitism in a community context: Trait-mediated interactions with competition and predation. Ecology 91:1900–1907.

Sandland, G. J., and C. P. Goater. 2000. Development and intensity dependence of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows (*Pimephales promelas*). Journal of Parasitology 86:1056–1060.

Sandland, G. J., and C. P. Goater. 2001. Parasite-induced variation in host morphology: brain-encysting trematodes in fathead minnows. Journal of Parasitology 87:267–72.

Sandland, G. J., C. P. Goater, and A. J. Danylchuk. 2001. Population dynamics of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows (*Pimephales promelas*) from four northern-Alberta lakes. Journal of Parasitology 87:744–748.

Schleppe, J. L., and C. R. Goater. 2004. Comparative life histories of two diplostomid trematodes, *Ornithodiplostomum ptychocheilus* and *Posthodiplostomum minimum*. Journal of Parasitology 90:1387–1391.

Seppälä, O., A. Karvonen, and T. E. Valtonen. 2007. Phenotypic variation in infectivity of *Diplostomum spathaceum* cercariae within a population. Journal of Parasitology 93:1244–1246.

Shirakashi, S., and C. P. Goater. 2005. Chronology of parasite-induced alteration of fish behaviour: Effects of parasite maturation and host experience. Parasitology 130:177–183.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman,

and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Stumbo, A. D., C. T. James, C. P. Goater, and B. D. Wisenden. 2012. Shoaling as an antiparasite defence in minnows (*Pimephales promelas*) exposed to trematode cercariae. Journal of Animal Ecology 81:1319–1326.

Suzán, G., E. Marcé, J. T. Giermakowski, J. N. Mills, G. Ceballos, R. S. Ostfeld, B. Armién, J. M. Pascale, and T. L. Yates. 2009. Experimental evidence for reduced rodent diversity causing increased hantavirus prevalence. PLoS ONE 4(5):e5461.

Tang, Z.-H., H. Wu, Q. Huang, L. Kuang, and S.-J. Fu. 2017. The shoaling behavior of two cyprinid species in conspecific and heterospecific groups. PeerJ 5:e3397.

Thieltges, D. W., M. D. Bordalo, A. Caballero Hernández, K. Prinz, and K. T. Jensen. 2008. Ambient fauna impairs parasite transmission in a marine parasite-host system. Parasitology 135:1111–1116.

Trautman, M. B. 1981. The Fishes of Ohio. Ohio State University Press, Columbus, Ohio.

Wojdak, J. M., R. M. Edman, J. A. Wyderko, S. A. Zemmer, and L. K. Belden. 2014. Host density and competency determine the effects of host diversity on trematode parasite infection. PLoS ONE 9:14–16.

Wood, C. L., A. McInturff, H. S. Young, D. Kim, and K. D. Lafferty. 2017. Human infectious disease burdens decrease with urbanization but not with biodiversity. Philosophical Transactions of the Royal Society B: Biological Sciences 372:2197–2223.

Table 3.1. Natural interspecific variation in mean *Ornithodiplostomum ptychocheilus* intensity in fish species collected from sites in Southern Alberta in 2018 as well as their relative susceptibility (measured as percent recovery) to *O. ptychocheilus* when exposed to 50 cercariae.

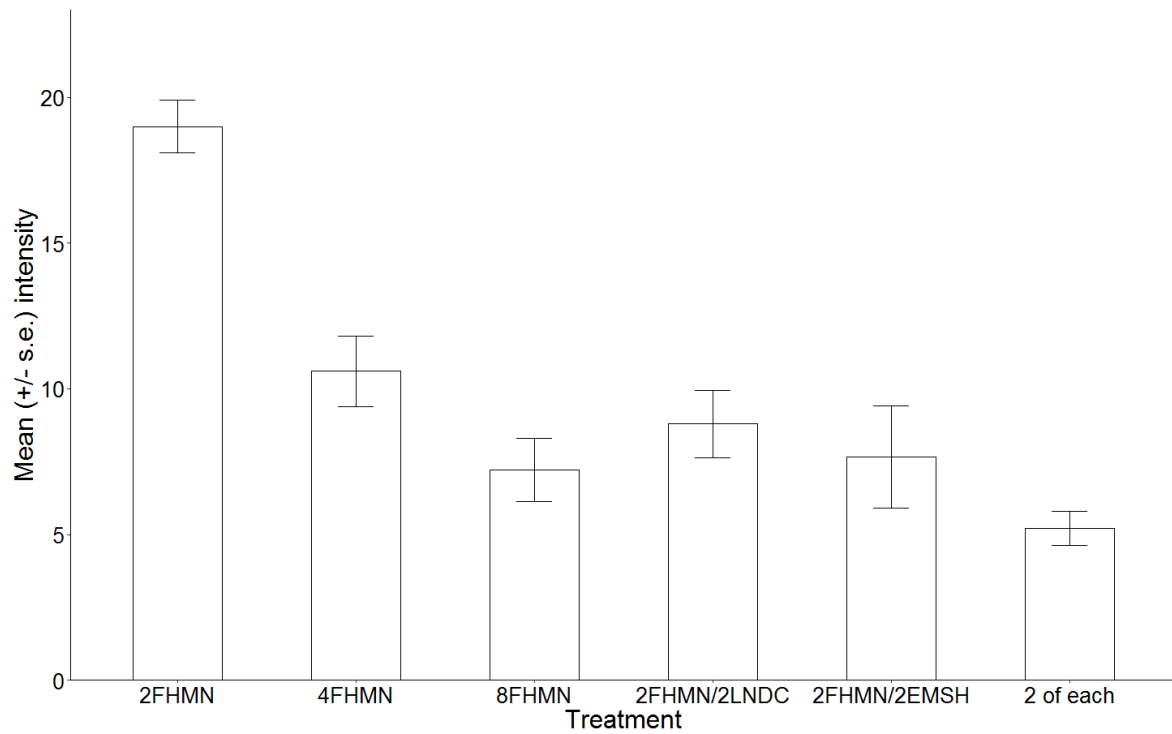|		Field collected fish (n=30)			Susceptibility experiment (n=14)
Host	Date of collection	Mean (±s.d.) *O. ptychocheilus* intensity	*O. ptychocheilus* prevalence (%)	Mean (±s.d.) standard length	Mean (± s.d.) percent recovery
Fathead minnow	11-25 Jul.	0.7 (± 0.2)	36.7	28.3 (± 2.2)	56 (± 21)
Emerald shiner	24 Jul. – 7 Sep.	1.0 (± 0.3)	46.7	48.4 (±5.1)	1 (± 0.03)
Longnose dace	14-25 Jul.	0	0	34.8 (± 5.9)	0

Figure 3.1. Effect of host density (total n = 2, 4, or 8 per container) and host diversity on mean intensity (± SE) of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows following exposure to 200 cercariae/3 L container. FHMN = fathead minnow; EMSH = emerald shiner; LNDC = longnose dace.
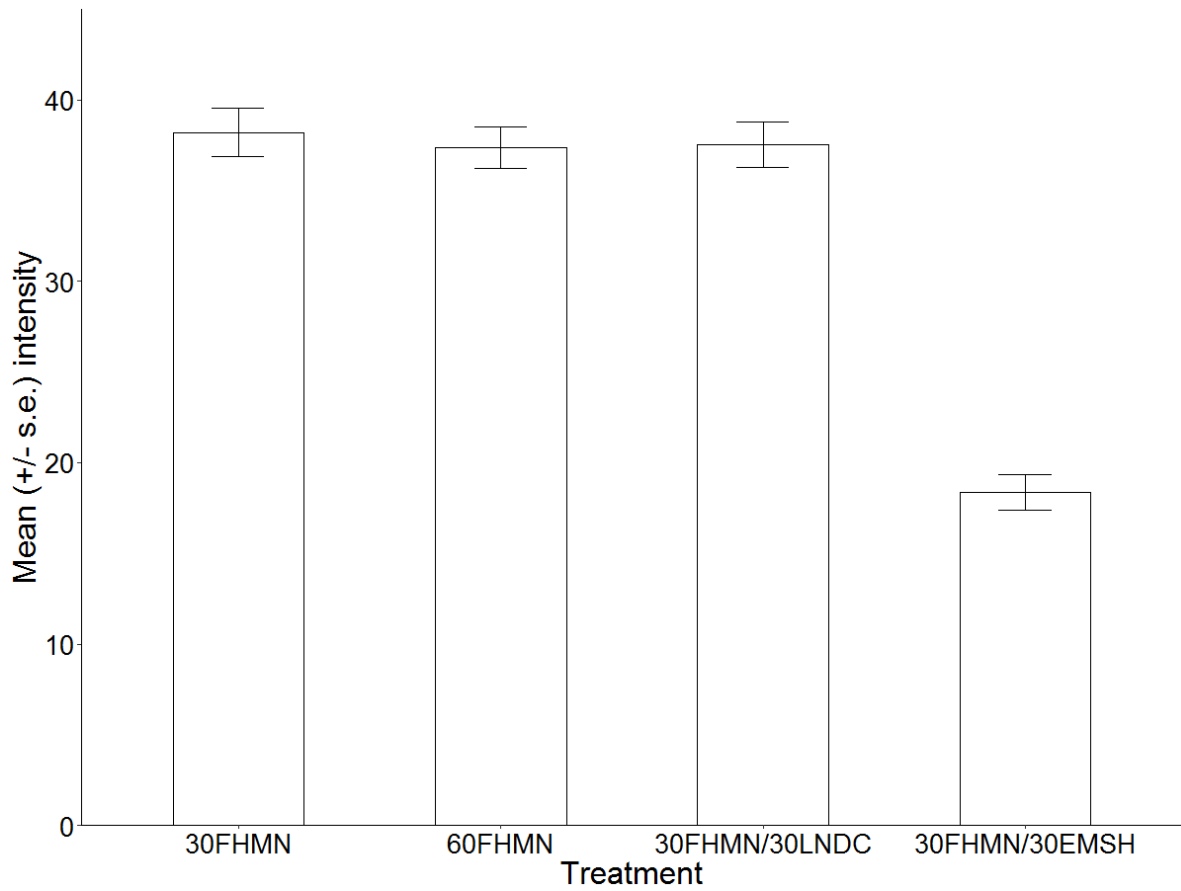
Figure 3.2. Effect of host density (total n = 30 or 60 per container) and host diversity on

mean intensity (± SE) of *Ornithodiplostomum ptychocheilus* metacercariae in fathead

minnows following exposure to 10000 cercariae/1200 L mesocosms. FHMN = fathead

minnow; EMSH = emerald shiner; LNDC = longnose dace.

CHAPTER 4: GENERAL CONCLUSION

My thesis aimed to determine whether parasite population sizes in a wild host are predictable relative to a suite of temporal, spatial, or ecological factors. On the one hand, the combined results from my two data chapters emphasized the challenges associated with identifying and generalizing how intrinsic and extrinsic factors affect parasite transmission rates, especially when comparisons are made between parasite species. On the other hand, the combined results of the field and empirical chapters can be synthesized into one concluding statement: parasite population sizes are highly variable because transmission rates are context dependent relative to host species, hosts populations, and habitats.

The results of the field survey in Chapter 2 show that over the long-term, every adult male minnow in the two ponds I studied is likely exposed to the larval stages of at least 5 species of trematode over its lifetime. Thus, the likelihood of exposure to cercariae of each of the 5 species can be considered very high during the life of a fathead minnow in Southern Alberta. However, the rate of cercarial exposure to fathead minnows is extremely variable between years, species, and sites. In the case of the two most common trematodes (both *Ornithodiplostomum* species), the magnitude of that variation exceeded two orders of magnitude relative to both site and year.

From an ecological perspective, characterizing the magnitude of variation in parasite prevalence and intensities is important (Kennedy 2009). I was unable to determine the underlying factors that determine rates of cercariae transmission into fish; however, it is clear from my analyses involving prevalence and mean intensity of the five species that there is a strong unregulated component to cercariae transmission in both space and time. These results are consistent with comparable field surveys studies involving a wide range

of fish/parasite interactions (Kennedy et al. 2001, Valtonen et al. 2004, Heins and Ecke 2012). Importantly, even with no regulatory factors (Kennedy et al. 2001) identified in Chapter 2, my results showed that closely-related parasite species (the two *Ornithodiplostomum* species) had similar patterns of spatial and temporal transmission.

The results of Chapter 2 are also important from an evolutionary perspective. The inherently antagonistic interaction between hosts and their parasites are expected to result in evolutionary arms races, often described by the 'Red Queen hypothesis'. Hosts adapt to combat the negative consequences of infection, to which parasites respond by evolving evasion or suppression mechanisms (Dawkins and Krebs 1979, Brockhurst et al. 2014). However, in most natural and long-standing host/parasite interactions, and as evidenced in the fathead minnow/trematode system, individual hosts are commonly infected by a diversity of parasite species (Petney and Andrews 1998, Cox 2001, Goater et al. 2005). Thus, individual minnows are commonly exposed to at least 2 species of trematodes at some point in their lives (Chapter 2, Schleppe 2002). When a host is infected by multiple parasite species, host defence are constrained by trade-offs (Stearns 1989) because hosts may not have the resources for independent responses against each parasite species, resulting in the evolution of more general defences, instead of optimal species-specific defences (Goater and Holmes 1997). Therefore, evolutionary constraints due to the finite resources of a host may be the reason mixed infections by parasites are commonly observed and maintained in the fathead minnow populations. Furthermore, the evolutionary constraints that result in these mixed infections may limit resources for fathead minnows to fight novel parasites and may have been a contributing factor in the dramatic emergence of the new species of myxozoan in the fathead minnow populations that was first observed in

2017. However, my thesis did not investigate the evolutionary or ecological consequences of mixed-species infections in fathead minnows. The amenability of the minnow/trematode interaction to controlled laboratory infections in the lab and field (e.g. Chapter 3), possibly with the addition of the new myxozoan, makes it an attractive model system to assess these important consequences.

In Chapter 2, I discussed the importance of factors such as host snail diversities (Johnson et al. 2009) and final host bird visitation rates (Kennedy and Burrough 1977) in determining the observed variation in mean trematode intensities. It is intriguing that the crash in population sizes of both *Ornithodiplostomum* species occurred in McQuillan Reservoir in the year following the introduction of an invasive snail species, *Radix*. Anecdotal observations suggest that the invasion of *Radix* snails may have been associated with a parallel collapse in the population size of the snail *Physa gyrina* in the reservoir. Studies in the Goater lab have shown that both species of *Ornithodiplostomum* use this species of snail as first intermediate host (Matisz and Goater 2010, Matisz et al. 2010). One intriguing follow-up study to come out of my work lies in the possibility of experimentally-testing the role of snail species diversity on altering the transmission rates of *Ornithodiplostomum* miracidia into *Physa* snails, similar to the work done by Johnson et al. (2009) with amphibian larvae as well as my work in Chapter 3.

The results provided in Chapter 3 are the first test of the host dilution hypothesis in a fish/parasite system. In addition to testing the DEH in a novel host/parasite system, this chapter provided two further contributions. First, the results in this chapter provide the first direct test of the DEH with a host specialist. As such, they contrast markedly with similar tests completed with aquatic trematodes that are host generalists in amphibian larvae

(Johnson et al. 2013, 2019, Wojdak et al. 2014) and with those completed with vector/arthropod (Ostfeld and Keesing 2000, Allan et al. 2009) systems, which that also tend to involve generalists. My empirical results from the host susceptibility assays show that *O. ptychocheilus* is a host specialist in fathead minnows; other cyprinids such as emerald shiners and longnose dace are poor hosts (1% of cercariae successfully penetrated) or completely incompatible. Therefore, in addition to confirming that a dilution effect can occur in fish/parasite systems, these results show that host specific parasites can exhibit dilution effects with changes to host biodiversity.

Secondly, my results highlight the role of host species composition versus host species diversity in determining the observed reduction in transmission (Johnson et al. 2019). Simply stated, the DEH describes a negative association between host biodiversity and parasite risk (Keesing et al. 2006, Johnson and Thieltges 2010). But the underlying mechanism behind the linkage between host biodiversity and reduced parasite transmission is still not understood (Wood et al. 2014, Halsey 2019). My results from Chapter 3 show that host biodiversity did not directly alter rates of cercariae transmission because fathead minnows only had a decrease in parasite transmission when co-occurring with emerald shiners, but not longnose dace or sticklebacks. Therefore, the identity of host species in a community was a better predictor of the dilution effect than host biodiversity. In Chapter 3, I suggested that the species-specific effect of emerald shiners modulated the transmission of *O. ptychocheilus* into fathead minnow. This result suggests that, for the proper prediction of disease risk, ecologists and epidemiologists need to understand what species-species interactions are being added or lost as animal biodiversity changes. Therefore, the results in Chapter 3 provide the basis for follow-up tests to determine the manner in which the

presence of a non-compatible host (emerald shiners) can so drastically reduce transmission rates into fathead minnows. Assays that compare behaviours that are known to be associated with risk of cercarial transmission (e.g. overall host activity, positioning in the water column, shoaling) in minnow only versus minnow/shiner containers would be a useful direction. Furthermore, the amenability of the fathead minnow/*Ornithodiplostomum* system to experimental manipulation (e.g. Chapter 3) means that comparative tests of the DEH with a host specialist (*O. ptychocheilus*) and a host generalist (e.g. *Ornithodiplostomum* sp., *Posthodiplostomum minimum*) would be possible.

The unique combination of field survey and empirical approaches I used in this thesis has contributed to a greater understanding of how highly variable rates of parasite transmission can be explained by factors such as host community structure, host identity, year, and site. My thesis presents the results of one of the few long-term studies on variation in the population sizes of multiple parasite species in a model aquatic host. My results are particularly relevant in the context of shifting ecosystems due to anthropogenic changes and the increased awareness of emerging diseases. Furthermore, my thesis is the first to experimentally test the Dilution Effect Hypothesis on a fish host system, with implications on predicting disease risk in environments that are currently changing at an unprecedented rate. As contemporary research continues to advance parasite ecology, my results will act as a steppingstone to lead to a greater understanding of how parasites interact with host individuals and population as well the environments within which they infect their hosts.

4.1 Literature cited

Allan, B. F., B. R. Langerhans, W. A. Ryberg, W. J. Landesman, and N. W. Griffin. 2009. Ecological correlates of risk and incidence of West Nile virus in the United States. Oecologia 158:699–708.

Brockhurst, M. A., T. Chapman, K. C. King, J. E. Mank, S. Paterson, and G. D. D. Hurst. 2014. Running with the Red Queen: The role of biotic conflicts in evolution. Proceedings of the Royal Society B: Biological Sciences 281.

Cox, F. E. G. 2001. Concomitant infections , parasites and immune responses. Parasitology 122:S23–S38.

Dawkins, R., and J. R. Krebs. 1979. Arms races between and within species. Proceedings of the Royal Society of London - Biological Sciences 205:489–511.

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

Goater, C. P., and J. C. Holmes. 1997. Parasite-mediated natural selection. Pages 9–29 Host-parasite evolution: general principals and avian models. Oxford University Press, Oxford.

Halsey, S. 2019. Defuse the dilution effect debate. Nature Ecology and Evolution 3:145–146.

Heins, D. C., and J. K. Ecke. 2012. The rise and fall of an epizootic of the diphyllobothriidean cestode *Schistocephalus pungitii* infecting the ninespine stickleback. Journal of Parasitology 98:1–5.

Johnson, P. T. J., D. M. Calhoun, T. Riepe, T. McDevitt-Galles, and J. Koprivnikar. 2019. Community disassembly and disease: realistic-but not randomized-biodiversity losses enhance parasite transmission. Proceedings. Biological sciences 286:20190260.

Johnson, P. T. J., P. J. Lund, R. B. Hartson, and T. P. Yoshino. 2009. Community diversity reduces *Schistosoma mansoni* transmission, host pathology and human infection risk. Proceedings. Biological sciences / The Royal Society 276:1657–1663.

Johnson, P. T. J., D. L. Preston, J. T. Hoverman, and K. L. D. Richgels. 2013. Biodiversity decreases disease through predictable changes in host community competence. Nature 494:230–233.

Johnson, P. T. J., and D. W. Thieltges. 2010. Diversity, decoys and the dilution effect: How ecological communities affect disease risk. The Journal of Experimental Biology 213:961–970.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485–498.

Kennedy, C. R. 2009. The ecology of parasites of freshwater fishes: The search for patterns. Parasitology 136:1653–1662.

Kennedy, C. R., and R. Burrough. 1977. The population biology of two species of eyefluke, *Diplostomum gasterostei* and *Tylodelphys clavata*. Journal of Fish Biology 11:619–633.

Kennedy, C. R., P. C. Shears, and J. A. Shears. 2001. Long-term dynamics of *Ligula intestinalis* and roach *Rutilus rutilus*: A study of three epizootic cycles over thirty-one years. Parasitology 123:257–269.

Matisz, C. E., and C. P. Goater. 2010. Migration, site selection, and development of *Ornithodiplostomum* sp. metacercariae (Digenea: Strigeoidea) in fathead minnows (*Pimephales promelas*). International Journal for Parasitology 40:1489–1496.

Matisz, C. E., C. P. Goater, and D. Bray. 2010. Migration and site selection of *Ornithodiplostomum ptychocheilus* (Trematoda: Digenea) metacercariae in the brain of fathead minnows (*Pimephales promelas*). Parasitology 137:719–731.

Nelson, J. S., and M. J. Paetz. 1992. The Fishes of Alberta. 2nd edition. The University of Alberta Press, Edmonton.

Ostfeld, R. S., and F. Keesing. 2000. Biology biodiversity and disease risk : The case of Lyme disease. Conservation Biology 14:722–728.

Petney, T. N., and R. H. Andrews. 1998. Multiparasite communities in animals and humans : frequency , structure and pathogenic significance. International Journal for Parasitology 28:377–393.

Sandland, G. J., C. P. Goater, and A. J. Danylchuk. 2001. Population dynamics of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows (*Pimephales promelas*) from four Northern-Alberta lakes. The Journal of Parasitology 87:744–748.

Schleppe, J. L. 2002. The ecology of two larval parasites in fathead minnows. University of Lethbridge.

Stearns, S. C. 1989. Trade-offs in life-history evolution. Functional Ecology 3:259–268.

Valtonen, T. E., E. Helle, and R. Poulin. 2004. Stability of Corynosoma populations with fluctuating population densities of the seal definitive host. Parasitology 129:635–642.

Wojdak, J. M., R. M. Edman, J. A. Wyderko, S. A. Zemmer, and L. K. Belden. 2014. Host density and competency determine the effects of host diversity on trematode parasite infection. PLoS ONE 9:14–16.

Wood, C. L., K. D. Lafferty, G. Deleo, H. S. Young, J. Peter, A. M. Kuris, C. L. Wood, K. D. Lafferty, G. Deleo, H. S. Young, P. J. Hudson, and A. M. Kūris. 2014. Does biodiversity protect humans against infectious disease? Ecology 95:817–832.