INTERACTIONS BETWEEN A BRAIN-ENCYSTING TREMATODE AND ITS INTERMEDIATE HOST, THE FATHEAD MINNOW

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ABSTRACT

Determining the extent to which parasites influence natural populations of hosts is a major focus of studies in parasitology. Addressing this issue requires host-parasite systems that can be monitored under natural conditions and can be manipulated in the laboratory. I study a model system involving the larval trematode *Ornithodiplostomum ptychocheilus* that encysts in the brains of its intermediate host, the fathead minnow (*Pimephales promelas*). This parasite was the most common and abundant of 13 other parasites found in minnows in four boreal lakes in Alberta, Canada. In two of these lakes, prevalence of infection reached 100% in most years and mean intensity ranged from 4 to 40 parasites/host.

Field and laboratory experiments showed that the size, the rate of parasite development, and time to encystment were intensity-dependent. However, parasite intensity had no effect on host or parasite survival after a simulated winter in the laboratory. One effect of infection was that infected fish had significantly greater cranial heights and widths than controls. The expression of this parasite-induced alteration in host phenotype was dependent on the size of the fish at infection and on parasite intensity. The cranial distortion led to significantly higher mortality of fish maintained on poor diets and altered the host's phototactic response.

PREFACE

On Being a Parasite

"A predator is an animal that eats other animals, generally those smaller and weaker than itself. We respect predators, paint them on our coats of arms and use their names as adjectives of quality. To be termed lion-hearted, cat-footed and as strong as a bear offends no person. A parasite is an animal that eats other animals that are always larger and more powerful than itself. We despise parasites and to call a person a louse, which is, after all, a courageous little animal in its own way, is to ask for trouble."

The Australian Museum

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Chapter 1. General Introduction

Determining the extent to which parasites influence individual hosts has always been a central aim in parasitology. Initially, researchers focused on describing the more obvious effects of parasitic infection on their hosts, such as those on growth, physiology, immunocompetence, and survival. Describing such effects, especially from an anthropogenic perspective, has provided the framework for most classical textbooks in parasitology (e.g. Schmidt and Roberts, 1989).

Over the past quarter-century, two extensions of this early focus on individual-level effects have dominated subsequent inquiry. One is the recognition that parasites can affect individual hosts in much more subtle ways than previously thought. Although the subtle interactions between parasites and host immunity continue to play a central role, those between parasites and host behaviour (e.g. Poulin, 1995), host life-histories, host reproduction (Minchella, 1985), and even host morphology (Poulin and Thomas, 1999) have become increasingly common.

Another important extension of earlier studies is the recognition that individual-level effects can translate to effects on host populations. Indeed, since Crofton (1971) and Anderson and May (1979) theoretically showed that the negative effects of parasites could impact host populations, the question has been a central focus of the discipline. Whether or not their models apply to natural populations has been the

source of controversy since the early 1980's (Holmes, 1982; Anderson and May, 1982). Resolving the controversy has been difficult and is far from complete, despite its focus in at least five edited textbooks over the past decade.

Supportive evidence for the subtle effects of parasites on host individuals, and for the role of parasites on host populations has come from studies which combine field, laboratory, and modeling approaches (Holmes, 1995). Not surprisingly, the inherent difficulties involved with investigating natural host-parasite interactions in a laboratory setting makes such studies rare. However, it is the handful of exceptions that have provided the most convincing evidence. Thus, using a combined modeling and experimental approach, Hudson et al. (1992) demonstrated that subtle reductions in the birth rate of red grouse, *Lagopus scoticus*, infected with cecal threadworms (*Trichostrongylus tenius*) reduced the host population the following year. By experimentally reducing worm intensity in a semi-natural population, they verified that it was indeed the parasite, and not other factors, that controlled host population size over a 10-year interval (Hudson and Dobson, 1998). Similar, although less intensive, studies have shown that native Hawaiian birds are killed by infection with avian malaria (*Plasmodium* sp.), with drastic effects on host populations (van Riper et al., 1986). Detailed laboratory and field studies by Schall (1990) show similar effects of lizard malaria on their hosts, although the population-level consequences are unclear. Thus, evidence is

accumulating that the effects of infection on individual hosts, albeit subtle in many cases, can influence host populations.

However, our ability to generalize from these few systems is hindered by several shortcomings. First, the best examples come from systems involving either direct life-cycle parasites or those that utilize a single insect vector. Their applicability to more complex systems, such as those involving most helminths, is restricted. Second, the majority of model host-parasite systems are restricted to terrestrial interactions. Those that have investigated aquatic host-helminth systems have focused exclusively on laboratory manipulation (Webber et al., 1987) or have examined field-infected fish (Lemly and Esch, 1984). Lastly, most of the model host-parasite systems come from areas that have been anthropogenically altered. For example, the exceptional work by Hudson and his coworkers involves a host that is managed in Great Britain for maximum density.

One way to address these shortcomings is to select model host-parasite systems that meet several requirements. Most importantly, the system should be amenable to controlled infections in the laboratory. It is only by satisfying this requirement that comparisons between infected and uninfected hosts can be made, thus allowing tests of specific hypotheses. Second, the system should be amenable to detailed field study under natural conditions. In this way, natural patterns of transmission can be monitored and realistic infection levels can be set in the laboratory. Also,

studies incorporating aquatic hosts, particularly those with complex life-cycles, could help evaluate the generality of the conclusions reached by Hudson, van Riper, Schall and others. The objective of this thesis was to use an aquatic host-parasite system, amenable to both field and laboratory experiments, to assess the subtle effects of parasites on host individuals and to make a preliminary assessment of how such affects might influence host populations.

The host-parasite system

Investigating the degree to which parasites affect their hosts and host populations requires a parasite that has the potential to induce host pathology. Such systems often involve parasites that invade the central nervous systems (CNS) of their host (Holmes and Zohar, 1990). Larval trematodes comprise a high proportion of the parasite species that invade the nervous tissues of fish hosts (Holmes and Zohar, 1990). In many of these systems, metacercariae can reach high intensities in critical regions of the CNS such as the brain (Hoffman, 1958; Barber and Crompton, 1997). Metacercarial brain infections have been shown to alter host behaviours (Bibby and Rees, 1971; Lafferty and Morris, 1996; Radabaugh, 1980b), decrease host survival (Szidat, 1969; Ballabeni and Ward, 1993) and distort both the brain (Hoffman and Hoyme, 1958; Heckmann, 1992) and the cranium itself (Mueiler, 1972). Therefore, systems involving brain-

encysting metacercariae hold high promise for elucidating the potential of parasites to negatively affect individual hosts and host populations.

I studied a model fish-parasite system involving the brain-encysting trematode *Ornithodiplostomum ptychocheilus* (Digenea: Diplostomidae) and its second intermediate host, the fathead minnow, *Pimephales promelas*. This species has a typical trematode life-cycle involving a snail first intermediate host, fish second intermediate hosts and piscivorous bird definitive hosts. Adult parasites reside in the intestinal tracts of birds such as mergansers and herons (Hoffman, 1960) and release eggs with the host feces. These hatch into ciliated miricidia which actively penetrate freshwater snails of the genus *Physa* (Hoffman, 1958; Hendrickson, 1986). After a period of asexual reproduction in the snail, cercariae are shed. These penetrate the epidermis of the fish, enter the peripheral nerves and eventually migrate up the spinal chord to the brain (Hendrickson, 1979). After a period of development within the brain tissue, the larvae encyst, typically in the optic lobes and cerebellum (Radabaugh, 1980a). Parasites can remain encysted for the life of the host or until the fish is consumed by a bird.

Previous work on the metacercarial stage of *O. ptychocheilus* provides a strong foundation for further study. Details of its geographical range (Hoffman, 1958, 1960; Amin, 1982; McDonald and Margolis, 1995; Amin and Minckley, 1996; Gibson, 1996), life-cycle (Hoffman, 1958; Hendrickson,

1986), the migration of cercariae in fathead minnows (Hendrickson, 1979), cyst ultrastructure (So and Wittrock, 1982) and site selection in the brain (Radabaugh, 1980a) are well known. In addition, ecological studies have assessed the effect of infection on host stamina (Sogandares-Bernal et al., 1979) and host schooling (Radabaugh 1980b).

The taxonomic status of the genus *Ornithodiplostomum* is unclear and confusing (Gibson, 1996). Part of the problem arises from the fact that the parasite is regularly reported from both the body cavity and the brain (Hoffman, 1958; Sogandares-Bernal et al., 1979). Although results from experimental infections provide evidence that the parasites from the two sites are distinct (Hendrickson, 1979; Radabaugh, 1980a), the survey literature continues to recognize them as a single species. This leads to difficulty in determining the geographical range and host specificity of *O. ptychocheilus*. Based on the experimental results of Hendrickson (1979) and Radabaugh (1980a), I treat brain infections of *O. ptychocheilus* separately from those in the body cavity.

Study sites and characteristics of host populations

The four study lakes (South Calling Lakes (SCL) 20, 100, 200, and 800) are located in Alberta's Boreal Plains Ecozone (55^0 43′ - 113^0 17′) (Fig. 1). This is a region of continuous, aspen-dominated forest that stretches across the three Canadian prairie provinces. The area is bordered on the north by the Boreal Taiga Ecozone and on the south by the Aspen-parkland Ecozone.

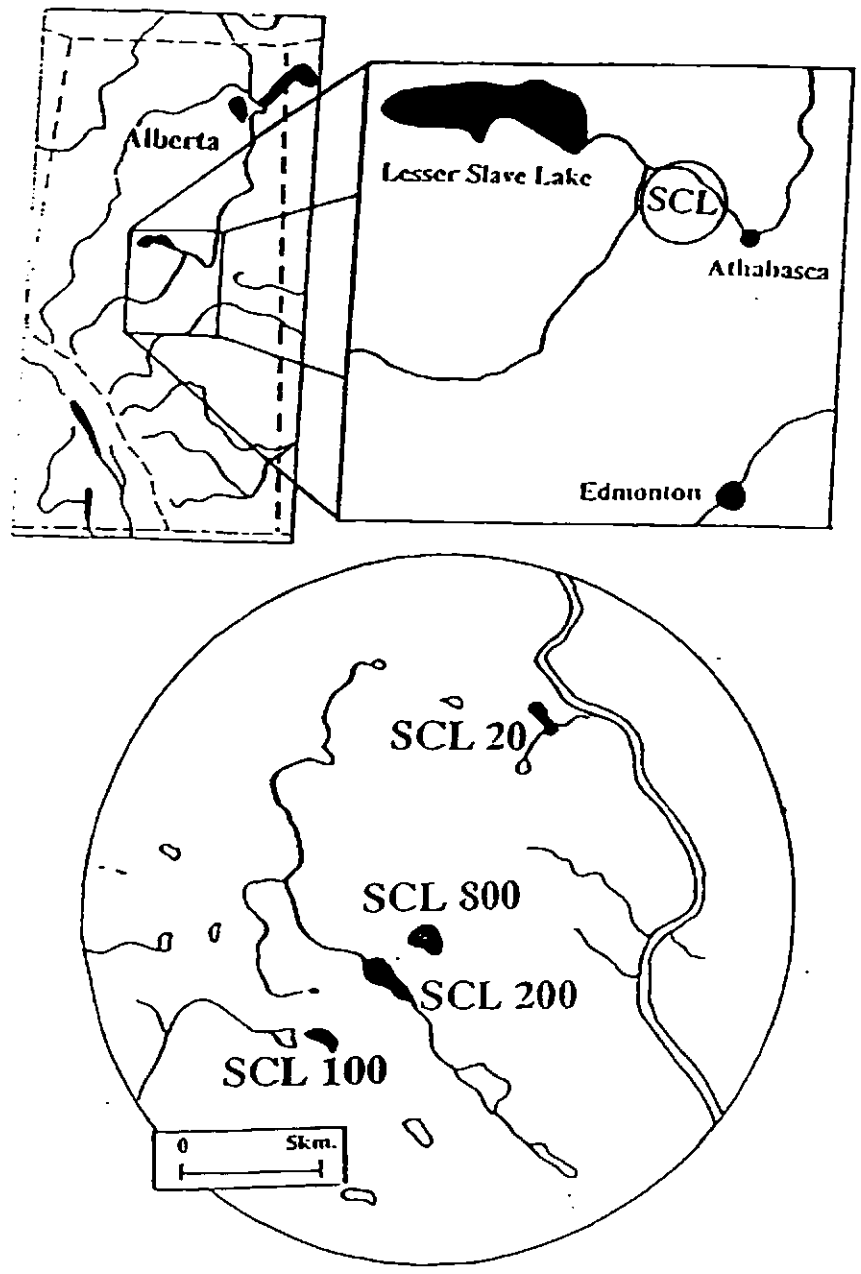

Fig. 1. A map of the four South Calling Lakes (SCL) in north-central Alberta, Canada.

The Boreal forest is dominated by trembling aspen (*Populus tremuloides*), although extensive stands of spruce (*Picea* spp.) are also common (Mitchell and Prepas, 1990). Unlike the Eastern boreal forest, this region is characterized by a low amount of surface water, low topographic relief, and prevalence of fire (Fisheries and Oceans, 1992).

Due to the unique soil geochemistry of the glacial boreal plain, lakes in the region are rich in phosphorous and are eutrophic (Mitchell and Prepas, 1990). The highly productive nature of the lakes has consequences for their aquatic flora and fauna (Fisheries and Oceans, 1992). For example, the diversity of fish species is lower than equivalent-sized lakes in Eastern Canada (mean = 2.0 species/lake vs. 3.9 species/lake), but the diversity of aquatic birds is higher (mean = 7.0 species/lake, range 2-12 vs. 2.0 species/lake, range 0-6; W. Tonn, U. Alberta, pers. comm.). Communities of zooplankton, phytoplankton, macrophytes and benthos are also species rich (E.E. Prepas, U. Alberta, pers. comm.).

Fish communities in this region are dominated by brook sticklebacks, *Culea inconstans* and fathead minnows, *Pimephales promelas* (Nelson and Paetz, 1992). Both of these small-bodied species are adapted to the short summer growing seasons and to the depletion of dissolved oxygen in winter (Tonn et al., 1990). However, both species are highly susceptible to predation, and they rarely co-occur with species such as northern pike, *Esox lucius*, and yellow perch, *Perca fluviatilis* (Tonn et al., 1990). Because

fathead minnows are so well-adapted to fluctuating ranges of pH, salinity and oxygen, they are an ideal species for laboratory experimentation (Arthur and Dixon, 1994).

The life-history of fathead minnows has also been well studied. They are sexually dimorphic and populations can be separated into three life-history categories: mature males, mature females and juveniles (Price et al., 1991). Breeding normally commences in May, continues through June, and subsides in July (Price et al., 1991; A. Danylchuk, U. Alberta, pers. comm.). The end of the breeding season often coincides with high mortality in adult fish (Price et al., 1991). The life span of breeding adults is not well known (Nelson and Paetz, 1992). Ongoing studies (A. Danylchuk, U. Alberta, pers. comm.) indicate that in the four SCL lakes, breeding adults are from 2 to 4 years old.

Objectives of the thesis

This thesis is divided into three general components. The field component (Chapter 2) summarizes the transmission dynamics of *O. ptychocheilus* in the four SCL lakes. Because of my interaction with the Terrestrial Riparian Organisms, Lakes, and Streams (TROLS) project, samples of minnows were available for the two years prior to the start of my thesis. Thus, Chapter 2 describes changes in the population dynamics of *O. ptychocheilus* in these four pristine lakes over the ice-free period between 1995 and 1998. I also report on the infection intensities of all of

the other parasites in the lakes, in each of the other co-occurring hosts. The field study was designed to provide enough scope to enable me to characterize natural patterns of infection and to provide the background for the laboratory experiments.

The second component attempted to provide the necessary background for subsequent experiments. Many parasitologists consider simple 'dose-response' experiments to be a necessary pre-requisite for further study. Thus, only by exposing hosts to known and varying doses of worms can we expect to understand features such as parasite recovery, mean intensity, and intensity-dependence. Chapter 3, describes a simple, dose-response experiment that was designed to evaluate the effect of metacercarial intensity on the growth and survival of both the parasites and the hosts.

The third component involved experiments designed to test hypotheses generated by the observations from the field data (Chapter 2) and the dose-response experiment (Chapter 3). In Chapter 4, the experiment was designed to test whether infection with *O. ptychocheilus* influenced the overwinter survival and growth of juvenile minnows. Hosts were infected with two doses of worms, and then subjected to a period of artificial winter in the laboratory. In Chapter 5, the experiment was designed to assess the effects of infection on the cranial morphology of minnows, to determine the conditions under which such effects were

manifested, and to determine their consequences on host behaviour, growth, and survival.

The TROLS program

The region encompassing the four SCL lakes (Fig. 1) is experiencing large-scale forestry operations for the first time. As a result, two comprehensive, field-based projects are currently underway which were initiated to understand the effects of logging and fire on aquatic ecosystems. The TROLS project is a multidisciplinary study established in 1995 to evaluate the role of buffer strip width in determining the stability and resilience of boreal aquatic ecosystems in Alberta. The Sustainable Forestry Management (SFM) Network was established in 1996 to study the effects of natural vs. logging disturbance on aquatic and terrestrial biodiversity. At least 70 scientists are involved in these projects, including economists, sociologists and biologists. It should be noted that prior to the initiation of the TROLS and SFM projects, this region of boreal Canada was virtually unstudied.

My study was integrated into the overall aims of the aquatic component of TROLS and SFM. Since 1995, a large team of fisheries biologists has been studying the effects of clear-cut logging on fathead minnow populations. The objectives of their work were to determine 1) the landscape-level distribution of minnows across the boreal forest, 2) the effects of watershed disturbance on reproduction and population sizes of minnows in 12

selected lakes, and 3) the mechanistic roles of factors such as overwinter mortality and size-selective predation in determining minnow population sizes. The latter objective is met through the use of an array of experimental dugouts situated at the Meanook Biological Research Station, Alberta (54^0 38′ - 113^0 17′). The availability of monthly samples of minnows, the access to the isolated lakes, and the experimental dugouts used in this study would not have been possible without my association with the TROLS project.

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Chapter 2. Parasite assemblages in forage fish from four boreal lakes of central Alberta, with emphasis on the population dynamics of the trematode, *Ornithodiplostomum ptychocheilus*

ABSTRACT

Annual, seasonal, and spatial variation in the population size of the trematode *Ornithodiplostomum ptychocheilus* was assessed in fathead minnows (*Pimephales promelas*) collected from four boreal lakes in north-central Alberta, Canada. Samples of the two other fish species that inhabit the lakes, finescale dace (*Phoxinus neogaeus*) and brook stickleback (*Culaea inconstans*), were also examined. A total of 11 species of trematode metacercariae was recovered from the host communities; 6 were specific to a particular host species. *Ornithodiplostomum ptychocheilus* was specific to fathead minnows and it was an important component of the parasite assemblages in all four lakes.

Ornithodiplostomum ptychocheilus intensity, prevalence, and variance to mean ratios fluctuated spatially and temporally in young-of-the-year fathead minnows. Much of this variation could be attributed to the effects of lake, year, season, and host size. In two of the four lakes, prevalence often reached 100%, with mean intensity ranging between 4 and 40 metacercariae/host. Mean intensity peaked in late fall in both lakes. The significant two-way interactions between the main effects (Lake*Year; Lake*Month; Year*Month) suggests that extensive variation in local,

intra-lake factors (e.g. temperature, depth, snail density) leads to variation in mean intensity.

INTRODUCTION

Determining the extent and causes of spatial and temporal variations in parasite populations provides parasitologists and epidemiologists with three useful forms of information. First, tracking parasite recruitment into hosts allows insight into where and when these organisms have the greatest potential to affect their hosts (Hudson and Dobson, 1997). Second, combining recruitment patterns with environmental parameters can allow investigators to determine the factors responsible for natural variation in parasite transmission (Valtonen et al., 1997). Third, population-level studies provide the important background information necessary for further, preferably experimental, investigations into parasite population dynamics (Lemly and Esch, 1984a, b).

The population dynamics of trematode metacercariae in fish has been extensively studied (reviewed by Chubb, 1979; Kennedy, 1981, 1987; Aho et al., 1982, Camp et al., 1982; Lemly and Esch, 1984a; Coleman and Travis, 1998). Many of these investigations have documented extreme spatial (Olsen, 1966) and temporal (reviewed by Chubb, 1979; Aho et al., 1982; Camp et al., 1982) variation in parasite intensity and prevalence between samples. Explaining the underlying factors responsible for this variation has been a major aim of parasitologists for decades.

Ornithodiplostomum ptychocheilus is a trematode (Strigeida: Diplostomidae) whose metacercariae encyst in the brains of fathead minnows (Hoffman, 1958; Hendrickson, 1978; Radabaugh, 1980). This parasite has been reported throughout the geographical range of its host (Hoffman, 1960; Hendrickson, 1978; McDonald and Margolis, 1995), in locations where its two other hosts (the snail, *Physa gyrina* and fish-eating birds) are found. In preliminary surveys of fathead minnows from boreal lakes of north-central Alberta, C. Goater (U. Lethbridge, pers. comm.) found *O. ptychocheilus* intensities an order of magnitude greater than those reported in other natural populations (Hendrickson, 1978; Radabaugh, 1980).

The purpose of this study was to monitor annual, seasonal, and spatial variation in mean *O. ptychocheilus* intensity, prevalence and dispersion in four lakes in Alberta's boreal forest. In addition to providing information on the population dynamics of this metacercarial infection in four boreal lakes, the study was also designed to provide the basis for a long-term study on the factors influencing *O. ptychocheilus* intensities (Goater and Tonn, pers. comm.). Lastly, the sampling protocol was designed to provide the background information required to support our laboratory and field experiments (Chapters 3, 4 and 5) .

MATERIALS AND METHODS

Study sites and collection procedures

Monthly sampling trips were made to the four South Calling Lakes (Chapter 1) during the ice-free period from 1995 to 1998. Prior to 1997, fish were collected from these lakes by researchers involved in the TROLS program (see Chapter 1) and preserved for later necropsy. Sampling on the same date within each month was not possible due to yearly fluctuations in the duration of the ice-free period and the isolated nature of the lakes. However, over the four years, monthly collections were performed within two weeks of each other.

Fish were collected from four to five randomly selected sites around each lake. Randomization procedures followed those established by Danylchuk and Tonn (U. Alberta, pers. com.). Adult and young-of-the-year (YOY) were collected from each site. Adult fathead minnows (*Pimephales promelas*), finescale dace (*Phoxinus neogaeus*) and brook stickleback (*Culaea inconstans*) were captured using standard minnow traps set overnight. YOY were caught with a specialized net (diameter: 60 cm) which was cast from a boat a total of ten times at each site. The net was then drawn back at approximately 1 meter per second. Once captured, all fish were anesthetized in MS 222. Adults were frozen and YOY were preserved in 80% EtOH for transport back to the laboratory.

Necropsies

All fish were thoroughly examined for metazoan parasites using standard necropsy techniques. For the general survey component, 15 adults of each fish species were necropsied from each lake in order to identify the parasite species present, their prevalences, and their intensities. Dace and sticklebacks were necropsied from a collection in June of 1998 and fathead minnows were necropsied from May of 1995. After dissection, parasites were prepared and identified using standard procedures. YOY fathead minnows were necropsied from each lake, for each month during the ice-free periods of 1995 and 1996.

Although complete necropsies were performed, emphasis was on the population dynamics of *Ornithodiplostomum ptychocheilus*. For the 1997 and 1998 collections, YOY were necropsied in the order of the latest sampling date (September/October) to the earliest (May/June). Necropsies were performed until the date at which no infections were detected. Thirty individuals were examined from each sample, and attempts were made to necropsy equal numbers of fish from each site within a sample. When sample sizes were limiting, I necropsied the maximum number of hosts available (n = 10-30). Total lengths, standard lengths, and weights were determined for all fish. Sex was determined for adult fish. Otoliths were also collected from all fathead minnows (adult and YOY) for future age-related studies.

Analyses and terminology

Data were analyzed using JMP software (Sall and Lehman, 1996). Prior to analysis, *O. ptychocheilus* intensities were log transformed to satisfy the normality assumption. Variations due to lake, year, and season were analyzed with ANCOVA where log host size was the covariate. Each lake was considered independent since detailed studies on fish populations provided no evidence of minnow migration among lakes (Danylchuk and Tonn, pers. comm.). Definitions of mean intensity, prevalence, and variance to mean ratio follow Bush et al. (1997).

RESULTS

Parasite composition and host specificity

Parasite prevalence and mean intensity varied extensively among species of host (Table 1). Of the 15 parasite species present, eight were between 80 and 100% prevalent in at least one of the four lakes. All of these were larval trematodes. Larval nematodes and cestodes were never present in more than 53% of the individuals within a single sample and mean intensities were consistently less than 5 metacercariae/host. Ectoparasites were absent from all samples.

Four species of trematode dominated the parasite assemblages in fathead minnows. Of these, *O. ptychocheilus* from the brain had the highest prevalence (93 to 100%) in all four lakes and had the highest mean intensity. *Ornithodiplostomum ptychocheilus* from the body cavity,

Bolbophorus confusus, and *Posthodiplostomum minimum* showed less consistent prevalence, and mean intensity was rarely greater than 10 parasites/host within a lake.

Most species were specific to one particular fish taxon, with very little overlap between hosts within a lake (Table 2). *Ornithodiplostomum ptychocheilus*, *P. minimum*, Metacercariae C, and *Tylodelphys* sp. 1 were each found in fathead minnows alone. Similarly, both *Apatemon* sp. 2 and *Schistocephalus solidus* were found exclusively in brook stickleback. Other species such as Nematode A, *Diplostomum spathaceum*, *Bolbophorus confusus*, *Apatemon gracilis*, *Apatemon* sp. 1, and *Tylodelphis* sp. 2 showed overlap between hosts but did not overlap among all three fish species within a lake (Table 2).

A full community-level analysis was beyond the scope of this paper. For the purposes of this study, three results are relevant to the population dynamics of *O. ptychocheilus*. First, this species was a major component of the parasite assemblages in all lakes and in all years. Second, intensities varied dramatically among lakes, suggesting that there were differences in parasite recruitment. Third, *O. ptychocheilus* was restricted to fathead minnows. Therefore, analysis of annual, seasonal, and spatial variations of *O. ptychocheilus* in this single host provides an accurate representation of its population dynamics.

Population dynamics of *O. ptychocheilus* in fathead minnows

Analyses of differences in mean intensity between samples of fathead minnows were restricted to SCL 200 and 800 where prevalences and intensities in YOY were relatively high in all years (Table 1). July, August, and September samples were included in the analysis as an October sample was unavailable for SCL 200 in 1998.

Mean *O. ptychocheilus* intensity (Fig. 1), prevalence (Fig. 2) and dispersion (Fig. 3) varied extensively between samples. ANCOVA results on mean intensity showed that lake, year, and season contributed to this variation (Table 3). Interactions between these factors also explained much of variation. For example, the Lake*Year interaction explained a significant amount of the variation in mean *O. ptychocheilus* intensity, indicating that annual fluctuations in parasite intensities were not consistent among lakes. Host size was also responsible for much of the variation in *O. ptychocheilus* intensity. In general, larger fish were more heavily infected than smaller fish ($r = 0.50$, $df = 722$, $P < 0.001$; Table 3).

The relationship between log variance and log mean parasite intensity was approximately linear ($r = 0.9$, $P < 0.001$) with a slope of 1.8 (data combined from 24 samples). In addition, r^2 was 0.81, indicating that approximately 80% of the variance in parasite counts could be explained by differences in mean intensity.

Mean intensity fluctuated erratically among lakes. In SCL 20 and SCL 100, mean intensity remained below 2 metacercariae/host. There were significant differences in mean intensity between fish from the heavily-infected lakes (Fig. 1). Annual recruitment in SCL 200 reached a peak in September of 1998 when mean intensity averaged 34.4 metacercariae/host (Fig. 1a). Mean intensities in SCL 800 were usually much lower than those in SCL 200, reaching a maximum intensity of 14.4 metacercariae/host in September of 1995 (Fig. 1b).

Patterns of increasing prevalence and overdispersion generally followed patterns of increasing mean intensity (Fig. 2a, b; Fig. 3a, b). Parasites in minnows from both SCL 200 and 800 were highly overdispersed in the latter periods of each year, with variance to mean ratios always much greater than one (Fig. 3). Parasites from SCL 200 demonstrated greater overdispersion than those from SCL 800. The distribution of parasites in YOY from SCL 20 and 100 were either underdispersed or close to random.

No consistent patterns were observed in parasite recruitment among years. For example, in SCL 200, mean intensity averaged 5 metacercariae/host in September of 1995 yet averaged 34.4 metacercariae/host in September of 1998 (Fig. 1a). There were also differences in annual parasite recruitment in SCL 800 where mean intensity reached a maximum of 14.4 metacercariae/host in October of 1995 compared to 4 metacercariae/host in October of 1998 (Fig. 1b).

There was also variation in the magnitude and duration of parasite recruitment both among lakes and among years (Table 3). In SCL 200, recruitment usually started in June whereas infections in SCL 800 were not detected until July (Fig. 1 a, b). The magnitude of subsequent parasite acquisition was erratic in SCL 200. In 1998, mean *O. ptychocheilus* intensity rose rapidly after initial infections in June as opposed to the more consistent increases observed in 1995 and 1997. SCL 800 showed a greater consistency among years although mean intensity increased more rapidly in 1995 than in subsequent years.

DISCUSSION

Parasite communities in fathead minnows

Parasite assemblages in forage fish are usually dominated by one or two species of helminth (Janovy and Hardin, 1987; Kennedy, 1990). For example, fathead minnows collected from the Platte River system in Nebraska had two species of helminth (McDowell et al., 1992). Plains killifish (*Fundulus zebrinus*) from Nebraska (Janovy and Hardin, 1987) and common minnows (*Phoxinus phoxinus*) from Wales (Bibby, 1972) harboured a total of one and four species, respectively. In contrast to these depauperate systems, fathead minnows in four lakes on the northern Boreal plain were infected with a total of 13 species of helminth, some of which demonstrated extremely high mean intensities. Thus, communities of helminths in fathead minnows collected from lakes in

the boreal forest of Alberta are relatively complex with high species richness.

The parasite community in fathead minnows was dominated by strigeid metacercariae. For example, of the four helminth species reported from European minnows, two were found as adults (a trematode and an acanthocephalan, Bibby, 1972). Similarly, six of the nine species reported from longnose dace matured in that host (Muzzall et al., 1992). These contrasting patterns lead to two questions concerning the parasite communities of fathead minnows: 1) what factors lead to high species richness and dominance by larval strigeids? and 2) what factors restrict the colonization of adult acanthocephalans, nematodes, cestodes and trematodes?

The answers to these questions are speculative. One striking aspect of the South Calling Lakes, in particular SCL 200 and 800, is the high density and diversity of pisciverous birds (Paskowski and Pierre, U. Alberta, pers. comm.). Indeed, the sites in this study have over six times the number of potential definitive hosts reported by Janovy and Hardin (1987) or Muzzall et al. (1992). Esch (1971) reported that larval parasites dominated eutrophic lakes where birds, as opposed to fish, were the tertiary predators. In these types of aquatic systems, the predator-prey pathways favour larval forms that mature in birds rather than those that mature in fish (Holmes, 1979). Therefore, both the diversity of the avifauna and their dominance as

tertiary predators may explain why the three species of forage fish were only infected with larval parasites that mature in birds.

The low occurrence of other larval parasites (in particular cestodes and nematodes) was also surprising. One explanation is a lack of suitable intermediate hosts. There are large and diverse populations of snails in these lakes which explains the success of trematode transmission into the fish. The composition of zooplankton in these lakes is unknown, but it is possible that the species within this community are either unsuitable or are not at high enough densities for successful transmission of larval cestodes and nematodes.

Other factors may be important in decreasing the species richness of adult parasites in the forage fish. It is possible that the dynamic nature of these lakes prevents the establishment of adult parasites. Phenomena such as winterkill (Robinson and Tonn, 1989) and high host mortality after breeding (Price et al., 1991) could potentially reduce the colonization potential of adult forms. Another possibility is that adult parasites in this system are annual and were not detected because of host recruitment after the spring collection periods.

A final, unexpected result was that approximately half of the 15 parasites species were host specific. This degree of specificity was unexpected, given that both the survey literature and laboratory experiments have found strigeids to be broad host generalists (Olsen, 1966; Chappell et al., 1994;

McDonald and Margolis, 1995; Gibson, 1996). Although differences in host diet could explain the specialization of the larval cestodes and nematodes (Dogiel, 1964), this explanation is inadequate for trematodes which directly penetrate their fish hosts. Instead, factors such as habitat segregation, host behaviour, host immunity, and host physiology must be important in determining the patterns of specificity in this system. Each of these factors alone, or in combination, has been used to explain parasite specificity in other systems (Hart, 1992; Chappell et al., 1994; Haas, 1994).

Although a full community-level analysis was beyond the scope of this paper, these results describe two important and previously unrecognized features of helminth communities in fish. First, species richness was relatively high, although only a single group was dominant. Adult trematodes, acanthocephalans, nematodes and cestodes were absent. Second, the three dominant species, *O. ptychocheilus* (brain) *O. ptychocheilus* (body cavity), and *P. minimum* were strict host specialists in the lakes. Currently, the literature on fish parasite communities is dominated by studies that focus on low-diversity systems (1 to 3 species/host) and those which are dominated by host generalists that transmit through predator-prey pathways (Kennedy, 1990). The overall structure of metacercarial communities in freshwater fish, and the identification of factors which determine that structure, has never been studied.

Population dynamics of *O. ptychocheilus*

Orders of magnitude differences in intensity between samples of hosts, as shown in these results, seems to be a general feature of the population dynamics of metacercariae in fish (Aho et al., 1982; Camp et al., 1982; Lemly and Esch, 1984a; Barber and Crompton, 1997; Coleman and Travis, 1998). In addition, factors such as collection site, season, year and host size, all of which affected *O. ptychocheilus* intensities in this study, have also been shown to contribute to variation in metacercariae intensity in these same studies. Lastly, it is not unusual to find that both the variance in parasite counts, and the level of overdispersion, is positively correlated with mean intensity (Anderson and Gordon, 1982; Scott, 1987). Thus, an understanding of the variation in parasite counts between samples can be determined by understanding the factors that lead to differences in mean intensity.

One consistent pattern was the seasonal peak in *O. ptychocheilus* intensity prior to ice-on. Although this occurred in all four lakes, it was most apparent in SCL 200 and 800. Several studies have reported a similar peak in the fall (Chubb, 1979; Spelling and Young, 1986; Menard and Scott, 1987), while others show a second wave of recruitment in early spring (Kennedy, 1987). In this system, an early release of parasites may have been undetected as YOY were not present in the minnow population until late May or early June. However, reports from other northern systems suggest that infected snails either lose their infections (Sankurathri and Holmes,

1976b) or die (McKindsey and McLaughlin, 1995) during winter. In SCL-200, none of the *Physa* collected in spring over the past four years released cercariae of any species. These results suggest that in these northern lakes, there is a single wave of cercariae recruitment in early and late autumn.

Although this seasonal pattern was consistent among lakes and years, the duration and magnitude of parasite recruitment varied dramatically. This was especially noticeable in minnows from SCL 200 and SCL 800 that always had higher mean intensities compared to fish from the other two lakes. One explanation for the inter-lake variation is their inherent variation in abiotic factors such as surface area, watershed area and depth. Each of these factors will have an important impact on features such as lake temperature and wind patterns, both of which are known to affect cercariae release and survival (Chappell et al., 1994). There is also evidence that combinations of factors can act in a cascade to alter *O. ptychocheilus* dynamics. For example, SCL 200 reached its lowest water level in 1998. The reduction in water volume paralleled an early increase in water temperature, which, in turn, coincided with an early detection of *O. ptychocheilus* infections.

Concurrent studies on these same lakes by limnologists, hydrologists and aquatic biologists have documented unparalleled variation in the biodiversity of macrophytes, zoobenthos, zooplankton and birds (A. Danylchuk and W. Tonn, U. Alberta, pers. comm.; E.E. Prepas, U. Alberta,

pers. comm.). Each of these factors alone, or in combination, could influence parasite transmission through their effects on final hosts, snails, or the cercariae themselves. For example, differences in the density of *Physa gyrina* may be one factor responsible for the observed variation in *O. ptychocheilus* intensities. This species reaches high densities in SCL 200 and SCL 800 but has rarely been observed in the other two lakes. Likewise, the diversity and density of potential final hosts is also much higher in the two heavily-infected lakes (discussed above).

The localized nature of transmission is central to recent theoretical studies in parasitology and epidemiology. Mollison and Levin (1995) conclude that it is only by understanding the highly localized, small-scale processes that determine individual infection status, that we can fully understand large scale patterns of infection. In a series of extensive long-term field studies, Kennedy (1981, 1987) concluded that much of the variation in population sizes of strigeid trematodes in fish was the result of local factors within a lake, many of which were stochastic. I concur with this view and conclude that variation between the SCL lakes in factors such as host community structure, productivity and temperature best explain the highly variable intensities of infection in minnows. Such variation in parasite intensity, generated by local factors within a lake, has potential ecological (Chapters 3, 4 and 5) and evolutionary (Goater and Holmes, 1997) implications for both the parasite and its host.

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Table 1. Intensity (mean± s.d.), prevalence (%) and tissue location of helminth parasites in species of forage fish collected from 4 lakes in north-central Alberta, Canada (n = 15 fish/species).

Species	Organ	Lake			
		SCL 20	SCL 100	SCL 200	SCL 800
Fathead minnow					
Ornithodiplostomum ptychocheilus	B	5.6 ±5.0 (100%)	10.6 ±5.7 (93%)	210.0±138.8 (100%)	178.8±64.3 (100%)
Ornithodiplostomum ptychocheilus	BC,M	3.2±2.5 (87%)	1.0±0 (20%)	3.4±2.4 (53%)	1.0±0 (20%)
Bolbophorus confusus	BM,G	3.4±2.9 (80%)	1.4±0.5 (53%)	9.1±10.8 (80%)	1.0+0 (20%)
Posthodiplostomum minimum	BC	4.0±2.9 (73%)	94.4±64.5 (100%	15.3±19.9 (73%)	1.8±1.0 (60%)
Apatemon gracilis	BC,E,B	0%	0%	1.0 (7%)	0%
Apatemon sp. 1	BC,L,E	0%	1.0 (7%)	0%	0%
Metacercariae C	BC	0%	1.0±0 (13%)	1.0±0 (20%)	1.7±0.5 (47%)
Diplostomum spathaceum	E	2.0±1.4 (13%)	4.6±3.3 (80%)	1.8±1.7 (40%)	1.0±0.8 (20%)
Tylodelphys sp. 1	B	1.0 (7%)	1.0 (7%)	1.5±0.7 (13%)	1.5±0.6 (40%)
Tylodelphys sp. 2	E	0%	2.3±1.4 (47%)	2.0±1.2 (47%)	1.0±0 (27%)
Nematode A	M	2.3±1.4 (53%)	1.0 (7%)	1.0±0 (33%)	1.0 (7%)
Philonema sp.	H	1.0 (7%)	0%	0%	0%
Ligula intestinalis	BC	1.0 (7%)	0%	0%	0%
Finescale dace					
Bolbophorus confusus	BM	X	0%	7.2±8.6 (73%)	1.0±0 (40%)
Nematode A	M	X	1.3±0.6 (20%)	0%	1.0±0 (13%)
Philonema sp.	H	X	1.0±0 (27%)	0%	0%
Brook stickleback					
Apatemon gracilis	BC,E	X	2.5±1.5 (87%)	3.1±3.2 (53%)	2.2±1.1 (93%)
Apatemon sp. 1	BC,L,E	X	19.8±13.8 (87%)	27.9±29.8 (87%)	1.7±0.7 (60%)
Apatemon sp. 2	S	X	43±0 (7%)	77.5±40.3 (13%)	0%
Diplostomum spathaceum	E	X	7.3 ±5.7 (100%)	2.0±1.8 (80%)	1.6±0.5 (47%)
Tylodelphys sp. 2	E	X	1.9±1.3 (60%)	1.8±1.5 (27%)	0%
Schistocephalus solidus	BC	X	4.7±3.8 (47%)	0%	0%

B = brain, BC = body cavity, BM = body musculature, E = eye, G = Gills, H = heart, L = Liver, M = mesenteries, S = stomach, X = not present.

Table 2. Patterns in the exchange of helminths between three species of forage fish in three lakes in north-central Alberta (n = 15 fish/species/lake). Values represent the proportion of the total number of parasites (of each species) found in each lake, in each host.

Parasite species	Lake								
	SCL 100			SCL 200			SCL 800		
	FM	FSD	BS	FM	FSD	BS	FM	FSD	BS
Ornithodiplostomum ptychocheilus (B)	100% (153)	-	-	100% (3150)	-	-	100% (2682)	-	-
Ornithodiplostomum ptychocheilus (BC)	100% (3)	-	-	100% (33)	-	-	100% (3)	-	-
Bolbophorus confusus	100% (29)	-	-	55% (98)	45% (79)	-	40% (4)	60% (6)	-
Posthodiplostomum minimum	100% (1416)	-	-	100% (168)	-	-	100% (14)	-	-
Metacercariae C	100% (2)	-	-	100% (3)	-	-	100% (11)	-	-
Apatemon gracilis	-	-	100% (39)	4% (1)	-	96% (24)	-	-	100% (28)
Apatemon sp.1	1% (2)	-	99% (245)	-	-	100% (365)	-	-	100% (17)
Apatemon sp.2	-	-	100% (43)	-	-	100% (155)	-	-	-
Diplostomum spathaceum	31% (51)	-	69% (111)	31% (11)	-	69% (24)	15% (2)	-	85% (11)
Tylodelphys sp. (1)	100% (1)	-	-	100% (3)	-	-	100% (9)	-	-
Tylodelphys sp. (2)	58% (21)	-	42% (15)	67% (14)	-	33% (7)	100% (4)	-	-
Nematode A	43% (3)	57% (4)	-	100% (5)	-	-	100% (1)	-	-
Philonema sp.	-	100%(4)	-	-	-	-	-	-	-
Schistocephalus solidus	-	-	100% (24)	-	-	-	-	-	-

FM = fathead minnow; FSD = finescale dace; BS = brook stickleback

Table 3. Summary ANCOVA statistics for the effects of lake, year and month on the intensity of the trematode *O. ptychocheilus* in the brains of fathead minnows. Main effects were adjusted for the effects of the covariate, host size.

Source of variation	df	Mean square	F-value	P-value
Host size	1	4.98	96.4	<0.001
Lake	1	1.99	38.6	<0.001
Year	3	6.54	42.2	<0.001
Month	2	2.42	23.4	<0.001
Lake*Year	3	7.32	141.7	<0.001
Lake*Month	2	0.49	9.4	<0.001
Year*Month	6	0.15	2.9	0.01
Residual	359	0.05		

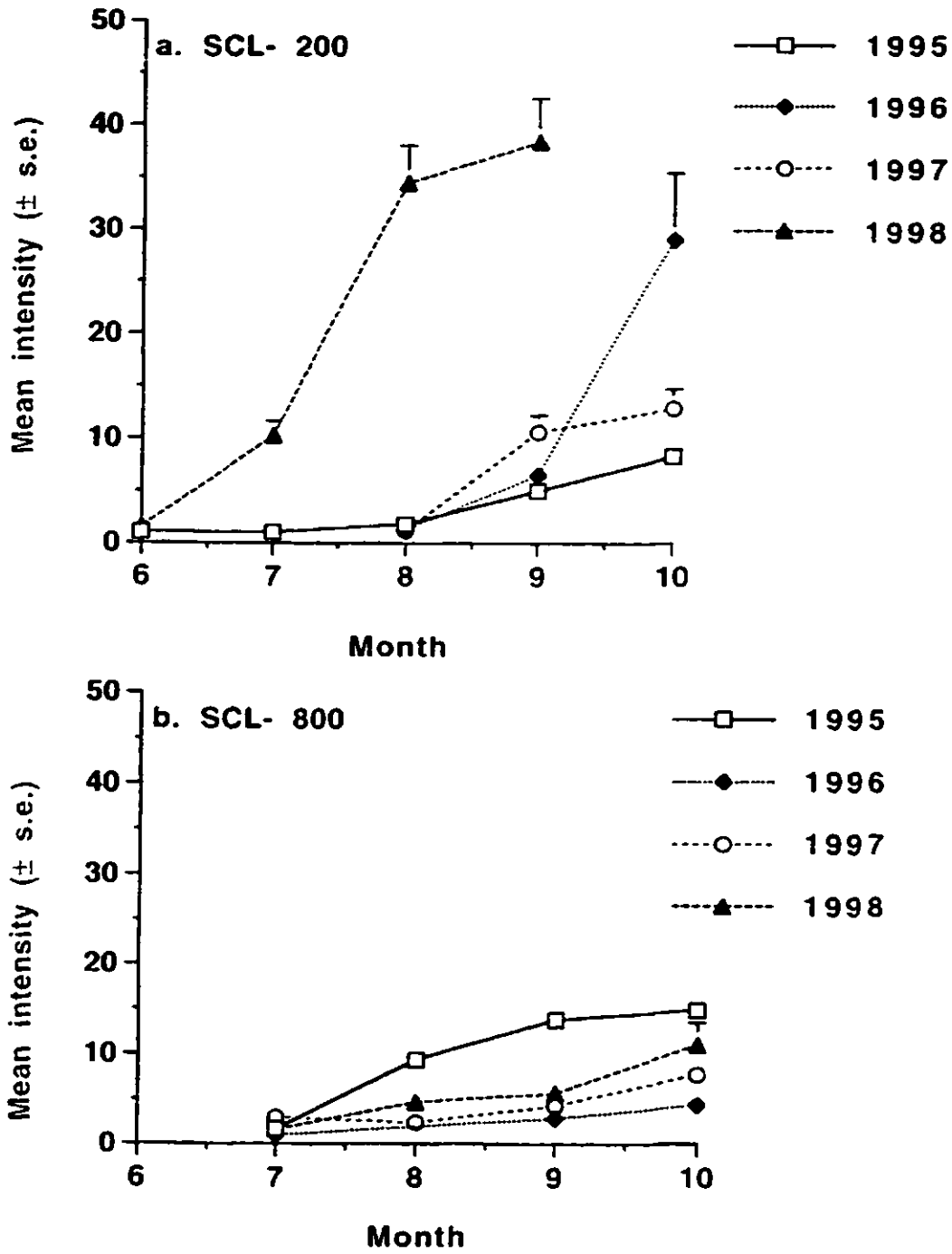

Fig. 1. Seasonal and annual changes in mean intensity of the trematode *O. ptychocheilus* in young-of-the-year fathead minnows from SCL-200 (a) and SCL-800 (b). Standard error bars are excluded from some points for clarity.

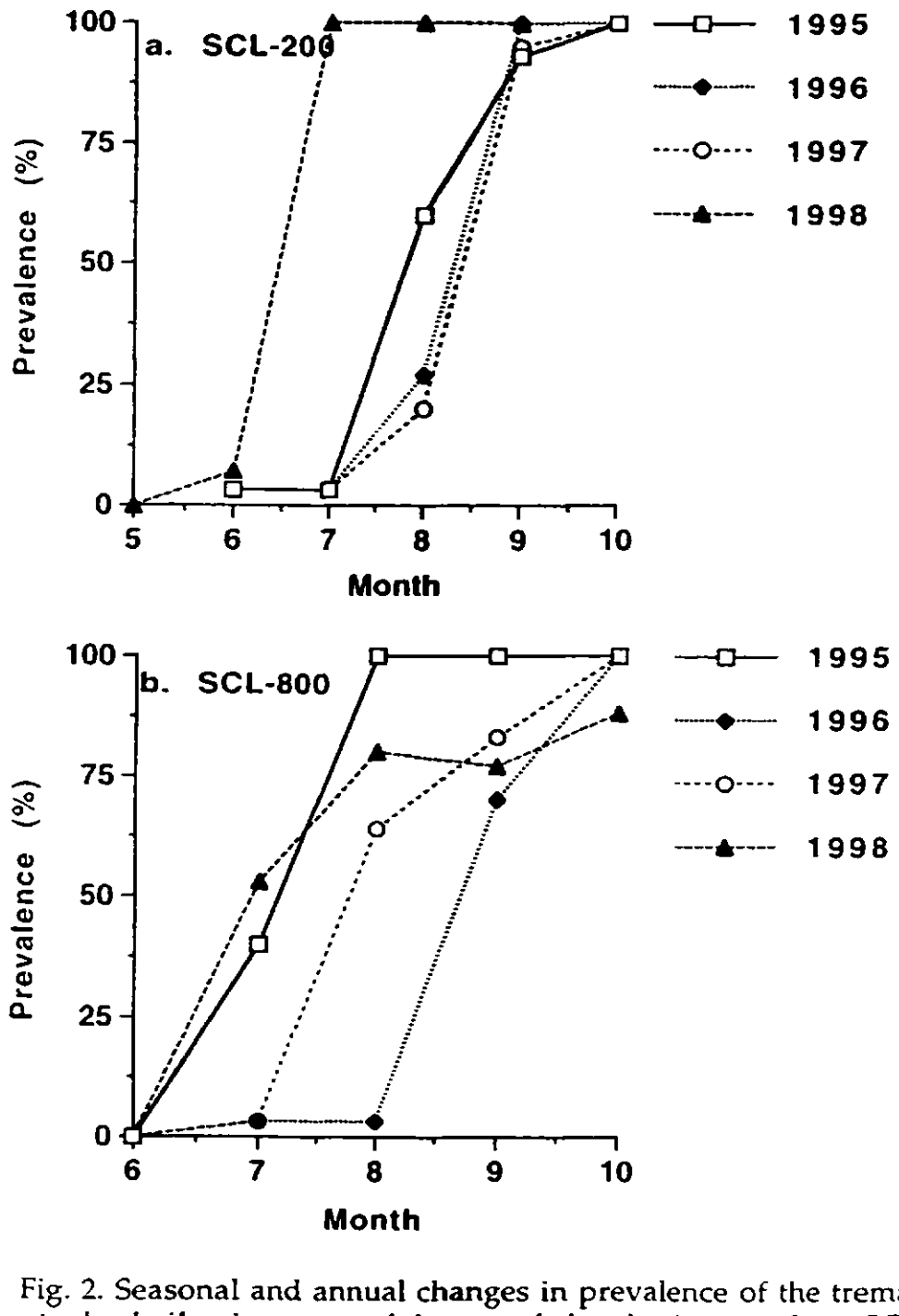

Fig. 2. Seasonal and annual changes in prevalence of the trematode *O. ptychocheilus* in young-of-the-year fathead minnows from SCL-200 (a) and SCL-800 (b).

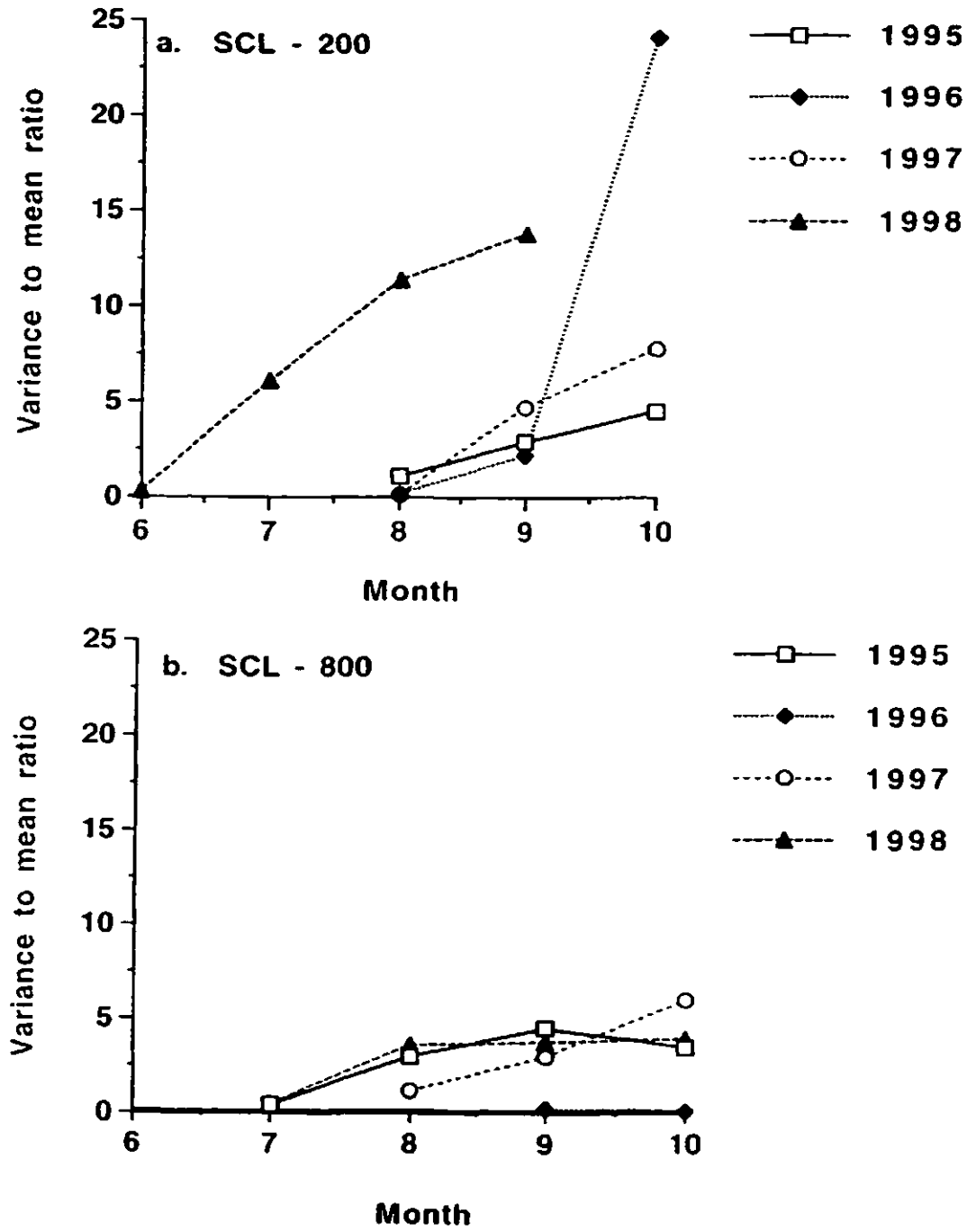

Fig. 3. Seasonal and annual changes in dispersion of the trematode *O. ptychocheilus* in young-of-the-year fathead minnows from SCL-200 (a) and SCL-800 (b)

Chapter 3. Interactions between *Ornithodiplostomum ptychocheilus* (Trematoda: Diplostomatidae) and fathead minnows (*Pimephales promelas*): Intensity dependence and host response

ABSTRACT

Dose-dependent recruitment, size, and time to encystment were studied for a brain-encysting trematode (*Ornithodiplostomum ptychocheilus*) of fathead minnows (*Pimephales promelas*). Fish were exposed once to 0, 20 or 120 cercariae and then maintained in individual containers in the laboratory, or in screened enclosures in outdoor ponds. Host response to infection was tested by comparing cercarial recruitment into fish exposed once to cercariae, with fish that received challenge doses at three-week intervals.

Increasing intensity resulted in reduced metacercarial growth and development (time to encystment) but there was no evidence of a response to challenge infection. In both the laboratory and enclosure experiments, developing metacercariae in low-dose fish were approximately 20% larger than those in high-dose fish. Metacercariae in low-dose fish also encysted 2-4 weeks earlier. By 8 weeks post-infection (p.i.), encysted metacercariae from high-dose fish were similar in size to those from low-dose fish, indicating that intensity-dependence acted primarily on the rate of development rather than on final metacercarial size. These results highlight the pre-encysting development stage as an important component of the life-cycle of this trematode. It is during this stage when metacercarial growth rate is highest and where the potential for intensity-dependent regulation is strongest.

INTRODUCTION

Density-dependent processes are well-recognized as important components of the population biology of parasitic organisms, especially helminths (Keymer, 1982; Quinnell et al., 1990; Shostak and Scott, 1993). Such processes have the potential to regulate parasite population sizes because increased density, or intensity in parasitological terms (Bush et al., 1997), is correlated with reduced parasite establishment, development and or reproduction. The processes are complex and context-dependent. Thus, in some parasite/host interactions, density-dependence results from interspecific competition between parasites for limited space or nutrients (Goater, 1992). In others, they result from host immune responses (Wakelin, 1996) or from direct antagonistic interactions between parasites (Cook and Roberts, 1985). Determining the relative importance of these processes is difficult, because each can play a role within any or all of the stages in the life-cycle of a particular parasite.

For trematodes, evidence for intensity-dependence comes primarily from studies involving the adult stage, such as *Fasciola* in sheep (Smith, 1984), *Echinostoma* in hamsters and birds (Yao et al., 1991) and larval stages in snail first intermediate hosts (Kendall and Ollerenshaw, 1963; Gerard et al., 1993). The metacercarial stage has largely been ignored, even though studies on the comparable life-cycle stages of cestodes (Rosen and Dick, 1989; Nie and Kennedy, 1993; Wedekind, 1997) and acanthocephalans (Brattey, 1986) have demonstrated intensity dependence. This is an important shortcoming since accumulating evidence suggests that intensity-dependent processes within

intermediate hosts can regulate parasite suprapopulations (Gemmell et al., 1987) and can also determine the structure of parasite communities in final hosts (Bush et al., 1993).

In this study, the metacercariae stage of a brain-encysting trematode (*Ornithodiplostomum ptychocheilus*) in fathead minnows (*Pimephales promelas*) was used to evaluate the relative importance of two potential regulatory processes affecting parasite recruitment, growth, and development. First, individual fish were exposed to one of two doses of cercariae and then dissected biweekly in order to assess potential intensity-dependent effects on parasite performance. This experiment was performed both under controlled laboratory conditions and under semi-natural field conditions. Second, the efficacy of a host response to infection was examined under laboratory conditions. This was done by comparing cercarial recruitment in laboratory fish exposed once or repeatedly.

MATERIALS AND METHODS

Parasite life-cycle and source of parasites and hosts

Metacercariae of *Ornithodiplostomum ptychocheilus* encyst on and in the dorsal surface of the brain, typically occupying the space between the optic tectum and the cranium (Hoffman, 1959; Hendrickson, 1979; So and Wittrock, 1982). As in most strigeid trematodes, there is a prolonged development period which occurs within the tissue of the optic lobes and cerebellum. Adults

infect the small intestine of piscivorous birds. Eggs are shed into water, and upon hatching, release miricidia that penetrate the snail, *Physa gyrina*.

Experimental infections required a source of metacercariae, uninfected F1 snails and surrogate definitive hosts (day-old chickens). The metacercariae were obtained from naturally-infected adult fathead minnows collected from Rochester Lake, north-central Alberta (Lat. 54° 22', Long. 113° 27') on 5 June, 1997. Prevalence of *O. ptychocheilus* in adult fish was 100% in this lake and intensities ranged between 55 and 600 metacercariae/host (n=25). To obtain the F1 snails, adult *Physa gyrina* were collected on 25 July, 1997 from an unnamed lake (SCL-200) near South Calling Lake, Alberta (approximately 100 km north-west of Rochester Lake). *Ornithodiplostomum ptychocheilus* is the most common trematode infecting *P. gyrina* in SCL-200 (Sandland, unpublished). The adult snails were bred in the laboratory following the methods of Ward et al. (1997). Lastly, to provide a source of uninfected fish for experiments, adult minnows were captured from Rochester lake and bred in parasite-free dugouts at the Meanook Biological Research Station (15 km south of Athabasca, Alberta). Young-of-the-year (YOY) minnows were collected from these dugouts in late summer to be used for experimental infections. YOY minnows were 4 to 6 weeks old at the time of collection.

Infection procedures

The methods used to infect juvenile fish follow Ward et al. (1997). Snails were infected by exposure to miricidia that were obtained from infection of six, day-old chickens. The birds were fed brains from eight naturally-infected

minnows from Rochester Lake. Between four and nine days p.i. (post-infection), host faeces were collected, pooled and placed into a 400 mL beaker containing tap-water (22°C). All water had been aerated within a 50 L tank for at least 48 h prior to being used in the experiments. The faecal solution was spun with a stir bar, allowed to settle and then decanted three times. Eggs were isolated from the solution by filtration through a 90 µm, followed by a 50 µm, mesh. They were then placed in 60 mm Petri dishes containing 20 mL aged tap water and incubated at 22°C in the dark. Under these conditions, miricidia hatched from eggs 10 to 16 days later. Snails (uninfected F1) were infected by enclosure in 1 mL micro-centrifuge tubes containing aged water, with five active miricidia for three hours. After exposure, snails were transferred to 1 L containers and maintained in the laboratory (at 22°C) on a diet of fresh lettuce and Tetramin. Snails released cercariae starting at approximately 28 days p.i.

Minnows were infected by placing one to three hr-old cercariae, from 8 to 10 infected snails, into a 500 mL Erlenmeyer flask and volume adjusted to 400 mL. Three, 1 mL aliquots were removed from the sample and the average number of cercariae/mL was determined. The volume of cercarial suspension required to contain either 20 or 120 cercariae was estimated, and then pipetted into 60 mm Petri dishes. Exposure doses were chosen to encompass the range of *O. ptychocheilus* intensities found in YOY minnows collected from SCL-200 in Sept. 1996 (range 10-113 metacercariae, n=25; Chapter 2).

For all laboratory experiments, minnows were maintained in individual 3 L plastic containers (20 cm long X 20cm wide X 10 cm high). The water in the containers was not aerated. Containers were maintained in an environmental chamber at 20°C with a constant 16h:8h L:D photoperiod. The water in each container was replaced every four days with aged tap-water. After each water-change, minnows were fed Tetramin fish flakes. Rations were adjusted every two weeks to equal 10% of the mean mass of the hosts necropsied at that interval (n = 15). This allowed for minnow growth rates similar to that observed in natural minnow populations (Danylchuk and Tonn, U. Alberta, pers. comm.).

Experimental design – Intensity dependence

Cercariae for this experiment came from snails exposed to miricidia on 19 August, 1997. Snails released cercariae starting on 19 September and the experiment was set up two days later. Sixty minnows were assigned at random (using random number tables) to individual Petri dishes, each of which contained 0, 20 or 120 cercariae (n = 20 minnows/treatment). Fish were exposed to cercariae for 2 hr. Prior to the beginning of the experiment, five fish from each treatment were assigned a necropsy date at 2, 4, 6 or 8 weeks p.i. On the date of necropsy, we determined host lengths (mm), host weights (0.01g), and metacercarial intensity.

For the determination of metacercarial growth and development, intact brains were removed from the host and placed on a microscope slide. After removal, the brain was immediately fixed with 1.0 mL of 70% ethanol and

then teased apart with dissecting needles. To ensure sufficient samples of linearly oriented metacercariae, a 24mm X 24mm cover slip was placed on top of the brain/metacercariae solution without additional pressure. Immediately after fixation, the slide was examined for the larvae under a compound microscope at 40X magnification. Maximum metacercarial lengths and widths were measured using an ocular micrometer. All undamaged metacercariae were measured from low-dose fish. For high-dose fish, the first 20 undamaged metacercariae observed, were measured.

The field experiment aimed to determine whether the results obtained in the laboratory experiment reflected those under semi-natural conditions. One hundred and eighty size-matched, YOY minnows were collected from the dugouts at the Research Station on 27 August, 1998. Sources of fish, snails and parasites were the same as in the laboratory experiment. The 180 fish were divided into three groups of 60, with each individual randomly selected for exposure to 0, 20 or 120 cercariae. After the two-hour exposure period, 15 fish per treatment were selected at random and added to each of 12 enclosures (3 infection treatments X 4 replicates/treatment). Each enclosure measured 1 m long X 1 m wide X 1 m deep and was constructed of mosquito-netting attached to a PVC frame. Styrofoam blocks were attached to the enclosure to ensure floatation. The tops of the enclosures were not covered in screen and thus remained open to dispersal by arthropod predators. The enclosures were anchored in haphazard positions within the dugout. The experiment was run for 4 weeks, after which the enclosures were drained and the survivors measured and weighed. Five of the survivors from each

enclosure were necropsied to provide estimates of metacercariae growth and development.

Experimental design – Host response

The sources of hosts and parasites used for this experiment were the same as those described earlier, as were the procedures used to infect individual minnows. Cercariae for this experiment came from snails exposed to miricidia on 10 Oct., 1998. The experiment (9 January, 1999) involved a dose (sham-infected control, primary infection with 120 cercariae, repeated infection with doses of 40 cercariae every three weeks) X diet (high, low) factorial design. The four infection treatments each contained 12 replicates; the sham controls contained eight (Total n = 64). In addition, to account for differences in the infectivity of each batch of cercariae, five minnows were exposed to 40 cercariae on each day of repeated infection. Containers with infectivity controls were interspersed haphazardly within the overall experiment. One half of the experimental fish and the infectivity controls received the diet regime described previously (10% of their averaged body weight every 4 days) the other half received 2% of their averaged body weights. Metacercarial intensity, size, and development were assessed at 8 weeks. Infectivity controls were necropsied at the end of the experiment.

Analysis

Maximum metacercarial length was used as the most conservative estimate of development (length and width of the metacercariae were strongly correlated, $n = 741$; $r = 0.758$; $p = 0.0001$). Data on metacercarial length were analyzed using a mixed model ANOVA with exposure dose as a fixed effect. In the laboratory experiment, dose (exposure to 20 or 120 cercariae) was treated as a fixed effect; within each dose, the different fish and the different worms measured from each fish were treated as nested factors. In the field experiment, data on fish size and metacercariae development were pooled within each enclosure to avoid pseudoreplication prior to ANOVA. Dose was treated as a fixed effect; within each dose, fish from each enclosure and the parasites from hosts in each enclosure were treated as nested factors. The analysis of the repeated infection experiment compared the proportion of metacercariae recovered from the brains of minnows exposed to a single vs. multiple exposure to cercariae at the two different diets. The presence of a host response would be indicated by a reduction in the proportion of metacercariae after repeated infection, compared to the controls. A significant dose X diet interaction would indicate that host nutrition affected metacercarial recovery. I used a two-way ANCOVA (dose X diet), controlling for initial size at infection. Prior to all analyses, count and measurement data were log transformed and proportional data (for encystment) were arcsin (square-root) transformed.

RESULTS

Intensity-dependent metacercarial recovery, growth and development

Parasite recovery was estimated as the numbers of metacercariae encysted at 8 weeks p.i., divided by the exposure dose. At the high dose, 97 ± 6% of the 120 cercariae were recovered as encysted metacercariae. At the low dose, 149 ± 33 % of the estimated 20 cercariae were recovered suggesting inaccuracies in estimation of cercarial densities.

Measurements of metacercarial length and width were straightforward for unencysted, developing larvae. However, for encysted metacercariae, our measurements would be biased if 1) the cyst itself constrained metacercariae size and 2) the cyst interfered with the fixation process. To examine this possibility, I compared the lengths and widths of metacercariae within cysts, with metacercariae which we manually excysted. There was no difference in the sizes of metacercariae fixed within cysts, compared to those fixed outside of the cyst ($F_{1, 32} = 1.52$; $P = 0.227$, pooled from metacercariae measured from five fish). Thus, for our purposes, post-encystment estimations of metacercariae size were taken within the intact cyst.

Over the first four weeks of the experiment, metacercariae from the low-intensity treatment developed at a faster rate than those from the high intensity treatment. At 2 and 4 weeks p.i., metacercariae dissected from fish exposed to 20 cercariae were 30% and 21% larger, respectively, than those exposed to 120 cercariae (Table 1, Fig. 1a). From 6 to 8 weeks p.i., metacercariae from the low intensity treatment decreased in size and became

encysted (= infective) at a faster rate than those from the high intensity infections. At 6 weeks p.i. worms from the low-intensity infections were 21% smaller than those from the high intensity treatment. This decreased at 8 weeks p.i. when the difference between the high- and low-dose was 10% (Fig. 1a). Variation in metacercarial size between fish was also significant (Table 1).

Increased intensity of exposure significantly increased mean time to encystment (Table 1; Fig. 1b). At 4 weeks p.i., 24% of metacercariae at the low exposure were encysted, compared to only 4% in the high group. By six week p.i., all metacercariae from the exposure group had encysted, compared to 86% in the high-exposure group. By 8 weeks p.i., more metacercariae from the high-exposure group had reached encystment (99%), but there were still significantly fewer than the numbers encysted at the low dose (all at 100%).

For the field experiment, parasite recovery was estimated as the numbers of metacercariae recovered at the end of the experiment (4 weeks p.i.), divided by the estimated exposure dose. At the high dose, 80 ± 11% of the 120 cercariae penetrated the minnows and developed as metacercariae. At the low dose, 76 ± 13% of the 20 cercariae had developed by 4 weeks p.i. None of the metacercariae were encysted by the end of the experiment. Metacercariae measured from fish exposed to 20 cercariae were 18% larger ($\bar{X}$ = 0.24 ± 0.02 mm) than those exposed to 120 cercariae ($\bar{X}$ = 0.19 ± 0.03 mm; ANOVA on metacercarial length; dose – $F_{1,8} = 28.62$; $P < 0.001$; enclosure (nested within treatment) – $F_{8,137} = 2.90$; $P = 0.005$; Fig. 2).

Less than one half (44 ± 30%, range 0-12) of the 15 minnows placed into experimental field enclosures survived the four weeks. High variation in overall survival was a result of the loss of all 15 minnows in one control enclosure and one high-dose enclosure. There was no relationship between host survival and parasite dose ($F_{2,9} = 0.25$; $P = 0.781$) even when the two enclosures with 100% mortality were excluded from analysis ($F_{2,7} = 0.92$; $P = 0.443$).

Host response

There was a significant decline in cercarial infectivity between the first, second and third batches (one-way ANOVA on mean proportion recovery; Time – $F_{2,7} = 41.6$, $P < 0.001$; Table 2). Although minnows repeatedly exposed to cercariae harboured fewer parasites in the brain by 8 weeks p.i., percent recovery remained close to 100% after the decline in cercarial infectivity was taken into account (two-way ANOVA for effects of dose – $F_{1,23} = 0.7$, $P = 0.41$, Table 2). Metacercarial recovery was also not affected by host diet (two-way ANOVA for effects of diet – $F_{1,23} = 2.4$, $P = 0.14$, Table 2).

DISCUSSION

Metacercariae from minnows exposed to 120 cercariae were smaller during the initial development period and took longer to encyst. In the laboratory experiment and in the parallel field experiment, metacercariae from the low-exposure group were at least 19% larger at 4 weeks p.i. than those from the high-exposure group. Moreover, in the laboratory experiment, metacercariae

from the former group encysted as early as 2 weeks p.i., whereas those from the latter took up to 6 weeks. By 8 weeks p.i., metacercariae from both exposure groups converged to approximately the same size. Thus, the effect of intensity is primarily manifested as an increase in development time, rather than a permanent reduction in metacercarial size.

Similar patterns of reduced growth and delayed development are consistent with studies involving larval stages of cestodes (Rosen and Dick, 1983) and acanthocephalans (Brattey, 1986). They also confirm the general observations made by Hoffman (1959) that developing *O. ptychochelus* metacercariae were smaller at high intensities. These results support the idea that intensity-dependent processes are important during the metacercarial stage of trematodes (but see Zelmer and Esch, 1998). Together with results showing regulation of sporocyst and cercarial production in snails (Kendall and Ollerenshaw, 1963; Gerard et al., 1993) and of adult size and reproduction in definitive hosts (e.g. Smith, 1984), the potential for intensity-dependent regulation exists within all of the parasitic stages of trematode life-cycles.

However, an overall assessment of the role of intensity on *O. ptyochocheilus* metacercariae is complicated by the pattern of growth between 0 and 8 weeks p.i. Metacercariae from both the low and high-dose minnows grew linearly during the development period, but then decreased in size prior to encystment (Fig. 1a). This peaked-pattern in growth contrasts with other larval parasites that show linear or logarithmic growth prior to encystment (Rosen and Dick, 1983; Shostak et al., 1985). One explanation is that our methods were insensitive to changes in body shape which occur prior to

encystment. Thus, the decrease in body length may coincide with an increase in body width or height. To address this possibility, I analyzed changes in metacercariae width between 4 and 6 weeks p.i. and found a significant decrease at both high and low intensities. Similarily, our qualitative observations on histological sections of infected brains (Chapter 4) indicate that metacercariae maintain the same 'flatworm'-shape when they encyst. Thus, although it is recognized that encystment may involve small changes in body shape, such changes are minor compared to the 50% reduction in body length associated with encystment.

An alternative explanation is that the process of encystment involves complex physiological mechanisms, one of which involves the loss of water and/or solutes. Other invertebrates (Caceras, 1997) and protozoans (Manwell, 1968) with encystment stages undergo radical physiological and morphological changes during encystment. Such changes are linked to the maintenance of viability during unfavourable environmental conditions. For trematodes, the specific physiological mechanisms involved in the encystment process are unclear and explanations for their adaptive significance are speculative (Erasmus, 1972). For *O. ptychocheilus*, the metacercariae are an important overwintering stage and probably live for as long as the host (2-4 years in northern lakes, A. Danylchuk, U. Alberta, pers. comm.). Physiological studies on the nutrient requirements of developing larvae, followed by studies on mechanisms underlying the process of encystment are needed.

Regardless of the precise mechanism of encystment, it is clear that the process is delayed at high intensity (Fig. 1a). Such a delay may explain why

metacercariae from the high-exposure group were smaller at 4 weeks p.i., but converged to a size similar to metacercariae in the low-exposure group. Had the duration of the experiment been longer, a delayed intensity-dependent reduction in size may have been more obvious. However, based on the results from this experiment, I conclude that intensity-dependent effects on metacercariae size are restricted to the 0-4 week development period.

One explanation for the intensity-dependent reduction in size and time to encystment is intraspecific competition for limited nutrients and or space. Knowledge of the nutrient requirements for developing metacercariae is limited, although since metacercariae possess a mouth, esophagus and digestive cecae, they presumably feed directly on brain tissue. The absorption of small monosaccharides across the tegument is also important for trematode nutrition. For example, Bibby and Rees (1971) showed that encysted *Diplostomum phoxini* metacercariae absorbed labeled glucose from the brain tissue and cerebro-spinal fluid of European minnows (*Phoxinus phoxini*). Similarily, developing cestode procercoids have discrete membrane transport systems for the delivery of small amino acids across the tegument (Hurd and Arme, 1984) and it is likely that metacercariae have similar capabilities.

The space requirements of developing or encysted metacercariae are also poorly known. Developing *O. ptychocheilus* metacercariae occur within the optic lobes and cerebellum, while cysts form in the space between these structures and the cranium (Hendrickson, 1986; Chapter 4). Rosen and Dick (1983) suggested that the size and development of *Triaenophorus*

pleurocercoids were constrained by the rigid exoskeleton of its crustacean host. The same mechanism may lead to reduced development for acanthocephalan larvae (Brattey, 1986). In juvenile minnows, we have observed that high-intensity infections lead to an expansion of the cranium (Chapter 5), similar to that reported for another brain-encysting trematode in minnows (Mueller, 1972). This morphological consequence of infection implies, not surprisingly, that there is a finite space available for development in the brains of these small hosts, within which developing and or encysted metacercariae must establish.

There were two important results from the enclosure experiment. First, at 4 weeks p.i., there was a consistent 20% difference in the size of metacercariae between low-dose vs. high-dose minnows. This result verifies our findings from the laboratory experiment and provides clear evidence that intensity-dependence is relevant under semi-natural conditions. Second, metacercariae in minnows from the enclosure experiment were unencysted and were, on average, 66% smaller than those in the laboratory experiment. This result indicates that factors other than intensity play a role in determining the size and development of metacercariae. Temperature is one factor which is well known to affect the growth and development of metacercariae (Olsen, 1966) and other larval parasites (Tokeson and Holmes, 1982). The water temperature in the ponds that held the enclosures decreased from 15°C to 8°C between the beginning and end of the experiment. Thus, the marked difference in metacercarial size between the two experiments is probably best explained by differences in temperature, although factors such as host density

and host diet also could be important. Results from the enclosure experiment better reflect infection dynamics under field conditions, partly because conditions such as temperature closely paralleled those occurring in surrounding lakes and ponds. If this is so, these results imply that the intensity-dependent reduction in growth and development is likely prolonged under field conditions, possibly into the following spring.

Results from this study provide no evidence for an effective host response to infection by larval *O. ptychocheilus*. Though the numbers of recruited parasites decreased with each repeated infection, this decrease corresponded to similar reductions in the infection controls. This suggests that there was either a loss of cercarial infectivity or an increase in resistance with fish age. Regardless of the mechanisms generating the reduced infectivity, minnows repeatedly exposed to infection recruited the same proportion of cercariae as minnows exposed once. This result contrasts studies on other fish-metacercariae systems. For example, Chappell et al. (1994) reviewed several studies that document both a humoral and cell-mediated response in the well-studied system involving *Diplostomum spathaceum* in the eyes of rainbow trout. Similarly, in an experiment similar in design to ours, Aaltonen et al. (1997) identified an antibody-mediated response by roach (*Rutilus rutilus*) against cercariae of *Rhipidocotyle fennica*. However, before discounting the potential role of protective immunity in the brains of minnows to *O. ptychocheilus*, several caveats should be noted. First, my experiment had low power to detect a subtle host response, if present. Second, it is possible that the juvenile fish used in the experiment (< 4 months old) were too young to

mount an effective response or that the experiment was too short to detect an effect on parasite recruitment. Further study is required on this system to more accurately characterize a potential immune response against *O. ptychocheilus*.

In conclusion, these results provide evidence for intensity-dependent regulation, likely mediated by competition in the brain for nutrients and or space. The consequences of an intensity-dependent alteration in development time will depend on the timing of transmission to the next host. Since metacercariae are primarily recruited in autumn (Chapter 2), it is possible that intensity-dependence is irrelevant because the parasites have the entire winter to develop and encyst. This scenario is unlikely. Observations of large numbers of unencysted metacercariae in spring-collected minnows, combined with the results from the enclosure experiment, suggest that any intensity-dependent delay in encystment carries over to the following spring. Overall, these results point to the importance of the pre-encysting stage as a critical, but overlooked period in the life-cycle of this trematode. During this stage, metacercariae will have different (and probably increased) nutritional and space requirements compared to encysted forms. Thus, differences in physiology and morphology should be expected between the two forms. Differences should also be expected in how each stage interacts with the host.

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Table 1. Summary ANOVA statistics for the effects of exposure dose (20 or 120 cercariae) on the growth and development of *O. ptychocheilus* in fathead minnows. Degrees of freedom are in brackets.

	ANOVA effects		
	Metacercariae length (mm) [1]		Proportion encysted[2]
Weeks p.i.	Dose	Fish (dose)	
2	40.8 (1,8)***	3.3 (8,131)**	-
4	22.9 (1,8)**	3.4 (8,155)**	12.0 (1,8)**
6	95.1 (1,8)***	0.83 (8,145)	79.4 (1,8)***
8	12.1 (1,8)**	2.0 (8, 165)*	5.7 (1,8)*

1. F-values from nested ANOVA on metacercariae body length. Effects are those among different exposure doses, and among different fish receiving each dose.
2. F-values from 1-way ANOVA on percent of the total number of encysted metacercariae.
3. P-values are as follows: * = 0.01, ** = 0.001, *** = 0.0001

Table 2. Differences in metacercariae recovery between minnows infected singly or repeatedly with *O. ptychocheilus* cercariae and then maintained for 8 weeks on high or low diets. Percent recovery was calculated as the number of metacecariae found in infected fish divided by the expected metacercarial number.

a. Infectivity controls:

batch	n	x ± s.d.	% recovery
1	5	40.7±6.4	100
2	5	27.5±3.5	69
3	5	10.0±2.0	25

b. Repeated infection:

Treatment	n	x ± s.d.	% recovery
Single-Low	5	119.2±8.3	99
Single-High	10	121.8±9.8	100
Repeated-Low	4	72.1±6.2	92
Repeated-High	8	79.0±2.8	100

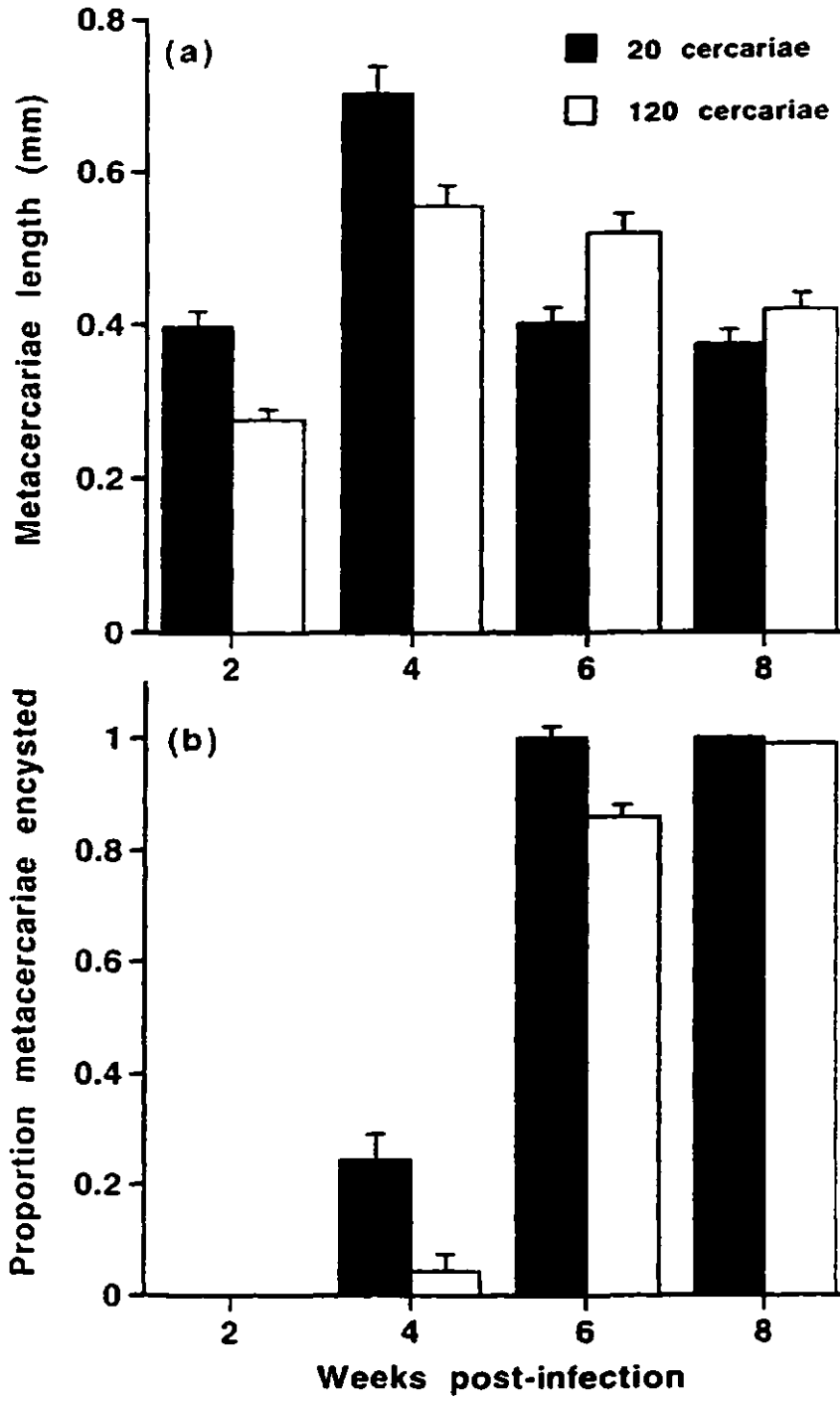

Fig. 1. Length (mean ±s.e.) (a) and proportion (mean±s.e.) encystment (b) of *O. ptychocheilus* metacercariae recovered from minnows exposed to 20 and 120 cercariae (n = 5 minnows/interval).

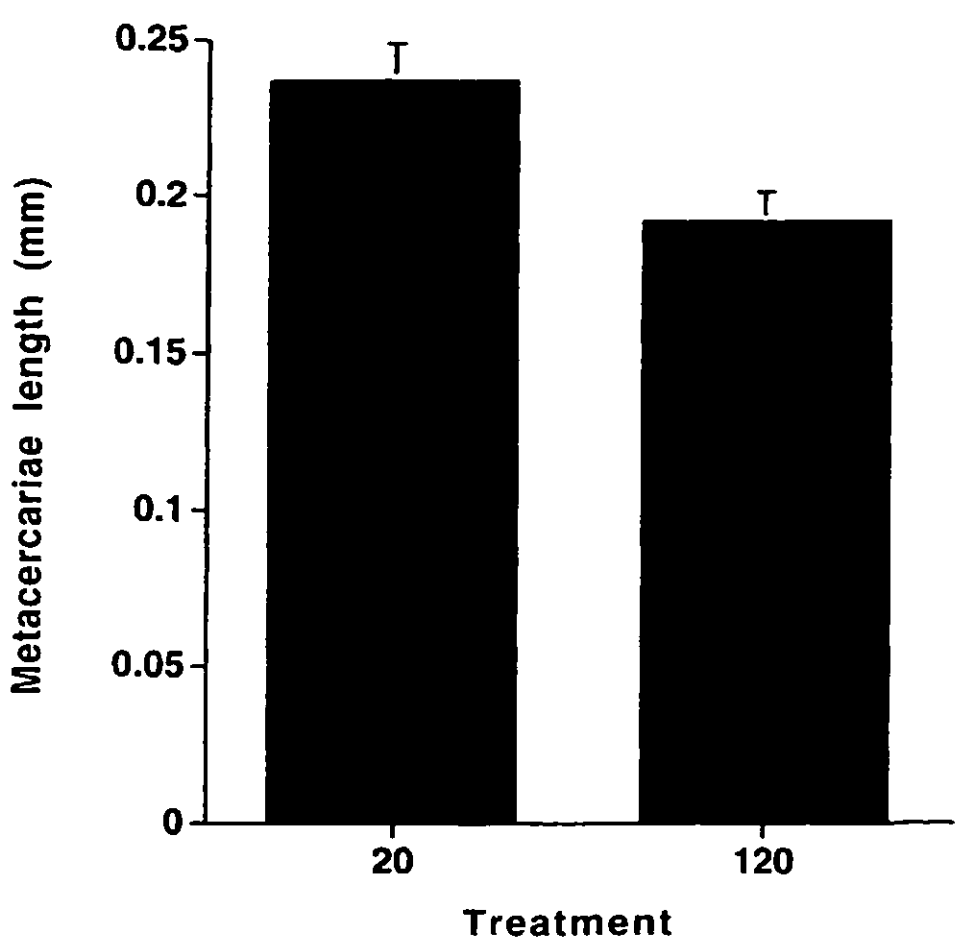

Fig. 2. Length (± s.e.) of *O. ptychocheilus* metacercariae recovered from minnows exposed to 20 or 120 cercariae and maintained in outdoor enclosures for 4 weeks (n = 5 minnows/treatment)

Chapter 4. The effect of simulated winter temperature on the interaction between the brain-encysting trematode, *Ornithodiplostomum ptychocheilus*, and fathead minnows

ABSTRACT

This study assessed the effect of parasite intensity and decreasing temperature on the survival of both fathead minnows and metacercariae of the trematode, *Ornithodiplostomum ptychocheilus*. Minnows were infected with 0, 20 or 120 cercariae and then dissected after a 16-week period of cooling. There were no differences in parasite or host survival over the simulated winter. Infection also had no effect on host growth. To assess parasite survival under natural conditions, minnows from one heavily-infected lake in north-central Alberta, Canada were collected before and after ice-on. There were no differences in *O. ptychocheilus* intensity between the two collection periods, supporting the experimental results. Thus, in contrast to some other metacercariae-fish interactions, simulated winter, at least with respect to temperature, does not appear to affect the interaction between *O. ptychocheilus* and fathead minnows.

INTRODUCTION

The outcome of parasite-host interactions is often mediated by environmental conditions. For example, the cecal nematode, *Trichostronglus tenuis*, reduces grouse natality on wet moors but not on dry moors (Hudson and Dobson, 1997). In addition, ectoparasites reduce the survival and growth of birds during a period of unseasonable cold, but not during average winters (de Lope et al., 1993). Munger and Holmes (1988) discovered that ground squirrels (*Spermophillis richardsoni*)

infected with trypanosome blood parasites had reduced growth compared to controls, but only when they were fed limited diets. Thus, the manner in which parasites affect their hosts can be considered context-dependent (Holmes, 1995).

Environmental temperature is well known to affect parasite-host interactions, especially in systems involving poikiliothermic hosts (Noble et al., 1989). Using a combined field and laboratory approach, Lemly and Esch (1984) showed that overwintering temperatures caused selective mortality of juvenile sunfish, *Macropterus salmoides*, infected with *Uvulifer ambloplitis* metacercariae. Similarly, Coleman and Travis (1998) and Hoglund and Thulin (1991) reported selective mortality of heavily-infected fish overwinter in natural populations. In addition, several studies have shown that overwintering conditions can also reduce the survival of the parasites themselves (Sweeting, 1974; Lemly and Esch, 1983; Kennedy, 1987). If such effects are widespread, they should be greatest at northern latitudes where winters are both long and severe.

Fathead minnows, *Pimephales promelas*, are a common component of fish communities in Canada's northern boreal forests (Robinson and Tonn, 1989; Nelson and Paetz, 1992, Chapter 1) where they periodically demonstrate the high overwinter mortality known as 'winterkill' (Price et al., 1991). Many of these populations also harbour high intensities of the brain-encysting trematode, *Ornithodiplostomum ptychocheilus* (Chapter 2). The main aim of this experiment was to determine the effects of infection intensity and decreasing temperature on the growth and survival of a laboratory population of juvenile fathead minnows. In addition, fish collected from one heavily-infected lake in Northern

Alberta were necropsied pre- and post-cooling to evaluate *O. ptychocheilus* survival over winter.

MATERIALS AND METHODS

The source of hosts and parasites, and infection procedures, followed those described in Chapter 3. Cercariae for this experiment were obtained from 24 infected snails that had been exposed to miricidia on 6 August, 1997. On 26 September, 1997, 90 young-of-year fish were randomly exposed to 0, 20 or 120 cercariae in Petri plates for two hours. Fish from similar treatments were then added to 5 L containers in groups of five (n = 6 containers/treatment) and maintained in a temperature-controlled room. Minnows were maintained for eight weeks at 22°C on an 12L:12D photoperiod. This development period was selected to ensure that all metacercariae would be encysted in the cranium prior to the period of cooling (Chapter 3). Thus, any effects of infection over winter would be due to the presence of encysted worms and not to developing ones. Every four days, water was exchanged and the fish were fed a weight-specific ration of Tetramin (Chapter 3).

At 8 weeks p.i., fish were separated into individual containers and placed haphazardly on shelves in a temperature-controlled cooler. Fish were allowed to acclimate in these containers for three days at 22°C on a 12L:12D photoperiod. The temperature was then reduced to 4°C over the next week and the photoperiod was altered to 8L:16D. During the period of artificial cooling, diets and the time between water changes remained the same. At 24 weeks p.i., the cooler was brought back to room temperature and the fish were necropsied.

Five fish from each treatment were selected for necropsy prior to cooling to determine *O. ptychocheilus* intensities and survival. Each brain was placed on a glass slide and overlaid with a coverslip. Survival was assessed by observing metacercariae movement within each cyst. After the 16 week period of cooling, surviving fish were measured, weighed, and necropsied. Post-cooling intensity and survival of metacercariae were assessed.

To evaluate overwinter parasite mortality in a natural population, *O. ptychocheilus* intensity was monitored in the 1996 minnow cohort until June, 1997. Minnows from SCL 200 were examined due to the high parasite intensity within this lake (Chapter 2) and its relatively easy access at ice-off. Collection procedures and sample sizes are described in Chapter 2. An attempt was made to repeat this precedure for the 1997 cohort, but the lake underwent a major winterkill. Because these fish were fixed in 80% EtOH, it was not possible to determine metacercarial survival.

Data were analyzed using JMP statistical software (Sall and Lehman, 1996). For the laboratory experiment, parasite intensities, before and after cooling, were analyzed using t-tests. One-way ANOVA was used to compare host sizes at the beginning and end of the cooling period. Host survival was assessed using chi-square analysis. Parasite intensities within a minnow cohort were compared between October, 1996 and June, 1997 using a t-test.

RESULTS

There were no significant differences in mean intensity before and after the cooling period (Table 1). Thus, decreased temperature did not affect

parasite intensity at either of the two exposure doses (High dose: $t = 0.80$, $df = 8$, $P = 0.460$; Low dose: $t = -1.9$, $df = 8$, $P = 0.101$). In addition, all metacercariae were motile at the beginning and end of the cooling period. Thus, metacercariae survival was 100% at both doses and there were no parasites lost due to the simulated winter. Parasite intensity also had no effect on minnow growth (1-way ANOVA - Dose: $F_{2,21} = 0.6$, $P = 0.550$) or survival ($X^2 = 2.9$, $df = 2$, $P = 0.240$).

Mean *O. ptychocheilus* intensity increased in the SCL 200 minnow cohort from August to October of 1996 (Fig. 1). This pattern was similar to those shown in other lakes for this parasite (Chapter 3). In spring, there was a trend towards increased mean intensity, but this difference was not significant ($t = 0.9$, $df = 45$, $P = 0.353$). Therefore, there was no evidence for parasite mortality under natural conditions.

DISCUSSION

Temperature can play an indirect role on host-parasite interactions if the negative effects of infection increase under adverse environmental conditions (deLope et al., 1993). Various studies investigating metacercariae-fish systems have reported high mortality in heavily infected hosts over the winter (Lemly and Esch, 1984, Hoglund and Thulin, 1991; Coleman and Travis, 1998). Lemly and Esch (1984) demonstrated that at least one component of the winter period, decreased temperature, was responsible for the death of fish with high metacercariae intensities. Conversely, results from this study showed that fathead minnow survival remained unaffected by *O. ptychocheilus* at low, overwintering temperatures.

There are several explanations for these contrasting results. Lemly and Esch (1984) suggested that maintenance of heavy infections through immune responses and tissue regeneration taxed important energy reserves required for fish survival at decreased temperatures. In this study, feeding regimes before and during the overwintering period may have been sufficient enough to support the homeostatic demands and the metabolic demands of *O. ptychocheilus*. It will be important to address the importance of host nutritional status in combination with parasite intensity and reduced temperature in future studies.

The specific characteristics of each host-parasite system may also explain the observed differences in parasite-induced host mortality. For example, because of the site of infection and their relatively small size, *O. ptychocheilus* may not have been as energetically costly in terms of immune (So and Wittrock, 1982; Mitchell et al., 1985; Chapter 3) and tissue (So and Wittrock, 1982) responses compared to that of *Uvulifer ambloplitis* (Lemly and Esch, 1984; Wittrock et al., 1991). Thus in this system, it is possible that the energetic costs of infection are not great enough to deplete the required energy reserves for survival at low temperatures even in situations where nutrients are limited.

Before the effects of winter temperatures and infection on host survival are discounted, several caveats should be noted. First, the phenomenon of winterkill is a complex phenomenon, involving numerous factors (Robinson and Tonn, 1989). For instance, this study excluded the role of reduced dissolved oxygen concentration (hypoxia), which is known to be an important mechanism of fish mortality in winterkill lakes (Price et al.,

1991). It seems intuitive that infection and temperature could interact with this factor to induce selective mortality.

Second, minnows from natural populations harbour metacercariae at various developmental stages as they enter the winter period. Because developing metacercariae tax fish energy reserves to a much greater degree than encysted worms (Lemly and Esch, 1984), fish in the field may be more susceptible to the effects of *O. ptychocheilus* at low temperatures than the fish in this study which harboured established infections. However, development of *O. ptychocheilus* is temperature-dependent (Chapter 3) and decreases in parasite growth at low temperature almost certainly correspond to decreases in the energetic costs to the fish. This suggests that the energy lost by fish to developing infections prior to temperature declines may be more important to fish survival than the numbers of developing larvae present during the winter.

Based on the experimental results of this study, *O. ptychocheilus* can survive up to eight months, independent of a period of overwintering temperature. Other experimental studies have demonstrated extreme variability in the life-spans of strigeid trematodes. Species such *Bolbophorus confusus* (Olsen, 1966) *Crassiphiala bulboglossa* (Hoffman, 1956), *Hysteromorpha trilobia* (Hugghins, 1954) and *Uvulifer ambloplitis* (Hoffman, 1965) can survive for years in their hosts. Others such as *Diplostomum spathaceum* and *Tylodelphys podicipina* undergo yearly mortality after winter periods (Sweeting, 1974; Kennedy, 1987). Interestingly, these short-lived metacercariae that are affected by winter temperatures are unencysted. Yet they are also site-specific to the eyes of their host. Thus, it is difficult to distinguish whether overwinter

mortality in metacercariae is correlated with their encystment strategy, or with habitat preference, or both. Future studies, on a variety of systems, could help to differentiate between these two possibilities.

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Table 1. Effect of simulated winter and infection with the trematode *O. ptychocheilus* on the growth and survival of fathead minnows exposed to low (20) and high (120) doses of cercariae.

	Weeks post-infecton	
	10	30
Intensity:		
Low	21.8±4.3	27.4±5.3
High	119.4±5.3	114.8±2.7
Host Survival (%):		
Control	100 (n = 18)	55 (n = 10)
Low	100 (n = 20)	30 (n = 6)
High	100 (n = 18)	44 (n = 8)
Host Size (mm):		
Control	28.5±3.3	31.1±1.7
Low	29.5±2.1	31.8±3.4
High	28.9±2.9	30.4±2.3

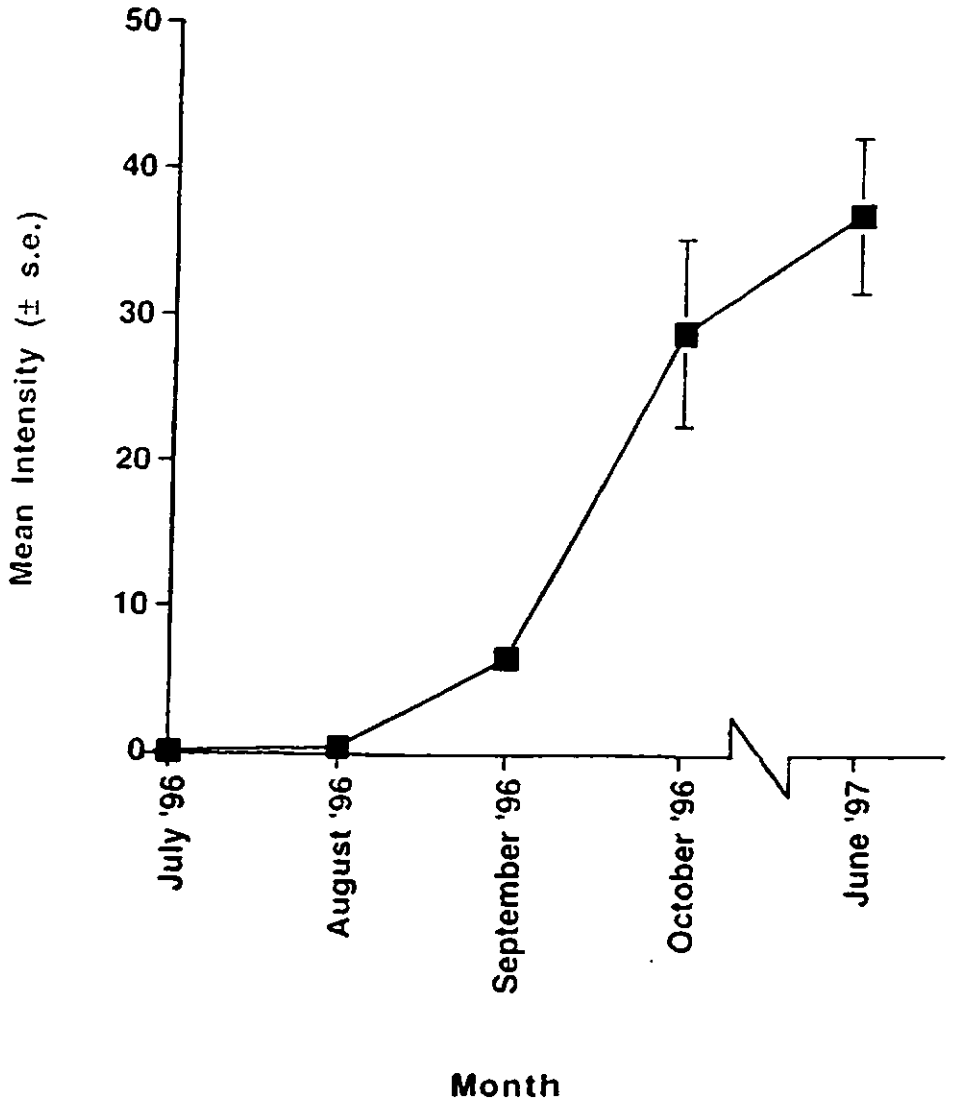

Fig.1. Parasite recruitment (mean intensity ± s.e.) in a cohort of juvenile fathead minnows from July of 1996 until June of 1997.

Chapter 5. Morphological and behavioural consequences of a brain-encysting trematode (*Ornithodiplostomum ptychocheilus*) on juvenile fathead minnows (*Pimephales promelas*)

ABSTRACT

Morphological and behavioural consequences of the trematode, *Ornithodiplostomum ptychocheilus*, were investigated in both laboratory and field-collected fathead minnows (*Pimephales promelas*). In fish sampled from two naturally-infected experimental ponds in Northern Alberta, distinctive deformations in the cranium were associated with this brain-encysting parasite. Results from whole-head histological sections showed that infection led to an approximate 10% increase in cranial height and width compared to reference controls and also led to distortion of the optic tecta and cerebellum.

A factorial experiment showed that the timing of exposure, host diet, and host size were important in generating the distortion. Thus, the expression of this infected phenotype is context-dependent, due mostly to complex factors associated with parasite transmission and to the growth of the host relative to the growth of the parasite. Infected fish maintained on a low-quantity diet had reduced survival (63%) compared both to their controls (88%-100%) and to infected fish kept on high-quantity diets (98%). Infected fish also displayed a greater phototactic response than did control fish in laboratory trials. Interference with phototaxis has been shown in

studies that removed or damaged the optic tectum. These results demonstrate a context-dependent morphological consequence of parasite infection that has direct effects on host survival and behavior.

INTRODUCTION

Characterizing and understanding the consequences of parasite infection to individual hosts is a central aim in parasite ecology. Whereas earlier studies documented some of the obvious consequences of parasites on host growth, mortality, and physiology (Schmidt and Roberts, 1989), more recent studies have identified phenotypic consequences of infection that are much more complex than previously recognized (Poulin and Thomas, 1999). Examples include parasite-induced alterations in host behaviour (Poulin, 1995), increased host conspicuousness (LoBue and Bell, 1993), altered host colouration (Hechtel et al., 1993) and altered host life-histories (Minchella, 1985).

Parasites can also change the morphological phenotypes of their hosts (Poulin and Thomas, 1999). For example, one outcome in humans infected with certain filarid nematodes is the massive disruption of lymph circulation that leads to elephantiasis (Schmidt and Roberts, 1989). Another human example involves the swelling of the neck area caused by infection with *Trypanosoma*. These swellings were apparently used by slave buyers to indicate future mortality, often resulting in the slaves being thrown overboard en route to the New World (Schmidt and

Roberts, 1989). In non-anthropogenic host-parasite interactions, larval cestodes such as *Schistocephalus* distend the body cavities of their fish intermediate hosts (LoBue and Bell, 1993). Sporocysts of trematodes such as *Leuchochloridium* convert the tentacles of terrestrial snails into colourful, pulsating sacks (Ulmer, 1971). Trematode metacercariae in fish have been shown to alter the morphology of internal organs such as the heart (Coleman, 1993) and the brain (Hoffman and Hoyme, 1958). In these systems, the extent of the altered phenotypes are due to both the intensity of infection and to the specific tissues damaged.

In this paper, I describe an unusual case of parasite-induced alteration in host phenotype. The alteration is caused by the trematode, *Ornithodiplostomum ptychocheilus*, which encysts on and in the brain of its second intermediate host, the fathead minnow, *Pimephales promelas* (Hoffman, 1958; Hendrickson, 1979). The distortion was first detected in experimentally-infected minnows as an enlarged cranium. Mueller (1972) reported a similar distortion in small forage fish (*Orestias* sp.) collected from Peru. He attributed the deformation to metacercariae of *Diplostomum mordax*, another strigeid trematode that encysts on the brain. Although I first dismissed the distortion as a laboratory artifact, it was subsequently discovered in minnows collected from naturally-infected ponds at a research facility in Northern Alberta.

Nothing is known regarding the factors which cause the distortion, its extent across different fish-metacercariae systems, or its consequences to fish behaviour and other aspects of fish ecology. The purposes of this study were to quantify the distortion phenotype in minnows, to identify factors leading to its formation in juvenile fish and to assess whether the distortion affected host behaviour, growth and survival.

MATERIALS AND METHODS

Experimental animals

The minnows used in these experiments originated from parents that were collected during the 1998 breeding season from Rochester lake, Alberta (see Chapter 3). Breeding adults were collected from the lake and then allowed to oviposit within the outdoor experimental ponds described in Chapter 3. Since we began studying this host-parasite interaction (1996), the network of ponds has been used for various experiments (e.g. Chapters 3 and 4; Danylchuk pers. comm.). Periodically, samples of fish are dissected from the ponds in order to monitor the infection status of 'controls'. Samples of minnows collected from two of four ponds in July, 1998 were the first to show infections. Thus, infected birds must have visited both ponds, resulting in the recruitment of miricidia into *Physa gyrina*. The migration of infected snails or infected fish between ponds is extremely unlikely.

On 5 Sept., 1998, prevalence of *O. ptychocheilus* in the two infected ponds was 100%; intensities ranged from 20 to 122 metacercaria/host. The third pond was uninfected, at least until ice-on of that year (October, 1998). Minnows were transported back to the laboratory at the University of Lethbridge on 5 Sept., 1998. They were maintained in aerated, 20 gallon fish aquaria (60cm X 30 cm X 30 cm) at 20°C on a 10:14 hr L:D photocycle. Densities were approximately 10 fish per gallon of water. Diets consisted of Tetramin fed *ad libitum* unless otherwise noted.

Histology

Histological sectioning followed the protocol developed by Prusky and Parker (U. of Lethbridge, unpublished). On 14 Sept., 1998, five fish from the uninfected pond (size: 26.30 - 30.10 mm) and five fish from one of the infected ponds (size: 27.48 - 30.10 mm) were anesthetized using MS 222 and immediately fixed in 4%, unbuffered paraformaldehyde (PFA). After four days in the fixative, fish heads were mounted on metal chucks with frozen section medium (Stephens Scientific, Riverdale, New Jersey), and stored overnight in a –70°C freezer. Chucks were secured in a cryostat microtome maintained at –25°C. Each head was sliced transversely into 40 µm sections from the snout to the posterior of the hindbrain. Sections were mounted on gelatin-coated slides (1%) and air dried for 1 day. Slides were not stained. Cranial dimensions were quantified using an ocular micrometer at a total magnification of 25X.

In order to compare the cranial dimensions of infected vs. uninfected fish, it was necessary to identify morphological "landmarks" which were consistent among hosts. I chose the anteriormost landmark as the cross-section where gill arches 1 and 2, on both left and right sides of the buccal cavity, were distinct and separate from other tissues. The posteriormost landmark was defined as the section where the vertical portion of gill arch 2 could be identified on both sides. The distances between these two landmarks varied between 480 and 600 µm, depending on minnow size (Fig. 1). Within this region, cranial heights and cranial widths were quantified per section. The height of the cranium was estimated as the distance from the top of the central gill stalk to the outermost tissue at the top of the head. Cranial width was quantified as the maximum distance from one side of the head to the other. The total number of measurable sections for each fish varied between 6 and 12. Damaged sections were excluded.

Laboratory experiment

Previous observations on this host-parasite system indicated that some infected minnows had distorted craniums and others appeared unaffected. Similarly, fish from infected ponds varied extensively in the expression of the distortion (Sandland, pers. obs.). The purpose of the laboratory experiment was to assess the role of host diet (and thus host growth rate) and infection regime on the expression of the distortion. Fish were fed on

either low- or high-quantity diets and exposed to either 120 cercariae once, or to 40 cercariae three times, over six weeks. The aim of this infection protocol was to produce two different types of infection dynamics. The primary infection would lead to an infrapopulation of metacercariae that would simultaneously develop over a relatively short period of time (Chapter 3). In contrast, the repeated infection would produce a similar intensity of infection, but development would proceed over a longer period of time, potentially allowing the host to accommodate for encysting worms. Thus, the 2 diet (high, low) X 3 dose (single, repeated, control) factorial experiment was designed to test the prediction that slow-growing minnows exposed to cercariae over a short period of time, would develop distorted craniums.

The methods used to infect the fish followed those outlined in Chapter 3. On 9 Jan., 1999, sixty-four uninfected fish of varying sizes were randomly assigned to one of the six treatments. Twelve fish were allocated to each infection treatment whereas control treatments included eight fish each. The repeated-infection treatment involved exposing minnows to doses of 40 cercariae at 0, 3, and 6 weeks. To assess differences in the infectivity of each batch of cercariae, five infection controls were exposed to 40 cercariae at each trickle dose (see also Chapter 3).

After exposure, individual fish were maintained in 1 L containers (15 cm X 14.5 cm X 8.5 cm) on one of two diets. One group received 10 % of their

body weight every fourth day, the second received only 2%. Water was changed every fourth day, prior to feeding. Survival was monitored daily. At 8 weeks p.i., fish were measured (standard length), weighed, and then sub-sampled to determine *O. ptychocheilus* intensity. In addition, three fish from each of the six treatments were randomly selected for histological analysis. Methods for fixing, sectioning and quantifying cranial dimensions followed those outlined previously for the field-collected fish.

Host behaviour

Minnows collected from the experimental ponds on 19 Aug., 1998 were studied in the laboratory to assess whether the cranial distortion influenced host behaviour. For these trials, minnows from one of the two infected ponds were used. Controls came from the uninfected pond. These field-collected minnows were transported to the aquatic laboratory at University of Lethbridge and were allowed to acclimate to large, laboratory aquaria for one week. On 19 Sept. 1998, infected and uninfected fish were separated into two size classes (small: 16-19mm; large: 21-24mm) and placed into individual, 1L containers for a second week. There were 10 replicates for each of the four treatments (small-infected; large-infected; small-control; large-control).

Behavioural trials (26 – 27 Sept., 1998) involved randomly selecting two of the 60 minnows and placing each into one of two 20-gallon, all-glass

aquaria (60 cm X 30 cm X 30 cm). The aquaria were placed end-to-end such that a video camera could capture events occurring in one-half of each tank. The camera was positioned such that 5 cm X 5 cm grid lines could be video-captured on both the back and the base of each tank. The grid on the bottom of each tank was captured by using a mirror positioned at a 45^0 angle above each aquarium. Each of the two tanks was separated into equal portions of light and dark by covering 50% of each one with black cardboard. Thus, the camera captured minnow behaviour along X, Y, and Z coordinates within the lit portion of both tanks. For each trial, minnows were allowed to acclimate in the dark for 15 min. A single, 90-watt incandescent light was placed 60 cm in the front of the two tanks and angled such that light entered the aquaria from the top. The light was turned on after the acclimation period. Fish were videotaped over the subsequent 30 minutes. The observer was not present in the room during video-taping.

The following three response variables were evaluated: 1) the total amount of time spent in the illuminated portion of the tank over the 30 minutes (measured in seconds), 2) the proportion of time each fish spent in the top 40% of the water column, relative to the total amount of time spent in light, and 3) overall activity. The latter was calculated by determining the total number of grid lines each fish crossed on the X, Y and Z axes. Mean *per capita* activity was evaluated over five, 30 s intervals which had been selected at random within each 30 minute trial.

Statistical analyses

All data were analyzed using JMP statistical software. The Shapiro-Wilk test was employed to test all data for normality (Sall and Lehman, 1996). For field collected fish, cranial heights and widths were compared with ANOVA. For fish infected in the lab, cranial dimensions were analyzed utilizing a two-factor ANCOVA (dose X diet), controlling for host size at infection. Differences in minnow survival were assessed utilizing the Kaplan-Meyer, log-rank analysis for survival curves (Sall and Lehman, 1996). Behavioural responses were analyzed using two-factor ANCOVA, controlling for host size at infection. Activity data were log-transformed to satisfy the normality assumption; phototactic and geotactic responses did not require transformation.

RESULTS

Histology

The metacercariae-induced cranial distortions in field-collected fish could be seen from the side as a vertical expansion of the head, directly posterior to the eyes (Fig. 2 a, b). Whole-head histological sections showed that all metacercariae were encysted. There was marked encystment site-selection for the cranial cavity surrounding the optic lobes and cerebellum (Fig. 2 c, d). In a small number of fish, 1-5 larvae encysted directly within the tissue of the optic lobes (Sandland, pers. obs.). Although histopathology was not

quantified in this study, there were obvious qualitative differences in the gross appearance of infected vs. uninfected brains. In particular, the optic tecta and valvula cerebelli were either displaced or completely destroyed by the encysted worms (Fig. 2 c, d). Encysted larvae tended also to fill the entire cranial cavity, thereby compressing brain tissue and forcing areas of the dorsal brain into the tegmentum.

Minnows from the infected ponds had significantly higher craniums than those collected from the control pond ($F_{1,8} = 30.9$, $P < 0.001$), after removing the effects of host size (host size – $F_{1,8} = 1.0$, $P = 0.341$). On average, the cranial heights of infected fish were 12.2% larger than their paired controls (Fig. 3 a). Cranial widths also differed significantly between infected and uninfected fish (parasite – $F_{1,8} = 13.7$, $P = 0.008$; Size – $F_{1,8} = 1.5$, $P = 0.260$) with infected fish having widths 7.1% greater than controls (Fig. 3b).

Laboratory experiment

General features of the population dynamics of metacercariae in minnows exposed once to cercariae were different from those exposed repeatedly. These results, and those dealing with aspects of host response, are detailed in Chapter 3. For the purposes of this chapter, the infection treatments provided two important results. First, by 8 weeks p.i., all metacercarcariae collected from hosts exposed to the single infection were encysted. In contrast, only 31% of the metacercariae were encysted in fish exposed to

trickle infections. Thus, the single infection produced a population of metacercariae that developed more completely in comparison to those in the trickle infection.

The second important result is that metacercarial intensities differed significantly between treatments at 8 weeks p.i. (Chapter 3, $F_{1,1} = 190.7$, $P < 0.001$); mean intensity was 120 metacercariae/host for the single infection and 76 metacercariae/host for the repeated infection. This result may have been due to differences in cercarial infectivity between batches or changes in resistance with host age (Chapter 3). This is important because the treatment effects (single vs. trickle infection) are confounded by differences in metacercariae intensities between treatments.

The cranial dimensions of fish sub-sampled at 8 weeks p.i. were affected by infection regime, host diet and initial host size (Table 1; Figs. 4, 5). Scheffe's post-hoc comparisons showed significant differences in cranial height between control fish and those exposed to the single dose ($P < 0.001$) and between fish exposed to the trickle dose and those exposed to the primary dose ($P = 0.003$). Fish that received a single dose of *O. ptychocheilus* had maximum cranial heights that were 10% greater than their controls. Despite this difference, there was no dose X diet interaction. Cranial width was affected by initial host size and diet but infection was non-significant (Table 1). Mean cranial widths were 5% greater in fish maintained on the high diet as compared to those on the low diet (Fig. 5).

The survival of minnows exposed to the single infection was the same as those exposed to the trickle infection ($X^2 = 0.01$, df = 1; P = 0.941). Thus, in order to increase sample sizes within the four remaining treatments, these fish were pooled together. There was a significant difference in host survival between the four treatments ($X^2 = 11.4$, d.f. = 3, P = 0.01) with the lowest survival in fish from the infected, low-diet category (Fig. 6). After 56 days, the proportion of fish surviving from this treatment was 63% whereas survival remained above 88% in the other three treatments.

At 8 weeks p.i, variation in host size was strongly affected by initial host size and by the diet regime, but was not affected by infection (two-way ANCOVA; dose – $F_{1, 22} = 0.24$, P = 0.63; diet – $F_{1, 22} = 46.3$, P = 0.0001; dose X diet – $F_{1, 22} = 0.022$, P = 0.891; host size at infection – $F_{1, 22} = 285.41$, P = 0.001).

Host behaviour

In general, individual minnows varied extensively in their overall activity and in their positions within the experimental aquaria (Table 2). Infection contributed little to this variation, although there was a significant effect on the amount of time small and large fish spent in the illuminated portion of the tank (Table 2; Fig. 7). Infected fish, regardless of their size, spent approximately 6.5% more time in the light compared to uninfected minnows (Fig. 7). Infected and uninfected fish did not differ in the amount of time spent in the upper 40% of the water column (Table 2).

However, host size affected overall activity (Table 2), with large fish being 27% more active per 30-second interval than small fish.

DISCUSSION

Infection with a brain-encysting parasite distorts the cranial structure of fathead minnows, at least under certain environmental conditions. This result adds to the growing evidence that parasites can alter morphological phenotypes of their hosts (Poulin and Thomas, 1999). More specifically, the results from the factorial experiment verify that cranial distortion in field-collected minnows was the result of infection, and not to other factors. This study, together with the earlier observation by Mueller (1972) on a related system, implies that the distortion may be a regular feature of brain-encysting trematodes. If this is so, then the consequences of the distortion which we have identified, such as reduced survival (when food was restricted) and altered phototaxis, may be relevant to other host-parasite interactions. The challenge ahead is to determine whether the consequences we have chararacterized for individual hosts, carries over to natural host populations.

However, the results also show that the expression of the phenotype was context-dependent (sensu Holmes, 1995). Factors such as the strength and duration of cercarial transmission, host growth rate and host size at infection were each important in determining the outcome of the interaction. Such complexity probably best explains why so few reports

exist on the deformation, despite the extensive attention these trematode-fish interactions have recently received (Coleman, 1993; Ballabeni, 1994; Lafferty and Morris, 1996). Indeed, it was only because we had the rare opportunity to compare size-matched fish from uninfected ponds, with those from adjacent infected ponds (the ponds are only 5 m apart), that we discovered the distortion at all.

One requirement for the formation of the distorted phenotype is the rapid development of large numbers (>20) of co-occurring metacercariae in the brain, especially in fish that are small at the time of exposure. This would explain the difference in cranial dimensions between small fish exposed once to infection, and those exposed repeatedly. Thus, one factor leading to cranial distortion appears to be the relative growth rate of the host compared to the growth rate of the metacercariae. If the collective growth of many metacercariae over a short interval of time exceeds the hosts' ability to accommodate for them, the result is cranial distortion.

However, explanations based on relative host and parasite growth rates are confounded by the results from the diet treatment. If the expression of the distortion was due simply to an imbalance in the relative growth rates of the parasites and hosts, then the hosts fed low-quantity diets should have had larger craniums than those fed the normal diets. One possibility is that parasites in slow-growing hosts are themselves slow-growing and thus do not produce severe constraints on the growth of the host's

cranium. However, host diet did not affect metacercarial recruitment, development or encystment (Chapter 3). These results suggest that in heavily-infected minnows, the distortion is detectable irrespective of host growth rate. Therefore, it is primarily the dynamics of transmission (especially intensity and duration), and metacercariae development, that lead to cranial distortion.

The development of cranial distortion could be caused either by the encysted stage of the larvae, or by the pre-encysted, developing larvae. One possibility is that the encysted forms interfere with the normal development of the skull by occupying the cranial cavity. However, an alternative, but not necessarily independent, mechanism is that the distortion occurs when the developing larvae presumably feed on brain tissue (2-6 weeks p.i.; Chapter 3). This period coincides with maximum parasite growth rate, maximum inter-specific competition for resources (Chapter 3) and most likely maximum damage to the brain. It is therefore possible that it is the developing larvae, and not encysted ones, which cause the cranial distortion. Distinguishing these alternatives would require monitoring the temporal sequence of expression of the distortion, in association with studies on the dynamics of encystment (e.g. Chapter 3).

It is also possible that the deformation is caused by an inflammatory immune response, similar to that described for at least some other brain-inhabiting parasites (Credille et al., 1993). Regardless of whether an

immune response is directed towards developing or encysted larvae, the resulting inflammation in the brain could lead to the distortion of the cranium. As reviewed in Chapter 3, the role that fish immunity plays in the dynamics of strigeid infections is poorly known. Based on evidence from other systems (see Chapter 3), the only functionally significant response is directed towards invading cercariae, and not towards encysted larvae. Thus, until a specific immune response to trematode larvae has been identified in fish, the role of immunity in producing the distortion can be discounted.

Interference with the production or circulation of cerebrospinal fluid (CSF) is also known to cause enlargement of the cranium in other vertebrates (Kolb and Wishaw, 1996). For example, in infant children (where the skull bones are not yet fused), obstructions in the flow of CSF leads to a conspicuous enlargement of the head known as hydrocephalus. This is caused by the massive buildup of intracranial pressure due to blockage of CSF circulation. CSF is produced by the choroid plexus in the ventricles, one of the specific sites of *O. ptychocheilus* encystment. Interestingly, one of the first signs of non-lethal hydrocephalus in children and murine rodents is visual disturbance (see below). Unfortunately, nothing is known regarding the association between infection and the production and circulation of CSF in fish.

Metacercariae-induced host mortality has been shown in several experimental systems (Olsen, 1966; Ballabeni, 1994; Lowenberger and Rau, 1994). The specific factors leading to mortality are rarely known, although mechanical distortion of critical organs such as the heart (Coleman, 1993), eyes (Szidat, 1969; Lester and Huizinga, 1977) and muscles (Olsen, 1966; Lauckner, 1984), and direct effects on host energy budgets (Lemly and Esch, 1984) have been implicated. As described in Chapter 4, such effects are often context-dependent, occurring as a result of synergistic interactions between infection and environmental factors (e.g. Lemly and Esch, 1984; Holmes, 1995). In this study, survival was only reduced in infected minnows on low-quantity diets. However, it should be noted that the survival analysis was unable to test the significance of the interaction between diet and infection. Thus, both factors may be additive in their effect on mortality, with neither alone being sufficient to kill the host.

In addition to trematode-induced reduction in minnow survival, the cranial distortion also altered host behaviour. Altered phototaxis has been shown for dace, *Leuciscus leuciscus* (Crowden and Broom, 1980) and brook trout, *Salvelinus fontinalis* (Brassard et al., 1982) infected with the strigeid *Diplostomum spathaceum*. In these two examples, the simplest explanation for altered behaviour involves direct mechanical damage due to encysting metacercariae in the eyes (Holmes and Zohar, 1990). In this study, results from whole-head histological sections showed that infection with *O. ptychocheilus* damaged the optic tecta and cerebellum. Previous

studies on fish brains have demonstrated that mechanical damage to tissues in and around the optic tecta alters vision (Springer et al., 1977). More specifically, Mark et al. (1973) showed that disruption of the dorsal tecta resulted in altered light-dark discrimination in goldfish. Fenwick (1970) demonstrated that ablation of the pineal organ (between the optic tecta) also modified the phototactic response of fish. Thus, direct damage to the optic tecta caused either by developing or by encysted metacercariae, appears to have direct consequences for light-dark discrimination.

However, I cannot discount the possibility that altered phototaxis is the result of the cranial distortion itself, and not directly to the parasite. This would explain why minnows experimentally-infected with 120 cercariae, but without cranial distortions, do not show differences in phototactic response compared to controls (Shirakashi and Goater, U. Lethbridge, unpublished). Given that infected minnows suffer brain damage and have high mortality, at least under some conditions (see above), it is possible that the phototactic response was a generalized response to poor host condition. However, the overall activity of infected fish was not different from controls, suggesting that their overall physical performance was not affected.

The altered phototactic response may also be due to parasite-induced alterations in host energy budgets (Milinski, 1990; Poulin, 1995). Lemly and Esch (1984) showed that encystment of the trematode *Uvilifer*

ambloplitis coincided with a reduction in host energy reserves. They speculated that the changes to the host's energy budget could lead to altered behavioural choices, possibly leading to changes in habitat preference, prey choice and predation risk (e.g. Milinski, 1990; Poulin, 1995). As discussed in Chapter 3, the details of the encystment process, especially those surrounding costs to the host, are unknown in this system. Alterations to the energy budget of minnows infected with *O. ptychocheilus* could result from costs incurred during encystment, during the regeneration of brain tissue (e.g. Springer et al., 1977; Schwassmann, 1975) or when mounting an immune response. Each of these processes alone, or in combination, could lead to altered host decisions with respect to light and dark.

Infection did not significantly influence overall activity or the proportion of time field-collected minnows spent in the top 40% of the water column. There was however, a significant influence of host size where larger minnows demonstrated greater overall activity than smaller minnows. This reflects a similar discovery by Sogandares-Bernal et al. (1979) who reported that fish stamina was dependent on host size and not the intensity of *O. ptychocheilus* infection. These results demonstrate the influence fish sizes can have on observed behaviours. Numerous studies of fish-trematode systems have not examined behavioural differences based on both infection status and host size (Crowden and Broom, 1980; Radabaugh, 1980, Lafferty and Morris, 1996). Results from this study

suggest that host size should be incorporated into behavioural analyses to prevent mistaking size-related effects for parasite effects.

Because many of the fish used in this study were collected from two separate ponds, it could be argued that the observed differences were due to environmental or genetic factors as opposed to infection. However, the two ponds are identical in their dimensions and in their history of use by fish biologists (Danylchuk and Tonn, U. Alberta, pers. comm.). Also, the adults giving rise to the YOY cohorts were all from Rochester Lake and were concurrently stocked into the ponds in 1998. Therefore, pond effects are unlikely to contribute to the differences in phototaxis between infected and uninfected minnows.

The implications of the parasite-induced change in host morphology to natural populations of minnows are speculative. We should expect direct effects of infection on minnow mortality, at least during conditions when parasite transmission is high and when hosts are nutrient stressed. However, the indirect effects of the altered phenotype may also be important. For instance, if the distortion is visible to human observers, it may also be conspicuous to predators, prospective reproductive partners and to potential school members. Studies on other metacercariae-fish interactions have shown parasite-induced effects on predation rates (Poulin, 1994), sexual selection (Milinski and Bakker, 1990), and school cohesion (Krause and Grodin, 1995).

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Table 1. Summary ANCOVA statistics for the effects of infection protocol (primary vs. trickle) and host diet on the cranial dimensions of fathead minnows infected with *O. ptychocheilus*.

		Cranial height		Cranial width	
Source of variation	d.f.	F-value	P-value	F-value	P-value
Initial host size (covariate)	1	47.6	<0.001	94.1	<0.001
Dose	2	10.2	0.003	0.7	0.533
Diet	1	68.8	<0.001	64.3	<0.001
Dose*diet	2	1.0	0.384	2.2	0.158
Total error	11				

Table 2. Summary ANCOVA statistics for the effects of the parasite *O. ptychocheilus* on three behaviours of juvenile fathead minnows. Main effects were adjusted for the effects of host size.

	d.f.	Mean Square	F-value	P-value
Proportion of time spent in top 40% of aquaria:				
Dose	1	0.032	3.293	0.080
Size	1	0.041	2.532	0.120
Dose*Size	1	0.006	0.496	0.486
Error	36	0.013		
Time spent in lit portion of aquaria:				
Dose	1	44.1	5.434	0.026
Size	1	$10.6X10^{4}$	0.002	0.963
Dose*Size	1	67.6	0.003	0.954
Error	36	$2.0X10^{4}$		
Overall activity:				
Dose	1	0.0003	0.011	0.919
Size	1	0.212	7.265	0.011
Dose*Size	1	0.011	0.361	0.552
Error	36	0.029		

Fig. 1. Profile of a juvenile fathead minnow showing the region examined for cranial dimensions.

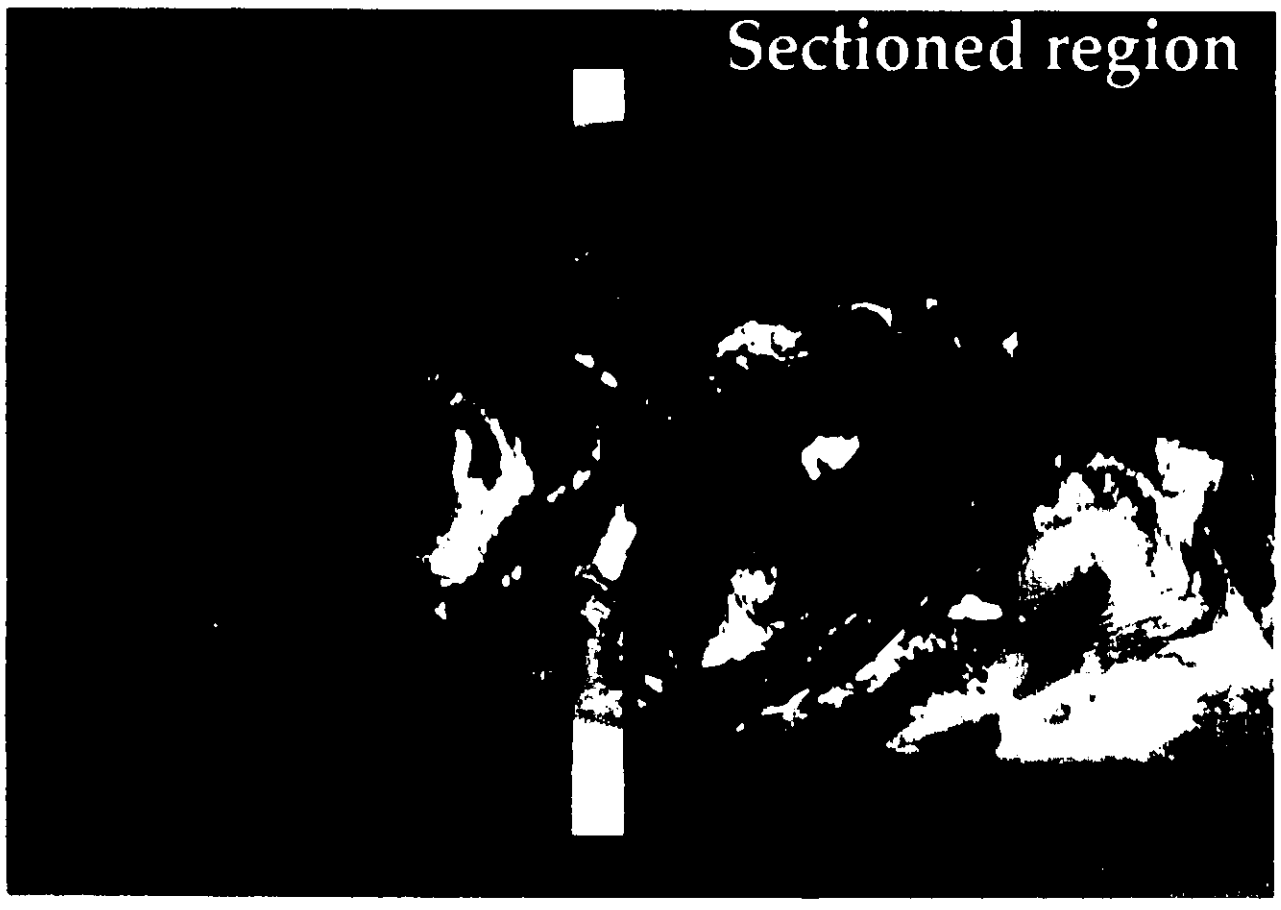

Sectioned region

Fig. 2a. Profile of an uninfected juvenile fathead minnow.

Figure 2b. Profile of a juvenile fathead minnow displaying the cranial distortion as a result of infection with *O. ptychocheilus*.

Figure 2c. Cross-section through the head of an uninfected fathead minnow. CGS, central gill stalk; FGA, first gill arch; SGA, second gill arch; T, tegmentum; TeO, optic tectum; VC, valvula cerebelli.

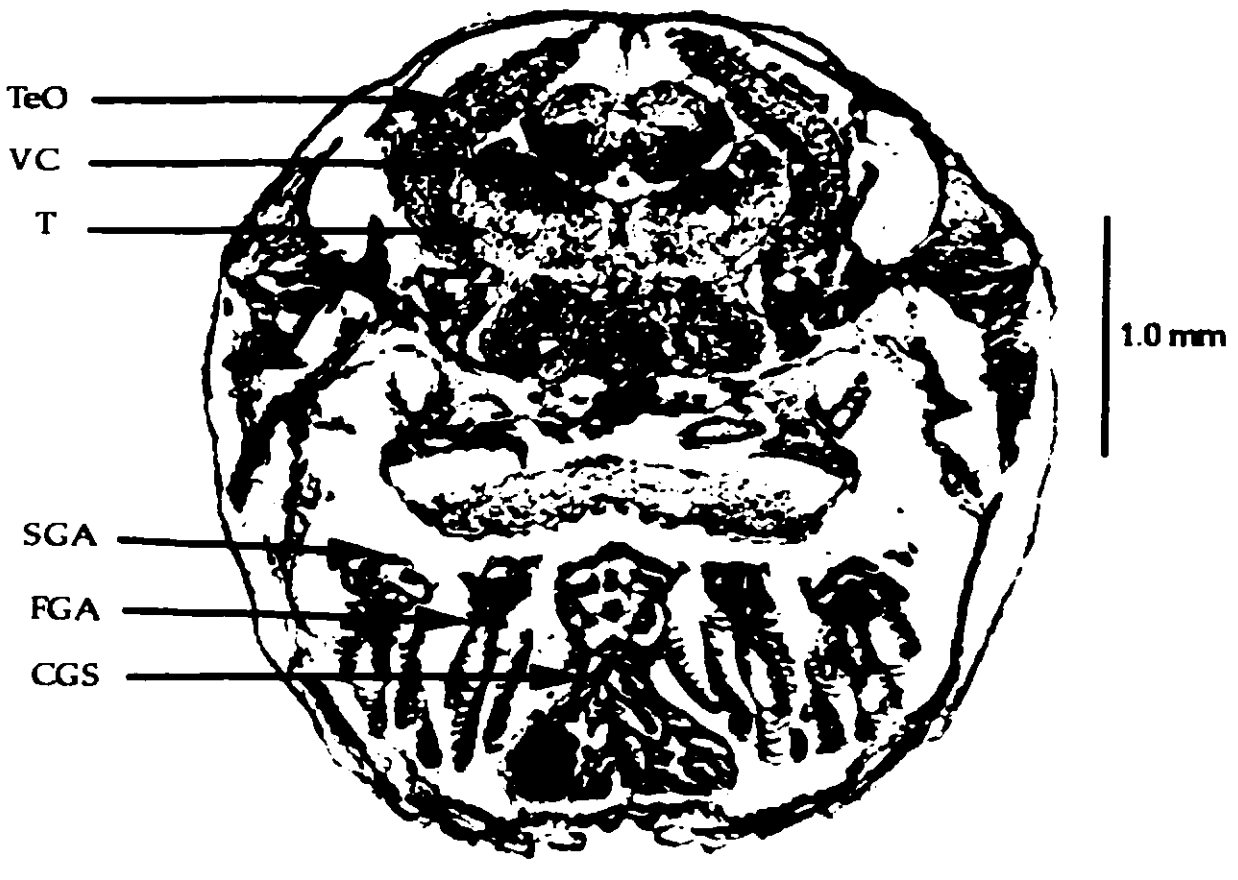
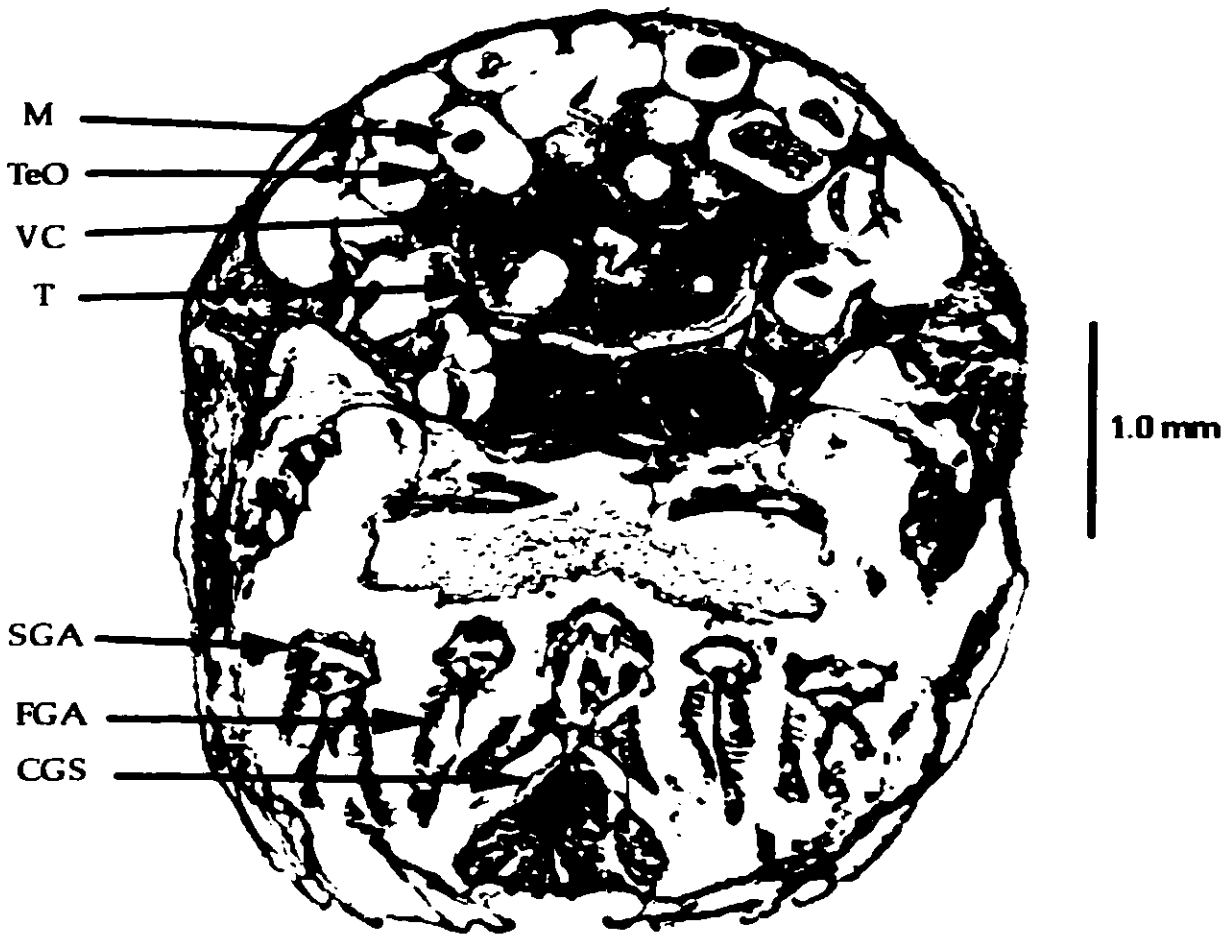

TeO
VC
T
SGA
FGA
CGS
1.0 mm

Figure 2d. Cross-section through the head of a fathead minnow infected with *O. ptychocheilus*. CGS, central gill stalk; FGA, first gill arch; M, metacercariae; SGA, second gill arch; T, tegmentum; TeO, optic tectum; VC, valvula cerebelli.


M
TeO
VC
T
SGA
FGA
CGS
1.0 mm

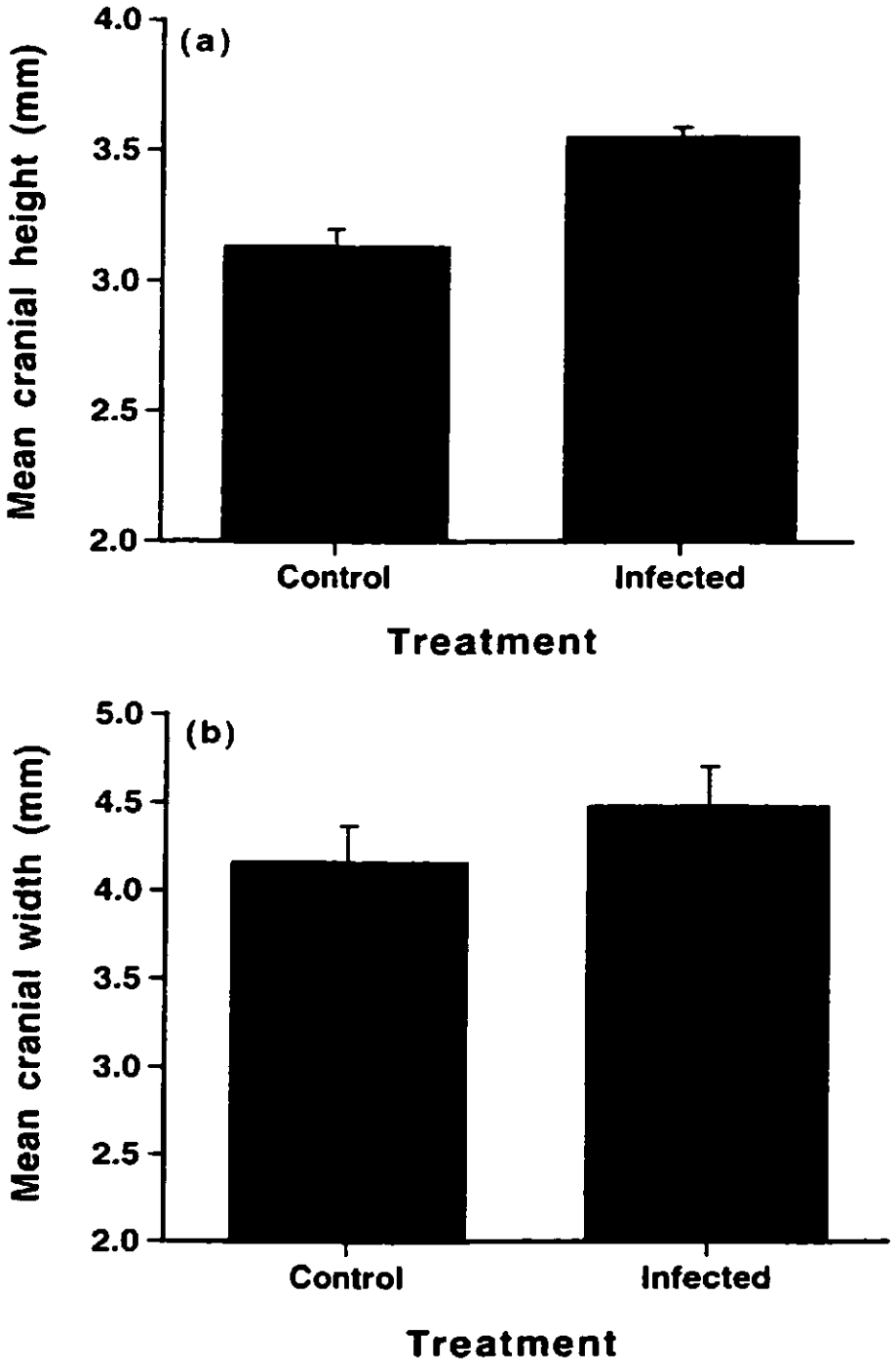


Fig. 3. Cranial heights (a) and widths (b) (means ± s.e.) of uninfected and *O. ptychocheilus* infected fathead minnows collected from two naturally-infected ponds in Northern Alberta.

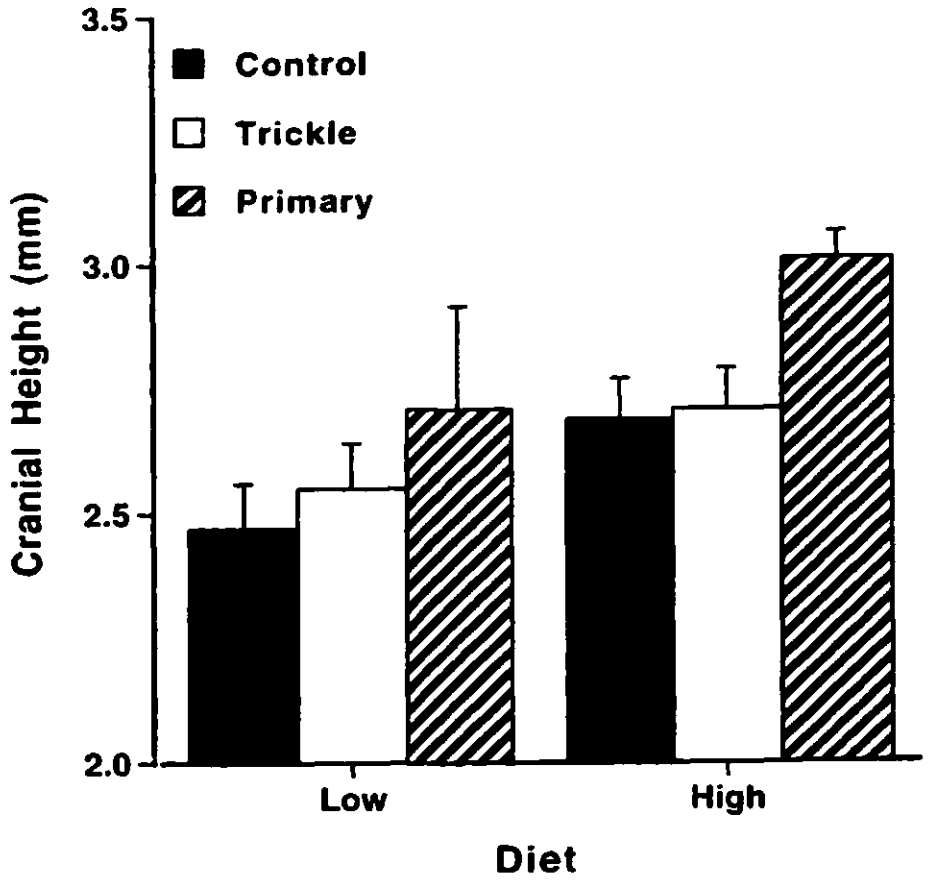


Fig. 4. Effects of infection with *O. ptychocheilus* and host diet on the cranial height (mean ± s.e.) of fathead minnows.

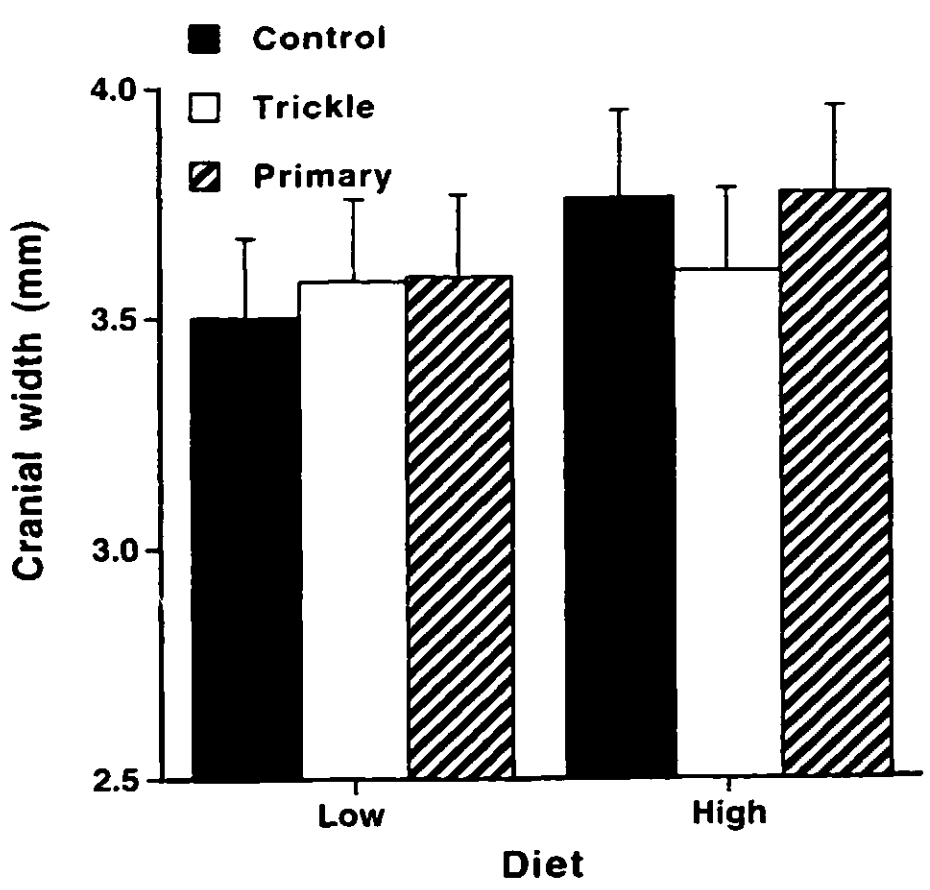


Fig. 5. Effects of infection with *O. ptychocheilus* and host diet on the cranial width (mean ± s.e.) of fathead minnows.

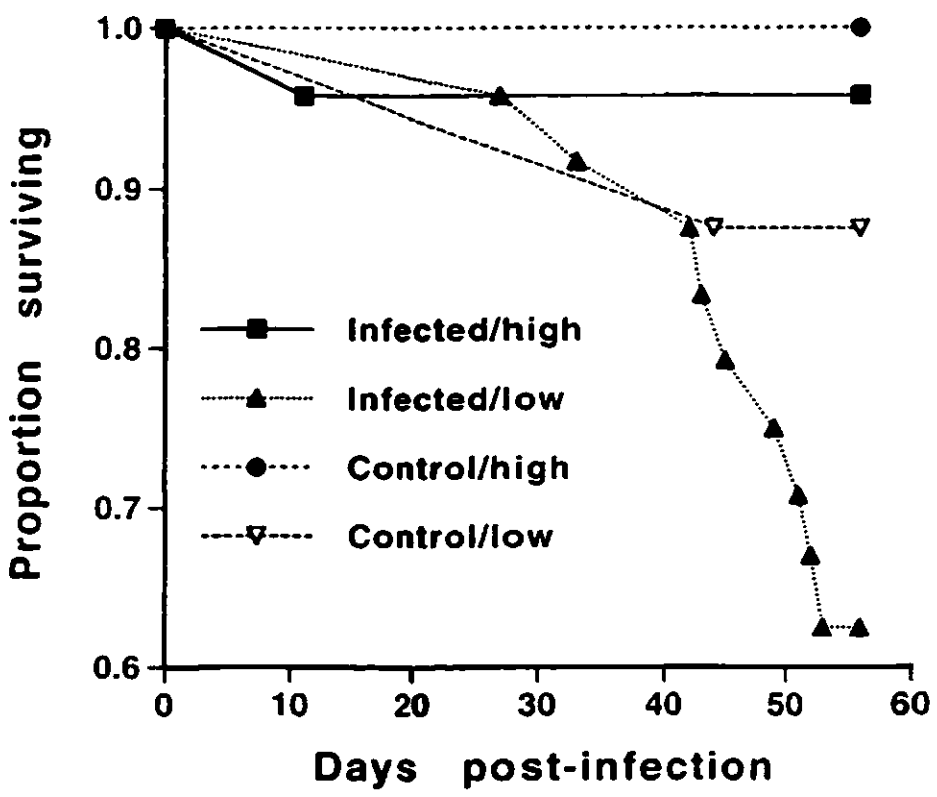


Fig. 6. Survival of minnows experimentally infected with metacercariae of *O. ptychocheilus* and maintained on low-quantity or high-quantity diets.

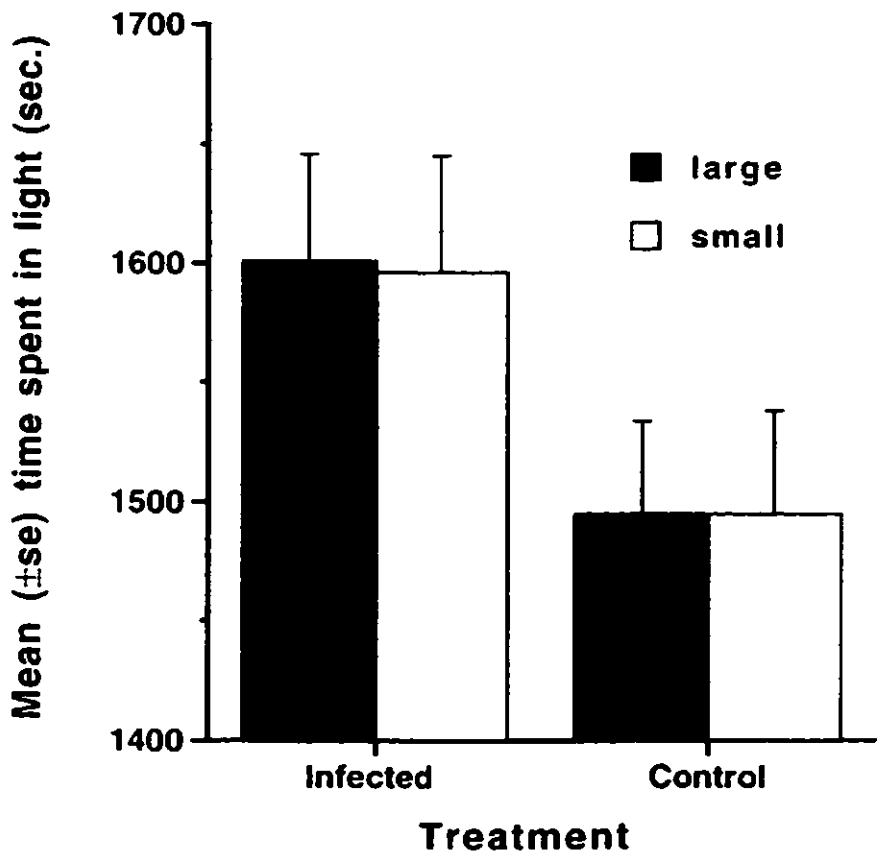


Fig. 7. Time (mean ±s.e.) that uninfected and *O. ptychocheilus*-infected fathead minnows spent in the lit portion of experimental tanks over replicate (n=10) 30 min. intervals (n = 10 minnows/treatment).

Chapter 6. General conclusions

Parasite ecologists have long-recognized that multifactorial approaches are required in order to understand the influence parasites have on their individual hosts (Poulin and Thomas, 1999) and on host populations (Minchella and Scott, 1991; Holmes, 1995). I have used a combination of both field observation and laboratory experimentation to evaluate the interaction between the trematode *Ornithodiplostomum ptychocheilus* and its second intermediate host, the fathead minnow. In so doing, this thesis has provided three main advances.

First, this study has demonstrated that parasite assemblages in boreal latitudes can be complex and species rich. Fathead minnows and brook stickleback had relatively species-rich assemblages, at least compared to those at southern latitudes. Indeed, fathead minnows had one of the richest helminth communities of any other forage fish on record. The majority of these parasites were larval trematodes, locally specific to one fish species. This is an important and unusual finding because many strigeids are reported as being broad host generalists (McDonald and Margolis, 1995; Gibson, 1996). It appears that factors specific to these lakes, such as the density and diversity of hosts, high frequency of winterkill, and high post-breeding mortality interact to generate these unusually complex patterns of helminth community richness (Esch, 1971; Holmes, 1979; Kennedy, 1990).

There was extreme spatial and temporal variation in the population dynamics of *O. ptychocheilus* in juvenile fathead minnows (Chapter 2). A single peak in parasite recruitment was observed from late summer to ice-on in each year of the study. Significant interactions between lake, year, and season suggest

that local characteristics of each lake are involved in producing the extensive variation observed in *O. ptychocheilus* intensities. Ongoing collaborative studies in Goater's laboratory, especially those involving the broader TROLS project (see Chapter 1), will provide an unparalleled opportunity to assess the role of factors such as water chemistry, hydrology and host density in determining this variation.

The second advance of this study was the documentation of *O. ptychocheilus* development in minnows and the effects of parasite intensity on this process (Chapter 3). In general, as intensity increased, metacercariae delayed their growth and their rate of encystment. Intensity-dependent processes have been described for other larval parasites and for adult trematodes, but this is the first report of such an effect limiting the development of metacercariae both in the laboratory and in the field. Subsequent studies will need to address the costs (if any) of these intensity-dependent delays by comparing the transmission of metacercariae from low-intensity and high-intensity infections into birds. In addition, this is also the first study to document the complex pattern of development associated with encystment. Unlike the development of most larval parasites, *O. ptychocheilus* metacercariae first increased, then decreased in size, as they encysted. Prior to this investigation, reports of such a process were limited to free-living invertebrates such as tardigrades and rotifers. I therefore consider the pre-encysted and encysted forms of *O. ptychocheilus* to be distinctive components of this host-parasite interaction. We should expect each form to have different physiological and nutritional requirements that could have different ecological and evolutionary effects on the host.

The identification of a phenotypic manifestation of infection in juvenile fathead minnows was, in my opinion, the most important advance of this thesis (Chapter 5). The discovery of the parasite-induced cranial distortion, in combination with that of Mueller (1972) demonstrates that the distortion occurs in natural populations, especially in small, heavily-infected fish. In addition, my thesis identified several consequences of the distortion, including altered phototaxis and reduced survival under conditions of low nutrition. This parasite-induced alteration of host phenotype could influence the strength and direction of natural selection on these infected hosts. An important area for future research will be to determine whether the manifestation is a parasite adaptation to increase transmission or is simply a pathological side-effect of infection. It will also be important to assess the frequency of the altered phenotype in natural populations of fish hosts.

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