

**BEHAVIOUR OF FATHEAD MINNOWS INFECTED WITH
A BRAIN-ENCYSTING PARASITE**

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ABSTRACT

A wide variety of parasites are known to cause changes in host behaviour. The altered behaviours range from simple changes in features such as activity and phototaxis, to the creation of behaviours that are new, and often bizarre. In this study, I investigated the effect of a trematode parasite, Ornithodiplostomum ptychocheilus (Strigeidae; Diplostomidae), on the behaviour of its intermediate host, the fathead minnow (Pimephales promelas). The larval stage (metacercaria) of this parasite resides within the central nervous system, specifically the optic lobes. In fish, one of the main functions of the optic lobes is to receive visual stimuli from the retina and then coordinate the optomotor response (OMR). This response is an innate component of rheotaxis that plays an important role in motion detection, navigation and orientation.

In an initial experiment, 16 wk-old metacercariae reduced minnow OMR by 42% compared to uninfected controls. However, in a follow-up experiment, it was 2- and 4-wk old metacercariae that caused the greatest (39 and 41% respectively) decrease in OMR. Because 2- and 4-wk old metacercariae are not infective to birds (the next host in the life-cycle), alterations in minnow OMR at this time are unlikely to be parasite adaptive. During this period, reduced OMR is more likely a result of pathology caused by developing larvae within the optic lobes. However, negative effects of infection on OMR performance persisted to 16 wk post-infection indicating that parasite-induced reduction in host performance could be an adaptive strategy to increase parasite transmission. Surprisingly, the magnitude of reduction in minnow OMR was only loosely linked to metacercariae intensity. Although both low (<5 parasites/fish), and high intensities (>100) led to large decreases in OMR, intermediate intensities had only a small effect. Such non-linearity between intensity and the magnitude of host behavioural

changes suggests that the mechanisms leading to altered host behaviours are varied, and complex.

PREFACE

"I have yet to see any problem, however complicated, which, when you looked at it in the right way, did not become still more complicated."

Poul Anderson

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Chapter 1. General Introduction

Parasitologists have long-recognised the ability for parasites to alter certain phenotypes of their hosts. Effects on traits linked to host physiology and immunity are especially well known. Yet, indirect and subtle parasite-induced changes in host phenotypes are also common. Textbook examples include the swellings of the stomach, legs and neck of humans infected with schistosomes, filarid nematodes and trypanosomes, respectively (Schmidt & Roberts, 1989). Non-anthropogenic host/parasite interactions provide especially graphic examples (review by Poulin and Thomas, 1999). Recent cases include the alteration in number and morphology of the limbs of anurans infected with an encysting trematode (Johnson et al., 1999), the expansion of the cranium of trematode-infected minnows (Sandland & Goater, 2001) and the grossly distended bellies of cestode-infected sticklebacks (Ness & Foster, 1999). Thus, in addition to the well-known effects of parasites on traits associated with host immunity, indirect effects of parasites on host individuals are probably legion, as first emphasized two decades ago by Dawkins (1982).

The most well-documented type of parasite-induced alteration in host phenotype is that on host behaviour, with approximately 150 described examples (reviews by Holmes and Zohar, 1990; Moore & Gotelli, 1990; Poulin, 1994). Studies on the phenomenon now receive attention not only from parasitologists and ecologists, but also from neurobiologists, pharmacologists and medical practitioners. This intensive interest is due to a number of features. One is the diversity of host/parasite interactions for which the phenomenon has been documented. Since its earliest roots in helminth/host interactions (Carney, 1969; Bethel & Holmes, 1973; Rau, 1983; Moore & Lasswell, 1986), the phenomenon has now been documented in groups as diverse as microparasites in insect

vectors (Koella, Sorensen, & Anderson, 1998), fungal infections in insects (Evans, 1988; Maitland, 1994), parasitoids in insects (Brodeur & Macneisl, 1992), and Toxoplasma in humans (Flegr et al., 1996). A second feature lies in the magnitude of some of the documented effects. For example, Bethel and Holmes (1973) showed that amphipods infected with a single cystacanth of Polymorphus paradoxus spent 88% of their time in the bright zone of an experimental tank, clinging to vegetation; uninfected controls spent only 2% doing so. Lastly, some examples of altered host behaviour are spectacular in their expression. Two examples include the altered behaviours of snails and ants infected with encysting trematode larvae (review by Poulin, 1995). In the first case, the trematodes exist within a brightly-coloured sac that emerges into one tentacle that pulsates when the snail climbs onto vegetation (Leweis, 1977). In the second, the brain-encysting parasite induces the ant's jaws to grasp the tips of blades of grass. Thus, given that the phenomenon seems to be widespread in nature, is often strong in its magnitude on hosts, and can be spectacular in its expression, the interest it receives from a wide variety of scientists should not be surprising.

Yet despite this attention, the precise nature of parasite-mediated host behavioural changes, and the mechanisms that produce them, remain controversial. Recently, Poulin (2000) has argued that the significance of the phenomenon has been over-emphasised in the literature. His central concern is that previous studies tended to over-emphasise the notion that the behavioural changes are parasite adaptations, without doing the critical tests (Moore & Gotelli, 1990; Poulin, 1995). On the one hand, examples such as the changes in snail and ant behaviour described above are difficult to interpret other than as parasite adaptations. On the other, most of the 150 cases of behavioural alterations used in Poulin's (1994) meta-analysis were simple changes in host activity associated with

infection. Such changes can be interpreted as parasite adaptations that enhance transmission, but they could also be interpreted as host adaptations that act to restrict the negative effects of current infection and (or) reduce the risk of future infection. Moreover, the behavioural alterations may not be adaptations of the parasite or the host, but may arise as simple consequences of infection (review by Poulin, 1995). Thus, pathology induced by infection may also lead to sick or moribund hosts that behave differently from non-infected hosts. Poulin (1995) emphasizes that although tests designed to distinguish among these alternatives are difficult to perform, they are a critical step in our understanding of the significance of the phenomenon of parasite-induced behavioural changes.

A further shortcoming of previous studies is that most do not involve experimentally infected hosts, but rather those collected from natural populations. A problem with this approach is that behaviours that might be interpreted as parasite adaptations might also be those that are associated with increased probability of infection (Poulin, 1995). For example, hosts that are naturally hyper-active may be more likely to be exposed to parasite larvae, either through direct contact or ingestion, and thus contain more parasites. If these hosts were used in subsequent behaviour assays, it would be difficult to distinguish effects caused by infection from those arising as a consequence of pre-collection hyper-activity, and thus exposure. In addition, field-collected hosts will almost certainly vary in their history of infection, and also in factors such as age, gender and diet. Not surprisingly, Poulin (1995) and others have urged that critical tests of the phenomenon utilize hosts with known histories and with varied and non-overlapping numbers of parasites in order to control for these confounding factors.

A further problem lies in the choice of the specific behaviours to be assayed. In many cases, examined behaviours are those that are convenient to evaluate, not necessarily those relevant to the biology of the parasite. For example, several examples exist that examine the effects of larval trematodes (eyeflukes) on various behaviours of their fish hosts (Lester & Huizinga, 1977; Crowden & Broom, 1980; Owen, Barber, & Hart, 1993). Although these eyeflukes are site specific in the retina or lens of the eye, few studies have examined for effects on behaviours that are linked to fish vision. Similarly, many studies examine for behavioural effects of larval parasites that encyst within the hemocoel of arthropods (Bethel & Holmes, 1973; Moore, 1983; Poulin, Curtis, and & Rau, 1992; McCurdy, Forbes, & Boates, 1999). Yet the behaviours that tend to be monitored are often those on general activity, phototaxis and geotaxis, where the link to site selection or parasite feeding strategies is unclear.

A model system that could usefully address these shortcomings would involve a system where hosts could be experimentally infected with known doses of worms and where the specific behaviour to be tested was closely linked to the biology of the parasite. Ideally, such characteristics should be combined with a system where features such as site selection, parasite development and parasite-induced pathogenicity are well-characterized. The purpose of my study is to determine the effects of a brain-encysting parasite (*Ornithodiplostomum ptychocheilus*, Faust) on specific behaviours of its second intermediate host, the fathead minnow (*Pimephales promelas*). Several features of this host/parasite interaction make it ideal to examine for effects on host behaviour. First, parasites such as *O. ptychocheilus* that reside within the nervous systems of their hosts have obvious potential to manipulate the behaviours of their hosts (review by Holmes & Zohar, 1990). Studies on fish infected with larval trematodes suggest that this potential

can be realized. Thus, metacercariae of Diplostomum spathaceum (eye flukes) alter the feeding behaviour and geotaxis of rainbow trout (Crowden & Broom, 1980) and also alter their susceptibility to predation (Brassard, Rau, & Curtis, 1982). Likewise, killifish collected from sites where infection with a brain-encysting metacercariae is high, increase their frequency of 'conspicuous' behaviours by 400% compared to fish collected from uninfected sites (Lafferty & Morris, 1996). Radabough (1980a), in a laboratory study, showed that fathead minnows formed less cohesive schools when they were infected with metacercariae of the brainfluke, O. ptychocheilus. Thus, evidence is accumulating that these parasites can affect a variety of behaviours. In one case, subtle alterations in behaviour resulted in a 30X increase in the rate of avian predation of infected fish, indicating that the phenomenon has important consequences for host fitness (Lafferty & Morris, 1996).

A second important feature of the brainworm/minnow system lies in the extensive background information that is available on the system that is relevant to tests of behavioural effects. Goater and his students provide information on O. ptychocheilus site selection (Sandland, 1999), development in the brain (Sandland & Goater, 2000), effects on the host (Sandland & Goater, 2001) and natural patterns of transmission (Sandland, Goater & Danylchuk, 2001). In addition to providing detailed methodology on the techniques used to experimentally infect minnows, this background work also allows me to set realistic infection levels and to time behavioural assays with periods when the parasites are in known locations within the brain, and when they are causing a known degree of pathogenicity. This work, together with earlier studies on this system (Hoffman, 1958; Hendrickson, 1979; Radabough, 1980b) and on systems involving related brain-encysting trematodes of fish (Ballabeni, 1994; Lafferty & Morris, 1996;

Barber & Crompton, 1997) provides a solid foundation for the examination of behavioural effects of O. ptychocheilus infection and to specifically test their adaptive nature.

Objectives of the thesis

The objective of my study is to evaluate the effect of O. ptychocheilus on visually-mediated behaviours of fathead minnows. Behaviours associated with vision were selected because earlier studies have shown that O. ptychocheilus larvae encyst on and in the optic lobes of the brain (Hendrickson, 1979; Radabough, 1980b; Sandland and Goater, 2000). A key function of the optic lobes is to receive visual stimuli (regarding movement, shape, colour and contrast) and integrate them to stimulate an appropriate motor response (Springer et al., 1977; Guthrie, 1986; Kotrschal et al., 1991). This connection between visual stimuli and motor response is well- described by neuroscientists as the Optomotor Response (OMR; Wallman, 1975). In fish, it is an innate component of rheotaxis (Rock, Tauber & Heller, 1964; de Peyster & Long, 1993) whereby even newly-hatched fry use visual landmarks to orient themselves within, and navigate through, their environment. The OMR has been shown to play an important role in a fish's ability to detect moving objects (Schaerer & Neumeyer, 1996), and to recognize conspecifics during schooling (Shaw & Tucker, 1965). Thus, a central theme of my thesis is that O. ptychocheilus encysts (often in large numbers) within a region of the brain that plays a critical role in host visually-mediated behaviours, and that any parasite-induced effects of infection on vision will ultimately impact host fitness.

The experiment in Chapter 2 was designed to evaluate the effect of O. ptychocheilus metacercariae on the optomotor performance of experimentally-infected minnows. The

main aim was to determine whether the encysted parasites could cause reductions in optomotor performance similar in magnitude to studies in which the optic lobes have been mechanically-damaged (Izowar & Aronson, 1987) or entirely removed (Springer, Easter & Agranoff, 1977). In this experiment, the behavioural assays were delayed until the metacercariae were 16 weeks old, ensuring that they would all be infective to birds.

In contrast, the experiment in Chapter 3 was designed to follow changes in optomotor performance throughout a 10-wk period of O. ptychocheilus development in the brain. Thus, the aim of Chapter 3 is to use this model system to distinguish the parasite-manipulation vs side-effect hypotheses for parasite-induced alteration in host behaviour. Supportive evidence for the manipulation hypothesis would exist if altered optomotor behaviour coincides with the onset of O. ptychocheilus infectivity. In contrast, supportive evidence for the side-effect hypothesis exists if the altered behaviours occur during the pre-infective period when pathogenicity is highest (Sandland & Goater, 2000; Sandland & Goater, 2001).

Lastly, the experiment in Chapter 4 tests the hypothesis that parasite-induced alterations in host behaviour are intensity-dependent. Despite the intuitive link between parasite intensity and the magnitude of behavioural changes, this hypothesis has been tested only rarely. This is an important shortcoming because establishing the link between intensity and behavioural alterations is a necessary first-step in understanding underlying mechanisms of behavioural changes. Thus, minnows were exposed to one of 5 doses of O. ptychocheilus larvae and then monitored for their optomotor performance.

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Chapter 2. Brain-encysting parasites affect visually-mediated behaviours of fathead minnows

ABSTRACT

Many populations of fathead minnows (*Pimephales promelas*, Rafinesque) in northern Alberta, Canada contain individuals with hundreds of trematode (*Ornithodiplostomum pychocheilus*, Faust) cysts on the surface of their brains. Most cysts are located on the optic tectum, a region known to play a role in integrating visual and motor stimuli, especially in schooling fish. I determined the effect of infection on visually-mediated behaviours of fathead minnows by evaluating host performance in an optomotor swimming task. Monitoring this task involved recording the time minnows spent following a spinning drum, onto which alternating black and white stripes had been painted. After controlling for host activity and host size, minnows containing an average of 18 (low-intensity) or 98 larvae (high-intensity) reduced their time spent following the spinning drum by 42 and 26%, respectively, compared to uninfected controls. Low-intensity minnows also took longer than controls to respond to a change in the direction of the spinning drum. Reduced optomotor performance has the potential to affect a host's ability to detect, and respond to prey, predators and conspecifics.

INTRODUCTION

Parasites are well-recognised for their ability to alter certain behaviours of their hosts (Moore & Gotelli, 1990; Poulin, 1995, 2000). Larval trematodes that have a required encystment stage within the nervous system of fish provide some of the best examples (review by Holmes & Zohar, 1990). For example, metacercariae of Diplostomum spathaceum (eye flukes) alter the feeding behaviour and geotaxis of dace, Leuciscus leuciscus (Crowden & Broom, 1980), and also alter the susceptibility of rainbow trout to predation (Brassard, Rau & Curtis, 1982). Likewise, killifish (Fundulus parvipinnis) infected with brain-encysting metacercariae of the trematode, Euhaplorchis californiensis, increase their frequency of 'conspicuous' behaviours such as flashing, surfacing and contorting (Lafferty & Morris, 1996). Lastly, Radabough (1980a) showed that fathead minnows (Pimephales promelas) formed less cohesive schools when they were infected with metacercariae of the brainfluke, Ornithodiplostomum ptychocheilus.

In each of these examples, the simplest explanation for host behavioural changes is mechanical damage caused by their presence in nervous tissue. For example, Owen, Barber & Hart (1993) showed that altered visually-mediated behaviours of eyefluke-infected fish could best be explained by direct damage to the retina. Likewise, it is possible that parasite-induced alterations in the schooling behaviour of fathead minnows (Radabough, 1980a) and the increase in conspicuous behaviours by killifish (Lafferty & Morris, 1996) may reflect damage caused by encystment in the optic centres of the brain of these fish. Whether this is so, and more generally, whether parasites affect traits that are under direct control of the nervous system (vision, olfaction, motor control), is poorly studied.

One commonly used method to assess an individual's visual performance is to monitor its optomotor response (OMR). In fish, the OMR is considered an innate component of rheotaxis (Rock, Tauber & Heller, 1964; de Peyster & Long, 1993), and is typically defined as a fish's response to stimuli resulting from its displacement relative to the position of natural landmarks in the environment (Harden-Jones, 1963; Arnold, 1974). The OMR can be simulated in the laboratory by spinning a background past a stationary fish. A large number of factors have been shown to affect the OMR of fish, including temperature and photoperiod (Dodson & Young, 1977), water quality (Dodson & Mayfield, 1979; de Peyster & Long, 1993), fish age (Veselov, Kazakov & Sysoyeva, 1998) and fish size (Hairston, Li & Easter, 1982). The effect of parasites on the OMR has not been evaluated. Since the OMR functions to integrate visual stimuli with motor responses, any reduction in OMR performance could influence an individual's ability to detect prey, predators and conspecifics.

The aim of this paper is to assess whether the trematode, O. ptychocheilus Faust, altered the OMR of its second intermediate host, the fathead minnow. This trematode encysts, often in large numbers (>600 worms/host), directly on, and in, the optic tecta of the brain of minnows (Hendrickson, 1979; Radabough, 1980b; So & Wittrock, 1982; Sandland & Goater, 2001). Since the function of the optic tecta in fish is to receive information from the retina, and in part, to integrate the OMR (Springer, Easter & Agranoff, 1977), I predicted that encystment of O. ptychocheilus causes impaired visual function in minnows.

MATERIALS AND METHODS

Experimentally-infected minnows were the F1 progeny of adults collected during the 1998 breeding season from Rochester Lake (100 km north-east of Edmonton, Alberta, Canada). Approximately 50 adults were collected from Rochester lake and then placed into artificial dugouts at Meanook Biological Research Station (54°38'-113°17'). Males actively defended territories on artificial nesting boards and females oviposited hundreds of eggs. Thousands of young-of-the-year (YOY) were collected from the dugouts on 4 Oct., 1998 and then maintained in the laboratory on Tetramin fish flakes.

Methods used to expose individual YOY to precise numbers of *O. ptychocheilus* cercariae are detailed in Sandland & Goater (2000). Cercariae were obtained from experimentally-infected snails, *Physa gyrina* (Say), that were exposed to miracidia originating from eggs shed by chickens (surrogate avian definitive host). The birds had been fed the brains of infected adult minnows from Rochester Lake. For infections, cercariae released from five snails over a 2-hr period were pooled together, their density estimated in 400 mL (Sandland & Goater, 2000), and then the volume required to contain 0, 20, or 120 cercariae was pipetted into 60 mm Petri dishes. A total of 72 size-matched YOY were removed from the stock tanks, placed into individual, numbered Petri dishes, and then assigned at random to one of two infection dates (Blocks 1 and 2; 23 or 24 Nov., 1998) and one of three exposure doses. Thus, there were a total of 24 replicates for each exposure dose (total N= 72 fish). Fish were exposed to cercariae for 3 hours. Fish from each exposure dose were maintained on Tetramin fish flakes in 20 L (60 cm long X 20 cm wide X 30 cm high) aquaria. Exposure doses of 20 (low-intensity) and 120 (high intensity) were chosen to incorporate the mean and maximum intensity, respectively, of YOY sampled between 1995-1999 from two lakes located 50 km north of Rochester lake

(Sandland, Goater & Danylchuk, 2001). In each of these lakes, all fall-collected YOY were infected with O. ptychocheilus.

The optomotor responses of control and infected minnows were monitored following the general methods of Springer, Easter & Agranoff (1977) and specifically applied to fathead minnows by de Peyster & Long (1993). The design of the optomotor apparatus followed Shaw & Tucker (1965). The apparatus consisted of a cylindrical glass aquarium (30cm diameter X 30cm high) mounted on a stationary platform. A cylindrical plastic screen (40 cm diameter X 30 cm high) was placed around the outside circumference of the stationary aquarium. The screen was attached by a rubber belt to a motor and a transformer that could regulate the rotation of the outer screen at 15 rpm. The rotating screen contained alternating bands of 25 mm black and white vertical stripes that subtended approximately 7 degrees at the centre of the drum (Springer, Easter & Agranoff, 1977). A video camera and a 60 watt incandescent lightbulb were mounted 50 cm directly above the optomotor apparatus. The rationale of the optomotor test is that fish tend to follow the direction of the moving screen and when the screen is reversed in direction, fish reverse their swimming direction.

The optomotor trials started on 15 March, 1999, corresponding to 16 wk post-infection (p.i.). I delayed the optomotor trials for 16 wk to avoid confounding the potential effects of developing vs. encysted larvae. Sandland & Goater (2000) showed that cercariae required up to 8 wk to encyst on the optic lobes of juvenile fathead minnows. Thus, all minnows used in the optomotor trials should have contained encysted metacercariae.

On the days of the trials, individuals were selected at random from their containers, measured for standard length, and transferred to the optomotor aquarium. Water in the

optomotor apparatus had been aerated at room temperature and aged for 7 days prior to the trials. Each trial lasted 12 min. During the first 6 min, the screen remained motionless. The first 2 min interval was considered a general acclimation phase, immediately after which the video camera was activated. The purpose of video-monitoring the following 4 min was to provide an overall assessment of pre-spinning activity and to determine whether individuals had a directional preference (i.e. left- vs right-handedness). This period was followed by a further 2 min acclimation period, during which the screen rotated in a randomly-selected direction. Swimming performance was monitored during the following 4 min. The spinning direction of the screen was chosen at random for the first 2 min, then reversed for the next 2 min. The main purpose of alternating the direction of spin was to determine whether fish swam in a preferred direction.

I analysed the effects of parasite intensity on two components of the OMR. The first, 'Following time', was defined as the time that fish spent following in the direction of the spinning drum. The second, 'Latency time', was defined as the time it took for fish to respond to the change in direction of the drum. To evaluate 'following time', the total available time that a fish could potentially follow the stripes was calculated as 4 min minus latency time. This removed any potential covariation between the two response variables. Thus, 'following time' was represented as the proportion of time that a fish followed the stripes, relative to the total time that the stripes moved after they were first detected. It was important in this experiment to distinguish parasite effects on host visual performance from those on host activity. The latter was evaluated as the proportion of time during the initial 4 min acclimation period that fish spent in motion. This value was used as a covariate in subsequent analyses.

Optomotor response data were tested for normality using Shapiro-Wilkes tests. Analyses involving 'following time' used proportional data that were arcsin(square-root) transformed. Analyses involving 'latency time' were log-transformed. The effect of parasite intensity on 'following time' was analysed with 1-way, repeated-measures ANCOVA. This analysis was used because the following time of individual fish was first monitored (over 2 min) as the screen spun in one direction, then monitored again (over 2 min) as the screen spun in the opposite direction. Thus, in this case, the repeated measure tests for the effect of 'direction', in addition to time. Analyses involving 'host activity' and 'latency time' used 1-way ANCOVA. The effect of infection period was accounted for in a 'Block' term in the ANCOVAs. The Tukey-Kramer HSD test was used to evaluate differences between infection treatments. Minnows from Block 2 were dissected after the trials to determine *O. ptychocheilus* intensity, whereas those from Block 1 were used in subsequent experiments not reported here. Parametric regression analyses were used to evaluate the association between parasite intensity and optomotor responses.

RESULTS

Seven of the 72 fish used in the experiment remained inactive during the 4 min acclimation period (when the screen was not spinning) and were removed from further analysis. During this 4 min period, minnows spent an equal proportion of time swimming in both directions ($F_{1,128} = 0.80$, $P = 0.373$). Because there was no evidence for a directional preference, host activity was evaluated as the total proportion of time spent active during the 4 min acclimation period. On average, fish spent $32.8 \pm 22.9\%$ of their time active during the acclimation period. Metacercariae intensity did not affect host activity ($F_{2,60} = 2.33$, $P = 0.106$) but small minnows were more active than large ones ($r = -0.30$, $P = 0.0206$, $N=65$).

Following time was affected by host activity, host size and parasite intensity (Table 1; Fig. 1). Minnows that were more active during the 4 min acclimation phase tended to spend more time swimming parallel to the spinning screen ($b=75.1$; slope = 0.897). After controlling for host activity, low-intensity minnows reduced their following time by approximately 42 %, whereas high-intensity minnows reduced it by 26 %. The Tukey-Kramer HSD test showed that high-intensity and low-intensity minnows had lower following times than controls, and that their following times also differed from each other ($P < 0.05$). For the 24 minnows dissected from Block 2, following time was not correlated with parasite intensity ($F_{1,23} = 1.00$, $P = 0.33$). The interaction between parasite intensity and direction was not significant (Table 1), indicating that fish from the different infection treatments responded similarly to a change in the direction of the spinning drum.

Latency time was affected by host activity and parasite intensity (Table 1; Fig. 2). Tukey-Kramer HSD comparisons showed that the greater than 3X increase in latency time between controls and low-intensity minnows was significant, but the difference between controls and high-intensity minnows was not. Fish that were more active during the acclimation period responded fastest to the change in spin direction ($b=4.24$, slope = -0.024). For minnows dissected from Block 2, latency time was not correlated with parasite intensity ($F_{1,19} = 3.63$, $P = 0.073$).

All minnows exposed to cercariae had encysted metacercariae in the brains at 16 wk p.i. Mean parasite intensity in minnows exposed to 20 and 120 cercariae was 18.2 ± 9.3 (range = 1-29; $N = 24$) and 98.4 ± 23.5 (range = 65-128; $N = 24$), respectively. All metacercariae were fully-encysted at necropsy.

DISCUSSION

Parasites that encyst on the optic tectum reduced the effectiveness of two visually-mediated behaviours in fathead minnows. Compared to uninfected controls, infected minnows followed the rotating stripes less often and took longer to first recognise, and respond to, a change in direction of the spinning screen. The optic tectum is recognized as the primary area for visual processing in the teleost brain, responsible for the reception and integration of visual stimuli (review by Guthrie, 1986). Moreover, there is also evidence that the optic tectum plays a role in integrating information received from the lateral line system (Weale, 1982). Thus, damage to the optic tectum could affect a wide range of traits that have unambiguous links to host fitness. For minnows, traits involved with the formation and maintenance of schools, the detection and discrimination of prey, the avoidance of predators and the recognition of conspecifics may be especially significant. Each of these traits has been shown to have a strong visual component in other teleosts (Harden-Jones, 1963; Shaw & Tucker, 1965; Hairston, Li & Easter, 1982; Sargent et al., 1998). Thus, the consequences of parasite-induced alterations to host OMR are potentially far-reaching in an ecological context.

The observed reductions in the OMR could be caused by parasite-induced effects on host activity or host visual function (or combinations thereof). Effects on host learning are unlikely because minnows had no prior experience with the optomotor apparatus. In a review of 114 examples of altered behaviour, Poulin (1994) concluded that hyper- and hypo-activity of infected hosts were the most common parasite-induced behavioural changes. These results provide no evidence that larval trematodes affected host activity, at least not during the 4-min pre-spinning interval. Moreover, in a concurrent experiment in which I tested for *O. psychocheilus*-induced changes in minnow activity over a 30 min

time-period, there was also no effect (Sandland, 1999). Thus, although following time and host activity were strongly correlated, variation in host activity was as a result of variation in minnow size, not infection.

The most likely explanation for the observed effects on minnow OMR is reduced visual function. Direct effects on host vision are unlikely, as encysted O. ptychocheilus would not affect the retinal surface. Rather, damage to the optic tecta must affect the ability of infected fish to process visual information received from the retina. In this study, the reduction in OMR associated with infection, even with low numbers of larval trematodes, is similar in magnitude to studies involving the surgical removal of the optic tecta of goldfish (Springer, Easter & Agranoff, 1977). The implication is that encysted larvae of O. ptychocheilus affect the centre that controls and integrates the OMR, resulting in reduced ability to follow the moving stripes. Thus, the key result of this laboratory study is that minnows infected with similar numbers of parasites to those found in natural populations (Sandland, Goater & Danylchuk, 2001) have a reduced ability to process visual information.

However, interpretations based on a direct link between histopathology and reduced visual functions are complicated by the non-linear relationship between parasite intensity and OMR. The tendency for high-intensity minnows to follow the spinning drum more than low-intensity minnows, and for the latter to have higher latency times, was surprising. Yet, despite the accumulating literature on the effects of parasites on host behaviour, most studies compare behaviours between 'infected' and 'uninfected' hosts and do not include dose-dependent effects. The few exceptions provide equivocal results. Crowden & Broom (1980) and Lafferty & Morris (1996) showed that as metacercariae

intensity increased, the extent of altered behaviour increased. In contrast, migrating larvae of Toxacara canis reduced the motor performance and activity of mice, but the extent of behavioural modification was not associated with intensity (Hay & Aitken, 1984). For minnows infected with O. ptychocheilus, the non-linear relationship between parasite intensity and effects on vision implies that factors in addition to (or other than) histopathological damage are important.

The possibility that density-dependence could restrict metacercariae size, and thus damage to the brain, is unlikely in this system. Sandland & Goater (2000) showed that O. ptychocheilus in high-intensity minnows took significantly longer to encyst, but were the same size at 8 wk post-infection as those in low-intensity minnows. Thus, the 16 wk-old metacercariae used in this experiment would all be the same size, regardless of intensity. A second possibility is that the mechanism responsible for altering host visual function in low-intensity infections differs from that operating in high-intensity infections. Thus, the presence of just a few worms may reduce visual function through, for example, the secretion of specific neuromodulators (Helluy & Holmes, 1990; Kavaliers & Colwell, 1993) or encystment in specific region within the optic tecta. On the other hand, the presence of many worms may lead to extensive pathological damage that either directly causes reduced vision, or masks the effects at low intensity. To distinguish these alternatives I need further experiments in which minnows are exposed over a wider period of time, to a wider range of exposure doses. I also need quantitative estimates of trematode-induced histopathology. Such experiments would help us understand whether the observed changes in the visual function have fitness consequences for the parasite, or are inconsequential side-effects of infection (e.g. Poulin, 1995).

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Table 1. Summary of ANOVA statistics for effects of *O. ptychocheilus* on two components of the optomotor response of fathead minnows

Source of variation	D.F.	MS	F	P
Response latency				
Block	1	1.494	2.157	0.147
Host size	1	0.874	1.262	0.266
Host activity	1	3.875	5.594	0.021
Parasite intensity	2	3.098	4.472	0.016
Error	59	0.693		
Following time				
Block	1	36.57	0.167	0.684
Host size	1	101.27	0.462	0.499
Host activity	1	2573.82	11.746	0.001
Parasite intensity	2	2067.81	9.437	<0.001
Error	59	219.12		
Direction	1	84.99	0.721	0.396
Direction * Parasite intensity	2	292.62	2.483	0.089
Error	62	117.87		

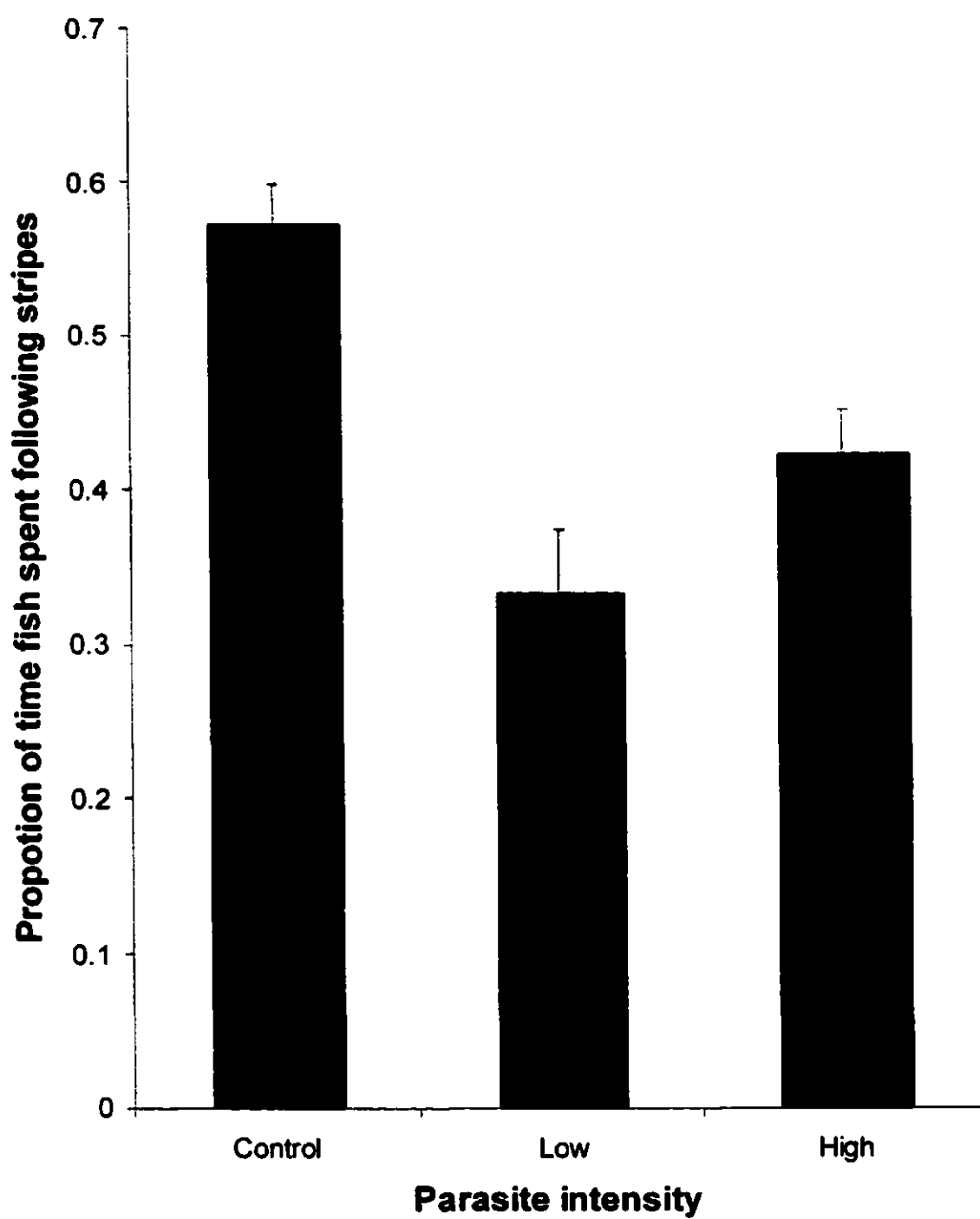


Figure 1. Proportion of time that infected fathead minnows spent following in the direction of moving stripes. Bars (\pm s.e.) represent means calculated from 18-24 fish containing 0 (control), 18.2 (\pm 9.3) (low intensity) or 98.4 (\pm 23.5) (high intensity) cercariae of the trematode *O. ptychocheilus*.

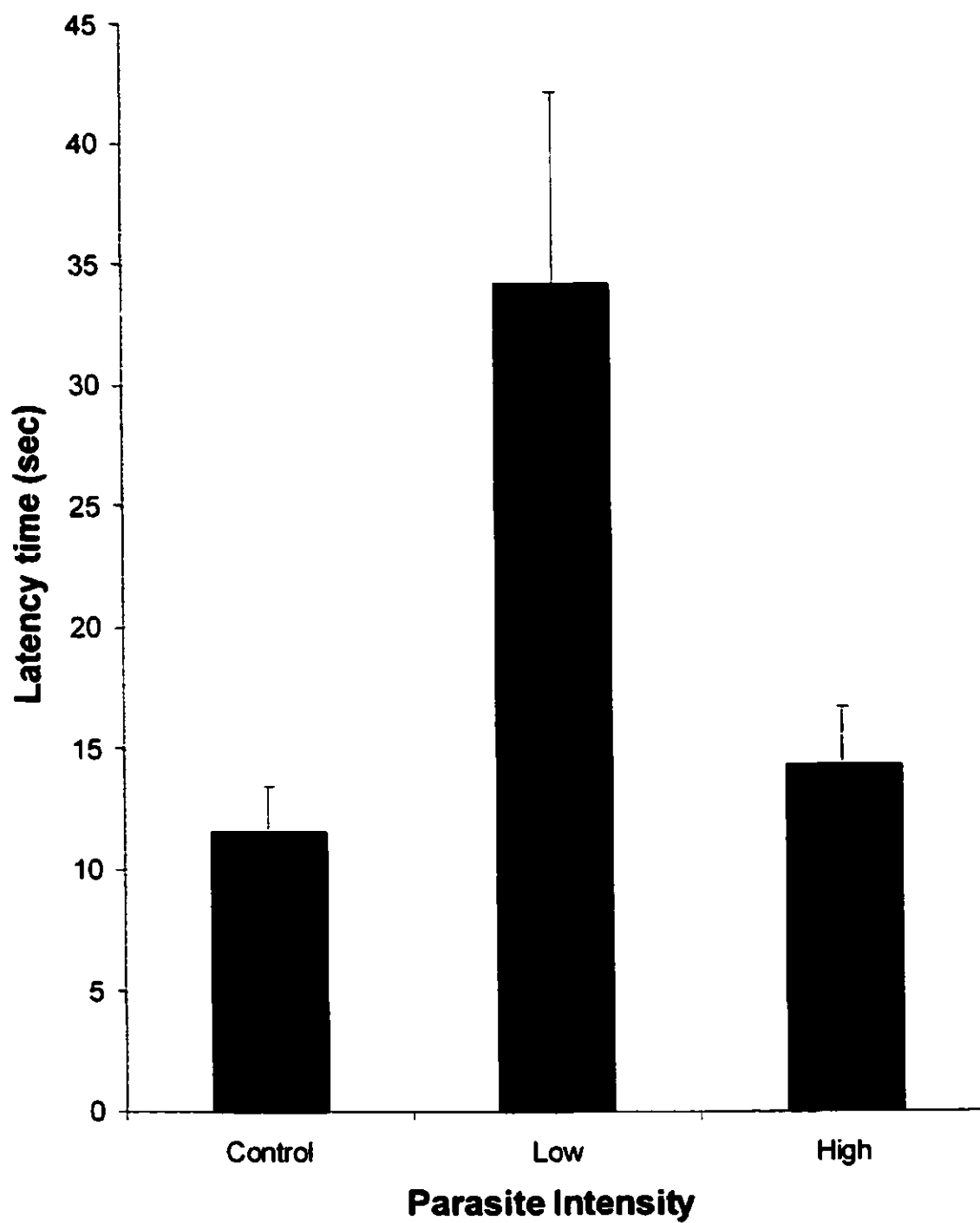


Figure 2. Time required for infected fathead minnows to respond to a change in the direction of a spinning screen. Bars (\pm s.e.) represent means calculated from 18-24 fish containing 0 (control), 18.2 (\pm 9.3) (low intensity) or 98.4 (\pm 23.5) (high intensity) cercariae of the trematode *O. ptychocheilus*.

**Chapter 3. Behaviour of minnows infected with brain-encysting trematodes:
a test of the parasite manipulation hypothesis**

ABSTRACT

The ability for parasites to alter certain behaviours of their hosts is well-documented. Such alterations are often considered parasite adaptations because they can lead to increased parasite transmission and thus increased parasite fitness (parasite manipulation hypothesis). Alternatively, the altered behaviours may be host adaptations, or simple side effects of infection. To test these alternatives, I monitored the development of a component of rheotaxis (optomotor response) in minnows (*Pimephales promelas*) infected with a parasite (*Ornithodiplostomum ptychocheilus*) that develops within the optic lobes. Optomotor performance was evaluated in a standard apparatus that monitored a minnow's ability to follow a spinning drum onto which alternating black and white stripes had been painted. Optomotor performance was evaluated prior to infection and then at 2-wk intervals up to 10 wk. Maximum reduction (39% and 41%, respectively) in performance occurred at 2 wk and 4 wk post-infection. Thereafter, the magnitude of the differences between infected and uninfected hosts was reduced, but still significant. Thus, the period of maximum parasite-induced altered behaviour coincided with the pre-infective period when parasites were developing within the optic lobes. These results are consistent with the side-effect hypothesis. However, because the negative effects of infection persist up to the time of parasite infectivity, the parasite manipulation hypothesis cannot be completely discounted.

INTRODUCTION

Despite the ever-increasing number of examples of parasite-induced alterations on host behaviour (reviews by Poulin, 1994), our understanding of their adaptive significance is limited. Three scenarios, or combinations thereof, are possible (Holmes & Zohar, 1990; Moore & Gotelli, 1990; Poulin, 1995). First, the altered phenotypes can be considered parasite manipulation if the changes lead to increased transmission and thus increased parasite fitness (parasite manipulation hypothesis). Good examples involve infected intermediate hosts where the behaviour changes are often reported to make the host more vulnerable to predation by the required definitive host (e.g. Bethel & Holmes, 1977; Moore, 1984; Lafferty & Morris, 1996; McCurdy, Forbes & Boates, 1999). Second, the alterations are host adaptations if they decrease the negative effects of current infection, or lead to decreased exposure to further infection (host adaptation hypothesis). Lastly, the changes are not adaptive to the parasite, or the host, if they arise as consequences of the normal life-cycle and feeding of the parasite (side-effect hypothesis). There are few model parasite/host interactions that can be utilised to distinguish between these hypotheses, in part because many of the parasites that alter host behaviours have complex life-cycles that are notoriously difficult to manipulate under laboratory conditions.

One method that can be used to provide evidence for a parasite adaptation is to determine whether the timing of behavioural alterations coincides with the period when the parasite is infective to its next host (Poulin, 1995). For example, supportive evidence for a parasite adaptation exists if the onset of the behavioural change coincides with the onset of infectivity. If the behavioural change occurs prior to parasite infectivity, and does not persist, then the change in host behaviour is unlikely to be parasite adaptive, and support the side-effect hypothesis. For example, altered behaviours may result from the

parasite's requirement to develop within particular sites prior to infectivity. Alternatively, the developing parasite may require specific types, or amounts, of host nutrients that result in host pathology. Thus, the altered behaviours may simply arise in sick or moribund hosts, in which case they may not favour parasite transmission at all.

In my earlier study, I showed that certain visually-mediated behaviours of fathead minnows (*Pimephales promelas*) were negatively affected by 16wk-old encysted larvae of the trematode fluke (*Ornithodiplostomum ptychocheilus*, Faust) (Chapter 2). Larvae of this parasite encyst on (and in) the optic lobes of the brain (Hendrickson, 1979; Radabaugh, 1980; Sandland & Goater, 2000), the primary vision-processing centre in teleost fishes. It is here where visual information concerning movement, shape, and colour are analysed (Guthrie, 1986) and it is here where stimuli from the lateral line system are received (Springer, Ester & Agranoff, 1977; Kortrschal et al., 1991). Specifically, a central function of the optic lobes is to coordinate visual stimuli with motor responses. In fish and other vertebrates, this reflex is termed the 'optomotor response' (OMR). It is an innate component of rheotaxis and is defined as a locomotory response to visual stimuli received as the animal moves relative to its background (Rock, Tauber & Heller, 1964; Rock & Smith, 1986; de Peyster & Long, 1993). The optomotor response has been shown to play important roles in detection of moving objects (Schaerer & Neumeyer, 1996), and the recognition of conspecifics (Shaw & Tucker, 1965). Thus, the 30-50% reduction in OMR caused by trematode infection in minnows (Chapter 2) could have widespread fitness and ecological consequences. However, whether such effects are adaptive to the parasite, the host, or are non-adaptive consequences of encystment in the optic lobes is not known.

The purpose of this experiment is to evaluate the adaptive nature of parasite-induced altered behaviour in minnows infected with larval O. ptychocheilus. Larvae of this parasite undergo a 4-8 wk of complex development stage in the optic lobes prior to infectivity (Fig. 1; Sandland & Goater, 2000). Thus, minnows can be experimentally-infected and then evaluated for their optomotor performance prior to infectivity and post-infectivity to evaluate the host manipulation vs. side effect hypotheses.

MATERIALS AND METHODS

Source of hosts and parasites

Uninfected juvenile fathead minnows (<20 mm total length) were collected from a pond (Sterling pond, located 30 km south of Lethbridge, Alberta, Canada) on 5 Aug., 2000. The fish were transferred in groups of 100 into 9, 1200L outdoor artificial ponds (e.g. Goater, 1994) for 8 wk. The fish grew at rates equal to, or greater, than in natural populations and they were never exposed to the larvae of O. ptychocheilus. One week prior to the start of the optomotor trials, surviving minnows were relocated to 9 indoor aquaria (60cm long X30cm wide X20cm high) that were held at 20C and on a constant 16:8 L:D photoperiod. Each aquarium was provided with a constant ration of Tetramin fish flakes three times daily for the duration of the experiment.

Because of the complex life-cycle of this trematode, experimental infections required a source of infected minnows, snails (Physa gyrina) and an avian definitive host. Infected minnows were collected (During July 17 to 20, 2000) from Rochester Lake located in central Alberta (Lat. 54° 22', Long. 113° 27') that was known to contain minnows with high intensities of larval O. ptychocheilus. Adult snails were collected from the same lake on the same date. Day-old chickens purchased from a local hatchery were used as

surrogate definitive hosts. For infections, I first fed the brains of adult minnows to chickens; 5 d later their feces were collected and sorted for parasite eggs (Sandland & Goater, 2000). The eggs were incubated in the dark for 12-14 d at 20C in tapwater. Miracidia hatched from the eggs and were exposed to F1 snails in groups of 5/snail for 3 hours in 3 mL Eppendorfer tubes. Exposed snails released larvae (cercaria) at approximately 28 d.

Minnows were exposed to cercariae following methods in Sandland & Goater (2000). On 27 (Block 1) and 28 (Block 2) Sept., 2000, 2-3 hr-old larvae from 3 infected snails were pooled together and their density in 500 ml water estimated using standard dilutions. From this cercariae/water solution, the estimated volume required to contain 120 cercariae, or equal volume of water for controls, was pipetted into 300 ml plastic containers. Individual minnows were selected haphazardly from the 9 stock aquaria and assigned at random into the containers containing cercariae for 3 hrs. A total of 110 size-matched fish (19-22 mm) were exposed to parasite larvae on the 2 infection dates. The exposure dose of 120 was chosen based on the maximum *O. ptychocheilus* intensity in juvenile fathead minnows sampled between 1995-1999 from 2 north-central Alberta lakes (Sandland, Goater & Danylchuk, 2001) and to parallel my earlier study (Chapter 2).

Optomotor Performance

For fish, the OMR can be simulated in the laboratory by monitoring the extent to which fish swim in the same direction, and with the same velocity, as a moving screen (Shaw & Tucker, 1965; Springer, Ester & Agranoff, 1977; Anstis, Hutahajan & Cavanagh, 1998). The optomotor apparatus was constructed based on the design by Shaw & Tucker (1965) and is detailed in Chapter 2. It consists of a cylindrical glass aquarium (30cm diameter X

30cm height) surrounded by a rotating cylindrical drum (40cm diameter X 30cm height), onto which alternating 25mm black and white stripes are painted. A small rubber wheel, attached to an electric motor, rotates the drum in either direction at approximately 15rpm while the aquarium remains stationary. Fish activity and behaviour were recorded by an 8mm video camera, mounted 50 cm directly above the aquarium. A 60-watt incandescent light bulb was placed above the apparatus.

The first optomotor trials were performed prior to infection (Sept 27 and 28, 2000) and then repeated at 2 wk intervals for 10 wk post-infection. I monitored the OMR of 12 size-matched infected and control fish following the general methods of Springer *et. al* (1977) and applied to fathead minnows by de-Peyster and Long (1993). The 10-wk period was selected to ensure that I encompassed the pre-encystment and post-encystment phases of O. ptychocheilus development within the optic lobes (Sandland & Goater, 2000). At each 2-wk interval, individuals were selected at random from their container and placed into the apparatus with aged tapwater at 20C. First, fish were acclimatized to the OMR apparatus for 2 min. During the following 4 min, the drum remained stationary, during which the fish's general activity and directional preference was video-monitored. The drum was then rotated in a randomly-selected direction for the following 2 min. Following this period of acclimation, the OMR was monitored for the following 2 min in one direction and then in the other direction for the subsequent 2 min.

The effects of O. ptychocheilus were evaluated on 2 components of the minnow OMR following methods outlined in Chapter 2. The first, 'Following time', was defined as the time that minnows spent following in the direction of the spinning drum. The second, 'Latency time', was defined as the time it took for fish to respond to a change in the

drum's direction of spin. To evaluate 'following time', the total available time that a fish could potentially follow the stripes was calculated as 4 min minus latency time. This removed any potential covariation between the two response variables. Thus, 'following time' was represented as the proportion of time that a fish followed the stripes, relative to the total time that the stripes moved after they were first detected. As in my previous experiment (Chapter 2), it was important to distinguish parasite effects on OMR performance from those on host activity. The latter was evaluated as the proportion of time during the initial 4 min acclimation period that fish spent in motion and was used as a covariate in the subsequent analyses. Lastly, minnows were measured for standard length and returned to the stock aquaria. After the 10 wk trial, the brain of each fish was removed from the braincase, squashed between 2 glass slides, and then observed under a dissecting microscope to obtain parasite counts.

In addition to parasite-induced effects on host vision and activity, it is possible also for the parasites to affect a minnow's ability to learn the optomotor apparatus. For example, Izower and Aronson (1980) showed that cichlid fish Aequidens latifrons improved their OMR after repeated trials. To test for an experience effect, the OMR of an additional group of 12 fish was evaluated at 10 wk. These fish were reared under the same conditions as all other fish, but had received no prior experience with the optomotor apparatus.

Parasite infectivity was assessed at 2, 4, and 10 wk post-infection. At each interval, 6, day-old chickens were each fed the brains of 3 minnows that had been exposed to 120 O. ptychocheilus larvae. Thus, each bird was exposed to approximately 360 metacercariae. At 120 hr, the chickens were killed and dissected. The small intestine of each bird was

removed, straightened lengthwise, and then cut into 20 equal sections following the methods of Bush and Holmes (1986). The mucosal surface of each section was scraped into a Petri dish, mixed with water, and then examined under a dissecting microscope. Parasites were counted and then examined under a compound microscope for the presence of eggs in the uterus.

OMR data were tested for normality using Shapiro-Wilkes tests. Analyses involving 'following time' used proportional data that were arcsin (square-root) transformed. Analyses involving 'latency time' were log-transformed. The focus of this experiment was to understand how the effects of infection on host OMR changed over the developmental period of the parasite. Thus, my focus is on the effects of 'parasite' and 'time' on OMR. In this experiment, I could not use a standard repeated measures ANOVA because individuals were not marked (in order to avoid any locomotory effects of the marks or tags) and thus not followed over the 10 weeks. I therefore used a standard ANOVA to examine the singular and interactive effects of parasites and time on host OMR. Both minnow length and host activity were covariates in the ANOVA. In this experiment, detection of differences in optomotor performance between infected and uninfected hosts at each time interval was a critical feature. Thus, to minimize the probability of making Type II errors, I conducted a series of 1-way ANCOVA's at each time interval to evaluate differences in performance between infected and uninfected hosts, while controlling for host activity

RESULTS

Fish grew an average of 6.9 mm over the 10 wk experiment, representing an approximate 130% increase in overall length. There was no effect of infection on host growth ($F_{1,11} =$

0.88, $P= 0.3494$). At 10 wk p.i., all fish exposed to cercariae harboured fully-encysted metacercariae in their brain. Mean parasite intensity was 108 ± 15 ($N=12$) representing approximately 90% parasite recovery. Metacercariae infectivity was extremely low at 2 and 4 wk p.i. At each interval, only 1 gravid specimen was recovered, representing <1% recovery. In contrast, the 6 birds dissected at 10 wk contained 118 ± 41 parasites, representing $36.5 \pm 12.7\%$ recovery.

During the experiment, both infected and control fish showed improvement in their optomotor performances. Compared to the pre-infection trial, following time of control and infected fish at 10 wk p.i. increased by 42.7% and 32.8% respectively. Host activity also varied between the trials. A Wilcoxon-test showed significant differences in activity level between the trials ($P = 0.014$). However, there was no effect of parasite infection on host activity ($P=0.087$), thus total activity was used as a covariate for later analysis.

Following time was affected by infection, time, direction of spin, and host activity (Table 1). The importance of parasite development is indicated by the significant interactions between direction and time, and infection and time. Although the following time of infected fish was always lower than that of controls (Fig. 2), maximum differences between control and infected fish occurred at 2 and 4 wk p.i. when following time of controls exceeded those of infected fish by 38.6% and 39.2%, respectively. The mean differences in following time between the 1st spin and the 2nd spin at 0, 2, 4, 6, 8, and 10 wk pi were -0.03 ± 0.15 , -0.02 ± 0.27 , 0.03 ± 0.29 , 0.18 ± 0.20 , 0.11 ± 0.20 , 0.11 ± 0.21 , respectively. The interaction between direction and the weeks was significant. Post-hoc 1-Way ANOVA's showed that uninfected minnows always significantly outperformed infected minnows except at 10 wk p.i. Differences were strongest at 2wk ($F_{2,24} = 15.99$, P

<0.001) and 4 wk ($F_{2,24} = 20.53$, $P < 0.001$), followed by declines in the differences at 6 wk ($F_{2,24} = 9.78$, $P = 0.005$), 8 wk ($F_{2,22} = 4.62$, $P = 0.044$) and 10 wk ($F_{2,22} = 3.14$, $P = 0.093$).

Response latency was highly variable between treatments and between weeks. The mean time (in seconds) fish took to respond to the changes of direction of drum spin at 0, 2, 4, 6, 8, 10 were 4.6 ± 5.1 ($N = 24$), 8.3 ± 5.2 ($N = 23$), 9.6 ± 12.0 ($N = 24$), 8.1 ± 5.4 ($N = 24$), 7.7 ± 5.2 ($N = 22$), and 8.2 ± 5.0 ($N = 22$), respectively. Total activity (the covariate) had no effect on latency ($F_{1,126} = 0.04$, $P = 0.835$), nor did infection ($F_{1,126} = 1.57$, $P = 0.212$). However, response latency changed significantly over the 6 trials ($F_{5,126} = 3.74$, $P = 0.003$). Tukey Kramer HSD test indicated that the only significant difference was the 41% increase between the pre-infection trial and the trial run at 2 wk pi.

Analyses restricted to the optomotor trials at 10 wk pi showed that following time was affected by infection ($F_{1,88} = 10.65$; $P = 0.0016$) and prior experience with the apparatus ($F_{1,88} = 36.56$; $P = < 0.0001$; Fig 1). The differences in following time between experienced and naïve fish were 28.6% and 48.2% for controls and infected fish, respectively. The interaction between infection and experience was not significant ($F_{1,88} = 2.98$, $P = 0.087$). The latency time was not affected by either experience ($F_{1,43} = 0.06$, $P = 0.810$) or infection ($F_{1,43} = 0.09$, $P = 0.766$).

DISCUSSION

These results confirm my earlier study (Chapter 2) by demonstrating that larval O. ptychocheilus reduced the optomotor response of fathead minnows, independent of effects on host activity. Because of the demonstrated link between a fish's optomotor performance and its ability to detect motion (Schaerer & Neumeyer, 1996) and its ability

to school (Shaw & Tucker, 1965), navigate and orientate (Clausen, 1931), O. ptychocheilus infection is likely to be important in an ecological context. However, the main result of this study is that parasite-induced reductions in optomotor behaviour are primarily restricted to the 2-4 wk development period when the parasite was not infective to birds. Transmission of infective metacercariae is an absolute requirement of this trematode and developmental time-lags within the final host have never been reported. This example of altered host behaviour is therefore unlikely to be adaptive for the parasite. Thus, these results support the predictions of the side-effect hypothesis of parasite-induced behavioural alteration.

The strong impairment in host behaviour at 2- and 4-wk p.i. are most likely caused by pathological damage imposed by O. ptychocheilus during its development within the optic tectum. Supportive evidence comes from the developmental sequence of O. ptychocheilus metacercariae in the brain. Sandland & Goater (2000) described a complex pattern of development involving distinct growth, encystment, and consolidation phases. Maximum metacercarial growth occurred between 0-4 wk p.i when larvae increased their maximum length by 40%. During this period, metacercariae were unencysted and motile. Although we know little regarding the nutrient requirements of metacercariae, and we do not know if they feed directly on brain tissue, this period coincides with pathogenic distortion of the cranium and associated tissues (Sandland & Goater, 2001). These effects decreased after 4 wks. coinciding with their metamorphosis to the encysted resting stage. In addition, in a laboratory study involving a related brain-encysting trematode in European minnows, Ballabeni (1994) showed that detectable host mortality was restricted to the pre-encystment stage. Thus, my observations that maximum reduction in optomotor behaviour coincides with a period of high host

pathogenicity supports the hypothesis that this altered host behaviour is primarily a pathogenic side-effect of infection. This result raises the possibility that the main effect of O. ptychocheilus infection on minnows lies in its direct effects on host survival, rather than on indirect traits associated with altered host behaviour. Other workers that have used a similar experimental protocol to the one used here have reached a similar conclusion (Thompson & Kavaliers, 1994; Kavaliers, Colwell & Galea, 1995; Hrda, et al., 2000).

However, unequivocal support for the side-effect hypothesis is premature for two reasons. First, there is some indication that the negative effect of infection on optomotor behaviour at 2- and 4 wk p.i. persists up to the time of encystment (Chapter 2). Although the differences in performance between controls and infected fish become progressively more reduced as the infection ages, it is conceivable that these small effects could influence parasite transmission, and thus fitness. It is also possible that the small differences at 6, and 8 wk p.i. are not due to persistence of earlier effects, but to new effects caused by encysted larvae. Regardless of which of the two mechanisms leads to differences in older infections, altered optomotor response anytime after 4 wk p.i. could be parasite adaptive. The second reason is that naïve infected fish tended to perform poorer than naïve controls at 10 wk p.i. Thus, parasite-induced effects on host learning (perhaps in the detection, and response to, novel stimuli) may be entirely parasite-adaptive. Further experiments that document effects on learning over the parasite's development cycle would be useful.

Moreover, these results must be interpreted in the context of at least two important features of O. ptychocheilus infection in natural populations. First, the infection

procedure in this study involved hosts exposed to a single dose of large numbers of infective larvae. Although Sandland, Goater & Danylchuk, (2001) showed that juvenile minnows can enter their first winter with >100 O. ptychocheilus metacercariae in their brains, these parasites would have accumulated over a period of approximately 10 wk at the end of summer. Thus, the single exposure to 120 cercariae used in my experiment is unlikely to reflect natural transmission. This is an important consideration in the interpretation of pathogenic consequences of infection, especially at 2 and 4 wks p.i. when all larvae would be undergoing rapid, simultaneous development within the optic lobes. Further experiments involving moderate exposure to cercaria over a longer interval could address this issue.

Second, natural populations of minnows contain metacercariae that are in various stages of development. At any one time, fish contain sub-populations of both fully-encysted and developing metacercariae (Goater, unpublished observations). In the experiment reported here, all metacercariae developed simultaneously and the main effects at 2 and 4 wks p.i. occurred in the absence of encysted (i.e. infective) forms. Because even small numbers of encysted worms affect optomotor behaviour (Chapter 2, Chapter 4), interpretations that emphasise the importance of effects of pure infections of un-encysted larvae should be treated with caution. The presence of mixed-stage infections complicates the interpretation of the adaptive nature of reduced optomotor performance because pathogenic side-effects due to developing larvae will be difficult to distinguish from concurrent and potentially adaptive effects due to encysted larvae. Moreover, if metacercariae within individual fish are genetically related, which is possible given the clonal production of cercariae, then host behaviours that are altered by uninfected

parasites could potentially be adaptive for encysted larvae that do not actively manipulate the host.

In conclusion, these results best support the side-effect hypothesis of altered host behaviour. Given the results from other experimental studies on this system (Sandland & Goater, 2000, 2001) the side-effects are likely to be a pathogenic outcome of the development of larvae within the optic lobes. However, interpretations regarding the adaptive nature of O. ptychocheilus effects on behaviour need to be set in the context of factors such as parasite intensity (Chapter 4), persistence of pathogenic effects, relatedness among parasites, and on the precise behaviour being affected. Context-dependent outcomes are frequently recognised within other features of host/parasite interactions (e.g. Goater & Holmes, 1997), but seldom within the context of parasite-induced effects on host behaviour.

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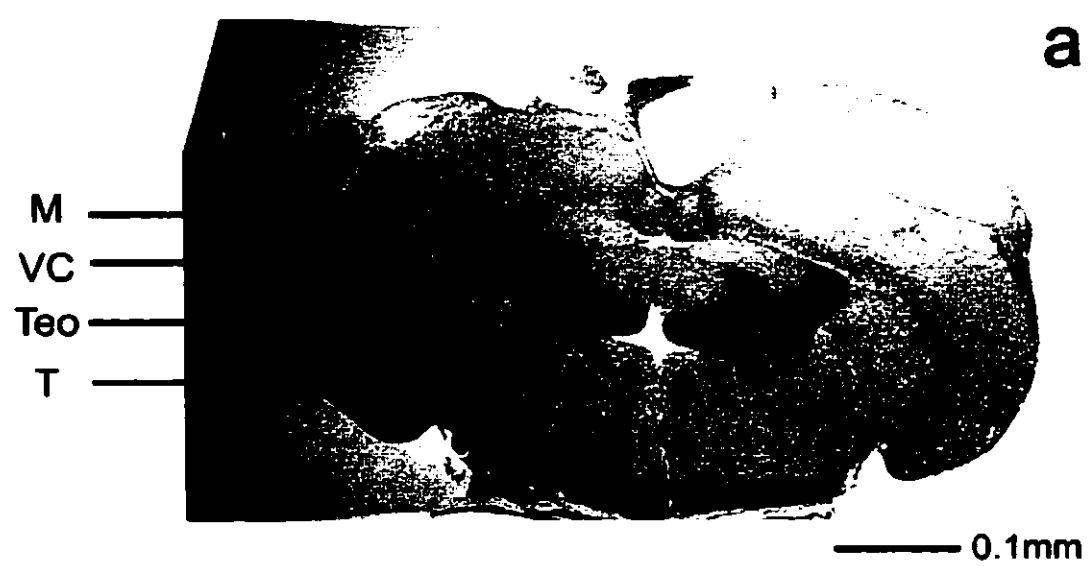


Figure 1. Cross section through the brain of a juvenile fathead minnow infected with O.ptychecheilus at 2 wk pi (a) and 8 wk pi (b). M, metacercariae; T, tegumentum; Teo, optic tectum; VC, valvula cerebelli.

Table 1. Summary of ANOVA statistics for effects of *O. ptychocheilus* on two components of the optomotor response of fathead minnow.

Source of variation	D.F.	MS	F	P
<i>Following time</i>				
Total activity	1	0.544	12.457	0.0005
Weeks	5	0.559	12.784	<0.0001
Infection	1	1.442	33.006	<0.0001
Direction	1	0.424	9.699	0.0020
Weeks*Infection	5	0.199	4.553	0.0005
Direction*Weeks.	5	0.122	2.783	0.0181
Direction*Infection	1	0.0155	0.356	0.5515
Total Error	260	0.0437	9.4214	
<i>Response Latency</i>				
Total activity	1	0.0185	0.044	0.8347
Weeks.	5	1.585	3.744	0.0034
Infection	1	0.666	1.573	0.2121
Weeks*Infection	5	0.502	1.185	0.3202
Total Error	126	0.423	2.241	

Table2. Summary of ANOVA statistics for effects of infection with *O. ptychocheilus* metacercariae and experience on optomotor performance of fathead minnows.

Source of variation	D.F.	MS	F	P
Experience	1	2.332	36.578	<0.0001
Infection	1	0.678	10.647	0.0016
Experience*Infection	1	0.145	2.273	0.1352
Total Error	88	0.0638	16.398	

Figure 1. Cross section through the brain of a juvenile fathead minnow infected with O.ptychocheilus at 2 wk pi (a) and 8 wk pi (b). M, metacercariae; T, tegumentum; Teo, optic tectum; VC, valvula cerebelli.

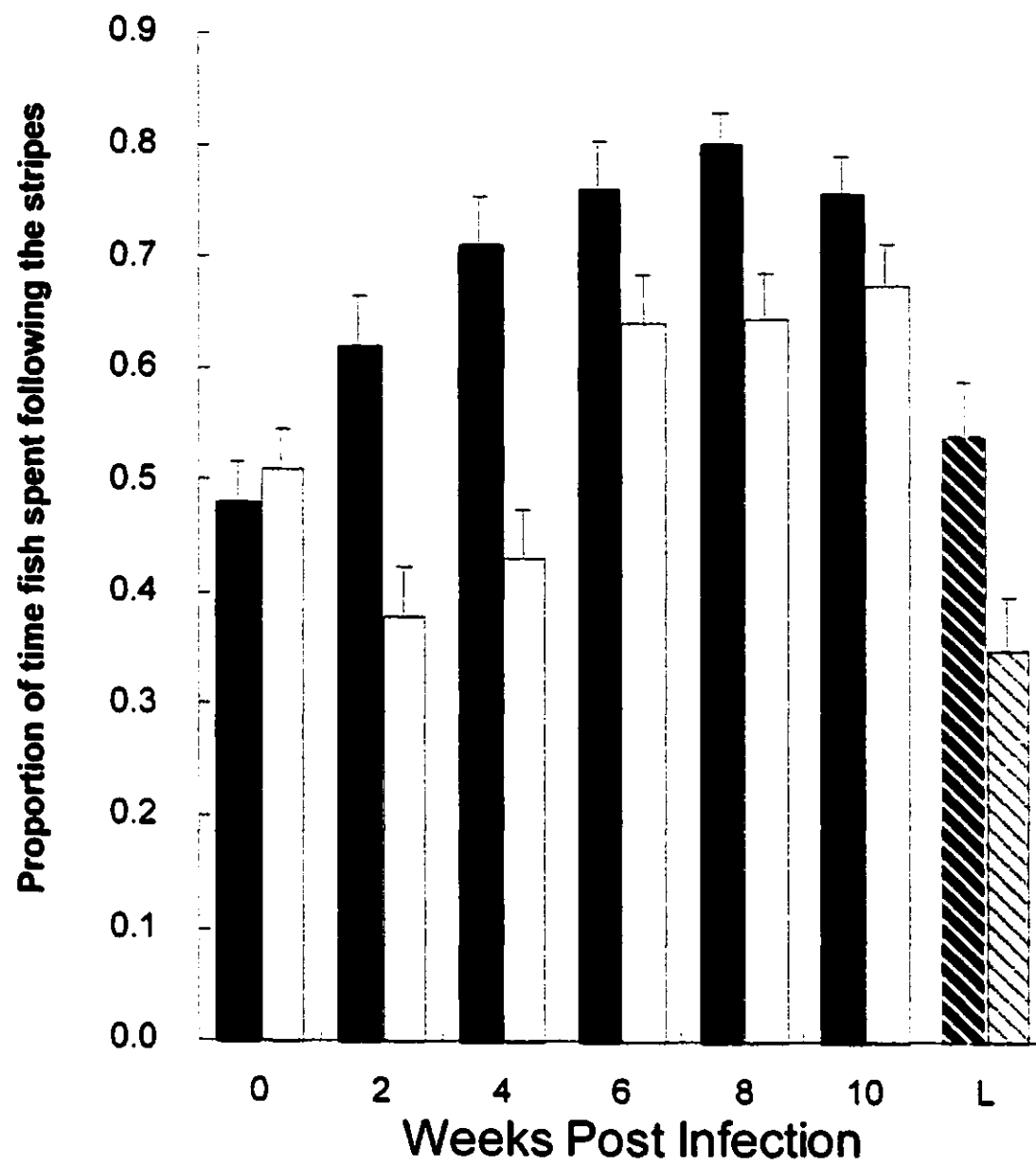


Figure 2. Proportion of time minnows spent swimming in the direction of moving stripes. Filled bars represent means (+SE) calculated for 10-12 uninfected controls. Empty bars represent means (+SE) calculated for 12 infected fish. Striped bars represent mean (+SE) following time of 12 non-experienced control (dark stripes) and 12 non-experienced infected fish (light stripes), respectively.

Chapter 4. Intensity-dependent alteration of fish behaviour by a brain-encysting parasite

ABSTRACT

Although there are many examples of changes in host behaviours caused by parasites, little is known regarding the factors that determine the magnitude of those changes. Intuitively, the numbers of parasites residing in a host should be correlated with the extent of behavioural changes, but this simple prediction has rarely been tested. In this experiment, I examined the relationship between the intensity of a brain encysting trematode (Ornithodiplostomum ptychocheilus) and the magnitude of altered optomotor performance in juvenile fathead minnows (Pimephales promelas). Minnows were exposed to 0, 5, 20, 120, and 300 larvae and then their performance in a standard optomotor apparatus was evaluated at 2 wk post-infection. Surprisingly, only those minnows containing either very high numbers of parasites (155 ± 31 worms/fish) or very low numbers of parasites (3 ± 3) differed significantly in their optomotor performance compared to controls. The non-linear relationship between parasite intensity and effect on host behaviour was consistent with an earlier study, but the underlying mechanisms producing this pattern are still unknown.

INTRODUCTION

Parasites are well-recognized for their ability to affect host behaviour (Moore & Gotelli, 1990; Poulin, 1994). Over the past decade, documentation of the numbers and types of parasites responsible for changes in host behaviour has increased dramatically (Poulin, 2000). Yet despite the accumulating number of examples of the phenomenon, little is known regarding the factors that lead to variation among individual hosts in the magnitude of parasite-induced behavioural changes. This is an important shortcoming for two reasons. First, the evolutionary significance of the phenomenon will depend, in part, on the extent to which the behaviour changes affect the fitness of both the host and parasite (Dobson 1988; Lafferty & Morris, 1996). Thus, it may well be the magnitude of the behavioural changes, not necessarily their presence, that ultimately influences the extent to which behaviour-altering parasites act as agents of natural selection on their hosts. Second, there is growing recognition that many of the diverse outcomes of parasite/host interactions are context-dependent (review by Goater & Holmes, 1997). For example, Yan et al. (1994) and Robb & Reid (1996) showed that the extent of behaviour alterations in cestode-infected beetles depended on the strain of the host, the numbers of parasites/host and the age of the parasites. Other factors such as host gender (Gotelli & Moore, 1992) and host age (McCurdy, Forbes & Boates, 1999) have been shown also to play a role within particular parasite/host interactions.

The numbers of parasites within a host (parasite intensity) should contribute towards variation in the magnitude of behavioural alterations. Thus, if the cause of the alterations is linked to parasite-induced pathology (Poulin, 1995), then more parasites should cause more damage, and therefore a higher degree of behavioural change. If the mechanism of

behaviour changes is associated with the secretion of neuromodulators (Helluy & Holmes, 1990; Kavaliers, Colwell, Choleris, 1999), then more parasites should secrete more substance, resulting in greater host manipulation. Unfortunately, most of the 150 examples of parasite-induced alterations reviewed by Poulin (1994, 1995) involved hosts that were classified as infected or not, so that these simple predictions regarding intensity-dependent effects have rarely been tested.

Supportive evidence for a link between intensity and behavioural alterations comes from two types of experiments. First, several correlational studies have associated parasite intensity with the magnitude of a behavioural change. Eye-fluke (Diplostomum spathaceum) intensity was negatively correlated with the feeding efficiency of dace, Leuciscus leuciscus (Crowden & Broom, 1980) and brain-fluke (Euhaplorchis californiensis) intensity was positively correlated with the numbers of conspicuous behaviours (flashing, surfacing, and contorting) in killifish, Fundulus parvipinis (Lafferty & Morris, 1996). Experimental studies involving hosts with manipulated numbers of worms also provide support. Arthropods infected with cestodes and acanthocephalans respectively, had higher hyper-activity when they were infected with >1 parasite (Wilson & Edwards, 1985; Urdal, Tierney & Jakobsen, 1995). Mice exposed to 200 nematode larvae did poorer in a maze test than those exposed to 50 larvae (Kavaliers and Colwell, 1995). These results support the notion that intensity and the extent of parasite alteration of host behaviour are linked.

Contrasting evidence also exists. Cezilly et al. (1999) showed that the altered phototaxis and altered vertical distribution of amphipods induced by acanthocephalans was not correlated with intensity. Similarly, Pulkkinen et al. (2000) showed that the alteration in

micro-habitat selection and activity of cestode-infected copepods was not intensity-dependent. Lastly, the intensity of Toxoplasma cysts in the brains of mice did not correlate with impairment of their motor performance (Hutchinson, Aitken & Wells, 1980). In these latter examples, the mere presence of the parasites within the host seemed to be more important in causing behavioural alterations than their actual numbers.

In an earlier study (Chapter 2), I showed that the brain-encysting trematode, Ornithodiplostomum ptychocheilus significantly reduced optomotor response in juvenile fathead minnows, Pimephales promelas. Specifically, minnows containing 18 ± 9 trematode metacercariae in their brains performed 42% poorer in an optomotor swimming task than controls; minnows containing 98 ± 23 larvae reduced their optomotor performance by only 26%. These results indicate that at least in this system, the link between parasite intensity and the extent of behaviour alteration is not as straightforward as the simple predictions outlined above would suggest. The aim of this follow-up study is to assess the relationship between the numbers of O. ptychocheilus metacercariae and the optomotor response of fathead minnows using a wider range of parasite intensities.

MATERIALS AND METHODS

Juvenile fathead minnows (15-20mm) were exposed to 4 doses of O. ptychocheilus cercariae to create 'low', 'medium', 'high' and 'very-high' infection levels, in addition to the uninfected control. Minnows were obtained commercially from a Biological supply house (Aquatic Research Organisms, NJ, USA) and were maintained in a large filtered stock tank (200 X 75 X 85 cm) at $20 \pm 3^\circ$ C with a constant 16:8 hr photoperiod. Fish were fed daily on Tetramin flake food. Methods used to expose minnows to O.

ptychocheilus cercariae followed Sandland & Goater (2000). In brief, metacercariae of O.ptychocheilus were obtained from infected adult minnows collected in June, 2001 from Rochester Lake, north central Alberta, Canada (Lat. 54° 22', Long. 113° 27'). Cercariae obtained from 5 experimentally-infected snails were added to a 500 mL volumetric flask and the volume required to contain 0, 5 (low), 20 (medium), 120 (high), and 300 (very-high) cercariae was pipetted into Petri dishes containing 50 mL of aged tap water. The exposure doses of 5, 20, and 120 were selected to incorporate the range of O. ptychocheilus intensities in juvenile fathead minnows sampled between 1995-1999 from two lakes in northern Alberta, Canada (Sandland, Goater & Danylchuk, 2001). The 300 exposure dose was intended to exaggerate any potential intensity effect. On infection day (16 July, 2001), 60 size-matched minnows (12 replicates X 5 infection doses) were removed from the stock tank and placed individually into the Petri dishes for 3 h. After exposure, fish were maintained in stock aquariums on Tetramin fish food until the day of the trials.

The effect of parasite intensity on optomotor performance was evaluated following the methods described in Chapters 2 and 3. The apparatus consisted of a glass cylindrical aquarium surrounded by a drum screen, onto which vertical black and white stripes (25 mm wide) had been painted (Shaw & Tacker, 1965; Springer, Easter & Agranoff, 1977). The drum was set to rotate at 15 rpm. Optomotor performance was video-monitored with the camera placed 50 cm directly above the apparatus. A 60 W incandescent bulb mounted above the apparatus was used as a supplementary light source.

The optomotor trials were performed between 30–31 July, 2001, at 2 wks post-infection (p.i.). This time period was selected because it corresponded to the period when the

effect of O. ptychocheilus on minnow OMR was strongest and because 2 wk-old metacercariae are not infective at any intensity (Chapter 3). It was also selected to avoid confounding the effects of intensity with development rate. Sandland & Goater (2000) showed that development rate of O. ptychocheilus is strongly density-dependent such that at 2 wk p.i. the parasites are un-encysted and un-infective. Beginning at 4 wk p.i., parasites begin to encyst, but their rate of development depends on intensity. Thus, in this experiment, I chose 2 wk, during which, despite the parasite intensity, all parasites would be undergoing development to the encysted stage and therefore un-infective.

The methods for evaluating optomotor performance followed those described in Chapters 2 and 3. Individual fish were randomly selected, placed into the apparatus, and video-monitored for a total of 12 min. During the first 6 min, the drum remained stationary. The first 2 min was considered an acclimation period, while the following 4 min was video-monitored to evaluate general host activity (proportion of time fish spent swimming within 4 min). The last 6 min was split into a similar sequence, except with the drum spinning. The first 2 min was considered an acclimation period, where fish could become accustomed to the moving stripes. During the following 2 min fish performance with respect to the spinning drum was video-monitored; the last 2 min were identical except the rotation of drum spin was reversed.

I examined two components of a fish's optomotor performance. First, 'following time' was defined as the proportion of time fish followed in the direction of the moving stripes (Chapter 2). The second, 'response latency' was defined as the duration of time it took a fish to respond to the change in direction of drum rotation. In order to separate the two response variables, 'following time' was considered as the proportion of time fish spent

following the stripes, minus 'response latency'. After each trial, fish were measured for standard length (the length between the tip of snout and end of the vertebrae) and then dissected to obtain metacercarial intensity and proportion encystment.

Analysis of variance (ANOVA) was used to determine the effect of metacercariae intensity on minnow behaviour. Metacercarial counts were transformed ($e^{0.2}$) to meet the assumptions of normality. Analyses involving 'following time' used proportional data that were arc-sin (square-root) transformed. The effect of parasite intensity on 'following time' was analysed using combined within-subject and between subject ANOVA with direction being a within-subject factor, and the intensity being a between-subject factor. Host size was used as a covariate. This analysis was employed because the same individuals were monitored during the first 2 min and in the subsequent 2 min after the change in direction of the screen. The repeated measure ANOVA therefore tested for the effect of spinning direction, in addition to time. Analyses involving host activity and response latency used 1-way ANOVA, and the data for 'Response latency' were log-transformed to meet the assumption of normality. Tukey-Kramer HSD tests were used to evaluate differences between infection treatments.

RESULTS

Of the 60 fish tested, 5 were not analysed: 1 contained no parasites at necropsy and 4 remained motionless during the entire OMR trial. Mean parasite intensities in minnows exposed to 5, 20, 120, and 300 cercariae were 3.4 ± 3.0 (Range = 1-11; N=11), 11.8 ± 3.6 (Range = 5-19; N=12), 88.5 ± 10.5 (Range = 76-101; N=11), and 155.6 ± 31.3 (Range = 111-209; N=11), respectively, representing a significant difference between infection levels ($F_{3,41} = 337.4$, $P < 0.0001$). At 4 wks p.i., the mean proportion of encysted

metacercariae for 'low', 'medium', 'high', and 'very-high' exposures was 0.74 ± 0.32 (N=11), 0.73 ± 0.2 (N=11), 0.79 ± 0.11 (N=10) 0.34 ± 0.33 (N=11), respectively. The proportion of encysted parasites at 4 wks p.i. was significantly reduced in the 300-dose fish compared to the other exposure doses ($F_{3,39} = 6.99$, $P = 0.0007$; Tukey Kremer HSD test). Mean host length did not differ between the 5 exposure doses ($F_{4,50} = 1.34$, $P = 0.267$). At the time of necropsy, I observed several of the 120-dose and most of the 300-dose fish to have mild to severe enlargement of their craniums, identical to that reported in this system by Sandland & Goater (2001). The enlargement was usually associated with distortion of the entire head region, including abnormal displacement of the eyes.

In contrast to my earlier experiments on this system (Chapter 2, Chapter 3), the effect of infection on host activity was analysed separately from following time. This is unfortunate because ideally my focus is on evaluating following time after controlling for host activity. However, in these data, there was a significant effect of parasite intensity on host activity ($F_{4,50} = 3.15$, $P = 0.02$), preventing the use of activity as a covariate in the ANOVA. Host activity was affected by parasite intensity but not by host size (Table 1; Fig. 1). Although 5-, 20-, and 120-dose fish spent approximately 55-75% of their time active, 300-dose fish reduced their activity by approximately 70% compared to controls. The Tukey-Kramer HSD test showed that the 300-dose fish spent significantly less time active than fish from the other exposure doses.

Following time was affected by parasite intensity, but not by host size (Table 1; Fig. 2). Tukey-Kramer HSD tests showed that the effect of parasite intensity on following time was complex. Although the 20- and 120-dose fish had reduced following times compared to controls, the differences were not significant. In contrast, the 5- and 300-

dose fish reduced their following times by 33% and 48% respectively, compared to controls.

To distinguish whether following time was associated with metacercariae intensity or host activity, I used partial correlation analyses. This analysis permitted me to evaluate the correlation between any pair of three variables (following time, host activity and metacercarial intensity) while controlling for the third. The test showed that metacercariae intensity and following times were negatively and significantly correlated ($r = -0.306$, $N = 55$, $t = -2.32$, $P = 0.024$) when controlling for host activity. The correlation between following time and host activity when controlling for metacercariae intensity was also significant ($r = 0.39$, $N = 55$, $t = 3.06$, $P = 0.0035$). There was no significant correlation between metacercariae intensity and host activity when controlled for following time ($r = -0.196$, $N = 55$, $t = -1.44$, $P = 0.156$). These correlation analyses indicate that metacercariae intensity was most closely associated with following time rather than host activity.

Mean response latency times (sec.) for control, 5-, 20-, 120-, and 300-dose fish were 13.5 ± 19.3 ($N=10$), 10.8 ± 8.9 ($N=11$), 8.0 ± 9.2 ($N=12$), 11.8 ± 13.1 ($N=11$), and 11.1 ± 10.1 ($N=11$), respectively. Response latency time was not affected by parasite intensity ($F_{4,49} = 0.22$ $P = 0.93$) or host size ($F_{1,49} = 0.01$ $P = 0.94$).

DISCUSSION

Results from this experiment support the idea that variation in the extent of altered host behaviours is associated with variation in parasite intensity. However, my expectation of a linear relationship between intensity and altered behaviour, based on correlational studies involving similar host/parasite interactions (Crowden & Broom, 1980; Lafferty &

Morris, 1996), was not met. Instead, it was the lowest intensity (3.4 ± 3.0 worms/host) and highest intensity (155.6 ± 31.3 worms/host) fish that performed poorest in the optomotor task, while those with intermediate intensities (11.8 ± 3.6 and 88.5 ± 10.5) performed similar to uninfected controls. Non-linearity in the relationship between intensity and behavioural alteration is consistent with my earlier study (Chapter 2), further confirming that at least in the brainworm/minnow interaction, mechanisms leading to altered host behaviour are complex.

The marked reduction in optomotor performance in fish containing only 1-3 metacercariae is important in the context of natural infections in minnows. In juveniles collected from four lakes between 1995-1999, prevalence usually reached 100% in samples of September-collected fish and intensity ranged from 3-10 parasites/host (Sandland, Goater, & Danylchuk, 2001). Thus, infection with 1-3 metacercariae is common in natural populations of minnows. The implication is that reduced OMR is likely to be a common consequence of O. ptychocheilus infection in nature. Reductions in optomotor performance have been shown to affect motion detection (Schaerer & Neumeyer, 1996), orientation (Clausen, 1931) and schooling (Shaw & Tacker, 1965) in other fish species. Effects on factors such as the recognition of prey, predators and potential mates should also be expected. In a similar host/parasite interaction, Owen, Barber & Hart (1993) showed that as few as four Diplostomum metacercariae per eye significantly reduced the feeding efficiency of sticklebacks, Gasterosteus aculeatus. They speculated that even small changes in feeding performance could lead to large changes in stickleback growth rate and thus host reproduction. Similar conjectures are possible in fathead minnows, even those exposed to fewer-than-typical numbers of parasites.

The simplest explanation for reduced optomotor performance in lightly infected minnows is the development of the parasite within the optic lobes. Site selection of O. ptychocheilus metacercariae has been well-characterised (Hendrickson, 1979; Radabough, 1980; So & Wittrock, 1982), although the distinction in micro-sites between pre-encysted and encysted larvae has only recently been recognized (Sandland & Goater, 2001; Goater, unpub. observ.). At 2 wk p.i., metacercariae are located directly under the outer edge of the optic lobes (Fig. 1, Chapter 3) where they appear to ingest brain tissue. Cyst development starts at 4 wk p.i., and the parasite becomes infective by 10 wk p.i. (Chapter 3). The ultrastructure of the optic lobes is extremely complex in cyprinid fish, consisting of at least 15 different tissue layers (Springer, Ester & Agranoff, 1977; Guthrie, 1986; Reichert, Wullimann & Rupp, 1996). Even single infections with 2 wk-old O. ptychocheilus appear to cause localised damage to the outer edge of the optic lobes (Fig. 1, Chapter 3). The specific function of this region within the optic lobes is unknown although it is likely to be tied to the processing of visual information received from the retina. My results suggest that even light metacercarial infections interfere with the normal functioning of this region of the optic tecta. Whether the interference is due simply to temporary histopathology caused by developing larvae, or as the result of more active mechanisms associated with manipulation, is unknown.

The significance of the optomotor results for hosts containing >100 parasites lies in the link between parasite effects on host activity and those on optomotor performance. Thus, minnows with >150 metacercariae reduced their activity by approximately 70% compared to controls and this reduction corresponded to a sharp reduction in following time. In experiments on this system that involving minnows with lower metacercarial intensities, parasite effects on host activity were independent of those on optomotor

performance (Chapter 2, Chapter 3). It is also noteworthy that it was minnows from this infection class that demonstrated clear pathological damage to the cranium, identical to that reported in high-intensity infections by Sandland & Goater (2001). Thus, at 2 wk p.i., fish with extremely high numbers of O. ptychocheilus metacercariae tend to suffer most from infection. This pathology is manifested primarily as reduced overall activity, which in turn is associated with reduced optomotor performance. Whether the pathology is due to simple pathology induced by large numbers of parasites in the optic lobes, or to an increased duration of the pre-encysted stage is unknown. In Poulin's (1995) review of over 100 known examples of changes in host behaviour, he describes parasite-induced alterations in host activity as the most frequent effect. These results do not support this generalization, because parasite-induced alteration in host activity only occurred at the most extreme infection levels.

Although minnows infected with either few or many metacercariae reduced optomotor performance, those with intermediate numbers outperformed formers. This non-linearity between intensity and the extent of behaviour alterations is surprising. However, these results confirm my earlier study (Chapter 2) that showed minnows infected with approximately 18 worms did poorer in optomotor trials than those infected with 98. Unfortunately, the results from this study did not clarify the relationship between intensity and optomotor performance. Instead, the results confirm the possibility that 1) mechanisms leading to altered behaviours at low intensity differ from those at high intensity and (or) 2) high intensity infections tend to mask the effects at low intensity (Chapter 2).

In summary, the key result of this experiment is the demonstration that the relationship between parasite intensity and altered host behaviours is not straightforward. However, such complexity has been described in at least one other trematode/host system. For example, the bizarre behaviour of ants infected with metacercariae of the trematode Dicrocoelium dendriticum is also independent of intensity (Carney, 1969; Wickler, 1976). Experimental studies have shown that only the one or two metacercariae that travel to the ant's brain induce altered geotaxis and subsequently cause the ant's jaws to clamp onto vegetation. The remaining metacercariae encyst within the hemocoel and apparently do not alter host behaviour. There are now many examples of subtle alterations in host behaviour (review by Poulin, 1995). Several, particularly those involving larval acanthocephalans in arthropods, involve complex behaviours that are altered by single worms (Bethel & Holmes 1973; Demont & Corkum, 1982). Perhaps it should not be surprising that multiple worm infections differ in complex and unpredictable ways from single worm infections. It should also not be surprising that multiple worm infections have the ability to mask the subtle effects of single worm infections.

Table 1. Summary of ANOVA statistics for the effects of metacercarial intensity on two components of the optomotor response of fathead minnows

Source of variation	D.F.	MS	F	P
<i>Following time</i>				
Host size	1	0.021	1.008	0.320
Exposure dose	4	0.129	6.322	<0.001
Error	49	0.020		
Direction	1	0.072	3.554	0.065
Direction * Exposure dose	4	0.044	2.167	0.087
Error	49	0.020		
<i>Activity</i>				
Host size	1	0.060	1.224	0.274
Exposure dose	4	0.180	3.713	0.010
Error	49	0.048		

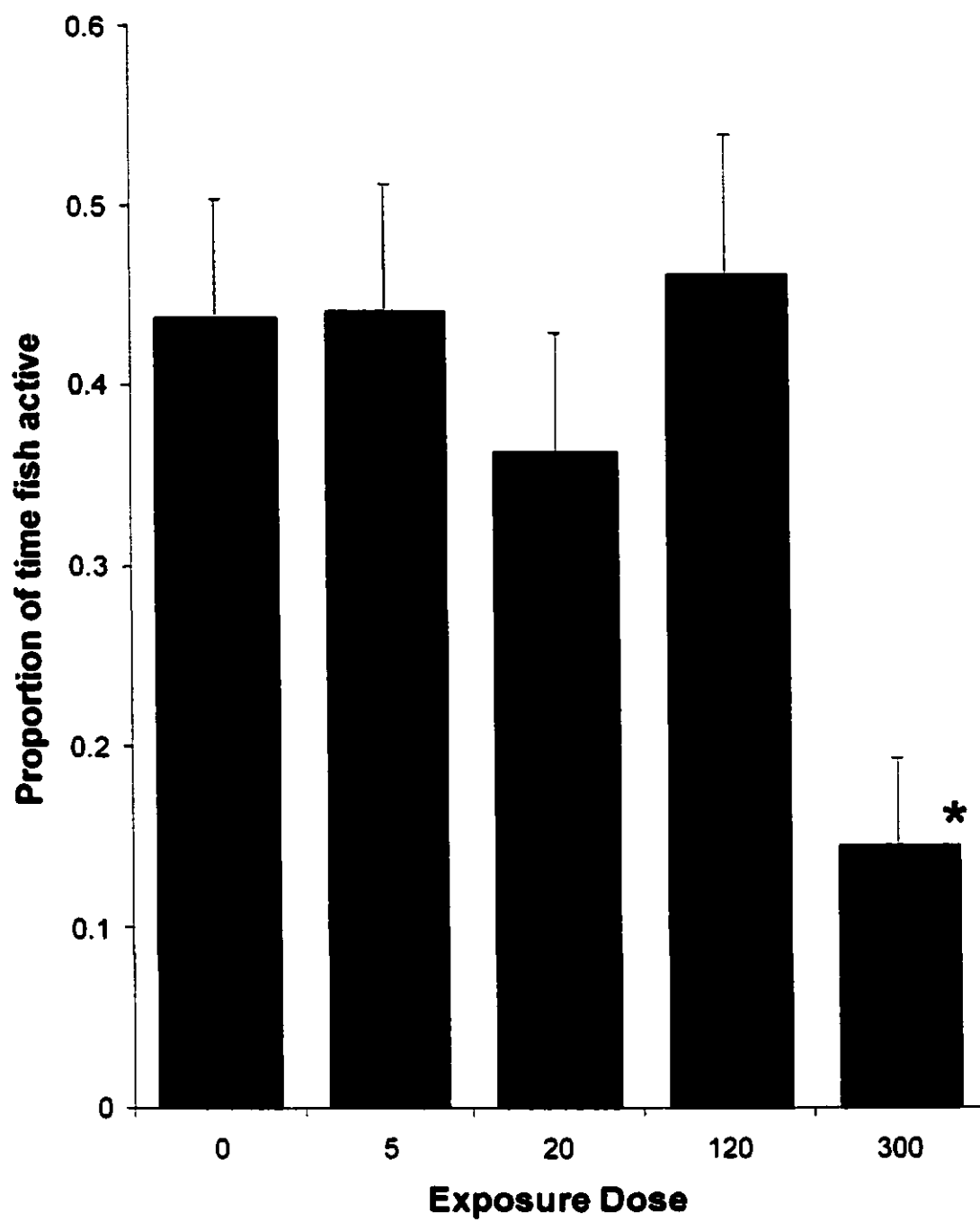


Figure 1. Proportion of time that fathead minnows spent swimming during a 4 min acclimation period. Bars (+ SE) represents means calculated from fish (N= 10-12) exposed to 0, 5, 20, 120, and 300 cercariae of *O. ptychocheilus*. Asterisk indicates $P < 0.05$ in Tukey-Kramer HSD test.

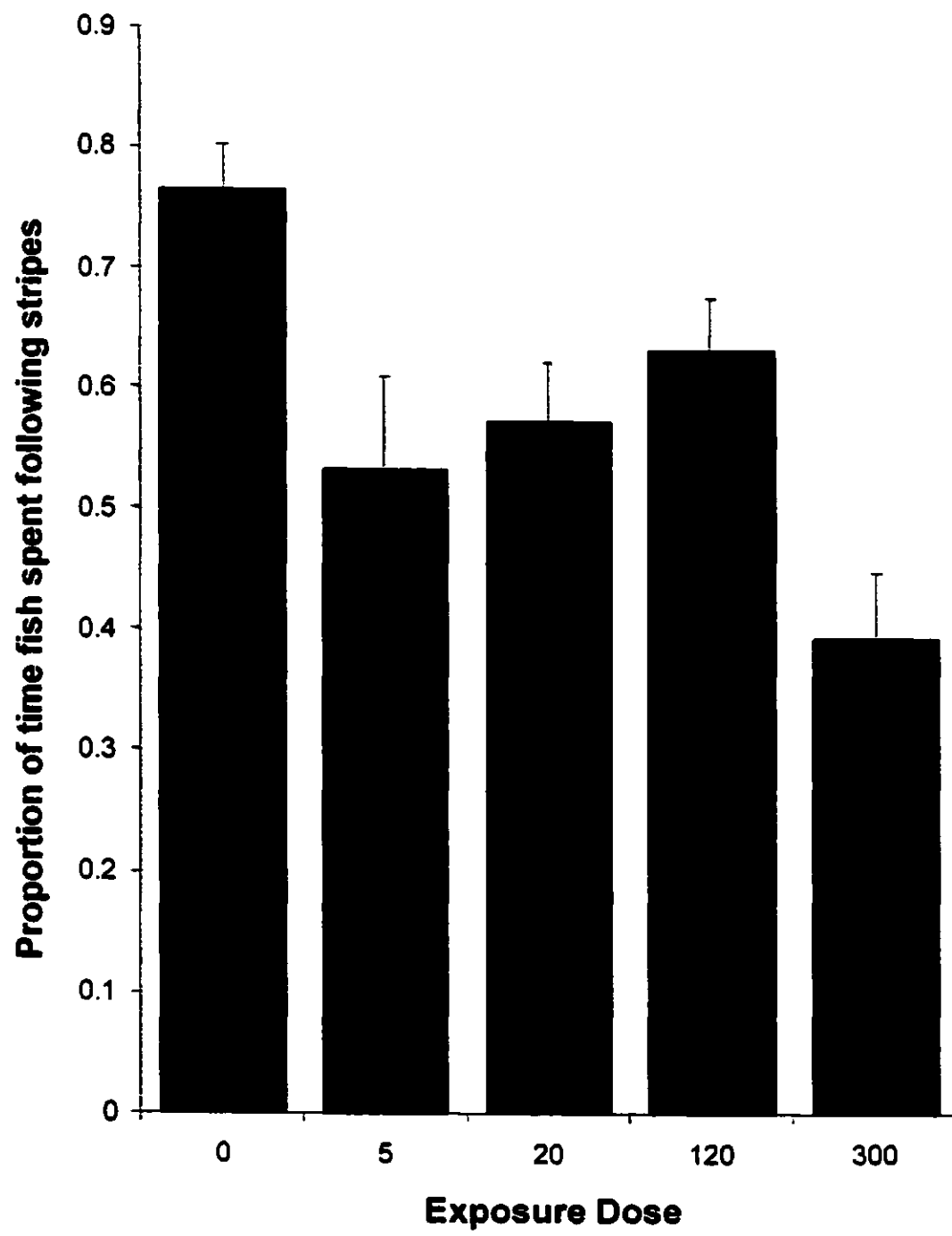


Figure 2. Proportion of time that infected fathead minnows spent following in the direction of moving stripes. Bars (+ SE) represents means calculated from fish (N= 10-12) exposed to 0, 5, 20, 120, and 300 cercariae of O. ptychocheilus.

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Chapter 5. General Conclusions

I used the *O. ptychocheilus*/minnow model system to investigate three features of the phenomenon of parasite-induced behavioural modification. One was to determine whether this parasite of the optic lobes affected a minnow's ability to detect and integrate visual stimuli. A second was to distinguish whether any observed changes in visually-mediated behaviour could best be explained as parasite adaptations, host adaptations, or as non-adaptive side effects of infection. A third was to determine whether variation in parasite numbers could help explain variation in altered behaviour. Significant advances have been made on all three features.

One central advance is the demonstration that *O. ptychocheilus* metacercariae cause a marked reduction in the optomotor response (OMR) of juvenile fathead minnows. Thus, under some conditions, uninfected fish out-performed infected ones in a visually-mediated swimming task by as much as 48%. Although the magnitude of altered OMR was variable between individual fish, an overall decrease was consistent for infected fish in each experiment (Chapters 2, 3 and 4). Although a large number of factors have been shown to affect the OMR of fish (review in Chapter 1), this is the first study to demonstrate the effect of parasitism. Thus, the present study provides another example of a behaviour that can be altered by parasites. Although this is a noteworthy advance in itself, it is especially significant because of the clear associations between infection, parasite site-selection and vision.

A second advance is the demonstration of an association between the magnitude of the reduction in optomotor performance and parasite development. The strongest effect of the parasite was observed when the parasites were 2-4 wk old. This is an important

finding because in a concurrent study, I showed that this period coincided with the period when *O. ptychocheilus* were developing their cysts, and were not infective to potential definitive hosts. This period also coincides with the period when metacercariae are undergoing rapid development in the optic lobes (Sandland & Goater, 2000) and when they are most pathogenic to young minnows (Sandland & Goater, 2001). Reduced OMR during this period is unlikely to be parasite adaptive, rather a consequence, or side-effect, of pathology induced by developing larvae. The greater than 70% reduction in host activity and following time of extremely heavily infected fish at 2 wk p.i. (Chapter 4) also supports the notion that the presence of many developing larvae in the brain is pathogenic. However, unequivocal acceptance of the side-effect hypothesis of parasite-induced behavioural modification is premature. Results from chapter 3 indicate that negative effects of infection are detectable as late as 8 wk p.i., although the effects seem to be much reduced. Whether these later effects are due to the persistence of earlier pathology or to 'new' (and adaptive) effects of encysted larvae is currently unknown and requires further investigation.

Lastly, my results show that variation in the magnitude of the reduction in minnow OMR is due, in part, to variation in parasite intensity. However, the precise nature of the link between intensity and the magnitude of the change in OMR was unexpectedly complex. Oddly, the extremes of low and high intensity caused the greatest reduction in OMR performance, whereas intermediate intensities had little effect. Moreover, this complex association between intensity and reduced performance was consistent between two experiments (Chapter 2, Chapter 4). Although several recent studies have shown that the link between intensity and the extent of host behavioural change is not always straightforward (Chapter 4), my results are the first to consistently show a non-linear

effect. One important implication is that the simple prediction of a linear effect of parasite intensity on host behaviour, most likely associated with pathology (e.g. Goater & Holmes, 1997), requires reconsideration. The results also indicate that the mechanisms that lead to reduced optomotor performance are probably very complex. One possibility, as discussed in Chapter 2 and Chapter 4 is that the mechanisms causing the behavioural changes at low parasite intensity are likely to be different from those at high intensity. Future experiments that carefully define the pathological changes in the optic lobes various doses of exposure and maturity of the parasite could address this issue.

One fundamental assumption of this thesis is that reduced OMR is likely to have consequences in an ecological context, particularly with respect to the transmission of O. ptychocheilus. This assumption was not tested in this study. However, reduction of optomotor performance in other fish has been shown to affect schooling (Shaw & Tucker, 1965), orientation and navigation within the environment (Clausen, 1931) general displacement with respect to conspecifics (Harden-Jones, 1963; Arnold, 1974), visual sensitivity (Cronly-Dillon & Sharma, 1968) and visual-locomotor coordination (Springer, Ester & Agranoff, 1977). Moreover, vision in fish is known to play an important role in feeding, mating, and predator avoidance (reviews by Guthrie, 1986). This study demonstrated that even small numbers of metacercariae (3-20/fish) are sufficient to cause significant reduction in minnow OMR (Chapter 2; Chapter 4). This is an important finding as the majority of young minnows in the lakes in north-central Alberta are infected with low intensities of O. ptychocheilus (Sandland, Goater & Danylchuk, 2001). Moreover, Laffery & Morris (1996) showed that even small changes in the behaviour of killifish infected with a brain-encysting trematode lead to large increase in the rate of predation by fish-eating birds. Thus, I conclude that parasite-induced reduction in

minnow OMR is likely to negatively affect various traits in minnows, some of which (schooling, predation rate, prey detection) are likely be linked to both parasite transmission and host fitness.

Although my study shows that a brain-encysting parasite can alter certain important behaviours of minnows, I know little regarding the precise mechanisms involved. The optic lobes are highly specialized and extremely complex structures, especially in schooling cyprinid fish. Detailed study has been conducted on the neuroanatomy of zebra fish, indicating that the optic tectum is one of the most complex structures in the brain, consisting of four differentiated zones, each of which can be further subdivided into at least 15 different layers (review by Reichert, Wullimann & Rupp, 1996). Moreover, the OMR itself is likely to be a complex phenomenon. It is conceivable that parasite-induced altered OMR could associate with one, or a combination, of at least three different components. Parasite infection could reduce the quality of the visual information received from the retina (i.e. acuity), the integration of the information, or their coordination with an appropriate motor response. Given that infection is associated with host activity (Chapter 4) and also pathology (Chapter 2, Chapter 4), both visual acuity and motor responses seem to be affected under some conditions. It is possible that the variation in OMR I observed between experiments, and between fish within experiments, could be due to the fact that different intensities, and different stages of metacercariae, affect different components of the OMR at different times. Sorting-out such complexity would be a necessary first step in determining the precise mechanisms involved in the effect of *O. ptychocheilus* on visually-mediated behaviours.