

**THE ECOLOGY OF TWO LARVAL PARASITES IN FATHEAD MINNOWS**

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B.Sc. Okanagan University College, 1999

A Thesis  
Submitted to the School of Graduate Studies  
of the University of Lethbridge  
in Partial Fulfillment of the  
Requirements for the Degree

**MASTER OF SCIENCE**

Department of Biology  
University of Lethbridge

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## General Abstract

The population dynamics and life histories of two larval parasites of fathead minnows were investigated, together with their effects on host reproduction in natural populations. In two lakes in northern Alberta, Canada, 100% of fathead minnows (*Pimephales promelas*) are infected with larval trematodes (*Ornithodiplostomum ptychococheilus* and *Posthodiplostomum minimum*) that encyst in their brains and mesenteries, respectively. The numbers of parasites in individual adult minnows varied extensively between and within two different lakes. Parallel laboratory studies indicated that selection imposed by common hosts in the life cycles of these species have shaped broadly similar life histories. Field collections of male minnows indicated that early in the breeding season, breeding males were longer than non-breeding males. Furthermore, breeding males had larger girths (independent of total length) and contained fewer numbers of three of 4 common larval trematodes than non-breeding males throughout the breeding season. These parasites most likely affect a male's ability to compete for or defend a nest.

## **Acknowledgements**

The writing of this thesis has been one of the more challenging tasks I have ever undertaken. I would not have completed such a daunting task without the scholastic guidance of Dr. Cameron Goater, my supervisor. Cam has “fueled”, inspired, and often focused my intellectual development throughout my tenure at the University of Lethbridge. I appreciate his patience with me while I experienced the “growing pains” that are typical of graduate students who are writing their first academic publication. Other members of Cam’s lab were also extremely helpful and encouraging throughout my tenure. Sho Shirakashi, Yukiyo, Kristi Jacobson, and last but not least Leslie Sellar all made “lab life” a little more interesting. I would like to extend an extra special thanks to Leslie, who would “troop” around with me in remote regions of Alberta, often for hours on end. I would never have been able to accomplish my goals without her help.

Several members of Department of Biological Sciences were also very helpful throughout my tenure. Thanks to Dr. Andy Hurly and Dr. Ralph Cartar who always had time to “chat” and always provided a helpful hint. I would also like to extend a special thanks to Bruce McMullin, head of Biology Stores, who was always helping with the “technical difficulties” of field and lab work. Finally, I thank three friends in the biology department. Katrina White, Keri Colwell and Sean Walker are honorable, intelligent and provided the “comic” relief that is needed for the highs and lows of research.

I also acknowledge all the members of the Graduate Student Association Executive, including Penny VandenBerg, Quintin Steynen, Claudia Gonzales,

Mike Crowe, Nicole Rabe and Melody Polych, for their hard work. We were the “first” GSA in Alberta’s history to incorporate under the Universities Act of Alberta. I hope that our goal of creating a “graduate community” is realized in years to come.

Thanks to each of my committee members, Dr. Drew Rendall, Dr. Gail Michener, and Dr. Tim Lysyk. Each of you helped develop my critical analysis skills. A special thanks to Tim for providing me with an “alternative view” to many ideas I had.

And finally, I thank my family and friends. The love and support of my parents, Mike and Jackie Schleppe, helped me stay on track during the course of this thesis. Also, my brother Ryan, sister Tara, and friends always pushed me to go forward, no matter how much I whined. Finally, I thank Misty Grimshaw for being there for me while writing this thesis. She probably understood more than anyone what a task this actually was. This thesis is dedicated to my family and friends who kept me going forward when the rigors of graduate school made it feel like I was not. Without their love and support I would never have seen the light at the end of the tunnel.

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## **Chapter 1: General Introduction**

### **General Introduction**

The study of parasitology has now become part of mainstream ecology and evolutionary biology. Most modern texts include specific chapters devoted to parasitism (e.g. Begon *et al.* 1990; Smith 1996) or contain parasite examples throughout the text (e.g. Cockburn 1991; Thompson 1994). In addition, the concepts of parasitology have figured prominently as 'special symposia' within meetings of several recent ecological and evolutionary societies over the past decade (e.g. International Congress of Ornithology, Ecological Society of America, American Society of Naturalists). Such attention points to the resurgence of parasitism as an important field of study in its own right (Poulin 1995; Price 1980) and in its applicability to several important concepts within mainstream ecology. Thus, the conceptual and empirical background underlying concepts such as co-evolution, speciation, specialization, evolution of sexual reproduction and population regulation almost always contain reference to the intimate interactions between parasites and their hosts (Combes 2001; Poulin 1998).

Yet an important shortcoming of this recent attention, as highlighted by Poulin (1998) and Combes (2001), is the shortage of appropriate model host/parasite interactions that can be applied to recent advances made in mainstream ecology. For example, the interaction between red grouse and their caecal nematode is probably the most often-cited example of parasite-induced regulation of host populations. In a study spanning 10 years, Hudson *et al.*

(1998) showed that the removal of parasites from host individuals lead to a cessation of host population cycles. However, the generality of this result is unknown because the hosts are maintained at artificially high densities to support local sport hunting operations. Similarly, the regulation of Soay Sheep numbers by gastro-intestinal nematodes occurs on an island where the sheep were introduced and where no predators have ever existed (Gulland 1992). Lastly, the idea that parasites can select for the maintenance of sexual reproduction in their hosts (Herre 1993) and can lead to altered host genotypes (Haldane 1949) is based upon systems involving nematodes that have direct life cycles or on systems that have been highly altered by humans. Unfortunately, similar types of questions are rarely asked in long-standing, relatively pristine systems where the most common parasites have more typical complex life cycles. The problem is that we are left with only a rudimentary (and perhaps biased) understanding of the extent to which the concepts of parasitology can be applied to questions related to host regulation, host-parasite co-evolution, and the evolution of host sexual reproduction.

An appropriate model system would be one that could be studied under relatively pristine natural conditions, yet could also be examined under controlled laboratory conditions where parasite 'dose' could be manipulated. Moreover, because multiple species infections are common under natural conditions, more than one species should be included in order to examine for general responses and to evaluate for synergistic effects. The interaction between the fathead minnow (*Pimephales promelas* Rafinesque) and its suite of encysting larval

trematodes is a candidate model system. Previous studies on this system provide a solid foundation for understanding natural patterns of trematode infection within pristine minnow populations in northern Alberta, Canada (Sandland *et al.* 2001). There is also background information on effects of one common trematode (*Ornithodiplostomum ptychocheilus* Faust) on host growth and survival (Sandland and Goater 2000), parasite development rates (Sandland and Goater 2000), over winter survival of parasites and hosts (Sandland 1999), and on behavioral effects of infection (Shirakashi 2002; Shirakashi and Goater 2001). The other common trematode of fathead minnows in northern Alberta is *Posthodiplostomum minimum* McCallum (Sandland 1999). Infections of this parasite result in inflammatory responses in minnows and sometimes parasite-induced host mortality occurs when intensities are high (Mitchell *et al.* 1982). These studies on fathead minnows, combined with parallel studies on various other cyprinid fish and their larval parasites in Finland (Valtonen *et al.* 1997), Scotland (Barber and Crompton 1997), and the USA (Lafferty and Morris 1996; McDowell *et al.* 1992; Radabaugh 1980) indicate that this interaction fulfills many of the requirements for an ideal model host/parasite interaction.

The purpose of my study was to utilize the minnow/trematode model system to further understand various features of the ecology of host/parasite interactions. My main aim was to evaluate the consequences of trematode infection on minnow reproduction (Chapter 4). Sandland (Sandland 1999; Sandland and Goater 2000; Sandland and Goater 2001) and Shirakashi (Shirakashi 2002; Shirakashi and Goater 2001) have documented effects of

infection on minnow survival and behavior, respectively, but it is unknown whether such effects transcend into effects on host fitness. For instance, although Shirakashi and Goater (2002) documented reduced visual acuity of minnows infected with *O. ptychocheilus*, it is unknown whether such effects ultimately lead to reduced reproduction at the population level. The question of parasite-induced effects on host reproduction is central in parasite ecology because such effects are a specific assumption of models of parasite-mediated natural selection (Goater and Holmes 1997), parasites and the evolution of sex (Hamilton 1980), and parasite/host co-evolution (Lively 1996). Only in very rare cases is this assumption explicitly tested.

Before undertaking an examination of parasite-induced effects on host reproduction, I aimed to further characterize natural rates of transmission within two populations of minnows in north-central Alberta (Chapter 2). This component of my study is a direct extension of the work by Sandland *et al.* (2001) on a similar system, in a neighboring locality. My work makes two important advances. First, I examined patterns of infection within snail intermediate hosts and second, I incorporated a second species of trematode (*Posthodiplostomum minimum*) into my monitoring study. The overall aim of this component of my study was to describe natural rates of transmission between snails and minnows, primarily to help interpret the results of the host-reproduction experiment.

A final aim of my study was to evaluate inter-specific differences in the life histories of the two most common species of trematode found in minnows in Alberta lakes. This study is made possible by my ability to maintain both of these

species in the laboratory. Because both species require the same sequence of hosts, it is possible to control confounding environmental factors so that realistic estimates of parasite reproduction rates (within two stages of the life cycle) can be obtained. There are almost no examples present in the literature that provide even crude estimates of lifetime reproduction for a parasite with a complex life cycle, and there are none that can compare such estimates between two species. This component of my study is motivated, in part, by Poulin's (1995) concern that the recent interest in parasitology by ecologists and evolutionary biologists tends to focus on the effects (ecological and evolutionary) of infection on hosts, rather than on the biology of the parasites themselves.

#### *General Ecology of Parasites and Hosts*

The parasites *Ornithodiplostomum ptychocheilus* and *Posthodiplostomum minimum* are the two most common larval strigeids found in fathead minnows (*Pimephales promelas*) of north central Alberta (Sandland 1999; Sandland *et al.* 2001). Both species have a typical trematode life cycle that involves a piscivorous bird as final host, the snail *Physa gyrina* Say as first intermediate host, and fathead minnows (*Pimephales promelas*) as second intermediate hosts. Mergansers or blue herons probably act as final hosts (Hoffman 1960), but it is unknown if other migratory birds also serve as hosts for these species. In northern Alberta, sympatric species of snail and fish are never infected with these parasites, indicating host specificity of first and second hosts in the life stages. Adult parasites reside in the intestinal tract of birds and release eggs that hatch into ciliated miracidia. Miracidia are small, short-lived (~ 36 h) larvae

that rely on nutrient reserves to actively seek and infect the snail host (Hoffman 1958). Once infected, miracidia reproduce asexually in the snail host and produce cercariae. Cercariae are free-swimming and seek and penetrate the epidermis of minnow hosts. Once cercariae have penetrated the minnow host, a period of density-dependent development (Sandland and Goater 2000) occurs before larvae encyst as metacercariae. Metacercariae of both species encyst in different regions of the minnow host. Thus, *O. ptychocheilus* cercariae migrate up the spinal cord to the brain of the minnow (Hendrickson 1979) and the larvae encyst in the optic lobes (Radabaugh 1980). Conversely, *P. minimum* cercariae encyst as metacercariae in the mesenteries of minnows. Once the metacercariae encyst in the brain or mesenteries, they presumably remain dormant until final hosts consume minnows. In north central Alberta, the prevalence of *O. ptychocheilus* and *P. minimum* is usually 100% and individual minnows can harbor as many as 600 worms (Sandland 1999; Sandland *et al.* 2001).

Fathead minnows are common in shallow, eutrophic lakes throughout most of North America (Scott and Crossman 1973). The general reproductive behavior, population biology, and community structure of fathead minnows is well documented. In particular, studies on minnow reproductive behavior include sexual behavior (Cole and Smith 1987; Isaak 1961; McMillan and Smith 1974), sex ratios (Shaw *et al.* 1995a), breeding density (Shaw *et al.* 1995b), and male parental care (Unger 1983; Unger and Sargent 1988). In Alberta, ongoing research on the community and population structure of minnows provides a solid

basis for this research (Price *et al.* 1991; Robinson and Tonn 1989). In north-central Alberta, adults of the snail, *Physa gyrina*, are typically 8-12 mm long and breed in the early spring after ice off (Sankurathri and Holmes 1976). New cohorts of snails typically appear in June or July and grow throughout the summer until ice covers the lakes. Periods of post-breeding mortality usually occur and snails migrate from shallow waters to deeper waters, prior to each winter (Sankurathri and Holmes 1976).

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and perch (*Perca fluviatilis*) in four lakes in central Finland. Canadian Journal of Fisheries Aquatic Science 54:572-585

## Chapter 2:

### Population dynamics of two species of strigeid trematode in snails and minnows from two contrasting habitats

#### Abstract

Previous studies on minnows from 4 lakes in north-central Alberta, Canada, found that the intensities of two common strigeid trematodes (*Ornithodiplostomum ptychocheilus* and *Posthodiplostomum minimum*) varied extensively between and within lakes. The purpose of this follow-up study was to determine the population dynamics of these two trematode species in their intermediate hosts to understand factors leading to variation in metacercarial intensities. Monthly collections of *Physa gyrina* (snail host) and *Pimephales promelas* (fathead minnow host) were obtained from a cool, oligotrophic lake and a neighboring, warm eutrophic slough. Intensities of both species varied between and within lakes. Components of the variation could be attributed to host size, habitat, species, and their interactions. Inter-lake variation in metacercarial intensities could not be attributed to observed differences in snail-density or size, even though snails were biannual in the eutrophic slough. Inter-lake differences in minnow life histories also played no role in determining variation in metacercarial intensities. No single factor could be identified to explain inter-lake variation in metacercarial intensities, although subtle differences in lake temperature, especially in the late summer, likely played a role.

## Introduction

A primary goal for parasitologists and epidemiologists is to understand the factors that lead to variation in parasite intensities between and within individual hosts populations. This focus is important, mostly because identification of factors that lead to high intensities, or 'worminess', is central to development of control strategies and to identification of conditions leading to high parasite exposure. Studies involving larval trematodes (metacercariae) in their second intermediate hosts provide good model systems because they demonstrate extremely high variation in intensity between individual hosts, and they can be studied under field and laboratory conditions. Factors such as habitat structure (Sousa and Grosholz 1990), host population structure (Kennedy *et al.* 2001), host genetics (Grosholz 1994), host size (Chubb 1979), and anthropogenic disturbance (e.g. Marcogliese and Cone 1997) have been shown to play a role in determining variation in metacercarial intensities, in particular parasite/host interactions.

Sandland *et al.* (2001) showed that metacercarial intensities of the trematode *Ornithodiplostomum ptychocheilus* Faust varied extensively in second intermediate host (*Pimephales promelas* Rafinesque) collected from 4 lakes in north-central Alberta, Canada. Intensities varied by up to 2 orders of magnitude, even between age- and size-matched fish (Sandland *et al.* 2001). Within-lake variation in parasite intensities was due mostly to seasonal and yearly differences in cercarial transmission from snails, although variation in minnow size was also important. Sandland *et al.* (2001) also found that *O. ptychocheilus*

intensities in minnows varied extensively between the 4 lakes. In particular, they found that *O. ptychocheilus* was more common in shallow eutrophic lakes that were warmer and contained more vegetation than cooler, oligotrophic lakes. In contrast, intensities of another common trematode *Posthodiplostomum minimum* (McCallum) were typically higher in the cooler oligotrophic lakes (Sandland 1999). The authors speculated that differences in water temperature and the densities and life histories of first intermediate hosts (*Physa gyrina* Say) could help explain this inter-lake variation.

The purpose of my study was to characterize the population dynamics of *P. minimum* and *O. ptychocheilus* metacercariae in minnows collected from two contrasting lake types. One aim was to test several of the predictions of Sandland *et al.* (2001) to better understand the factors that lead to high variation in metacercarial intensities in fish. My study focused on samples of snails and minnows collected from 2 lakes that represented the 2 most common habitats sampled by Sandland *et al.* (2001). Chain Lake is a relatively large, oligotrophic lake that contains little aquatic vegetation, whereas Rochester Lake is a small, highly eutrophic slough that by late summer is matted with extensive macrophyte growth. A further aim was to determine whether variation in the densities and life histories of snail and minnow hosts was associated with variation in metacercarial intensities.

## Materials and Methods

### *Parasites*

Both *O. ptychocheilus* and *P. minimum* use the snail, *Physa gyrina*, and the fathead minnow, *Pimephales promelas*, as first and second intermediate hosts, respectively. In northern Alberta, sympatric species of snail and fish have never been found to be infected with either of these species (Sandland 1999; Sandland *et al.* 2001). *Ornithodiplostomum ptychocheilus* metacercariae encyst in the brain of minnows, typically in the optic tectum (Radabaugh 1980), whereas *P. minimum* encysts in the mesenteries.

### *Snail Collections*

The protocol used to sample snails provided estimates of seasonal changes in snail density, size distributions, and trematode infections in the 2 lakes. Snail density was assessed in each lake as the number of snails recovered during a series of 12–20, 30-s sweeps of a standard sampling net (mesh size = 2 mm), following the methods of Sankurathri and Holmes (1976). I considered this method as the only one that could be repeated in both lakes, primarily due to differences in aquatic vegetation and substrate. Each 30-s sweep sampled approximately 1 m<sup>3</sup> of water and vegetation. All snails sampled from each sweep were counted and measured for maximum shell length using digital calipers. Sampling sites were chosen to encompass all microhabitats within each lake and, in total, always covered at least 30 - 40% of the perimeter of the lake. At each sampling site, the surface temperature of the water ( $\pm 0.5$  °C)

was measured within 50 cm of the surface. Collections were made in 2001 on 3-7 May, 2-7 June, 6 - 9 July, 30-31 July and 7-9 September.

Seasonal changes in the prevalence of larval trematodes were estimated by monitoring infections within the 2001 cohort. Samples of snails from each 30-s sweep were selected, until a total of 50 was reached, and then brought back to the laboratory. All samples of snails were checked for the release of cercariae using standard techniques (see Sandland and Goater 2001). If no cercariae were present, the snails were crushed and examined for immature infections under a dissecting microscope. Cercariae were exposed in batches of 30 to at least 5 fathead minnows to facilitate cercariae identification following the methods of Sandland (1999). Snails that were <5 mm were reared in groups of 10 in small plastic containers (25 X 15 X 11 cm) for 30 d to allow the development of pre-patent infections, then checked for infections. Snails were reared on a 12:12 light:dark photoperiod and fed *ab libitum* on lettuce and Tetramin fish flakes.

#### *Minnow Collections*

Collection protocols and sampling dates for minnows paralleled those used to collect snails. Monthly collections were designed to allow evaluation of minnow growth rates and to evaluate the rate of cercarial transmission into adult (> 1 yr.), juvenile (1 yr.) and young of the year (YOY, < 1 yr.) minnows. Thus, I attempted to collect the 1999 (adult), 2000 (juvenile), and 2001 (YOY) cohorts of minnows from Chain Lake and Rochester Lake. Sampling constraints severely restricted my ability to sample the 2000 cohort from Rochester Lake. Thus, I

collected adult minnows (1999 cohort) from Chain and Rochester Lake in May and June 2001 respectively, and had comparable collections of YOY (2001 cohort) minnows from August and September.

Adult, juvenile and YOY minnows were typically sampled at the same sites where snails were sampled. Juvenile and YOY minnows were sampled by sweeping vegetation with standard dip nets (same as snail nets), similar to the methods of Sandland (1999). Adult minnows were captured using 12 un-baited Gee minnow traps set for 12 h starting at 1700-1800 h. All captured fish were anesthetized in MS 222. Juvenile and YOY minnows were preserved in 70% ethanol for transport and adult minnows were frozen.

The eyes, brain, mesenteries, and body musculature were dissected for 30 randomly-selected minnows from each cohort in each month. Total length, standard length, and weight were determined for each necropsied fish.

### *Analyses*

Differences in snail density (number of snails/30-s sweep) were analyzed using a 2-way ANOVA, where lake and month were fixed effects. To meet normality assumptions, estimates of snail density were Box-Cox transformed. Growth of YOY minnows was compared for each lake using the non-parametric Kruskal-Wallis median test because these data could not be normalized. Adult minnow size did not need to be transformed and were compared using a 2-way ANOVA, where gender and lake were fixed effects. Finally, a 3-way ANOVA was used to examine the effects of lake, species, and host age on mean *O. ptychocheilus* and *P. minimum* intensities.

## Results

### *General*

The temperature profiles within the two lakes were similar (Fig. 1). In May, the mean temperature of Rochester Lake was slightly higher than Chain Lake, whereas in September the mean temperature was slightly lower. Although temperatures peaked in August in both lakes, the mean temperature in Chain Lake was 3 °C cooler and never exceeded 20 °C (Fig. 1). The mean temperature of Chain Lake ( $12.3 \pm 4.1$  °C,  $n = 36$ ) over all months was significantly lower than the mean temperature of Rochester Lake ( $16.2 \pm 4.9$  °C,  $n = 15$ ) (Kruskal-Wallis,  $\chi^2 = 9.3$ ,  $p = 0.002$ , d.f. = 1). There were also marked differences in macrophyte abundance and cover between the two lakes. In general, macrophytes tended to cover the entire perimeter of Rochester Lake in a thick mat that grew from the substrate to the surface. In contrast, macrophytes in Chain Lake were patchy in distribution and could rarely be seen on the surface of the lake.

### *Demography of Snail and Minnow Populations*

There were large differences between the two lakes in the growth and reproduction of snails. Most significantly, snails in Rochester Lake had two generations per year, whereas snails in Chain Lake were annual (Fig. 2). In Rochester Lake, the sub-population of large (approximately 12 mm maximum shell length) over-wintering snails began producing the F1 generation almost immediately after ice-off in late May. The growth rates of the F1 generation peaked in July and August, with the result that some of these snails produced a second generation in late summer or early fall. In contrast, over-wintering snails

in Chain Lake were 50% smaller than those in Rochester Lake and these did not begin breeding until at least one month later. This delay, coupled with the slower growth rates of F1 snails (Fig. 2), restricted *Physa* in Chain Lake to one generation per year.

Snail density varied between lakes and months (ANOVA, month X lake;  $F_{3,139} = 8.2$ ,  $p < 0.0001$ , Fig. 3). This interaction can be explained by the slower increase in the density of snails in June and July in Chain Lake compared to Rochester Lake. There were significant differences in densities of snails between lakes (ANOVA, lake;  $F_{1,139} = 31.4$ ,  $p < 0.0001$ , Fig. 3) and there was a seasonal pattern in snail density (ANOVA, month;  $F_{3,139} = 246.9$ ,  $p < 0.0001$ , Fig. 3) that could be explained by the appearance of the F1 generation commencing in June. Snail density declined in September in both lakes.

Breeding minnows from Chain Lake were significantly larger than breeding minnows from Rochester Lake (ANOVA, lake;  $F_{1,58} = 172.4$ ,  $p < 0.0001$ , Table 2) and in both lakes, males were significantly larger than females (ANOVA, gender;  $F_{1,58} = 29.9$ ,  $p < 0.0001$ , Table 2). September-collected YOY were the same size in the two lakes (Student's t-test,  $t_{55} = 0.086$ ,  $p = 0.93$ , Table 2) and there was a significant increase in YOY size between August and September (Chain Lake, Kruskal-Wallis;  $\chi^2 = 13.2$ ,  $p = 0.0003$ , d.f. = 1; Rochester Lake, Kruskal-Wallis;  $\chi^2 = 11.27$ ,  $p = 0.0008$ , d.f. = 1).

#### *Patterns of Infection in Snail and Minnow Populations*

Four species of larval trematodes were found in snails sampled from the two lakes; three occurred in Rochester Lake and one in Chain Lake (Table 1).

*Ornithodiplostomum ptychocheilus* was never found in any of the 429 snails sampled from either of the two lakes and *P. minimum* was found in only a single individual from Chain Lake. Therefore, the prevalence of infection of these two species in these lakes was less than 1% of the overall snail population. Peak prevalence of all trematodes in the overall snail population occurred in August, followed by a decline in September (Fig. 2). Overall, a total of 17.3% of September-collected snails from Rochester Lake were infected with larval trematodes, and approximately half of these could be attributed to an unidentified strigeid trematode (furcocercous cercariae). Infections in Chain Lake were much rarer.

Mean metacercarial intensities in minnows were significantly affected by species, size, and lake (Table 3). Interactions between these factors were also important (Table 3). For example, the lake by species interaction can be explained by the fact that *P. minimum* intensities were significantly higher in Chain Lake than in Rochester Lake, yet intensities of *O. ptychocheilus* were similar between Lakes (Fig. 4, Tukey-Kramer HSD). These results indicate that complex interactions between lake, age, and species all affect metacercarial intensities in these lakes.

### **Discussion**

Transmission of *O. ptychocheilus* and *P. minimum* was highest in late summer and early fall, especially into the largest minnows within a cohort. Also, metacercarial intensities were at least one order of magnitude higher in adult vs young-of-the-year fish, indicating accumulation with age. These general findings

are consistent with results from a large number of studies involving metacercariae in small-bodied fishes (Chappell 1995; Chubb 1979). Similarly, the demonstration of extensive inter-specific and inter-lake variation in cercarial transmission is also consistent with other studies (e.g., Aho *et al.* 1982; Bergeron *et al.* 1997; Coleman and Travis 1998; Marcogliese and Conmpagna 1999), including the study by Sandland *et al.* (2001) involving the same parasites in similar types of lakes.

Sandland *et al.* (2001) showed that intensities of *O. ptychocheilus* metacercariae were far higher in eutrophic lakes compared to those in adjacent oligotrophic ones. They speculated that the shallow, eutrophic nature of these lakes led to conditions that were ideal for the transmission of cercariae from *Physa gyrina*. Rochester Lake was selected for its similarity to the two high-intensity lakes of Sandland *et al.* (2001); it is a shallow, warm-water slough that contains high densities of snails and minnows and extensive vegetation cover. Yet intensities of infection in Rochester Lake were much lower than the lakes studies by Sandland *et al.* (2001), and they were also much lower than in the deeper, larger and oligotrophic Chain Lake. These results support Kennedy's (1990) contention that no single factor (e.g. host densities, aquatic productivity, waterbody size) can be used to predict variation in metacercarial intensities between populations of hosts. Instead, such variation is due to a complex suite of factors that affect magnitude and duration of cercarial transmission within localized habitats.

One explanation for the inter-lake differences in parasite intensities is temperature. Chubb (1979) identified temperature as the single most important factor affecting metacercarial intensities in fish because rates of cercarial development, release, and infectivity are strongly temperature dependent. Thus, the contrasting temperature profiles of Chain Lake and Rochester Lake likely contributed to the variation in intensities of *O. ptychocheilus* and *P. minimum*. Chain Lake remained at or near 15 °C for at least one month longer than Rochester Lake and this extended period of warmer temperature coincided with peak cercarial transmission. Also, *P. minimum* cercariae are more infective at 20 °C than *O. ptychocheilus* cercariae, which corresponds with the mean temperature in Chain Lake during cercarial transmission (Chapter 3). This subtle difference in temperature may have led to higher metacercarial intensities of *P. minimum* and *O. ptychocheilus* in Chain Lake and the inter-specific differences within Chain Lake. My laboratory study (Chapter 3) indicated that even small differences in temperature could lead to large increases in the numbers of cercariae shed from snails and in cercarial infectivity in minnows.

An alternative explanation (Sandland *et al.* 2001), is that inter-lake differences in intensities are due to events occurring within first intermediate hosts. My results do not support this suggestion. First, *O. ptychocheilus* infected snails were not more common in Rochester Lake, nor were *P. minimum* infected snails more common in Chain Lake. Rather, the prevalences of infection in both lakes were so low that differences could not be detected. Second, the occurrence of an additional generation of snails in Rochester Lake, coupled with

their much higher densities and larger size, seemed to have little effect on cercarial transmission. These results suggest that the marked differences between Chain Lake and Rochester Lake in snail demographics, densities, and life histories have little impact in determining inter-lake variation in metacercarial intensity.

A further explanation, as suggested by Sandland *et al.* (2001), is that inter-lake differences in intensity are due to differences in minnow densities and life histories. One explanation for the higher intensities in Chain Lake is that minnows in this lake are larger and older. Accumulation of metacercariae with host age is a typical finding (review by Chubb 1979), as is the increase in transmission into larger hosts (review by Chubb 1979). However, collections over the past 2 years in Chain and Rochester Lakes indicate that adult minnows in both lakes typically reach 3 years of age before senescing (unpub.) and it is unlikely that the large differences in intensity between the lakes are due to the small differences in host size. Thus, the explanatory power of differences in minnow densities and life histories between the two lakes also seems to be low.

Kennedy (1990) suggested that the difficulty in explaining variation in parasite intensities between populations of hosts is due to the fact that stochastic events could likely play an important role in determining variation in transmission. For example, Kennedy (1987) showed that the most important factor responsible for determining spatial and temporal variation in eyefluke intensity in perch was the visitation of a single pair of breeding grebes to a lake in southern England. The occurrence of this pair was completely unpredictable between years, and

between lakes. Various migratory birds may act as the final host for *O. ptychocheilus* and *P. minimum*, and variation in bird density may similarly affect metacercarial intensities. Several piscivorous bird species were observed at both lakes and fledgling birds were present during the period of trematode transmission into snails. The contributions of bird final hosts to overall patterns of transmission are completely unknown in this system.

Kennedy (1987) described the opportunity available for cercarial transmission as a transmission window. He further suggested that understanding the dimensions of this window could help explain variable infection levels. The results of my field collections, coupled with my results on the development rates of larval stages of *O. ptychocheilus* and *P. minimum* (Chapter 3), allows me to characterize the timing of various transmission events (Fig. 5). Blue herons and other piscivorous birds arrive on these northern lakes in approximately late May and are exposed to infective metacercariae with every ingested fathead minnow. After a short period of development in the gut (3 days, Chapter 3), eggs are released into the water. Development of miracidia within the eggs takes approximately 10–16 days at 20 °C. Thus, infective miracidia will be available in mid-June under ideal conditions (Fig 5.). This period coincides with the emergence of the new cohort of F1 snails. Infection studies (Sandland and Goater 2001) indicate that *Physa* is most susceptible when snails are less than 5 mm in length. Subsequent development of these parasites in snails takes at least 28 days under ideal laboratory conditions (Chapter 3), meaning that minnows cannot be exposed to cercariae until mid-July at the earliest. Esch and

Fernandez (1993) suggested that transmission windows of trematodes are narrow from a temporal standpoint. In this situation, the window available for the transmission of cercariae to minnows in these two lakes is quite restricted and is also likely to be extremely variable between seasons, lakes, and years. For example, any conditions that increase the growth rate of snails in Rochester Lake will restrict their exposure to miracidia. Likewise, any conditions that prolong the optimal period of cercarial release (long, warm periods in autumn) will increase the period available for transmission of cercariae to minnows.

In summary, the results from this study indicate that our understanding of the factors leading to variation in infection levels between hosts is still rudimentary. Although the design of this study was aimed to clarify the link between habitat type (eutrophic vs oligotrophic) and intensity (Sandland *et al.* 2001), my results indicate that the findings of Sandland *et al.* (2001) are not general. Thus, eutrophic lakes, even if they reach high peak summer temperatures and contain extremely high densities of snail intermediate hosts, are not necessarily most heavily infected with minnow parasites. Given the very low prevalences of infection in snails for both species of parasites in both lakes (<1%), it is possible that infection levels in minnows are determined primarily by highly localized, stochastic 'downstream' events that restrict the exposure of snails to these trematodes.

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Table 1: Prevalence (% of infected snails) of larval trematodes in snails sampled between June and September 2001 from Chain and Rochester Lake.

Species	Chain Lake Prevalence % (N = 213)	Rochester Lake Prevalence (N = 216)
Plagiorhid (leptocercous cercariae)	0	10.9
Unknown Strigeid (furcocercous cercariae)	0	10.5
<i>Posthodiplostomum minimum</i>	0.05	0
<i>Ornithdiplostomum sp.</i>	0	0.5

Table 2: Mean ( $\pm$ S.D.) total length (mm) of minnows sampled from Chain Lake and Rochester Lake in northern Alberta, Canada. Adults were sampled in May and June from Chain and Rochester Lake, respectively, whereas samples of young of the year (YOY) minnows were taken in September. Numbers in brackets represent sample sizes.

Gender	Chain Lake	Rochester Lake
Male	73.8 $\pm$ 6.15 (13)	59.4 $\pm$ 2.2 (19)
Female	67.9 $\pm$ 4.7 (19)	53.0 $\pm$ 3.5 (11)
YOY	26.3 $\pm$ 4.4 (29)	26.2 $\pm$ 3.4 (28)

Table 3: Summary ANOVA statistics for the effects of lake, species, and size on metacercariae intensity of the trematodes *O. ptychocheilus* and *P. minimum* in fathead minnows.

Source of Variation	df	Mean Square	F-Value	P-Value
Lake	1	53.6	89.5	<0.001
Species	1	1.3	2.2	0.14
Age	1	127.8	215.6	<0.001
Lake*Species	1	45.7	77.2	<0.001
Lake*Age	1	102.5	172.9	<0.001
Species*Age	1	11.8	19.9	<0.001
Lake*Species*Age	1	18.1	30.5	<0.001
Residual	162	0.59		

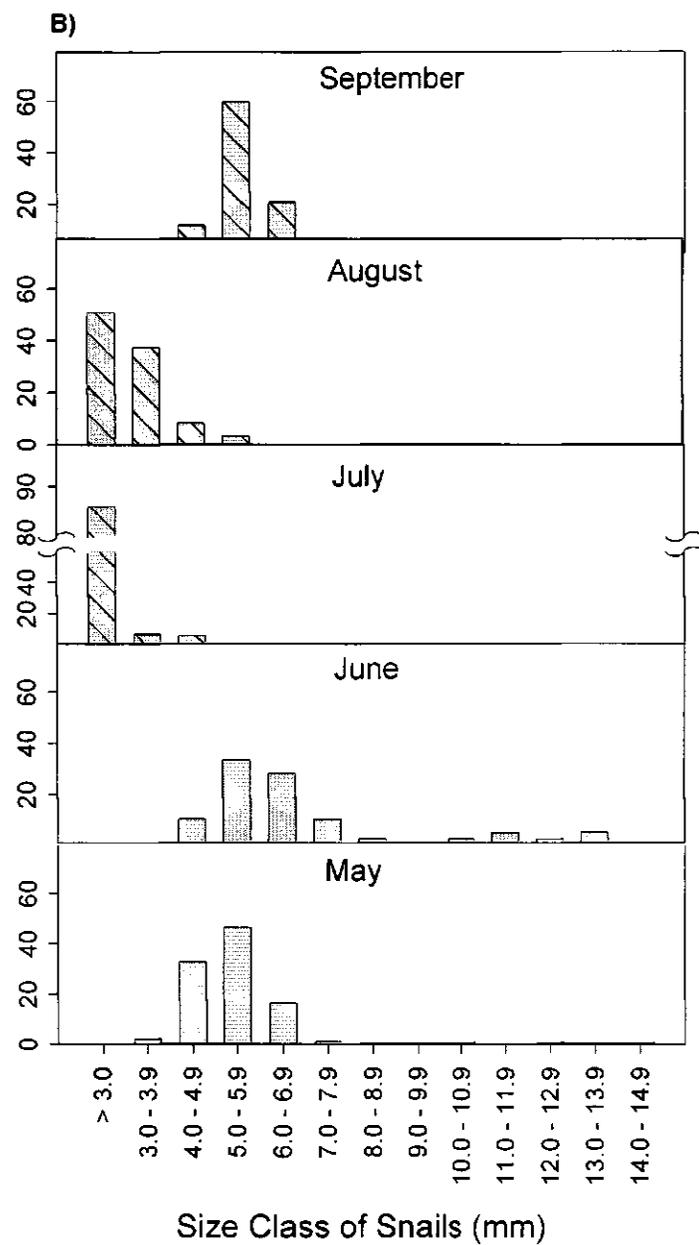
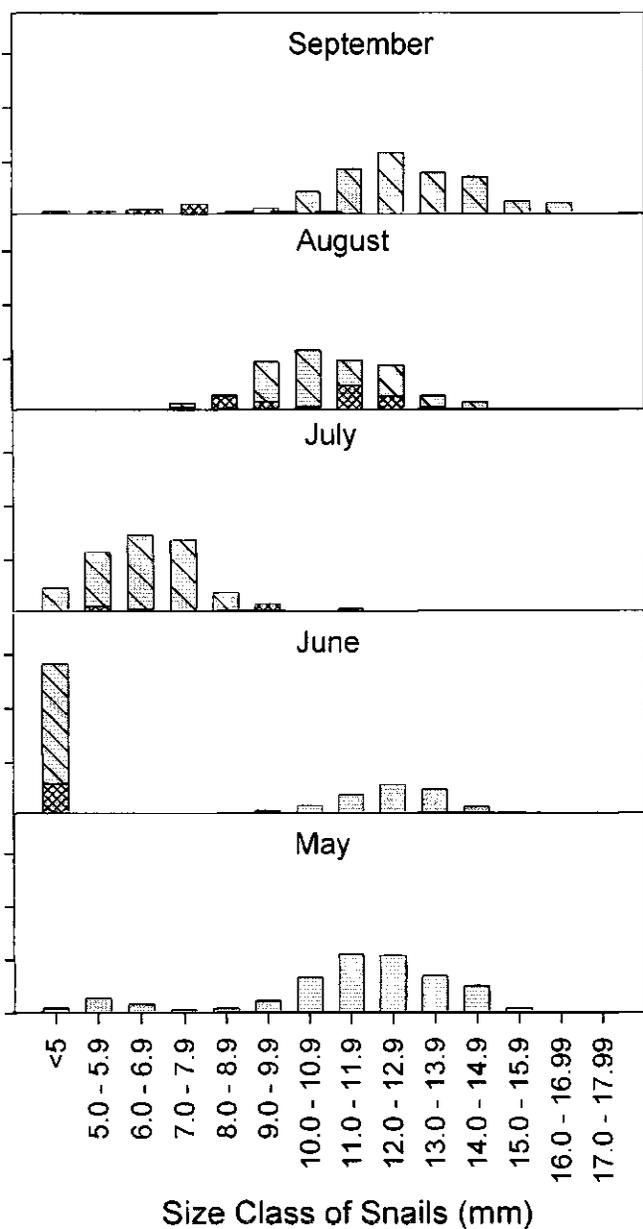


Figure 2: Size frequency distributions of *Physa gyrina* collected monthly between May and September from Rochester Lake (A) and Chain Lake (B) Lakes in northern, Alberta, Canada. Solid bars indicate adult snails and single hatching indicates juvenile snails. Cross hatched bars indicate the total prevalence of parasites in each size class of snails.

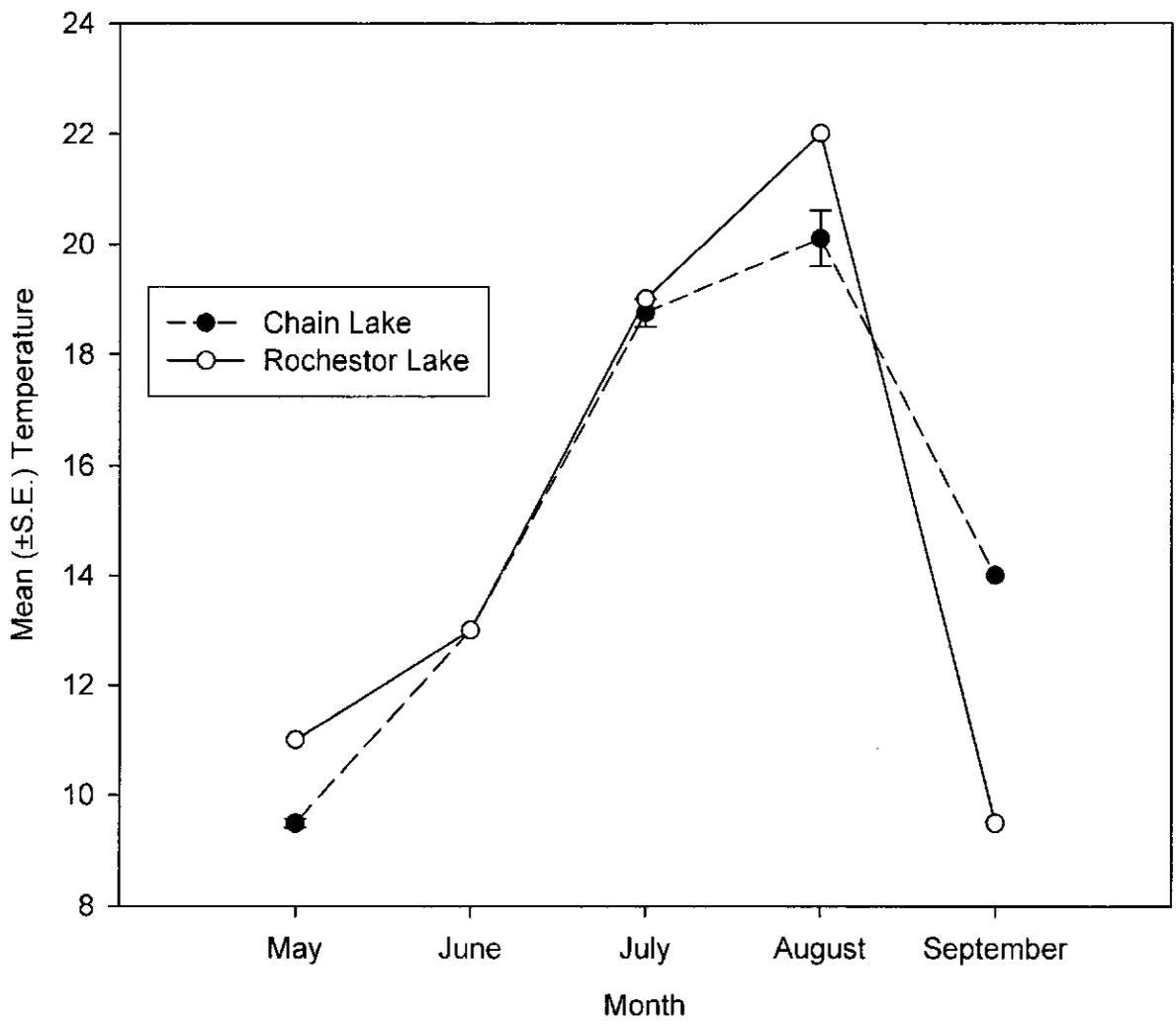


Figure 1: Temperature ( $\pm$ S.E.) profiles taken during the ice free period from May to September in Chain and Rochester Lake, Alberta, Canada

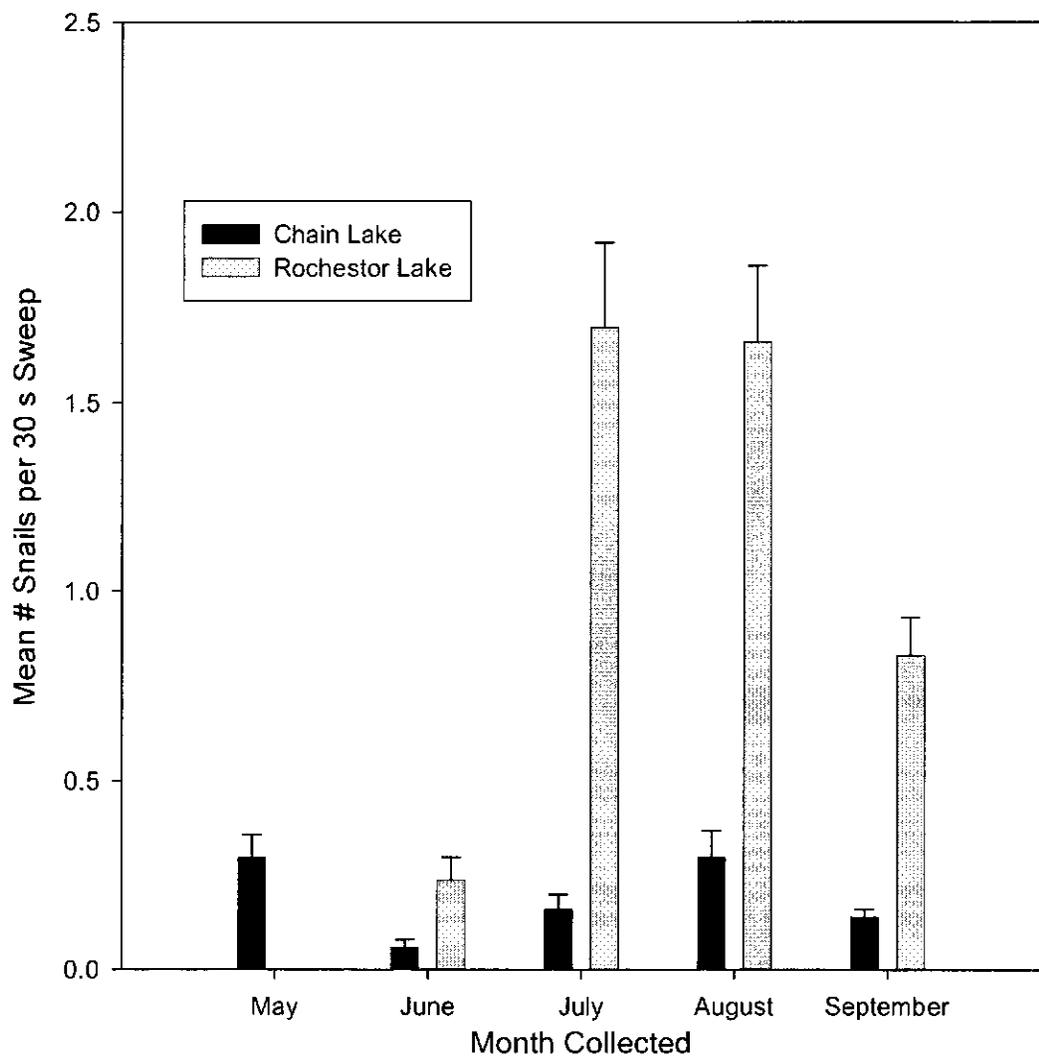


Figure 3: Mean number of snails (+SE) sampled during 30 s sweeps of a standard dipnet in Chain and Rochester Lake, Alberta, Canada.

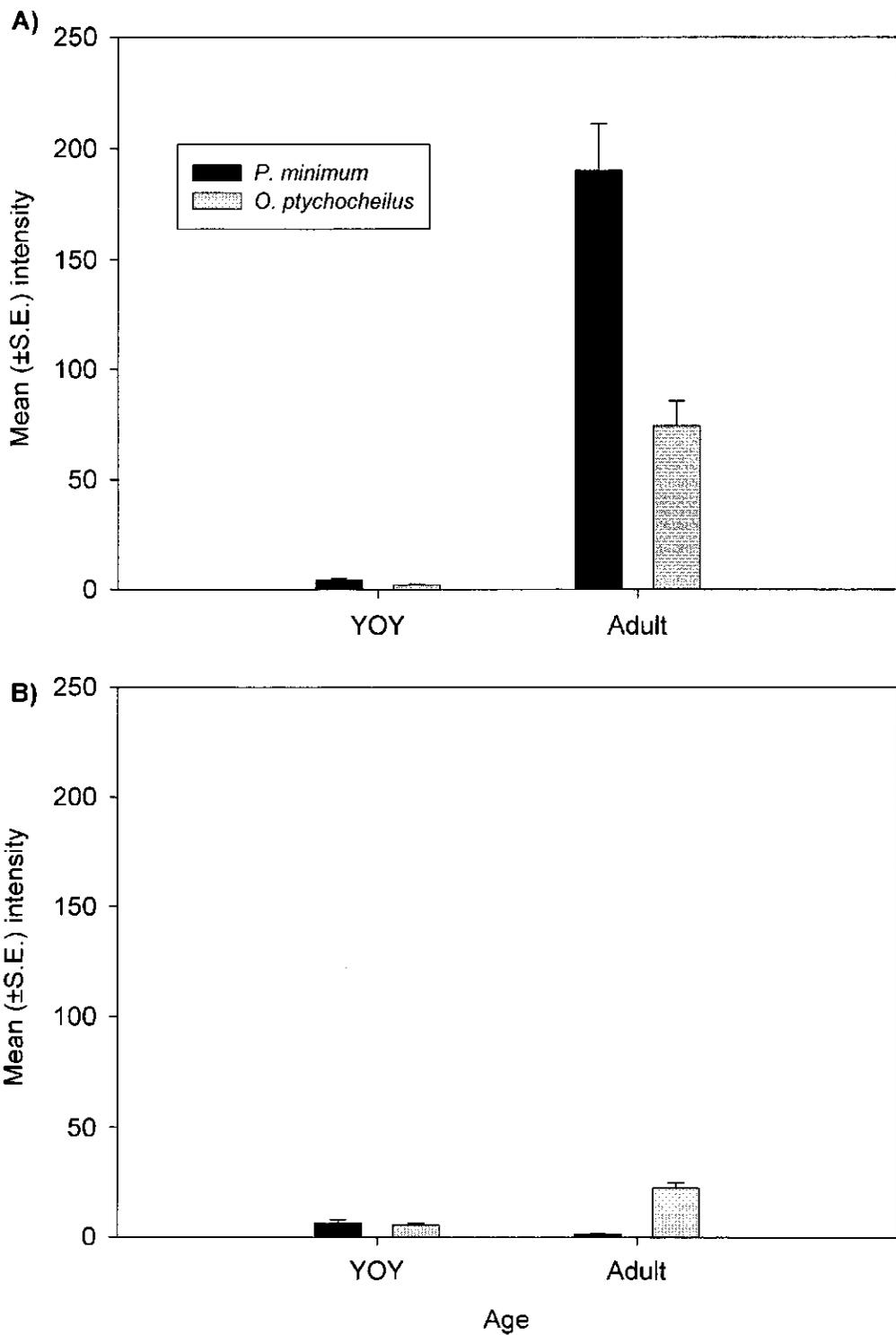


Figure 3: Mean ( $\pm$ S.E.) intensity of *P. minimum* and *O. ptychocheilus* metacercariae in adult and young of the year (YOY) minnows sampled from Chain Lake (A) and Rochester Lake (B).

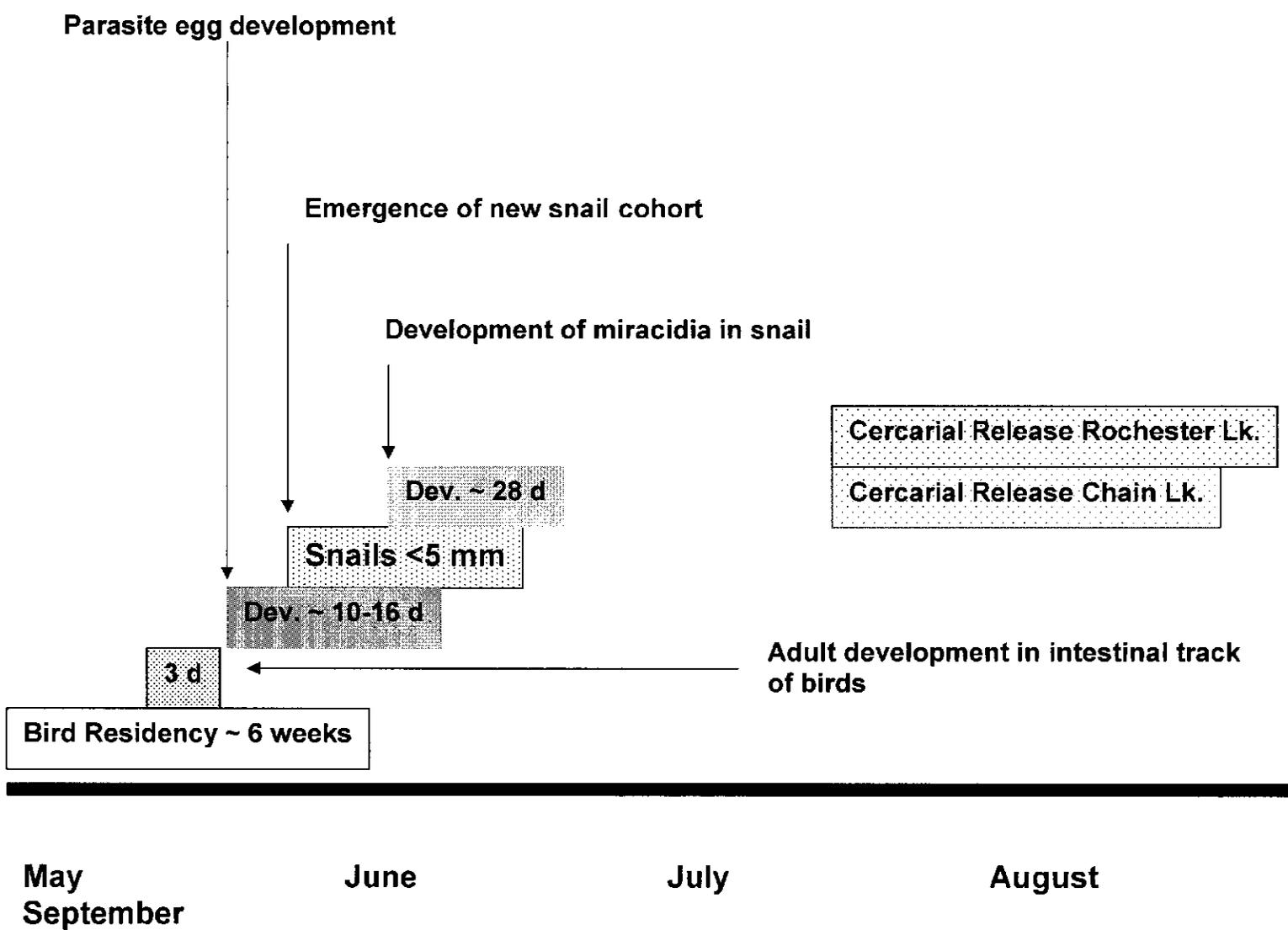


Figure 5: The timing of transmission events in the aquatic stages of the life cycles of *O. ptychocheilus* and *P. minimum* for populations in north-central Alberta, Canada.

### Chapter 3:

#### Comparative life histories of two sympatric species of strigeid trematode

##### Abstract

I evaluated inter-specific differences in the life-history characteristics of two species of strigeid trematodes (*Ornithodiplostomum ptychocheilus* and *Posthodiplostomum minimum*: Diplostomatidae) in their snail, fish, and bird hosts. Both species were small as adults (<2 mm), had short pre-patent periods in snail (~28 days) and bird hosts (<72 h), and the release of cercariae in response to changes in temperature and photoperiod were similar. Both species released more cercariae as temperature increased, similar to other trematodes, and both species released over 50% of their cercariae during the first 2 hours of daylight. Differences in adult worm size were correlated with fecundity, cercarial production, and metacercarial size. Most differences between these species could be explained by carry over effects of adult worm size. However, several differences could not be explained by adult size alone. For instance, peak cercarial infectivity of *P. minimum* was 5 °C lower than for *O. ptychocheilus*, and this temperature-dependent response could not be explained by adult or cercarial size. These results suggest that the use of similar hosts in their life cycle has selected for similar life histories in *O. ptychocheilus* and *P. minimum*. Subtle inter-specific differences may be explained by the contrasting habitats where the hosts of these parasites are most heavily infected.

## Introduction

The life history of an organism can be described as the manner in which it partitions its resources into growth and reproduction over its lifetime. Thus, characteristics such as adult size, the numbers and size of eggs and offspring, and the timing of current versus future reproduction are important life-history traits. Extensive attention from evolutionary biologists has demonstrated that the phenotypic expression of such traits is both variable and heritable, and therefore evolves by natural selection (reviews by Roff 1992; Stearns 1992). However, our current understanding of life-history variation is almost entirely based on free-living organisms (Roff 1992; Stearns 1992). This is a shortcoming for a number of reasons. First, at least 50% of all species adopt a parasitic life-style during at least one stage of their life cycle (Price 1980). Thus, life-history theory cannot be considered inclusive unless it includes organisms that adopt this common lifestyle. Second, parasitic organisms demonstrate a diverse array of life histories. Some species have simple life cycles involving a single host (e.g. many nematodes) whereas others have complex life cycles involving several hosts and several stages where either sexual or asexual reproduction can occur (e.g. many helminths, protists, copepods). Such variation provides an excellent opportunity to test specific predictions of life-history theory.

In theory, parasite life histories should be shaped by a combination of selection imposed by their hosts and also by their host's environment. A parasite's life-history strategy should therefore be one that maximizes reproduction within hosts and transmission between different hosts in the life

cycle (Poulin 1996). Two species that utilize the same sequence of hosts should presumably evolve similar life histories. Similarly, in the case where two species utilize the same hosts, most inter-specific differences in life histories should be due either to selection imposed by different environments inhabited by the hosts, to selection imposed by different micro-habitats within a given host, or to association by descent from common ancestors (review by Poulin 1995b). Poulin (1996) challenged workers to test these and other predictions by performing inter-specific tests similar to those done on a wide range of free-living organisms (e.g. Badyaev 1997; Clobert *et al.* 1998). Although there have been several attempts to meet this challenge, they all involve parasites with direct life cycles (i.e. single host) and they are restricted to comparisons among life-history characteristics that are available from literature surveys (e.g. Arneberg *et al.* 1998; Morand 1996; Poulin 1995a; Trouve *et al.* 1998).

*Ornithodiplostomum ptychocheilus* (Faust) and *Posthodiplostomum minimum* (McCallum) are strigeid trematodes that are sympatric within several lakes in north-central Alberta, Canada (Sandland 1999, Chapter 2). Both species utilize the snail *Physa gyrina* (Say) as first intermediate host, the fathead minnow (*Pimephales promelas* Rafinesque) as second intermediate host, and migratory birds (probably great-blue herons) as a final host. Sympatric species of snail and fish from these lakes have not been found to be infected with either of these 2 parasites (Sandland 1999). In the context of life-history variation, I predict that selection imposed by common hosts in the life cycles of these two species will

have resulted in similar life-history characteristics and that any differences will be attributable to differences in their hosts aquatic environment.

My aim is to compare selected life-history characteristics of the closely related trematode species *O. ptychocheilus* and *P. minimum*. Both species (and their hosts) can be maintained under constant laboratory conditions so that differences in morphological traits (adult, cercarial, and metacercarial size) and reproductive traits (cercarial production and adult fecundity) can be evaluated. In addition, a further aim of this study is to examine inter-specific differences in the production and pattern of cercarial shedding from snails due to temperature and photoperiod.

## **Methods**

### *Sources of parasites and uninfected hosts*

Analyses of various morphological and reproductive characteristics required hosts to be experimentally infected with one of the two trematode species. Methods used to infect snails, minnows, and birds with *O. ptychocheilus* are described in Sandland and Goater (2001). To initiate the laboratory infections, metacercariae were obtained from naturally infected minnows from Rochester Lake or Chain Lake, Alberta, Canada (Chapter 2). In those cases where uninfected minnows were required for infections, they were purchased from a commercial supplier (International Aquatic Organisms, NH, USA). Uninfected snails (*Physa gyrina*) were the lab-reared progeny of adult snails sampled at Rochester Lake. Day-old chickens (*Galus domesticus*)

purchased from Lilydale Hatchery (Lethbridge, Alberta) were used as surrogate final hosts to maintain infections and to provide a source of adult parasites.

#### *Inter-specific differences in trematode morphology and reproduction*

The aim of these experiments was to evaluate inter-specific differences in trematode life-history characteristics under constant environmental conditions. The initial focus was on evaluating differences in cercarial size. During the 11 - 15 June 2001, 40 snails (3-5 mm) were each exposed for 3 h to 5 miracidia of *O. ptychocheilus* or *P. minimum* following the methods of Sandland and Goater (2001). After exposure, snails were maintained under constant conditions until cercariae were released (20°C, 10 snails per container, 12-h photoperiod, fed lettuce and Tetramin fish flakes *ad libitum*). Snails were checked for the release of cercariae daily starting from 20 d post-infection. At 28 d post-infection, cercariae were collected over one 12-h light period (0900 – 2100) from 17 *O. ptychocheilus* and 30 *P. minimum* infected snails. The remaining snails did not release cercariae. Cercariae were collected by placing individual snails in small vials under an incandescent bulb. The vials (7 cm high X 2.5 cm diameter) were filled with approximately 15 ml of aged and aerated water. Cercariae were fixed in an approximate 1:2 10% formalin:water mixture and filtered using 50 µm mesh filter. Filtered cercariae were washed off the filter, pooled, and stored in 10% formalin. Initial investigations of this filtration method (counted directly before and after filtering) revealed that the number of cercariae recovered was within ±6% (n=3) of the actual number filtered. The maximum length of the head and

tail of the first 100 intact cercariae was measured at 400X on a compound microscope using an ocular micrometer to the nearest ocular unit.

Inter-specific differences in metacercarial size were evaluated from larvae (72 days old) that were dissected from 5 minnows exposed to 120 cercariae of each species on 16 August 2001. An ocular micrometer was used to measure the diameter of the entire cyst (1 parasite per cyst) and the maximum length of the intact parasite within the cyst. A cover slip (22 X 50 mm) was placed over the metacercariae before they were measured. The intensity of metacercariae found in minnow brains (for *O. ptychocheilus*) and mesenteries (for *P. minimum*) was used as an estimate of the rate of cercariae transmission into fish.

Inter-specific differences in adult trematode morphology and egg production were based upon adult parasites dissected from birds fed the brains or mesenteries of experimentally infected minnows. The minnows had been exposed to 120 cercariae 72 d prior to being used as sources of metacercariae and were infected according to the methods described above. Five birds were each fed 4 brains or mesenteries on 25 October 2001. The feces of each bird were collected on 29 and 31 October 2001 for 6 h beginning at 0900. Eggs were filtered from feces using the methods of Sandland and Goater (2001) and counted under a dissecting microscope.

Birds were killed by asphyxiation with CO<sub>2</sub> on 31 October 2001, frozen, and dissected 3 days later. The intestine of each bird was divided into 20 equal segments following the methods of Doster and Goater (1997). The contents of each section were removed and examined for adult parasites. The maximum

length of the hindbody and forebody, maximum width, and the maximum number of eggs within the uterus were determined for a maximum of 10 adults per section, using an ocular micrometer at 100X for *O. ptychocheilus* and 40X for *P. minimum*. Daily per capita trematode fecundity was estimated as the total number of eggs produced in 6 hr multiplied by 4 and divided by the total number of worms recovered at dissection.

#### *Effects of temperature on cercarial production and infectivity*

Methods used to evaluate the effects of temperature on cercarial production followed those described by Shostak and Esch (1990). Snails were exposed to larvae of both species of trematode using the techniques described above. After exposure, snails were maintained under standard conditions (20°C and 12:12 light:dark photoperiod) for 35 d. Four to nine infected snails were placed in plastic containers (25 X 15 X 11 cm) within 4 environmental chambers on 18 July 2001 and maintained at either 10, 15, 20, or 25°C. This range of temperatures was chosen because it encompassed the minimum and maximum temperatures measured during the ice-free period in an oligotrophic and eutrophic lake that were sampled during the 2001 field season (Chapter 2). Snails were acclimated for 5 d within each chamber before the collection of cercariae. Cercarial production was estimated for one 12-h light period using the techniques described above. To estimate the number of cercariae, I used one of three separate methods of volumetric sub-sample estimation, depending upon the number of cercariae released. Cercariae were diluted into 100, 50 or 0 ml of

tap water and two 5 ml aliquots were counted. If the second count was not within 20% of the initial count a third count was done.

Inter-specific differences in cercarial infectivity were evaluated at the 4 different temperatures. Twenty-four size-matched minnows were randomly assigned and maintained in two plastic containers (25 X 15 X 11 cm, 12 minnows/container) within 1 of the 4 environmental chambers 5 d prior to being exposed to cercariae. Minnows were then moved to individual containers and each was exposed to 30 cercariae of one species of trematode for 2 h following the methods of Sandland and Goater (2001). After exposure, minnows were kept in the same temperature chambers for 2 d in plastic containers (25 X 15 X 11 cm, 12 minnows/container). Minnows were then placed on a 12:12 light:dark photoperiod at 20°C at the same density per container. Minnows were humanely killed on 17 August 2001 using MS222 and stored in 70% ethanol until necropsy.

#### *Effects of photoperiod on cercarial production*

Inter-specific differences in daily cercarial production were investigated in individual snails exposed to 3 different photoperiods. Because my preliminary trials indicated that neither species released cercariae during periods of darkness, my focus was on altering the timing of the commencement of the 12 h light period. Thus, the response of snails to altered photoperiod was tested in snails exposed to 12 h of light commencing at 0900, 1200, and 1800 h. Eleven snails infected with *O. ptychocheilus* and 10 snails infected with *P. minimum* were used in this experiment. Infected snails were maintained at the same conditions described above, with the only exception being the commencement of

the photoperiod. Snails were acclimated to each new photoperiod for 3 d prior to the collection of cercariae. Cercariae were collected from individual snails during 6 consecutive 2-h intervals, during the 12-h light period, for each different photoperiod. Methods used to estimate cercarial production were the same as those described above.

### *Data analyses*

Student's t-tests were used to determine inter-specific differences in mean parasite size and egg production. Effects of temperature on cercarial production were evaluated with a 2-way ANCOVA, with temperature and species as fixed effects, and snail size as a co-variate. The effect of altered photoperiod was evaluated using a repeated measures ANCOVA because each individual snail experienced all 3 photoperiods and the only difference between treatments was the time at which the light period began. A 2-way ANOVA, where species and temperature were fixed effects, was used to evaluate the effect of temperature on the proportion of cercariae recovered from minnows. To meet the assumptions of these parametric tests, cercarial counts were Box-Cox transformed and log transformed for the temperature and photoperiod experiments, respectively. Means are presented as  $\pm 1$  SD.

## **Results**

### *Inter-specific difference in morphology and reproduction*

*Posthodiplostomum minimum* was larger than *O. ptychocheilus* throughout most stages of its life cycle. Adults, metacercariae, and eggs of *P. minimum* were 97%, 86%, and 5% longer, respectively, than for *O. ptychocheilus* (Table

1). In contrast, the cercarial heads of *O. ptychocheilus* were 15% larger and the eggs were 6% wider than *P. minimum* (Table 1).

There were also significant inter-specific differences in the production of cercariae and in per capita adult fecundity. After controlling for host size, snails infected with *P. minimum* released 95% more cercariae per snail per day than snails infected with *O. ptychocheilus* (Table 1). Furthermore, *P. minimum* produced approximately 197% more eggs per adult per day than *O. ptychocheilus*.

A total reproductive output index was calculated to determine if total reproductive output varied per unit adult length. This index was calculated by determining the average daily egg output and average adult size for trematodes from each infected bird. To calculate average daily egg output, per capita adult fecundity was multiplied by the average egg length and width (eggs are ellipsoid) (Poulin 1997). Average adult size was calculated by multiplying total adult length by maximum adult width (Poulin 1997). Based upon this crude approximation of total reproductive output, *P. minimum* invested significantly more in reproduction per unit adult length than *O. ptychocheilus* (Table 1).

Recruitment of metacercariae from minnows into birds was highly variable. For *O. ptychocheilus*, recruitment ranged from 6 to 45% and each of the 5 exposed birds harbored adult trematodes. In contrast, adult *P. minimum* were only found in 2 of the 5 exposed chicks'. Inter-specific differences in adult recruitment were not significant (Table 1).

Each species inhabited different, non-overlapping regions along the intestine of birds. Adults of *P. minimum* were located in the anterior-most region of the duodenum, at  $5.4 \pm 0.25\%$  along the length of the intestine. In contrast, *O. ptychocheilus* was found within the middle of the intestine ( $37.3 \pm 3.4\%$ ). Moreover, *O. ptychocheilus* ranged more broadly within the intestine, from 22.5 to 67.5 % along its length.

#### *Effects of temperature on cercarial production and infectivity*

Snails that released fewer than 110 cercariae during the 12 h collection period were considered outliers and omitted from analysis (2 *P. minimum* at 25°C, 1 *O. ptychocheilus* at 15°C, 1 *P. minimum* at 15°C). Thus, at 15, 20, and 25°C, there were 4, 4, and 4 snails infected with *O. ptychocheilus* at each temperature and 6, 9, and 5 snails infected with *P. minimum*, respectively. At 10°C only 1 of 4 snails infected with *O. ptychocheilus* and 2 of 8 snails infected with *P. minimum* released cercariae so this temperature was removed from statistical analysis. Cercarial production was strongly affected by snail size (ANCOVA, size;  $F_{1,25} = 31.9$ ;  $p < 0.0001$ ) and temperature (ANCOVA, temperature;  $F_{2,25} = 10.1$ ;  $p < 0.001$ ; Fig. 1), but not by species (ANCOVA, species;  $F_{2,25} = 0.47$ ;  $p = 0.49$ ) or by the species X temperature interaction (ANCOVA, species X temperature;  $F_{2,25} = 2.94$ ;  $p = 0.07$ ). Thus, both species responded similarly and positively to increases in snail size (raw regression coefficient = 0.62) and also to increases in temperature, with the maximum release occurring at 25°C (Fig. 1).

The infectivity of *P. minimum* and *O. ptychocheilus* cercariae was strongly affected by temperature and species (ANOVA, species X temperature;  $F_{2,55} =$

28.1;  $p < 0.0001$ ; Fig. 3). However, when the maximum infectivity of *O. ptychocheilus* (at 25°C) and *P. minimum* cercariae (20°C) was compared, no significant differences were found (Tukey-Kramer HSD). Overall, the infectivity of cercariae increased with temperature (ANOVA, temperature;  $F_{2,55} = 8.96$ ;  $p < 0.001$ ) and there was no species difference (ANOVA, species;  $F_{2,55} = 0.002$ ;  $p = 0.96$ ). For the few cercariae recovered at 10°C (approximately 20) and subsequently exposed to minnows, only *P. minimum* metacercariae were recovered.

#### *Effects of photoperiod on cercarial release*

Six of 11 *O. ptychocheilus* and 6 of 10 *P. minimum* infected snails survived the duration of the photoperiod trials. Daily cercarial production (number of cercariae shed per snail per day) of *O. ptychocheilus* and *P. minimum* varied extensively between the 2 species and over time. However, changing the commencement of the photoperiod did not affect the production of cercariae in either species (ANCOVA, photoperiod X species;  $F_{2,58} = 2.43$ ;  $P = 0.10$ ), and altering the photoperiod did not affect their pattern of cercarial release (ANCOVA, photoperiod X species X time period;  $F_{10,58} = 1.04$ ;  $P = 0.41$ ). *Posthodiplostomum minimum* and *O. ptychocheilus* released 57% and 55% of their daily cercarial production, respectively, during the first 2 h after the onset of light (ANCOVA, time period;  $F_{5,59} = 34.8$ ;  $p < 0.0001$ ; Fig. 3), and the pattern of cercarial release was the same for both species (ANCOVA, time period X species;  $F_{5,59} = 0.27$ ;  $p = 0.93$ ; Fig. 3). Snail size did not affect the number of cercariae released (ANCOVA, size;  $F_{1,59} = 2.5$ ;  $p = 0.12$ ).

## Discussion

Natural selection presumably shapes the life histories of all organisms. Parasites with similar life cycles that use identical hosts should therefore evolve similar morphologies and rates of reproduction. Observations from the current study and previous work on this system support this prediction. Both *O. ptychocheilus* and *P. minimum* are small as adults (<2 mm) and both have short pre-patent periods (<72 h) in birds. Cercariae are typically shed from snails 28 days after exposure and continue to do so until host senescence. Metacercariae take approximately 4 weeks to encyst within their minnow hosts, after which they become infective to final hosts (Shirakashi 2002). The response of cercariae to changes in photoperiod and temperature were identical for both species, with peak release occurring during the first 2 h of daylight. Additionally, the numbers of cercariae shed per snail per day increased with increasing temperature. Because both species are phylogenetically related, share 2 of 3 required hosts (snails and minnows), and are sympatric (at least within individual minnows) in most lakes, selection imposed by common environments has led to broadly similar life histories. For example, cercarial emergence patterns provide some of the best-documented examples of responses to selection favoring transmission (Combes 1991) and both *O. ptychocheilus* and *P. minimum* responded the same to changes in photoperiod, which is the most common stimulus for cercarial release.

Despite the many similarities in the life histories of *O. ptychocheilus* and *P. minimum*, there were still several striking differences. First, *P. minimum* was

larger throughout most stages of its life cycle. Thus, adult *P. minimum* were 97% larger than *O. ptychocheilus* and this size advantage appeared to carry over to an 86% difference during the metacercariae stage. The larger adults of *P. minimum* produced approximately 197% more eggs per adult per day than *O. ptychocheilus*, and snails released approximately 95% more cercariae per day. The link between body size and fecundity is well established for many invertebrates, including many parasite species (Loker 1983; Poulin 1995a; Poulin 1997). Poulin (1997) showed that body size was positively correlated with both egg size and egg numbers in trematodes and concluded that there was no tradeoff between egg size and egg numbers, unlike many non-parasitic species. My results suggest that adult size is positively associated with fecundity, cercarial production, and metacercarial size, but not with cercarial size. More experimental work is needed on more related species to evaluate whether these patterns are general for trematodes.

A second difference was in the details of response to temperature by *O. ptychocheilus* and *P. minimum*. In both species, the infectivity and production of cercariae increased with temperature, a result consistent with several other species (Evans 1985; Lo and Lee 1996; McCarthy 1999; Schmidt and Fried 1996). However, the temperature for peak cercarial infectivity of *P. minimum* was at least 5°C lower than for *O. ptychocheilus*. Also, at 10°C, some metacercariae of *P. minimum* were recovered from minnows exposed to cercariae, but no metacercariae of *O. ptychocheilus* were recovered. In northern Alberta, metacercarial intensities of *P. minimum* in minnows are greater in cooler

oligotrophic lakes whereas intensities of *O. ptychocheilus* are lower (Chapter 2). As predicted, differences in life histories of these sympatric trematodes may reflect subtle differences in the habitats of their hosts. Temperature-dependent differences in cercarial infectivity provide supportive evidence for this prediction. However, other factors such as pH or salinity can affect cercarial infectivity (e.g., Shostak 1993) and should not be dismissed when comparing minnows from different habitats.

It is not surprising that two species with similar life cycles, similar life-histories, and presumably similar resource requirements should segregate spatially within individual hosts. Thus, *O. ptychocheilus* metacercariae encyst in the brain, whereas *P. minimum* metacercariae encyst within the body cavity. Within-host site segregation is a common finding for metacercariae. For example, at least 3 species of *Diplostomum* can be found in the eyes of individual rainbow trout; one in the lens, one in the vitreous humour, and one on the retina (review by Chappell 1995). The life-history consequences of metacercarial site segregation are not known. However, in the case of *O. ptychocheilus*, Sandland and Goater (2001) have shown that space restrictions within the host cranium led to density-dependent metacercarial development. Thus, selection for increased size must be traded-off with space restriction within the cranium. The important point from a life-history perspective is that site selection within intermediate hosts may impose 'downstream' constraints on other stages within the life cycle.

The sites selected in final hosts were also different and did not overlap. This was not surprising because theory predicts that each species should inhabit a niche that reduces inter- and intra-specific competition for resources (Holmes 1990). However, the difference in the range that *O. ptychocheilus* and *P. minimum* inhabited along the intestine of their final hosts was surprising. *Posthodiplostomum minimum*'s range was very narrow and this species was found exclusively along the first 10% of gut (in the duodenum). In contrast, the range of *O. ptychocheilus* in the intestine was broad and this species used as much as 40% of the intestine. Several authors have speculated about factors that lead to intestinal site selection in several different helminth taxa, including trematodes, and several hypotheses have been proposed, such as regional variation in nutrient availability and quality (reviews by Esch *et al.* 1990; Sukhdeo and Sukhdeo 1994). However, there are few empirical data to support these hypotheses and parasitologists can only speculate about why parasites inhabit certain sites in the intestine (Sukhdeo and Sukhdeo 1994). Thus, explanations for why one species has a narrow range and the other a broad range remain unclear and highly speculative.

My data suggest that adult size is positively associated with fecundity, cercarial production, and metacercarial size. This is an important result because it implies that selection for size during one stage of the life-cycle may carry over to subsequent stages. However, there are likely several constraints that operate to limit the size of adult *P. minimum* and *O. ptychocheilus*. In northern latitudes, migratory birds act as final hosts for these parasites. Thus, the time available for

the transmission of metacercariae into hosts whose residence time on lakes is brief (approximately 6 weeks) is probably an important constraint on adult size. Because development time and adult size are positively correlated in a large number of trematodes (Poulin 1995b; Trouve *et al.* 1998), the fecundity advantages of large size must be traded-off with delays in adult development. Such delays are likely to be strongly selected against in trematodes that require transmission into migratory birds. Our unpublished results suggest that the window available for trematode transmission is further narrowed by the restricted opportunity for transmission between worm eggs and snails. This stage must occur within approximately 2-3 weeks in mid-July. Earlier than this, F1 *Physa gyrina* are not available (Chapter 2); any later, snails are too large to be infected (Sandland and Goater 2001). Thus, metacercariae must be recruited rapidly after birds arrive on breeding grounds and the release of eggs must be rapid in order to synchronize with the annual cycle of *Physa* (Chapter 2). This constraint for rapid egg release is likely to be a key factor in selecting for short pre-patency and therefore small adult size. My results imply that constraints operating on adult size may carry-over to other stages in the life cycle. Similar comparative studies on a larger number of species, on trematodes of non-migratory and low-latitude birds, and on other parasite taxa would address the generality of these important tradeoffs.

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Table 1: Interspecific differences in life history characteristics ( $X \pm SD$ ) and in the life cycles of *O. ptychocheilus* (Op) and *P. minimum* (Pm). Morphological measurements are in mm. If N is not given it is the same as the previous value.

Observation	Op		Pm		Test Statistic	P
	N	$X \pm SD$	N	$X \pm SD$		
<b>Morphological Characteristics:</b>						
adult hindbody	128	0.27±0.03	128	0.68±0.10	t= 36.4	P<0.0001
adult forebody		0.62±0.11		1.08±0.13	t= 18.6	P<0.0001
adult width		0.29±0.03		0.48±0.10	t= 18.6	P<0.0001
egg length	53	0.095±0.0036	52	0.10±0.0056	t= 6.6	P<0.0001
egg width		0.068±0.0021		0.064±0.0027	t= -8.4	P<0.0001
cercariae head	104	0.15±0.011	81	0.13±0.020	t= -8.2	P<0.0001
cercariae tail		0.19±0.016		0.18±0.018	t= -0.56	P=0.57
metacercariae length	20	0.37±0.04	21	0.69±0.067	t= 18.5	P<0.0001
metacercariae cyst <sup>A</sup>	25	0.53±0.055				
<b>Reproduction and Recruitment:</b>						
eggs in utero	128	1.14±0.68	28	1.39±1.47	t=1.38	P=0.17
per capita worm fecundity (# eggs/worm/day)	5	42±19	2	125±53	t=3.31	P=0.03
total reproductive output index <sup>B</sup> (average daily egg output / average adult size)		0.19±0.11		0.97±0.52	t=3.59	P=0.02
cercarial production <sup>C</sup> (#/snail/day)	6	2119.6±915.9	6	4135.4±2072.5	$F_{1,59} = 7.69$	P=0.007
adult recruitment (%)	5	30.7±17.3	2	21.2±3.8	t=-0.72	P=0.49
metacercarial recruitment (%)	7	80.9±15.4	5	57.5±30.4	t=-1.8	P=0.10

A = Maximum length

B = Average daily egg output was calculated by multiplying per capita fecundity by average egg length and width. Average adult size was calculated by multiplying average total adult length by width for each bird. See results for more details.

C = Average number of cercariae per snail. Average was calculated from 3 collections per snail over the same photoperiod starting at 0900, 1200, and 1800. See photoperiod experiment for details.

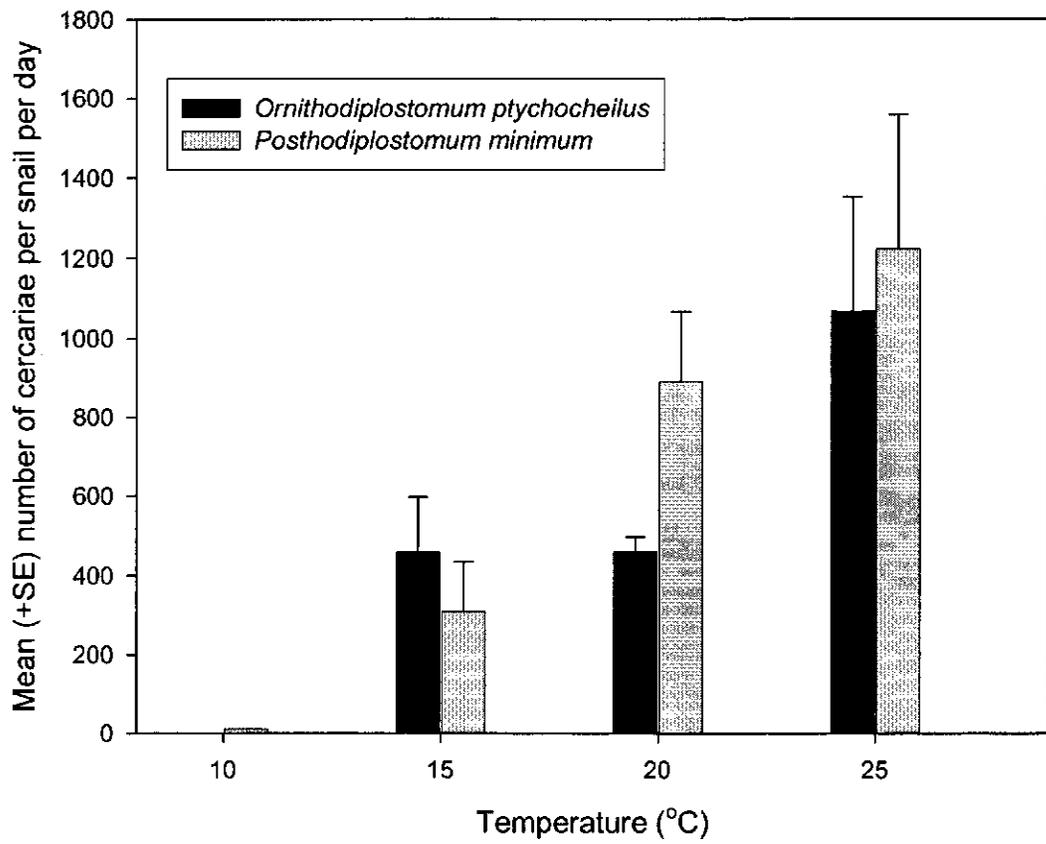


Figure 1: The mean (+SE) number of cercariae released from snails infected with *O. ptychocheilus* and *P. minimum* at 10, 15, 20 and 25 °C.

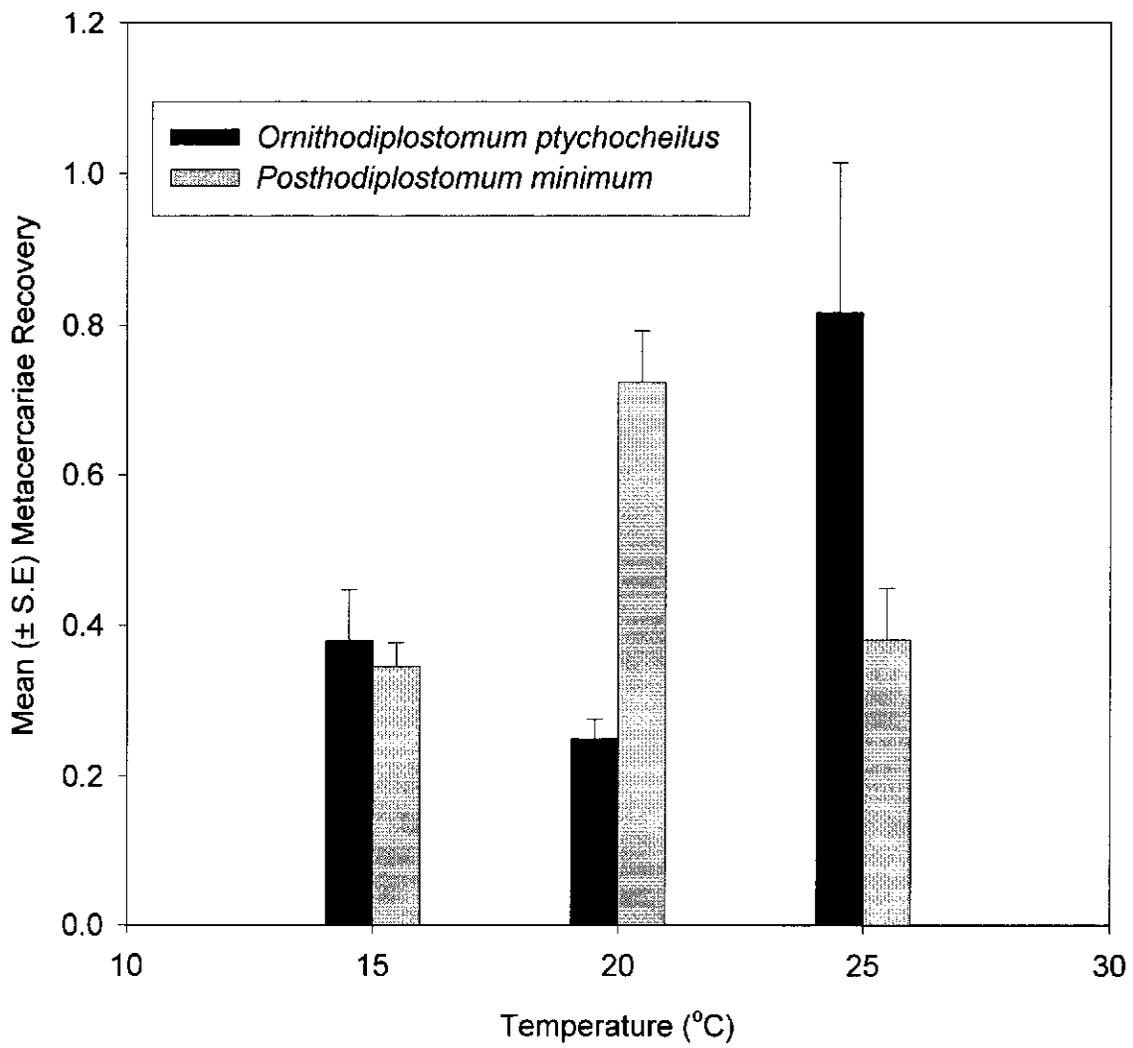


Figure 2: Mean ( $\pm$ S.E.) cercarial infectivity based upon the recovery of metacercariae from minnows exposed to 30 cercariae of *O. ptychocheilus* and *P. minimum* at 15, 20 and 25 °C.

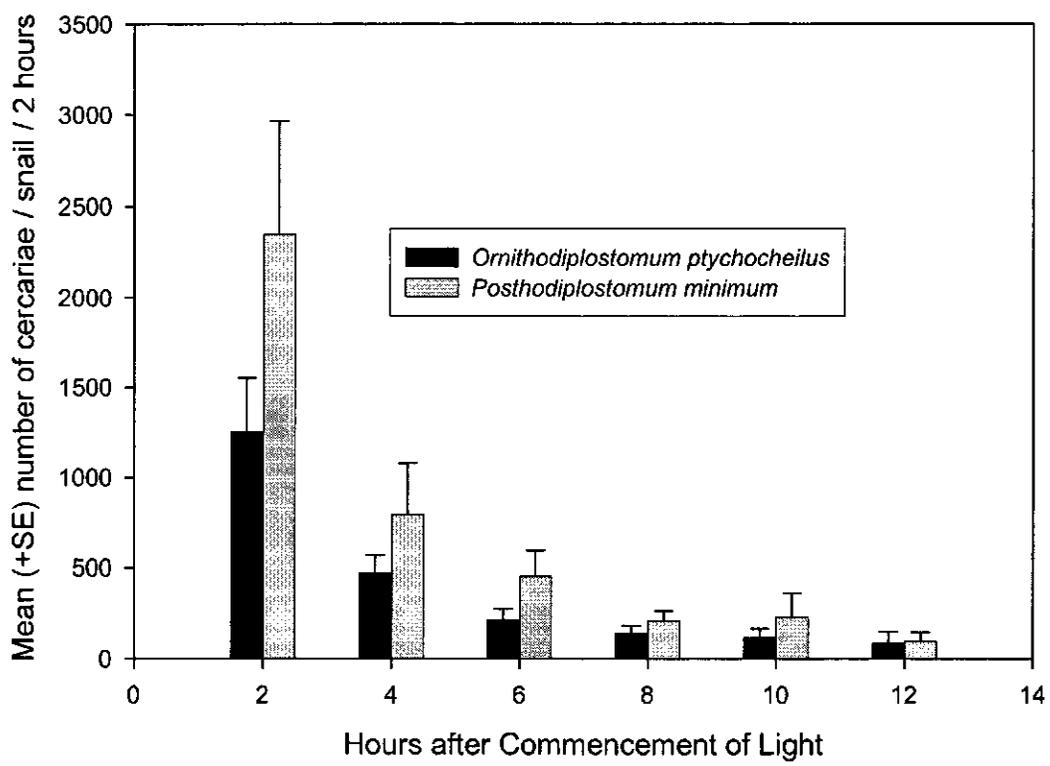


Figure 3: Inter-specific differences in daily cercarial production rates from *Physa gyrina* exposed to miracidia of *O. ptychocheilus* and *P. minimum*

## **Chapter 4:**

### **The effects of parasites and male morphological characteristics on the nesting status of male fathead minnows (*Pimephales promelas*)**

#### **Abstract**

I investigated correlates of nesting success of male fathead minnows (*Pimephales promelas*) in a population in north central Alberta, Canada. Morphological characteristics and parasite burdens were compared between breeding and non-breeding males sampled in the beginning, middle, and end of the 2001 breeding season. Males collected from nests were longer and wider than non-breeders and contained fewer numbers of 3 out of 4 common larval trematode parasites. Breeding males were 6% longer than non-breeding males early in the breeding season, when male competition for nesting sites was presumably highest. In addition, mean abundance of 3 of 4 species of larval trematode was 18-49% lower in minnows collected from nests compared to non-breeding males. Differences in parasite intensity between breeding and non-breeding males could not be explained by differences in host length or girth, or by differences in parasite transmission into the two male types. Rather, the evidence supports the idea that infection with larval trematodes interferes with a male's ability to obtain or defend a nest. Thus, in this population of minnows, the most important correlates of male nesting success were girth, total length, and parasite intensity.

## Introduction

Parasites can influence the reproduction of their hosts through direct or indirect mechanisms (review by Moller and Jennions 2001). Thus, parasites can directly reduce host fecundity through suppression of gonadal development (e.g. Arme 1997), reduced clutch size (e.g. Hudson *et al.* 1992; Kraak and Bakker 1998), or alterations to host hormonal levels that affect breeding behavior (Morales *et al.* 1996). The effects of parasites on host reproduction can also be indirect, for example, through interactions between the sexes, such as mate choice (e.g. Hamilton and Zuk 1982). There are now several convincing examples of cases in which females use male ornaments to select lightly parasitized or uninfected males as mates (e.g. Milinski and Bakker 1990; Rosenqvist and Johansson 1995). Examples such as these are the most common and provide the best supporting evidence for the effects of parasites on host sexual selection.

Parasites also have the potential to affect intra-sexual selection. Competition between males typically arises when they vie for a particular resource that is required for reproduction. Such males possess morphological and/or ornamental characteristics that help them compete for these resources. In many species, competition for resources is a mechanism of intra-sexual selection and often involves male-male competition for nesting sites. Intra-sexual selection in fishes is often particularly important when females gain more than sperm from males,

such as parental care (Howard and Minchella 1990). Howard and Minchella (1990) and Read (1990) provided convincing examples of cases in which parasite-induced effects on male stamina and energy utilization were more important determinants of parasite-mediated sexual selection than ornamentation and female choice. However, the few studies that have examined the effects of parasites on intra-sexual selection provide equivocal results. For example, parasites had no effect on male-male competition or male nesting status in several fish species (Candolin and Voigt 2001; Fitzgerald *et al.* 1993; Hamilton and Poulin 1995; Warner and Shultz 1992). In contrast, cestode infection was important in determining the outcome of male-male competition for nest sites in a natural population of sticklebacks, but not in a laboratory population (Candolin and Voigt 2001). Results from the latter study are particularly important because they indicate that the effects of parasites on male-male competition may best be detected in natural populations.

Fish exhibit a wide array of conspicuous sexual ornaments, many of which play a role in intra- and inter-sexual selection (Barber *et al.* 2000). The reproductive behavior of the fathead minnow (*Pimephales promelas* Rafinesque) has been well studied in this context. At the northern edge of its range, males undergo sexual maturation and acquire sexual ornamentation in late May, approximately one month after the ice melts off of lakes. Males are ornamented with tubercles and a lateral band. They tend to compete vigorously for nests under submerged logs and small

rocks to which they attract females for egg laying (Isaak 1961; Shaw *et al.* 1995; Wynne-Edwards 1932). Males provide parental care against conspecifics or egg predators such as dragon fly naiads or crayfish (Sargent 1988), and this care increases egg survival. The reproductive behavior of males (Cole and Smith 1987; McMillan and Smith 1974) and the effects of male-male competition for nesting substrate (Sargent 1988; Unger 1983; Unger and Sargent 1988) have been especially well documented. In several lakes in northern Alberta, fathead minnows are also heavily infected with larval trematodes (Sandland 1999; Sandland *et al.* 2001, Chapter 2). One species, *Ornithodiplostomum ptychocheilus* (Faust), has been shown to reduce minnow growth, reduce activity, alter visual acuity (Shirakashi and Goater 2001), and reduce minnow survival (Sandland 1999; Sandland and Goater 2001). It follows that features such as reduced activity and altered visual acuity could directly affect a male's ability to fight for, or guard, nests.

Knowledge of the extent to which parasites may affect male-male competition for nesting sites is important because host fitness and reproductive success are dependent upon males obtaining a nest. The purpose of this investigation was to determine the associations between a male's breeding status and his morphology and parasite intensity from a population in north central Alberta, Canada. I collected nesting and non-nesting male minnows in the beginning, middle, and end of the breeding season to evaluate differences between morphological characteristics and

parasite intensities throughout the breeding season. If infection affects male-male competition, then heavily infected males should be under-represented in the sample of breeding males or heavily infected males may breed later in the summer.

## **Methods**

### *Study site*

Sandland (1999) and Sandland *et al.* (2001) indicated that the types and numbers of parasites in minnows from Chain Lake were characteristic of minnows sampled from other lakes in the region. Chain Lake was selected for this study because the population size of minnows was high, the lake was accessible by vehicle, and because water clarity in the lake was high. Moreover, the sandy substrate that surrounded the shallow perimeter of the lake allowed me to easily observe and collect breeding minnows.

### *Collection procedures*

All male minnows (nesting and non nesting) were considered to be sexually mature if they had well-developed tubercles on the snout. Males in Chain Lake tended to defend nests that were located under submerged wood approximately 25-100 cm below the surface. Nests were located within 2-5 cm of the surface of the substrate and were scattered unevenly within 2-15 m of the shoreline in all areas that contained a sandy substrate. Nest density appeared to be dependent upon the amount of submerged woody vegetation available to nest beneath. These

characteristics are similar to those described for other populations of fathead minnows (Isaak, 1961; DeWitt, 1993).

Collections were made on 5 June, 7 July, and 31 July 2001, representing the beginning, middle, and end, respectively, of the breeding season that year. Breeding males were collected using a large, open-topped metal cage (122 X 122 X 122 cm). The sides of the cage were constructed from 2-mm wire-mesh screening framed with metal rods. This trap design facilitated placing the trap over nesting sites and capturing males from above using standard dip nets.

Wooden nest boards (25 long X 20 wide X 11 cm high) were used to supplement the sample of nesting males. The nesting surface upon which eggs could be deposited was 18 X 20 cm and was approximately 9 cm from the substrate. Nests had two open sides that were 20 cm wide. A 30-cm metal spike that passed through the surface of the nest board (offset from the middle) held the nest submerged throughout the study. Five artificial nest boards were placed amongst natural nesting sites in each of 4 locations along the perimeter of the lake (Fig. 1) on 4 May. Artificial nests were placed at least 100 m apart and at an average depth of  $32.5 \pm 4.46$  cm ( $X \pm SD$ ). Artificial nests remained in the same location throughout the duration of the study. These boards were designed to mimic natural nests as much as possible, and the density and placement of the boards were based upon observations made during the previous breeding season. Methods used to capture males that were defending

nest boards were the same as those used to collect males from natural nests.

Non-nesting males were collected using 12 un-baited Gee minnow traps placed arbitrarily around the perimeter of the lake, following the methods of Candolin and Voigt (2001) and Fitzgerald *et al.* (1993) (Fig. 1). The traps were set the night before the collections of nesting males and then retrieved exactly 8 hours later. Traps were placed a minimum of 100 m from the nest boards. Traps averaged  $143 \pm 25.8$  ( $n = 36$ ) minnows (over entire breeding season), and usually contained non-nesting males, females and juveniles. The first three to seven non-nesting males were selected from each trap using small fishnets, until a total of 30 was reached. Samples of non-breeding minnows collected on 31 July contained more juvenile minnows than other samples but these juveniles could easily be discriminated from non-breeding males because they lacked tubercles and were much smaller.

#### *Host characteristics*

The clutch size of each nesting male was evaluated by removing his nest, photographing the entire clutch with a digital camera (Nikon Coolpix 700) and replacing his nest. Immediately thereafter, males were immersed in concentrated MS222, and then frozen until subsequent examination in the laboratory. Although I attempted to count the number of tubercles and measure their length, this character was too unreliable to be accurately measured. Total minnow length (to the nearest, mm) was

measured with digital calipers and measured from the tip of the snout to the fork in the caudal fin. Wet weight, which includes the mass of the parasites, was measured using a balance to the nearest 0.01g. Host girth was measured by encircling a fine thread around the maximum circumference of the male at the anterior end of the dorsal fin and measuring the thread length to the nearest mm with digital calipers.

All males were necropsied in the laboratory using standard techniques. The eyes, brain, and viscera were removed from each host, pressed between two glass slides, and thoroughly examined for endoparasites. The body musculature was also teased apart with forceps and thoroughly examined. Thus, the intensity of each parasite species was determined by directly counting the number of parasites present within each male. A male's clutch size was evaluated from direct counts of eggs from computerized camera images.

#### *Data analyses*

Because numbers of adult male, female, and juvenile minnows caught within the minnow traps could not be normalized, non-parametric statistics were used to compare the number of minnows captured during the beginning, middle, and end of the breeding season. Total length, girth, weight, and clutch size were transformed using the Box-Cox transformation to meet normality assumptions. Parasite intensities of *Posthodiplostomum minimum* (McCallum) and *O. ptychocheilus* were square root transformed and *Bolboforus confusus* intensity was Box-Cox

transformed. One species (*Tylodelphys* sp.) could not be transformed to normality so non-parametric statistics were used for hypothesis testing.

Initial analyses used 2-way ANCOVA's to assess the effects of breeding status (NB = non-breeder, ANB = artificial nest boards, NN = natural nests) on morphological characteristics and parasite intensities between and within each collection period (early, middle, late). For girth and weight, total length was used as a co-variate to adjust for effects of total length. Nesting males (ANB and NN) were pooled where possible so that the focus could be placed on comparing breeding and non-breeding minnows. Pair-wise correlations were used to evaluate associations within and between host morphological characteristics and parasite intensities. Data that met normality assumptions were evaluated using Pearson's product-moment correlation coefficients. Data that did not meet normality assumptions were compared using non-parametric Spearman's Rho coefficient of rank correlation. To evaluate correlations between girth and weight, independent of total length, the residuals of a linear regression with total length were used. All post hoc comparisons were done using the Tukey-Kramer HSD test.

## **Results**

### *Host characteristics*

The number of adult male and female minnows caught in Gee minnow traps decreased between the 3 collection periods (Males, Kruskal-Wallis,  $\chi^2 = 22.6$ ; df = 2;  $P < 0.0001$ , Females, Kruskal Wallis,  $\chi^2 = 23.2$ ; df

= 2;  $P < 0.0001$ , Table 1). The number of juvenile minnows caught in minnow traps was more variable, and appeared to increase across the 3 collection periods, although the trend was not significant ( $\chi^2 = 1.56$ ;  $df = 2$ ;  $P = 0.46$ ).

A combined total of 177 breeding and non-breeding males were sampled, from a total of 972 males that were collected over the 3 collection periods (Table 2). Thus, less than 20% of the total number of males collected were removed from the breeding population during the course of the study. Each artificial nest board contained at least one male during the 3 collection periods. Two or more males were caught from 11 of 97 nests sampled; these data were omitted from analyses because I could not identify the resident male defending the nest. *Post hoc* comparisons showed that there were no significant differences in morphological characteristics or parasite intensities between minnows sampled from natural nests and those sampled from nest boards. Thus, minnows sampled from natural nests and artificial nest boards were pooled and these data hereafter are referred to as breeding males because males require a nest in order to obtain a clutch of eggs.

The total length of ornamented males varied among nest types and collection periods (2-way ANOVA, nest type X time;  $F_{4,157} = 7.13$ ;  $P < 0.0001$ , Table 2). Tukey-Kramer *post hoc* comparisons indicated that there were no differences in host length between ANB and NN within a collection period. The total length of breeding and non-breeding minnows

was affected by the collection period and breeding status (2-way ANOVA, breeding status X time;  $F_{2,160} = 25.1$ ;  $P < 0.0001$ ; Fig. 2) . Breeding males decreased in size throughout the breeding season, whereas the size of non-breeding males did not change significantly. Thus, breeding males were larger than non-breeding males early in the season, were of equivalent size in the middle, and smaller near the end of the season (Fig. 2).

Girth of adult male minnows varied among nest types and collection periods (2-way, ANCOVA, nest type X collection period;  $F_{4,155} = 2.86$ ;  $P = 0.03$ ). There was no significant difference in girth between ANB and NN males. The slightly significant breeding status by time interaction (2-way ANCOVA, breeding status X time;  $F_{2,158} = 3.02$ ;  $P = 0.05$ ; Fig. 3) indicated that changes in girth over time were not consistent between breeding and non-breeding males. Because girth was significantly positively correlated with total length (2-way ANCOVA, total length;  $F_{2,158} = 238.3$ ;  $P < 0.0001$ ), I used the least square means to show the relationship between girth and collection period (Fig. 3). Thus, independent of total length, the girth of non-breeders declined rapidly over the 3 collection periods; this decline was markedly delayed in breeders (Fig. 3). Overall, adjusted girth was larger in breeding than non-breeding males (ANCOVA, breeding status;  $F_{1,158} = 14.4$ ;  $P < 0.0002$ ).

Weights of adult male minnows varied among collection periods (2-way ANCOVA,  $F_{2,156} = 3.05$ ;  $P = 0.049$ ) but not nest types (2-way

ANCOVA,  $F_{2,156} = 1.27$ ;  $P = 0.28$ ). The interaction between nest type and collection period was not significant (2-way ANCOVA,  $F_{2,156} = 1.42$ ;  $P = 0.22$ ). Males collected early in the breeding season were significantly heavier than those collected in the middle or end.

The morphological characteristics of naturally nesting males, non-breeding males, and males sampled from artificial nest boards were highly correlated (Table 3). Total length was significantly positively correlated with girth and weight (Table 3). The adjusted girth of males was positively correlated with adjusted weight (Table 3). Clutch size was negatively correlated with total length, weight, and girth (Table 3). Clutch size was not correlated with adjusted girth or weight.

#### *Patterns of parasite infection in males*

*Posthodiplostomum minimum* and *O. ptychocheilus* were found in all adult minnows in all collection periods (Table 4). *Bolboforus confusus* and *Tylodelphys* sp. had lower prevalences but they were always present in more than 50% of the hosts within a sample. Mean abundances were highest in the 2 most prevalent species and were lowest (<10 parasites/host) for the other 2 species. Total length, girth, and weight of minnows were positively correlated with abundances for *P. minimum* and *B. confusus*. Only total length and weight of minnows were positively correlated with *O. ptychocheilus* abundance (Table 3). Clutch size was significantly negatively correlated with the abundance of larval *P. minimum* (Table 3).

Mean *P. minimum* abundance varied among nesting types (2-way ANCOVA, nest type;  $F_{2,156} = 9.62$ ;  $P < 0.0001$ ). *Post hoc* comparisons indicated that there were no differences in mean abundance between natural and artificial nests or between collection periods. However, in data pooled across collection periods and across the two types of nests, mean *P. minimum* abundance was affected by total length and breeding status of male minnows (2-way ANCOVA, total length X breeding status;  $F_{1,162} = 5.5$ ;  $P = 0.02$ ; Fig. 4). In general, breeding males had fewer parasites per unit length than non-breeding males, and for every unit increase in male length, non-breeding males had higher *P. minimum* abundance. Overall, breeding males had lower *P. minimum* abundances than non-breeding males (2-way ANCOVA, breeding status;  $F_{1,162} = 21.5$ ;  $P < 0.0001$ ; Fig. 5).

Mean *O. ptychocheilus* abundance varied among nest types (ANCOVA, nest type;  $F_{2,156} = 6.37$ ;  $P = 0.002$ ). *Post hoc* comparisons indicated that there were no differences in mean abundance between natural and artificial nests or between collection periods. However, in data pooled across collection periods and across the two nest types, mean *O. ptychocheilus* abundance in breeding minnows was lower per unit length than non-breeding minnows (2-way ANCOVA, total length X breeding status;  $F_{1,162} = 4.7$ ;  $P = 0.03$ ). Breeding males had a significantly lower abundance of *O. ptychocheilus* than non-breeding males (2-way ANCOVA, breeding status;  $F_{1,162} = 4.8$ ;  $P = 0.03$ ; Fig. 4).

Mean *B. confusus* abundance differed between the nesting types (2-way ANCOVA, nest type;  $F_{2,156} = 8.64$ ;  $P < 0.001$ ) and among collection periods (2-way ANCOVA, collection period;  $F_{2,156} = 3.48$ ;  $P = 0.03$ ). However, *post hoc* comparisons indicated that no differences in *B. confusus* abundance were found between artificial and natural nesting males or between collections period (2-way ANCOVA, nest type X time;  $F_{4,156} = 0.94$ ;  $P = 0.44$ ). In data pooled across collection periods and across nest types, *B. confusus* abundance in breeding males was lower than non-breeding males (2-way ANCOVA, breeding status;  $F_{1,162} = 36.11$ ;  $P < 0.0001$ ; Fig. 4). The total length of males was positively correlated with mean *B. confusus* abundance (ANCOVA,  $F_{1,162} = 40.1$ ;  $P < 0.0001$ ). However, mean abundance of *B. confusus* of breeding and non-breeding minnows did not change differentially with length (ANCOVA, breeding status X total length;  $F_{1,162} = 0.01$ ;  $P = 0.91$ ).

### Discussion

Male fathead minnows collected from nest sites tended to be longer and wider than males collected away from nest sites. The former also tended to contain fewer numbers of larval parasites. The overriding importance of male size in determining the outcome of male-male competition, either directly for females, or indirectly for oviposition sites or territories, is a common feature of studies involving the reproductive biology of several fish species (e.g. Bisazza and Marconato 1988; Brown 1981; Forsgren *et al.* 1996; Kraak *et al.* 1999; Kuwamura *et al.* 2000;

Takahashi *et al.* 2001; Warner and Shultz 1992; Wiegmann *et al.* 1992). In general, larger males tend to attain breeding sites first, have a longer residency time at those sites, and tend to receive more egg clutches than smaller males (Brown 1981).

In contrast, the magnitude and consistency of the differences in parasite intensity between breeders and non-breeders was surprising. Breeding males contained up to 49% fewer larvae of 3 out of 4 common species of encysting trematode compared to non-breeders. As noted by Howard and Minchella (1990), there are few comparable studies, and none have evaluated inter-specific differences in parasite intensities over an entire breeding season. However, one other field study provides similar results to those found here (Candolin and Voigt 2001). Candolin and Voigt (2001) showed that in a sub-population of 33 breeding male sticklebacks, only 2 were infected with a larval cestode, whereas 15 of 33 non-breeders were infected. Thus, the main result of my study is the finding that the longest, widest, and least-infected males were over-represented at breeding sites, especially early in the breeding season. This result suggests that it is these males that would have the highest reproductive success in this lake.

The reproductive biology of male fathead minnows seems to be particularly closely associated with male girth. Thus, males with the widest girths tended to be sampled most often from nests compared to those away from nests. This pattern held independent of total length.

Similarly, Warner and Schultz (1992) showed that the body depth of male blue-headed wrasse (*Thalassoma bifasciatum*) was related to their ability to defend a nest against conspecific males. In minnows, non-nesting males preferentially attack nesting males with smaller girths, possibly because they are easier to evict than males with larger girths (Unger 1983). Moreover, breeding fathead minnows also have a lateral banding pattern that is commonly associated with male-male aggression (Unger 1983). The expression of this lateral band also changes rapidly depending upon the presence or absence of an intruder. Unger (1983) speculated that the male banding pattern of minnows is functionally analogous to the 'Beau Geste' behavior of red-winged blackbirds and may enhance the perception of robustness of a nesting male to conspecific intruders. The position of this lateral band on the body surface of minnows was in the same location that male girth was measured. Thus, males that had a larger girth also appeared more robust, due to the combination of larger girth and the lateral band enhancing this appearance.

Girth may be a fairly flexible trait in male minnows because they have the ability to alter their water retention during the breeding season and appear more robust (Unger 1983). In my study, weight and girth were highly correlated, independent of total length. This indicates that increases in weight, independent of total length, result in increased girth. Unger (1983) found that individual minnows increased in wet weight

during the breeding season and, according to my data, this increase in weight may result in increased girth. Thus, this unique ability of fathead minnows to retain water (Unger 1983) is the most logical explanation for the observed increases in girth later in the breeding season, when breeding and non-breeding minnows were of similar lengths.

The significant differences in parasite intensity between breeding and non-breeding males are not likely due to differences in the magnitude of trematode transmission between the two groups of hosts. In the case of *O. ptychocheilus* and *P. minimum*, transmission occurs in late summer and fall (Sandland 1999, Chapter 2; Sandland *et al.* 2001), a period when it is improbable that breeders and non-breeders would be segregated in the lake. It is also unlikely that breeding minnows were older and potentially more resistant. Our earlier field (Sandland *et al.* 2001) and laboratory studies (Sandland and Goater 2001) indicate that larger minnows within a single cohort always harbor more *O. ptychocheilus*. Given this strong correlation between length and trematode intensity, large breeders collected at nest sites should harbor more parasites than non-breeding males. Moreover, the significant interaction between breeding status and host length in determining parasite intensity indicates that for similar-sized minnows, those with the lowest intensity infections are most likely to be found at nest sites. These results indicate that the intensity differences between breeders and non-breeders are unlikely to be due to a sampling artifact related to transmission differences.

An alternative explanation for males with fewer numbers of trematodes found nesting is that the parasites may affect a minnow's ability to compete for, or defend, a nest. Each of the four common parasites found in minnows in Chain Lake has been shown to cause reduced growth and survival, and/or alterations in host behavior. *Ornithodiplostomum ptychocheilus* is associated with reduced survival in outdoor ponds (Sandland and Goater 2001) and causes reduced visual acuity and learning capacity (Shirakashi and Goater 2001) even in lightly (<20 cysts/host) infected hosts. Trematodes that cause blackspot (e.g. *B. confusus*) have been documented to cause a wide range of ecological and physiological effects. For example, Lemly and Esch (1984) showed that developing metacercariae caused a marked increase in the rate of metabolism of sunfish, leading to decreased body condition and reduced survival. This result is important in the context of my results because the maximum rate of development of metacercariae coincides precisely with the onset of the reproductive season in minnows. Thus, although minnows become infected in fall, metacercariae do not develop to encystment until water temperatures increase the following spring. The implication is that the high intensities of larval parasites in Chain Lake may leave fish with such a large energy deficit that heavily infected non-breeding males are compromised in their ability to compete for nests. Thus, differences in intensity between breeders and non-breeders should be greatest early in the season, coinciding with the period when

competition for nests is highest and when the rate of metacercariae development is highest. This hypothesis remains to be tested experimentally. A further direction for future work is to determine whether such effects are due to high intensities of individual parasite species or to the additive intensities from multi-species infections.

These results may also have important implications for sexual selection via female choice. For example, Howard and Minchella (1990) suggested that females might choose lightly infected males based upon their ability to defend resources. In the case of fathead minnows in Chain Lake, if a female were to choose a large male that was defending a nest, or conversely a large nest that contained a male, she would indirectly be selecting a male with fewer parasites. Likewise, if she selected a male with a large girth, she would also be selecting a male with fewer parasites. For male minnows collected during the middle of the breeding season, their girth (independent of total length) was negatively correlated with abundances of *P. minimum*, *O. ptychocheilus*, and *B. confusus* whereas it was positively correlated with clutch size. Thus, females that select males with larger girths would be selecting those that can defend nests and yet also contain fewer parasites. Recent reviews that cover the importance of parasites in host sexual selection tend to emphasize systems in which either inter- or intra-sexual selection takes precedence (e.g. Howard and Minchella 1990; Read 1990). It is also possible, and perhaps likely, that

several systems exist in which parasites simultaneously influence both processes of sexual selection.

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Table 1: Mean ( $\pm$ SD) number of adult male, adult female, and juvenile minnows per trap collected from 12 traps set overnight in Chain Lake, Alberta, Canada, during the 2001 breeding season.

		Fish Density		
Collection Period	# of Traps	Male	Female	Juvenile
Beginning	9	85.2 $\pm$ 46.7	98.8 $\pm$ 47.6	1.2 $\pm$ 1.9
Middle	12	8.25 $\pm$ 8.8	31.0 $\pm$ 51.9	0.6 $\pm$ 0.8
End	11	0.8 $\pm$ 1.7	0.09 $\pm$ 0.3	97.0 $\pm$ 169.8

Table 2: Differences in the morphologies and clutch sizes (# of eggs per defended nest) of breeding (ANB = artificial nest board, NN = natural nest) and non-breeding (NB) male minnows collected during the 2001 breeding season in Chain Lake, Alberta, Canada. Data represent  $X \pm SD$

Morphological Data						
Collection Period	Nest Status	N	Total Length (mm)	Girth (mm)	Weight (g)	Clutch Size (eggs/male)
Beginning	NN	19	78.7 $\pm$ 3.2	42.2 $\pm$ 2.1	5.60 $\pm$ 0.58	291.8 $\pm$ 59.1
	ANB	17	79.8 $\pm$ 4.3	40.3 $\pm$ 3.2	5.53 $\pm$ 0.99	200.2 $\pm$ 150.2 (16)*
	NB	30	74.9 $\pm$ 4.0	39.2 $\pm$ 2.8	4.74 $\pm$ 0.82	0
Middle	NN	35	71.3 $\pm$ 3.0	37.2 $\pm$ 2.3	4.09 $\pm$ 0.66	469.0 $\pm$ 314.3 (31)
	ANB	17	71.4 $\pm$ 5.1	38.0 $\pm$ 3.5	4.00 $\pm$ 0.87	580 $\pm$ 454.7 (15)
	NB	30	72.6 $\pm$ 5.2	36.0 $\pm$ 3.4	4.12 $\pm$ 1.17	0
End	NN	2	71.5 $\pm$ 2.1	35.8 $\pm$ 3.9	3.78 $\pm$ 0.74	1035 $\pm$ 65.8
	ANB	7	68.0 $\pm$ 8.17	33.0 $\pm$ 5.3	3.40 $\pm$ 1.66	547.7 $\pm$ 443.8
	NB	9	75.5 $\pm$ 5.6	35.4 $\pm$ 4.1	4.39 $\pm$ 1.13	0

\* numbers in brackets refer to sample sizes when they are different from total N

Table 3: Correlations of morphologies and parasite burdens of male minnows sampled during the 2001 breeding season from Chain Lake, Alberta, Canada. Data above the diagonal represent correlations of all data and data below the diagonal represents correlations with girth and weight, independent of total length. *Tylodelphys* sp. did not meet normality assumptions and was compared using non-parametric correlations. Significance levels: + P < 0.1; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001

X	Total Length	Girth	Weight	Clutch Size	Op	Pm	Ty	Bc
Total Length	X (166)	0.81*** (165)	0.93*** (166)	-0.33** (89)	0.21** (166)	0.52*** (166)	-0.01 (166)	0.43*** (166)
Girth	0.00 (166)	X (165)	0.91*** (166)	-0.21* (89)	0.09 (165)	0.20*** (165)	-0.08 (165)	0.26*** (166)
Weight	0.00 (166)	0.75*** (165)	X (166)	-0.27** (89)	0.16* (166)	0.49*** (166)	0.00 (166)	0.38*** (166)
Clutch Size	-0.33*** (89)	0.15 (89)	0.14 (89)	X (89)	0.09 (89)	-0.20* (89)	0.28** (89)	-0.03 (89)
Op	0.21** (166)	-0.16* (165)	-0.11 (166)	0.09 (89)	X (166)	0.47*** (166)	0.06 (166)	-0.12 (166)
Pm	0.52*** (166)	-0.10 (165)	0.03 (166)	-0.20* (89)	0.46*** (166)	X (166)	0.00 (166)	0.38*** (166)
Ty	-0.11 (166)	-0.07 (165)	0.02 (166)	0.28** (89)	0.06 (166)	0.00 (166)	X (166)	0.02 (166)
Bc	0.28*** (166)	-0.14* (165)	-0.06 (166)	-0.03 (89)	0.18* (166)	0.39*** (166)	0.02 (166)	X (166)

Op = *Ornithdiplostomum ptychocheilus*, Pm = *Posthodiplostomum minimum*, Bc = *Bolboforus confusus*, Ty = *Tylodelphys* sp.

Table 4: Mean ( $\pm$ S.D.) parasite abundance of breeding (ANB = artificial nest board, NN = natural nest) and non-breeding (NB) male minnows collected during the 2001 breeding season from Chain Lake, Alberta, Canada. Data in brackets represents parasite prevalence.

Collection Period	Nest Status	N	Abundance (Prevalence)			
			Op	Pm	Bc	Ty
Beginning	NN	19	60.1 $\pm$ 20.5 (100)	184.8 $\pm$ 100.5 (100)	4.6 $\pm$ 4.4 (94.7)	2.6 $\pm$ 2.6 (73.7)
	ANB	17	61.5 $\pm$ 43.0 (100)	170.4 $\pm$ 124.5 (100)	4.9 $\pm$ 4.6 (96.4)	1.1 $\pm$ 1.7 (50.0)
	NB	30	65.6 $\pm$ 31.1 (100)	235.8 $\pm$ 94.4 (100)	6.7 $\pm$ 4.9 (96.7)	1.7 $\pm$ 2.3 (60.0)
Middle	NN	35	74.3 $\pm$ 46.1 (100)	124.9 $\pm$ 79.1 (100)	1.3 $\pm$ 2.0 (48.6)	3.9 $\pm$ 3.3 (89.2)
	ANB	17	41.2 $\pm$ 23.5 (100)	96.9 $\pm$ 65.1 (100)	1.8 $\pm$ 2.3 (58.8)	4.7 $\pm$ 5.3 (82.4)
	NB	30	73.2 $\pm$ 47.4 (100)	157.9 $\pm$ 110.9 (100)	4.1 $\pm$ 3.4 (100.0)	3.7 $\pm$ 2.6 (93.3)
End	NN	2	62.0 $\pm$ 29.7 (100)	120.5 $\pm$ 96.9 (100)	1.0 $\pm$ 2.0 (50.0)	4.0 $\pm$ 1.4 (100.0)
	ANB	7	46.4 $\pm$ 23.2 (100)	96.8 $\pm$ 123.4 (100)	3.0 $\pm$ 1.4 (85.7)	4.3 $\pm$ 2.6 (100.0)
	NB	9	107.4 $\pm$ 45.1 (100)	258.2 $\pm$ 187.4 (100)	6.0 $\pm$ 6.5 (88.9)	9.6 $\pm$ 10.9 (88.9)

Op = *Ornithodiplostomum ptychocheilus*, Pm = *Posthodiplostomum minimum*, Bc = *Bolboforus confusus*, Ty = *Tylodelphys* sp.

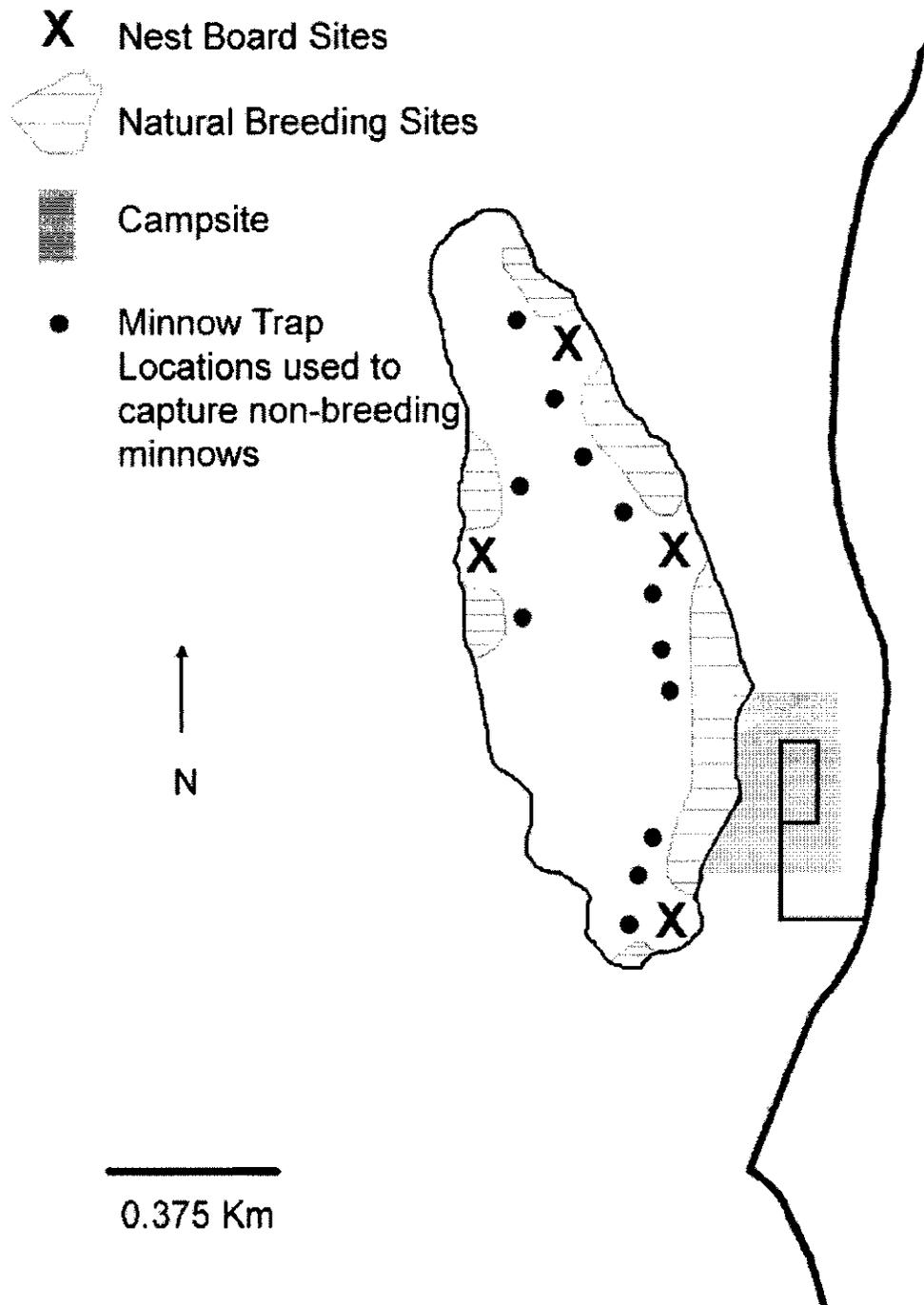


Figure 1: A map of Chain Lake, Alberta indicating the placement of artificial nest boards, natural breeding sites, and traps used to collect non-breeding minnows.

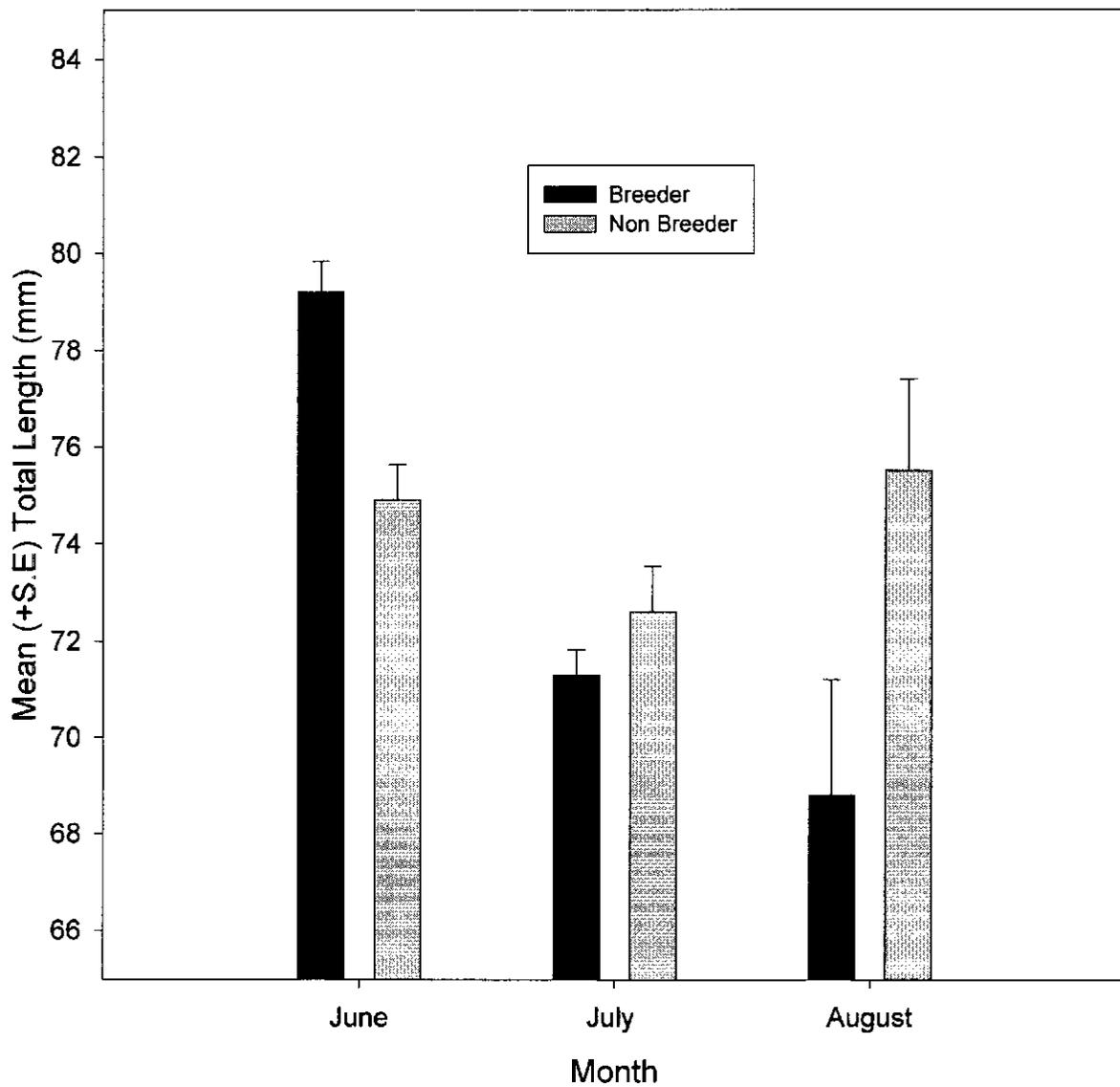


Figure 1: Mean total length ( $\pm$ S.E.) of breeding and non-breeding minnows sampled during the beginning, middle, and end of the 2001 breeding season from Chain Lake, Alberta, Canada.

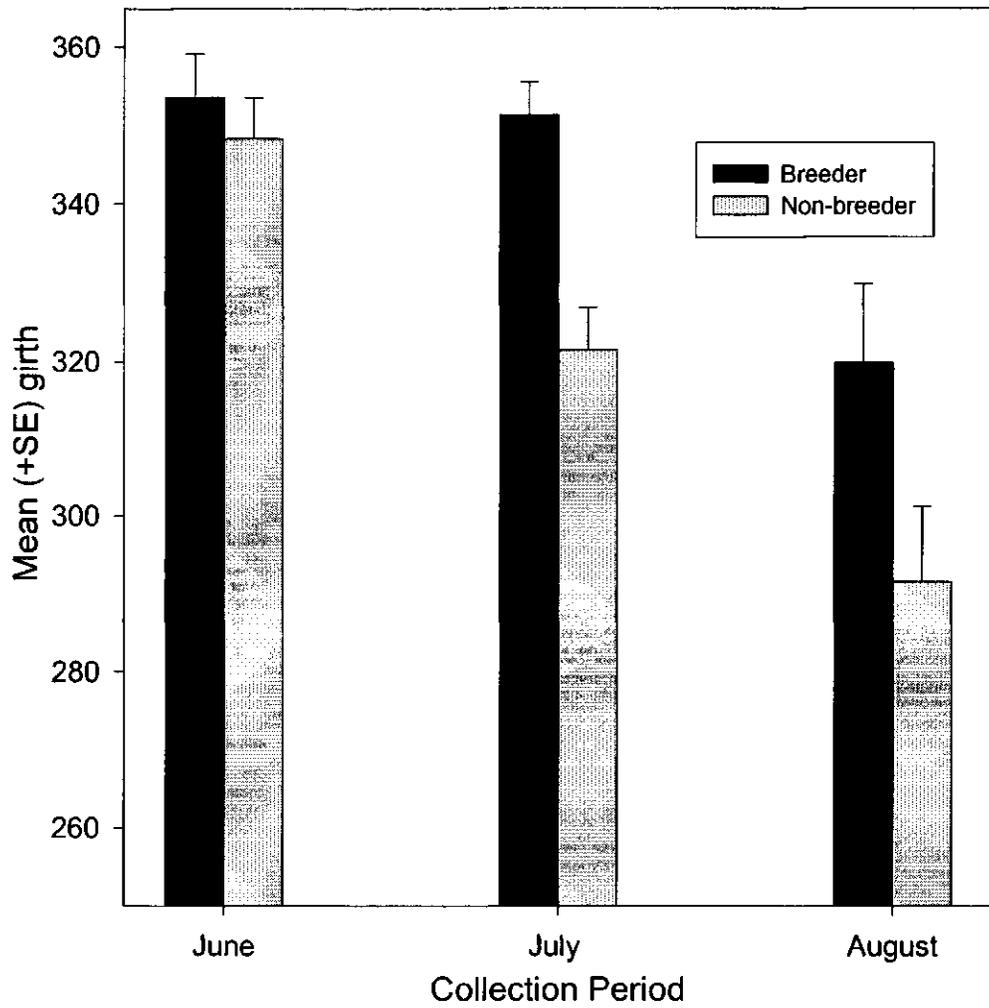


Figure 3: The girth (+SE) of male minnows, independent of total length, sampled during the beginning, middle, and end of the 2001 breeding season from Chain Lake, Alberta, Canada.

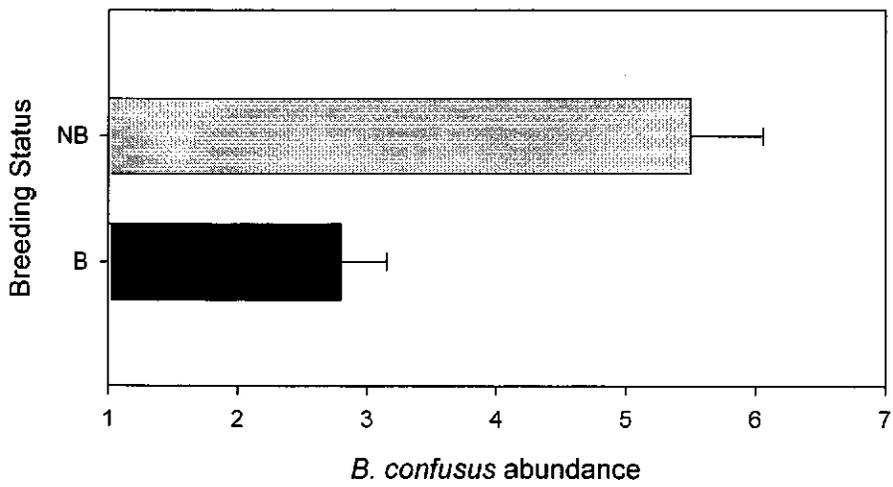
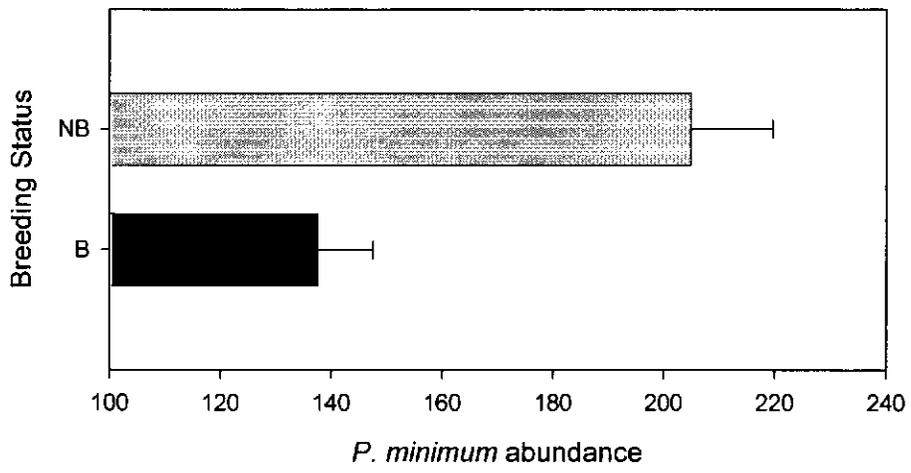
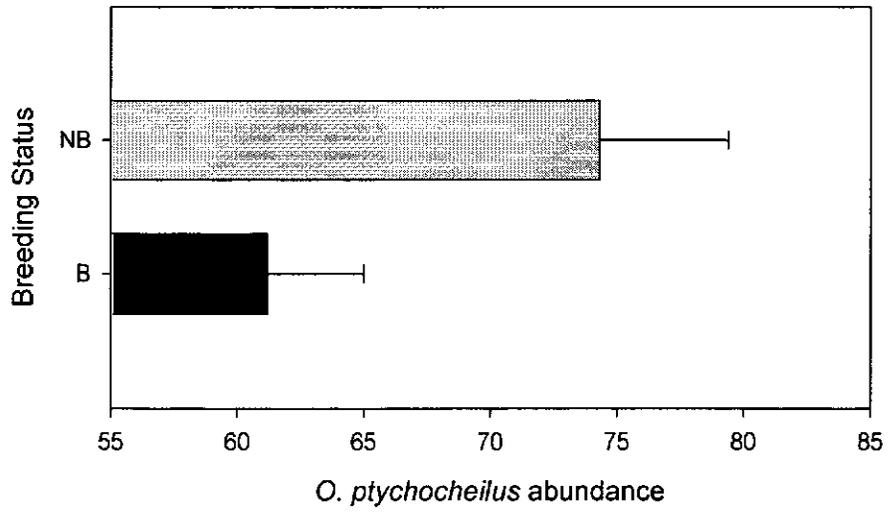


Figure 4: Mean (+SE) abundances of *O. ptychocheilus*, *P. minimum*, and *B. confusus* metacercariae in samples of breeding (B) and non-breeding (NB) male minnows.

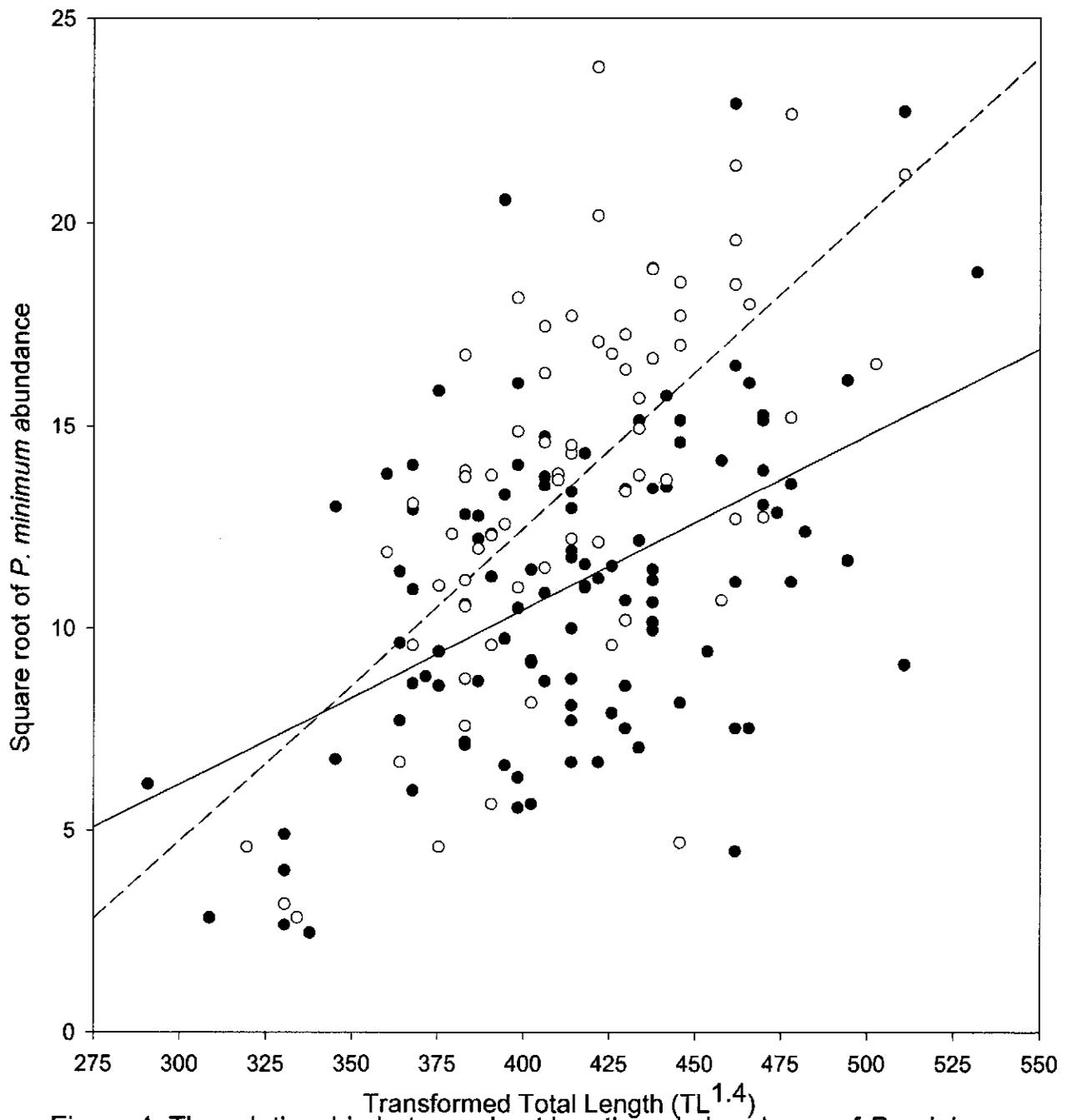


Figure 4: The relationship between host length and abundance of *P. minimum* metacercariae for samples of breeding (solid circles) and non-breeding (hollow circles) male minnows collected from Chain Lake, Alberta, Canada. Regression line equations are  $y = -18.4 + 0.08x$  for non-breeders (dashed line) and  $y = -6.7 + 0.04x$  for breeders (solid line), where,  $y$  = transformed *P. minimum* abundance, and  $x$  = transformed total length.

## Chapter 5:

### General Conclusions

#### *Host populations from different habitats*

The main advance of the field monitoring study (Chapter 2) over the earlier study by Sandland *et al.* (2001) was the description of the population dynamics of a second species of trematode (*Posthodiplostomum minimum*) and the description of trematode infection characteristics within snail intermediate hosts. In general, the transmission dynamics of *P. minimum* were found to be similar to those of *O. ptychocheilus*, and similar to other trematodes studied in north-temperate habitats (reviews by Chappell 1995; Chubb 1979). Thus, transmission of cercariae peaked in late summer and early fall, especially into larger hosts. There was extensive inter-lake variation in metacercariae intensities in minnows, probably reflecting differences in features such as temperature. Surprisingly, events occurring within first intermediate hosts had little impact on metacercariae recruitment into fish.

However, by monitoring seasonal changes in infection levels in snails and in minnows, I was able to approximate the manner in which various transmission events are synchronized with the availability of hosts in the two lakes. I was therefore able to qualitatively describe the windows available for the transmission of free-living stages of both of these species. Several interesting predictions can be made that may provide the key to understanding the notoriously high spatial and temporal variation in infection levels seen in natural host populations. First, the transmission window for infections of snails may be larger during years with

below average temperatures in May through July because slower-growing snails will be exposed to miricidia for longer periods of time. Thus, this longer period of transmission may result in increased numbers of infected snails, leading to increased intensities in minnows. Similarly, extended warm periods between August and October may also result in higher-than-average intensities in minnows because cercarial release from infected snails would be extended. Each of these predictions would be straightforward to test in long-term field studies designed to monitor infection levels in snails, fish, and ideally also in birds. Such tests are important because they would be the first to specifically evaluate the link between variation in transmission potential and variation in parasite intensities. Clearly, such an approach on Schistosomes or other anthropogenic trematodes would have applied implications.

#### *Parasite life histories*

The finding that two specialist trematodes that share all of their hosts and have similar population dynamics (Chapter 2) should also have similar life-histories (Chapter 3) is not surprising. The results from Chapter 3, to my knowledge, are the first attempt to compare the reproductive output of two closely-related trematodes in several stages of the life-cycle. As predicted, the two species had very similar patterns of growth and reproduction, during both the adult stages and the asexual larval stages. The carry-over effects of adult size to other stages in the life cycle could explain almost all of the differences in life-history traits. Thus, *P. minimum*, which is the larger of the two parasites, had larger metacercariae, higher daily cercarial production, and higher per capita

fecundity. The over-riding importance of body size was also shown in a comparative study involving 7 species of schistosomes (Loker 1983) and 35 species of nematodes (Morand 1996).

One surprising difference between *O. ptychocheilus* and *P. minimum* was cercarial head size, particularly since both species use the fathead minnow as a host. Cercariae are a free-living motile stage and do not obtain energy from external sources. Thus, dispersal and penetration of the host require large energy reserves (e.g. Lawson and Wilson 1980; Lowenberger and Rau 1994). *Ornithodiplostomum ptychocheilus* cercariae may require more energy to penetrate, reach and encyst in the optic tectum of minnows than *P. minimum* cercariae need to penetrate, reach and encyst in the mesenteries. The difference in the number of *O. ptychocheilus* cercariae produced, compared to *P. minimum*, suggests that small differences between these species may be related to the cost of producing individual cercariae. Cercariae may be analogous to sperm because they do not have means to obtain energy. Also, during development cercariae must be provided with sufficient resources to survive in a variety of environments and encyst within their hosts, similar to sperm development. Thus, investment in cercariae may vary between and within parasite species, like sperm (Parker 1993; Parker and Begon 1993). An important area for future research is to determine whether there are intra-specific differences in cercariae between or within hosts of one species and whether small differences such as cercarial size can affect recruitment.

### *Sexual selection and male-male competition*

The host reproduction study demonstrated that parasites have the potential to impact male competitive ability because heavily-infected male minnows experienced reduced success in obtaining and/or defending nests. An important finding of this study was that the 3 most common parasites of minnows had consistently lower intensities of trematodes in breeders compared to non-breeders (Chapter 4). These results could not be explained by differences in sampling protocol, or by differences in size between breeders and non-breeders. However, the mechanisms that underlie these results remain unclear. Perhaps these parasites reduce male nesting success by altering the energy budget of heavily infected individuals. Thus, a useful follow-up study would aim to determine physiological costs of infection, especially during the period of metacercariae development. Lemly and Esch (1984) found that the trematode, *Uvulifer ambloplitis*, reduced total body lipid and body condition of juvenile bluegill sunfish, *Lepomis macrochirus*, during the encystment phase. Recruitment of cercariae into juvenile minnows is high in these northern lakes (Chapter 2) and encystment does not typically occur until the following spring (Sandland 1999). Thus, encysting metacercariae may reduce energy reserves during times when competition for nesting sites is strong.

Male-male competition in Chain Lake is likely strong because many males, particularly early in the season, are not found within nests (Chapter 4) and nesting substrate is probably limiting (per. obs). Thus, the availability of nesting substrate may limit or reduce reproductive success in male minnows. However,

in many lakes (Rochester?) nesting substrate may not be a limiting factor and the effect of parasites may be absent or reduced. In sand gobies (*Pomatoschistus minutus*) strong intra-sexual selection exists when nesting substrate is limiting, but is weaker in areas where nests are plentiful (Forsgren *et al.* 1996). Thus, male–male competition for nesting substrate is likely context dependant, and dependent upon the availability of nesting substrate. It follows that future studies should examine the effects of parasites on male nesting success in lakes with different densities of nesting substrate or through experimental manipulation of nest density within one lake.

Lastly, the results of the host reproduction study have important implications for studies of parasite-mediated sexual selection. These results, together with the field study by Candolin and Voigt (2001) provide the strongest evidence to date that parasites can affect the outcome of male-male competition. Regardless of the precise mechanism leading to this effect, lightly-infected males have a greater probability of attaining and defending a nest than heavily-infected males, and therefore have higher reproductive success. Importantly, females that select defending males (or their nests), will also be selecting lightly-infected males. If a male's ability to obtain and keep a nest is a heritable trait (perhaps associated with body size or his ability to increase girth), then females that mate with these males will be attaining genes that ultimately lead to decreased parasite intensities. It is possible that such traits are directly linked to genes for resistance to these trematodes, but contrary to the predictions of Hamilton and Zuk's (1982) model of parasite-mediated sexual selection, resistance genes are

not an absolute requirement. Follow-up studies on the minnow/trematode system could ideally address these issues, particularly since minnows can be bred under laboratory conditions. Moreover, the system provides an ideal opportunity to experimentally evaluate the relative roles of parasites on male/male competition and/or female choice.

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