TREMATODE INFECTION OF AN INTERMEDIATE FISH HOST: IMPLICATIONS FOR HOST ENERGETICS AND VISUALLY-MEDIATED ESCAPE RESPONSES

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Abstract

Trophically transmitted multiple host parasites have been known to have significant effects on their intermediate hosts through factors such as histopathology related to parasite development, as well as the well-known host behavioural alterations which are associated with increased transmission to the next host in the parasite life cycle. *Ornithodiplostomum ptychocheilus* is a multihost parasite which is trophically transmitted from its intermediate fathead minnow (*Pimephales promelas*) host to the definitive hosts, piscivorous birds. Within fathead minnows, this parasite causes histological damage of host tissue at its infection site – the superficial layers of the optic tectum and cerebellum – and is associated with the disruption of visually-mediated behaviours. Similar to many multihost parasites, the degree of host physiological and behavioural alterations during *O. ptychocheilus* infection has been found to be dependent upon parasite developmental stage and parasite burden.

The described pathologies suggest that infection represents a direct cost to host energy budgets as well as survival costs related to host antipredator responses to visual stimuli. Therefore, I investigated the influence of *O. ptychocheilus* infection on host metabolic rates as well as during visually-mediated escape responses – an antipredator behaviour which requires rapid visual processing. First, I compared standard metabolic rate of uninfected and infected fish during the obligate growth period – a parasite developmental stage associated with significant *O. ptychocheilus* growth as well as host histopathology. I found that infection significantly increased metabolic rates at very low parasite loads, but standard metabolic rate gradually decreased with parasite numbers until it was comparable to or below that of uninfected fish. The following year, I measured fast-start response latencies of both newly infected hosts and minnows infected from the previous year to fast-moving visual stimuli approaching from above and the front (vertical and horizontal planes) across parasite developmental stages. I found that infection significantly reduced host abilities to respond to the stimulus, but this was dependent upon parasite load as well as the direction of stimulus approach. Surprisingly, the influence of infection was similar regardless of days post parasite exposure indicating that parasite development did not alter visual capabilities. Overall, my results suggest that, while infection-induced pathologies are costly for hosts and may persist overtime, there appears to be significant host compensation to mitigate the pathologies associated with the obligate growth period.

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Chapter 1 - Introduction: Phenotypic Alterations of Infected Hosts

1.1 Infection-induced modifications to host physiology and behaviour

Parasites, organisms which rely on a symbiotic relationship with a second, "host" organism, for resource acquisition and through this relationship cause harm to the host organism, have become recognized as influential components of ecosystems (Raffel et al., 2008). The discovery of complex networks of ecto- and endoparasites spanning a wide taxonomic range suggest a framework for a widespread influence of ecosystem dynamics (Lima et al., 2012). The significance of the parasite "role" has been primarily described through the direct modification of host phenotype and the resulting alterations in host intra- and interspecific interactions (Mikheev et al., 2010; Poulin, 2010). It is well-understood that the underlying physiological modifications of the host phenotype are responsible for the more conspicuous alterations associated with infection such as modified behaviour and morphology (Klein, 2003; Barber & Wright, 2005; Kelly et al., 2010). Although many of the specific physiological mechanisms targeted by infection are relatively unknown, previous investigations have attributed observed phenotypic alterations to parasite-induced neuro- (Shaw et al., 2009; Perrot-Minnot et al., 2014) and endocrine modulation (Trubiroha et al., 2010). This strongly suggests that parasite control of physiological pathways of their host lead to the observed behavioural and morphological outcomes and likely contribute to the alteration of other physiological functions such as metabolism (Wagner et al., 2005; Seppänen et al., 2009).

Among the described phenotypic alterations, conspicuous infection-induced behaviours have been given considerable attention (Poulin & Maure, 2015). Fish are a well-studied group which has demonstrated significant alterations in behavioural patterns during infections (Crane et al., 2011; Curtis, 2014; Poulin & Maure, 2015). Often these changes can be attributed to unavoidable consequences of parasite presence known as pathology (Griffin et al., 2014), but

some parasites modify host behaviour, specifically antipredator responses, as an adaptive strategy to facilitate transmission (McElroy & de Buron, 2014). Multihost parasites require more than one type of host to complete their life cycle and are a well-known example of a parasite group associated with modifications of host behaviours. In these systems, the life cycle of the parasite includes development in one or more intermediate hosts before reaching a final, definitive host in which the parasite sexually reproduces (Shaw et al., 2010; Seppälä et al., 2011; Fredensborg & Longoria, 2012). Many multihost parasites life cycles include an ontogenetic stage reliant on current host consumption by the parasite's next host for transmission, forming a complex predator-prey-parasite system (Anderson, 1986; Bairagi & Adak, 2015). In these systems, parasite "manipulations" target antipredator responses of the current host to increase its vulnerability to the next host in the parasite life cycle. In general, complex parasite-induced modifications of host phenotypes which directly increase the probability of transmission to the next host in the parasite's life cycle are accepted as examples of adaptive manipulation (Worth et al., 2013). However, Poulin has raised the possibility that any parasite induced-alteration which increases transmission may be considered adaptive if the parasite's characteristic underlying the host change is inherited (Poulin, 2010). One well-known example of adaptive manipulation is the behavioural modification of Longnose (*Fundulus similis*) and California killifish (*Fundulus parvipinnis*) infected with *Euhaplorchis californiensis*, a trematode developing on the pial surface of the brain. Infected fish demonstrate conspicuous swimming behaviours near the water's surface which attract the parasite's final host: piscivorous birds – increasing the probability of fish consumption by birds 30-fold (Shaw et al., 2010; Fredensborg & Longoria, 2012). The modifications of host behaviours during infection are associated with specific physiological alterations. In the California killifish, altered behaviours of infected fish correspond to changes in the levels of the neurotransmitters: serotonin and dopamine (Shaw et al., 2009; Shaw & Øverli, 2012).

1.2 Infection conditions and associated host phenotypic alterations

Infection conditions have been found to influence the parasite-induced modifications to host physiology and exhibited host behaviour. Two infection conditions which have been shown to significantly influence host functions and, thus, behaviours are parasite burden (Shirakashi & Goater, 2002; Santos & Santos, 2013) and parasite developmental stage (Shirakashi & Goater,

2005). Although multihost parasites are well-known to increase intermediate host vulnerability to the next host in the parasite's life cycle, handicapping hosts during non-infective stages is detrimental to the parasite (Parker et al., 2009). As a result, ontogenetic parasite stages are essential in determining behavioural outcomes during multihost parasite infections. Some hosts have even shown increased antipredator behaviours during non-infective parasite stages which have been proposed as parasite manipulations to avoid premature host consumption (Gopko et al., 2015). However, developing parasites have an increased reliance on host resources to facilitate growth. Consumption of host resources generally results in the deterioration and modification of host tissues, and, consequently, the disruption of normal host functions (Barber & Crompton, 1997; Sandland & Goater, 2000). Further, complicating infection-induced alterations associated with parasite ontogeny are the complex effects of parasite burden on host physiological and behavioural outcomes (Shirakashi & Goater, 2002; Fredensborg & Longoria, 2012). This suggests that investigations of the combined influence of parasite development and burden are essential to understanding the role of infection in both parasite manipulations of host behaviour as well as infection-induced pathology.

1.3 Model System: Ornithodiplostomum ptychocheilus infection of Pimephales promelas

The three-host life cycle of the trematode, *Ornithodiplostomum ptychocheilus*, is an example of a multihost parasite system which relies upon trophic transmission. The first intermediate host of *O. ptychocheilus* is the snail, *Physa gyrina*. This host is followed by a second intermediate fish host, the fathead minnow, *Pimephales promelas*. The final, definitive, hosts are piscivorous birds (Sandland & Goater, 2001). The infective stage of *O. ptychocheilus* to *P. gyrina*, the miracidium, is motile and hatches from eggs released into the water with the feces of piscivorous birds. After development and asexual reproduction within the snails, a second motile ontogenetic stage, the cercaria, is released to locate fish hosts. After infecting fathead minnows, the stage of *O. ptychocheilus* is known as the metacercaria. Metacercariae are trophically transmitted to piscivorous birds through consumption of infected minnows by the birds. Within the definitive bird host the parasite develops into an adult stage and undergoes sexual reproduction (Schleppe & Goater, 2004).

As the host of the trophically transmitted stage of this parasite, fathead minnows and their infection-induced phenotypic alterations, have been a focus of previous investigations. *O.*

ptychocheilus is a prevalent parasite of fathead minnows and wild minnows experience a wide range of parasite burdens with ≥ 400 metacercariae recorded in hosts (Sandland & Goater, 2001). Minnows experience a peak exposure period to cercariae during the months of August and September (Sandland et al., 2008). This parasite infects hosts by penetrating the epidermal layer and migrating through host tissues to reach the superficial layers of the optic tectum, and cerebellum (Radabaugh, 1980; Matisz et al., 2010b). The optic tectum has been established as an integrative center for visual stimuli and information from the lateral line system (Arnold, 1974; Springer et al., 1977; Vissio et al., 2008) while the cerebellum is considered a center for sensory-motor function (Hodos, 2009). In vertebrate visual systems, the superficial layers of the optic tectum primarily receive topographically-organized visual information, while the deeper layers play a greater role in the integration of other sensory stimuli (Vissio et al., 2008).

Within hosts, this parasite develops for approximately 10 weeks before becoming infective to piscivorous bird (Shirakashi & Goater, 2001). During development, *O. ptychocheilus* undergoes three distinct stages: obligate growth, differentiation and consolidation, and encystment (Sandland & Goater, 2000). During the obligate growth period, the metacercariae develops within the tissues of the optic lobes and cerebellum and doubles in size. At the start of this period, the tegument of the metacercaria develops into a network of microvilli and microlaminae which extends into host tissue (Goater et al., 2005; Conn et al., 2008). Host cells in direct contact with this network show significant disruption and appear deteriorated (Goater et al., 2005). After this initial stage of development, the metacercaria undergoes encystment (development into a double-walled cyst) and consolidation. Following encystment, the parasite begins to migrate into the endomeniux above the optic tectum and cerebellum (Matisz et al., 2010b). The onset of metacercariae migration to the endomenix generally occurs between 28-42 days post infection (p.i.) and is associated with significant inflammation of the meninges. After this transition, the metacercariae completes development and becomes infective to definitive bird hosts (Goater et al., 2005).

1.4 Primary objectives

Previous investigations of this host parasite system have revealed a repertoire of phenotypic alterations of fathead minnow hosts including visuomotor processing disruption (Shirakashi & Goater, 2001; 2002; 2005), oxidative stress of liver cells (Stumbo et al., 2012), and distortion of

cranial morphology (Sandland & Goater, 2001). The degree of the observed changes correspond to distinct parasite developmental stages (Shirakashi & Goater, 2005) as well as the number of parasites within hosts (Shirakashi & Goater, 2001; 2002). Known host modifications associated with infection have significant implications for the existence of other infection-induced alterations of host phenotypes. The purpose of this thesis was to further explore the influence of *O. ptychocheilus* infections on physiological and behavioural modifications of fathead minnow hosts.

Experiment 1: Infection-induced modification of host metabolism

Metabolic alterations are a common byproduct of infection as both the consumption of host resources and associated tissue damage are energetically expensive for hosts. However, the direction of metabolic change during infection is specific to the parasite-host system (Robar et al., 2011), thus, the effects of *O. ptychocheilus* infection on fathead minnow hosts are difficult to predict.

Objective: I investigated the relationship between host metabolic rate and parasite burden during the obligate growth period to determine if the high energetic demand of this ontogenetic stage significantly influences host metabolism.

Hypothesis: I hypothesized that hosts would experience a modified metabolic rate associated with the number of developing parasites within the fish.

Experiment 2: Infection-induced visuomotor processing disruption and predator escape

As described, trophically transmitted parasites are often associated with parasite development-dependent alterations of host antipredator behaviours. Although antipredator responses have not be investigated in this parasite system, the reduced visuomotor capabilities observed during infection suggests that visually-mediated antipredator behaviours of infected fish may be less successful. Infection-induced visual processing disruption may be particularly costly during host responses to imminent threats when appropriate timing of escape onset is vital. Moreover, reduced visuomotor processing may increase the possibility of parasite transmission if it results in selective disruption of host responses to aerial predators.

Objective: I explored the latency of fast-start escape responses of infected fish to vertically and horizontally approaching stimuli to determine the influence of infection on host antipredator abilities to imminent aerial and aquatic threats in the context of parasite development and parasite load.

Hypothesis: I hypothesized that infected hosts would exhibit delayed fast-start responses to threatening visual stimuli and that the degree of increase in response latency would depend upon parasite ontogeny, parasite burden and, the direction of stimulus approach. I predicted that the resulting patterns of response latencies would provide evidence for one of two scenarios. First, if infection-induced behavioural modifications are adaptive, then I expected longer response latencies to vertically approaching stimuli (i.e. aerial predation) in fish during the final stage of parasite development. Second, if alterations to host behaviour are a result of pathology, I predicted the response latencies to stimulus approaching from both planes would be similarly disrupted, and likely associated with the obligate growth period.

Chapter 2 - Infection-induced Modification of Host Metabolism

2.1 Introduction

Metabolism reflects host mitigation of infection-induced pathology

Energetic cost associated with infection is a common pathology experienced by infected hosts due to parasite use of host tissues (Barber & Crompton, 1997; Raffel et al., 2008) as well as the energetic requirements of host compensatory responses such as immune function (Nilsson et al., 2007). Metabolism, as the physiological function which mobilizes host energy for other physiological processes, is often used to measure the extent of infection-related costs in host energy budgets. In many host-parasite systems measures of host oxygen consumption at rest have been used as a proxy for measures of metabolic rate during periods of otherwise low energetic demand. Studies of metabolic rate at rest revealed a range of responses to infection including increases (Powell et al., 2005; Jones et al., 2007; Seppänen et al., 2008; Filipsson et al., 2017), decreases (Powell et al., 2005; Seppänen et al., 2009), and, most often, no change (Walkey & Meakins, 1970; Meakins & Walkey, 1975; Wagner et al., 2005; Leef et al., 2007; Seppänen et al., 2008; Lapointe et al., 2014; Thomas et al., 2014; Bruneaux et al., 2017; Powell & Gamperl, 2016). Fish even demonstrate species-specific metabolic alterations to infection by the same parasite (Powell et al., 2005). These results strongly suggest that the metabolic outcome of infection is dependent upon host's ability to modify physiological processes to compensate for the energetic demand posed by the parasite. For example, fish hosts demonstrating comparable metabolic rates to uninfected individuals have exhibited physiological compensatory responses such as increased oxygen transport capacity of the gills and circulatory system (Fisk et al., 2002; Powell & Gamperl, 2016; Filipsson et al., 2017) as well as increased reliance on anaerobic metabolism (Powell & Gamperl, 2016).

As previously described, changing infection conditions have been shown to significantly influence the intensity of infection-induced metabolic changes. Current discussions related to the effects of infection conditions have been restricted to modified metabolism associated with parasite burden. Similar to the overall pattern of metabolic changes during infection, the relationship between host metabolic response and parasite burden vary across host-parasite systems. Under ambient oxygen and temperature conditions, fish hosts have demonstrated increased metabolic rate (Jones et al., 2007), decreased metabolic rate (Filipsson et al., 2017), and no significant change in metabolism in response to higher parasite burdens (Fisk et al., 2002). However, the greatest implications of parasite burden may be the influence of parasite numbers on hosts' ability to mobilize energy to respond to external stressors. Higher parasite burdens have been associated with reduced ability to acclimate to hypoxia (Fisk et al., 2002; Lapointe et al., 2014), reduced thermoregulatory capabilities (Lapointe et al., 2014; Lawlor, 2016; Bruneaux et al., 2017), and increased recovery time following a period of stress (Thomas et al., 2014). Unlike parasite burden, metabolic changes associated with parasite development have not been directly investigated in fish hosts. However, in systems where the parasite exhibits different energy requirements during distinct developmental periods, host metabolism may change to meet the energetic demands of the parasite's ontogenetic stage.

Trematodes are an example of a parasite group in which parasite development influences the energetic challenge imposed by infection (Ballabeni, 1994; Goater et al., 2005). Surprisingly, although the early stage of development described in many of these systems has been associated with significant host damage and parasite growth, changes associated with metabolism have not yet been observed. One study of metabolic rates in a fish host revealed that Brown trout (*Salmo trutta*) and roach (*Rutilus rutilus*) infected with *Diplostomum pseudospathaceum* demonstrated ventilation rates similar to that of uninfected fish during the early stage of parasite development (Laitinen et al., 1996). Another investigation demonstrated similar relationship with host metabolism during infections with *Diplostomum sp.*, a trematode which causes cataracts in the eye lens of fish, during the early stages of parasite development (Voutilainen, et al., 2008). In this study, changes in metabolic rate only occurred in chronic infections and coincided with increased foraging activity of infected fish presumably due to the impaired feeding abilities associated with cataract formation. These studies suggest that infected fish are able to compensate for the costs of infection associated with earlier infection periods.

Fish metabolic responses to infection suggest that metabolism is not a measure of the direct energetic costs of infection such as parasite consumption of host resources. Instead, it appears to be a useful indicator of the physiological compensation of hosts to the energetic demands associated with parasite presence and function (Fisk et al., 2002; Powell & Gamperl, 2016; Filipsson et al., 2017). Therefore, measures of host metabolism can be considered a guide to understanding host strategies to mitigate the addition of infection-induced costs to host energy budgets. It also highlights the efficacy of host compensatory responses during different infection conditions such as parasite burden and parasite developmental stage.

O. ptychocheilus-induced phenotypic alterations and host metabolism

Direct measures of metabolism during *O. ptychocheilus* infections have not been investigated. However, studies have revealed infection-induced physiological changes, including oxidative stress (Stumbo et al., 2012), immune response (Matisz et al., 2010a), and tissue damage and repair (Shirakashi & Goater, 2005) which have been previously been linked to metabolic alterations. Similar to other parasite systems, these infection-induced changes are modified by parasite load and development, suggesting that these factors influence the degree of physiological change. Specifically, the pathologies associated with infection have demonstrated a non-linear relationship with parasite burden as well as increased pathology associated with the obligate growth period. This period is considered to be a particularly energetically demanding time in parasite development, illustrated by the formation of a microvillar network associated with resource acquisition which extends into and disrupts host tissue (Ballabeni, 1994; Goater et al., 2005). The increased energetic requirements of the parasite as well as the assumed cost of host tissue repair likely present a direct drain on host resources during this developmental stage. The disintegration of the microvillar network during later parasite stages indicates that the energetic demands of the parasite are reduced following the obligate period (Goater et al., 2005). This, along with the reduced pathology experienced by hosts during later developmental periods, suggests that infection becomes less energetically expensive overtime.

The previous pathologies associated with *O. ptychocheilus* infection suggest that infection is energetically costly. It follows that these additional energetic demands elicit specific host responses to mitigate the effects of infection and that these responses are reflected in host metabolism. Therefore, investigations of metabolic rates during *O. ptychocheilus* infections will

provide a greater understanding of how fish hosts compensate for the energetic costs associated with infection-induced alterations in host physiology. To quantify the possible metabolic alterations associated with infection, I compared metabolic oxygen consumption between infected and uninfected fathead minnows during a period of rest. Due to the importance of parasite ontogeny and parasite burden in determining the degree of pathology experienced by hosts, both the number of parasites as well as the developmental stage of the parasite during experiments were recorded.

3.2 Methods

Ethics and safety statement

All fish housing and experimental procedures were conducted following Canadian animal research standards as outlined by the Animal Research Ethics Board at the University of Saskatchewan. Biosafety Containment Risk Level 2 protocols for parasite containment procedures were approved by the appointed University Workplace Safety & Environmental Protection Biosafety Officer.

Fish collection and maintenance

To ensure that the minnows used during experiments were parasite free, I raised fathead minnow fry in the laboratory. To my knowledge, cercariae have not been known to infect fathead minnow embryos, therefore, fry were obtained from eggs collected from a nearby wild population. Egg collection followed a previously established protocol (Chivers & Smith, 1994): artificial spawning sites (i.e. 3-4.5 cm diameter clay pots) were provided to fathead minnows at Feedlot Pond, a drainage pond bordering the University of Saskatchewan's Beef Research Station, during May-July 2016. Spawning sites were spread along the surrounding shore in an effort to maximize genetic variability of eggs collected. Sites were gently inspected at least twice a week to determine the presence or absence of eggs and the maturity of clutches. Eggs were only collected near hatching time to increase hatching success in the lab. A total of 24 clutches were collected and stored in holding tanks maintained at approximately 20°C. To discourage the growth of harmful pathogens in holding tank water during egg storage and the initial hatching period, approximately 0.5-1 ml of methylene blue was added for every 3.8 liters of water in holding tanks. After hatching, the fry were raised in smaller tanks and fed a diet of larval brine

shrimp. Upon reaching 3-5 weeks of age, they were transferred to several larger holding tanks, where they gradually transitioned to a diet of Tetramin commercial fish food and maintained at a slightly lower temperature of approximately 19°C in a 14:10h light/dark cycle.

Collection of infected snails

Due to the multiple host life cycle of *O. ptychocheilus*, the infective stage of this parasite to fathead minnows (the cercaria) was obtained via infected *Physa gyrina* snails. In a wild system containing a heavily infected minnow population approximately 2% of *P. gyrina* are infected (Cameron Goater, pers. comm.). Therefore, snails were also collected from Feedlot Pond, a minnow population known to have high parasite burdens, to increase the probability of finding infected snails. This had the added benefit of exposing the young of the year in the lab to parasites released from a snail population that they would have encountered in the wild. *P. gyrina* were collected during August and September – months associated with a peak in parasite release. Following collection, snails were maintained in a Biosafety Level 2 containment zone.

Infected snails release thousands of cercariae a day for a period of approximately a month. In order to determine if wild caught snails were infected, individual snails were placed in 100 ml of water and exposed to direct artificial light to induce cercariae release. Following a period of 1-2 h, the surrounding water was examined to determine if cercariae were present. In snail holding containers with visible cercariae, 1-3 ml of the water was examined under a dissecting scope to determine if the cercariae were likely to be *O. ptychocheilus*. Between 3-5 fish were exposed to the remaining cercariae-filled water from potential *O. ptychocheilus*-infected snails to verify the parasite species. To my knowledge, only *O. ptychocheilus* is known to infect the brain tissue of fathead minnows. Therefore, a week following exposure, when the metacercariae were large enough to be readily visible within the brain tissue under a dissecting microscope, minnows were euthanized with a Tricaine (MS 222) overdose and the fish brains were inspected for parasite presence (see *Parasite counts*). After confirmation, infected snails were placed in separate holding containers and kept until fish infection procedures. Due to the significant physiological cost imposed on the snail during *O. ptychocheilus* infection, only one infected individual survived for cercariae collection during experiments.

Fish exposure to O. ptychocheilus

Minnow infection protocol followed a previously established method (Shirakashi & Goater, 2005). Minnows were 3-5 months old during exposure procedures – an age range representative of wild young of the year during the first *O. ptychocheilus* exposure (Sandland et al., 2008). In preparation for fish exposure to cercariae, the infected snail was placed 100 ml of fresh water for 5-7 h to ensure sufficient concentration of parasites in the water. The infected snail was then removed and returned to the holding container. Cercariae density within the water was estimated by counting the total number of cercariae within 1 ml 3 times and averaging the results of all counts. Counts were conducted under a dissecting scope in a small petri dish over a 1 x 1 cm grid. To facilitate count accuracy, 3 drops of 70% ethanol was added to slow parasite movement. Estimates of parasite numbers within the cercariae-filled water were recorded and used to determine the parasite exposure doses for the fish. Two fish exposures staggered one week apart were completed prior to the conclusion of the cercariae release period.

Forty-eight similarly-sized minnows were arbitrarily chosen from holding tanks for the first parasite exposure period and transported to a larger Biosafety Level 2 containment area. Within this zone, individual fish were placed in 100 ml of system water and minnows were transferred to 3 rows of 6 individual exposure containers. Each row was randomly assigned and then exposed to 1 of 3 cercariae doses – low (5-30), high (75-100), or zero (clean system water) – for a 1.5-2 h period. These doses are within the range of parasite loads found in minnow hosts of two Alberta lakes with a high prevalence of *O. ptychocheilus* infection (Sandland et al., 2008) as well as infection levels found in wild-caught minnows from Feedlot Pond. A 1-ml pipette was used to add the exposure doses into the individual holding containers. After exposure, fish were separated according to the assigned infection levels and placed into their original holding tanks with other minnows in their infection group. The remaining cercariae-filled and fish exposure water was sterilized with a 1% bleach solution and disposed of and all items and surfaces used during infection procedures were disinfected in accordance with Biosafety Level 2 protocols. A second exposure of 18 minnows (6 minnows per treatment) was conducted the following week. This was the final exposure period of minnows in this experiment as cercariae release ended soon after.

Respirometry system set up

A Loligo ® Systems intermittent flow respirometer and AutoResp™ version 2 software was used to measure the aerobic metabolic rate (MO_2) of minnows. Specifically, the standard metabolic rate (SMR) was compared across minnow infection groups. SMR is considered an estimation of metabolic rate at rest and is a common measure used to determine metabolic responses to stressors such as parasitic infection (Seppänen et al., 2008; 2009). During experiments, individual fish were housed in four cylindrical chambers. Each chamber was 50-mm long with a diameter of 33 mm and had a volume of 43 ml. Fish included in the experiment maintained the mass (g) to water volume (ml) ratio within the desired range (between 1:20 and 1:100) (Svendsen et al., 2016). The chambers were covered in opaque black electrical tape to visually separate the fish during trials. MO_2 was measured via a fiber optic probe connecting an oxygen sensor within each chamber to a single oxygen regulation unit (OXY-REG). During experiments, the chambers were connected to individual water recirculation loops, each with a peristaltic pump to control water flow. This apparatus was then added to a water bath and the recirculation units were attached to a single flush pump which regulated the addition of water from the bath to the system. The water bath also contained an air pump, heating coil, and a temperature probe monitored by the temperature control unit (TEMP-REG). As metabolic rate is significantly influenced by even small temperature changes (Svendsen et al., 2016), the water bath was maintained at 19˚C with a hysteresis of 0.04 to match fish holding tank temperatures.

Prior to fish introduction into the system, the oxygen sensors in each chamber were calibrated through exposure to a maximum and minimum concentration of oxygen, following previously established methods (Svendsen et al., 2016). The sensor was aerated by maximally bubbling an air stone in the water bath surrounding the oxygen sensor over a several-hour period to create the maximum oxygen saturation value ($MO_{2,\,max}$). The minimum oxygen concentration ($MO_{2,\,min}$) was accomplished through multiple-hour exposure of the oxygen sensor to a Na_2SO_3 solution.

Respirometry trials

All infection groups were represented during each trial and all testing was completed between days 4-28 post exposure to remain within the time frame of the obligate growth period. One day prior to trials, the mass of each of four minnows was measured and the fish were

transferred individually into one of four empty 37-L holding tanks. Here they underwent a 24-hour food deprivation period at holding tank temperatures (19°C) in an effort to avoid digestion-related effects on MO_2 measures during trials (Chabot et al., 2016).

Prior to fish introduction the next day, the chambers and associated water recirculation loops were thoroughly cleaned with a 1% bleach solution to remove significant microbial growth. Microbial oxygen consumption can strongly influence measurements, especially when measuring the MO_2 of smaller fish (Clark et al., 2013). To ensure microbial oxygen consumption (i.e. background respiration) did not become a confounding factor during trials, MO_2 within the chambers was measured prior to fish introduction. Trials only commenced if resulting MO_2 values were less than 10, indicating that background respiration was low. Following trial conclusion and fish removal from chambers, microbial oxygen consumption was measured again. These pre and post measurements of background respiration, known as blanks, were later used to correct MO_2 data collected during trials prior to analysis.

Upon completion of the first blank, minnows were placed into the chambers prior to 18:00 hours. This ensured a sufficient acclimation period was included in trials to allow fish to recover from handling. Minnows spent at least 14 h in the respirometer chambers at 19°C in a quiet, undisturbed room to facilitate an eventual resting state in the fish. It was essential that fish reach a period of "calm" during trials – marked by low MO_2 – for an accurate measurement of SMR. During the overnight period, multiple measurements of MO_2 were recorded as the intermittent flow respirometer cycled through three periods: a flow through (flush), a measurement, and a wait. During flush periods, deoxygenated water in the chamber is replaced with oxygenated water. Then the chambers close and a recirculation pump mixes the water inside the chamber to allow for a more accurate estimation of oxygen saturation, and, thus, MO_2. For a brief "wait" period after the chamber closes and the flush pump stops, measurements are not recorded. After this delay, MO_2 (i.e. the decline in dissolved oxygen concentration within the chamber) is recorded continuously while the chamber is closed. In this experiment, oxygen in the chamber was measured for 480 sec followed by 300-sec flush and 90-min wait periods for approximately 4 MO_2 measurements per hour. The trial was concluded at 10:00 hours the following day and fish were removed from the chambers, transported to individual holding tanks and fed. If a fish did not reach a period of calm during the trial period, it was given at least a 2-

day recovery prior to a second trial. Any fish (n=1) that failed to become calm following the second trial attempt was removed from experiments.

Parasite counts

After the completion of trials, fish were euthanized with a Tricaine (MS 222) overdose. Fish were removed from the solution after several minutes upon the cessation of all gill movement and frozen at -20ºC until dissection. During dissection, sharp, curved tweezers were used to peel back the cranium of minnows. The intact brain was then carefully removed by detaching the spinal cord and gently scooping and lifting the tissue out of the brain case with the curved tweezers. The brain was transferred to a glass slide and then gently mushed with second glass side. The two slides were held together with small metal clamps to ensure that the brain tissue was not significantly moved after the second slide was applied. The brain was then observed against a 1x1 cm grid under a dissecting scope. In place of the right microscope lens was a Dino-Lite Eyepiece HR 5 Megapixel which allowed for images and videos of the brain to be taken with DinoCapture 2.0 software. The slides were cleaned thoroughly between uses and inspected under the microscope to ensure that no parasites from the previous brain were transferred and later counted erroneously. Finally, the images from each brain were pieced together and enhanced in GIMP version 2.8.4. A small black dot was placed over each parasite during counts to avoid double counting.

Statistical analysis

MO_2 values during the period of calm were corrected for background (microbial) respiration and the lowest 10 values during this period were averaged to obtain a mean oxygen consumption value for each fish. To avoid the influence of hypoxia on fish metabolism, M_{O2} measurements were removed from analyses if oxygen saturation within the chambers fell below 90% (Svendsen et al., 2016). One fish was removed from analyses because it did not reach a resting period during either of two trial attempts it experienced.

Statistical analyses were conducted in R 3.4.3 (www.rproject.org). Changes in metabolic function were analyzed with a mixed-effect model (package lmerTest) with respirometry chamber and trial period designated as random effects and time period post infection (first and second half of the obligate growth period) and infection status (uninfected, low, and high) run as

fixed. The influence of infection on MO_2 (in mg of O_2 per kg of fish per hour) were analyzed with the model: MO_2 = infection status * time period + (chamber/run). Due to low infection success (see results) and a few instances of fish rerun through the respirometer, trial period could not be run by day and was split into sequential 2-day periods. A Tukey's HSD test was used for *post hoc* analyses of significant fixed effects and a $p < 0.05$ was considered significant. The regression function in R was used to analyze the relationship between MO_2 and parasite load, mass, and days p.i. as well as the relationship between mass and parasite load in infected fish.

2.3 Results

Infection success

Parasite counts revealed that overall infection success was lower than that of previous studies which reported wide ranges of parasite numbers in infected minnows: 3-209 metacercariae (Shirakashi & Goater, 2002) and 1-128 metacercariae (Shirakashi & Goater, 2001). This resulted in fewer infected fish (n = 12) than expected and the scope of the results was limited to low parasite burdens (2-24 metacercariae). To avoid possible confounds related to parasite exposure or missed metacercariae during parasite counts, minnows which were exposed to cercariae, but were not observed to have metacercariae were removed from analysis (n = 11).

Infection-induced modifications of SMR

Infection was not found to have a significant overall effect on SMR and MO_2 between infected and uninfected fish were remarkably similar ($F_{1,19} = 0.004$, $p = 0.948$; Figure 2.1). However, a linear regression analysis revealed that infection resulted in an initial increase in MO_2 at very low infection rates followed by a significant downward trend with higher parasite numbers ($F_{1,10} = -3.84$, $p = 0.003$). The negative relationship between parasite load and MO_2 indicates that the metabolic rate of infected fish decreased with the number of metacercariae to levels comparable to and below that of uninfected fish (Figure 2.2). Although low infection rates limit possible conclusions of metabolic modifications at higher parasite burdens, the results suggest that infection has a depressive effect on metabolic rate at more intermediate parasite loads. This parasite undergoes significant developmental changes over the course of the obligate growth period. However, time period post parasite or sham exposures did not influence SMR regardless of infection status ($F_{1,19} = 1.08$, $p = 0.312$). This was further illustrated by a cubic

regression analysis which indicated no influence of days p.i. on SMR of infected fish ($F_{3,8}$ = 0.31, p = 0.816; Figure 2.3). Fish mass is known to influence metabolic rates and parasite burdens; however, a quartic regression analysis showed no significant effect of mass on the MO_2 of infected fish ($F_{4,7}$ = 2.67, p = 0.122) and a cubic regression analysis demonstrated no significant relationship between fish mass and parasite burden ($F_{3,8}$ = 2.35, p = 0.149). The chamber housing the fish during experiments and the trial period (i.e. "run") did not influence fish MO_2 (p > 0.99 for both factors).

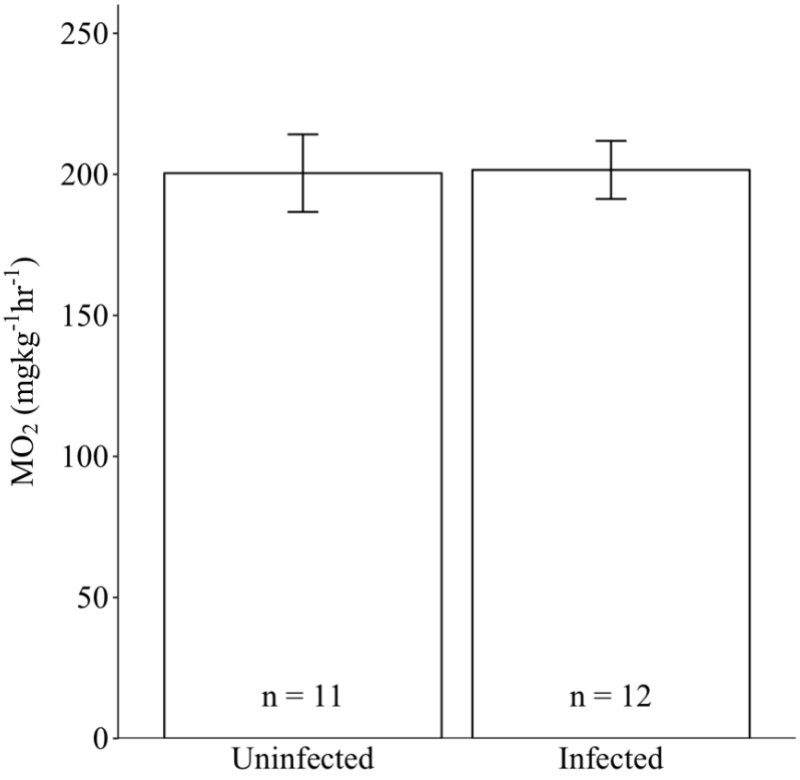

Figure 2.1 Mean ± SE MO_2 of uninfected and infected minnows.

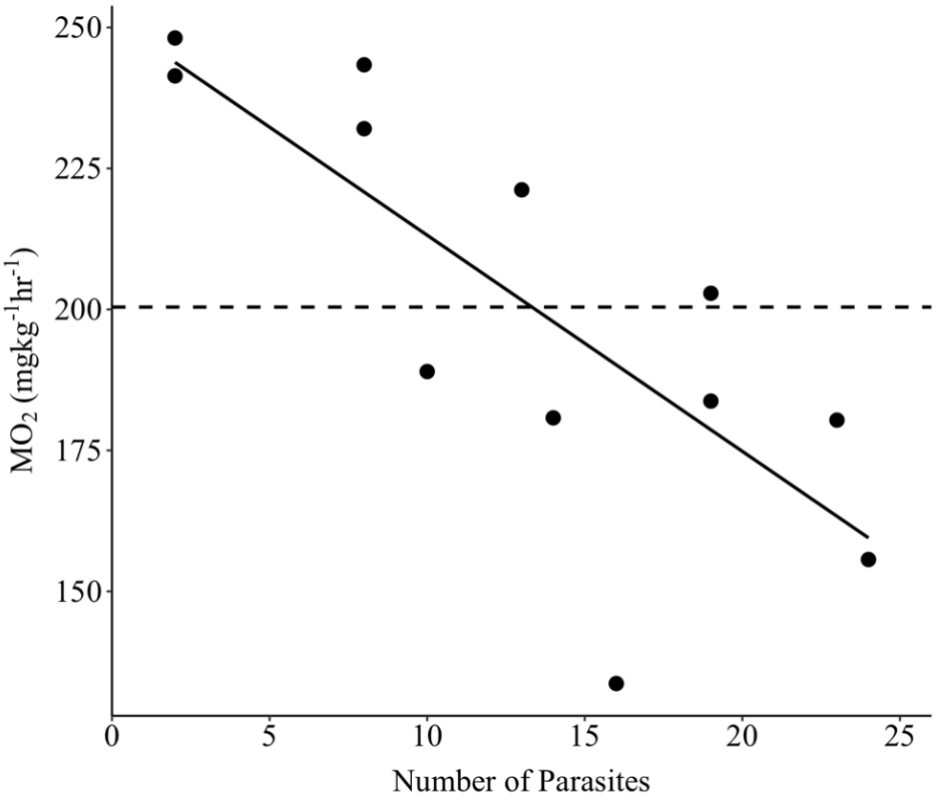

Figure 2.2 The relationship between the MO_2 of infected minnows and parasite number (n = 12, R^2 = 0.56). Mean MO_2 of uninfected fish is represented by a dashed line (n = 11).

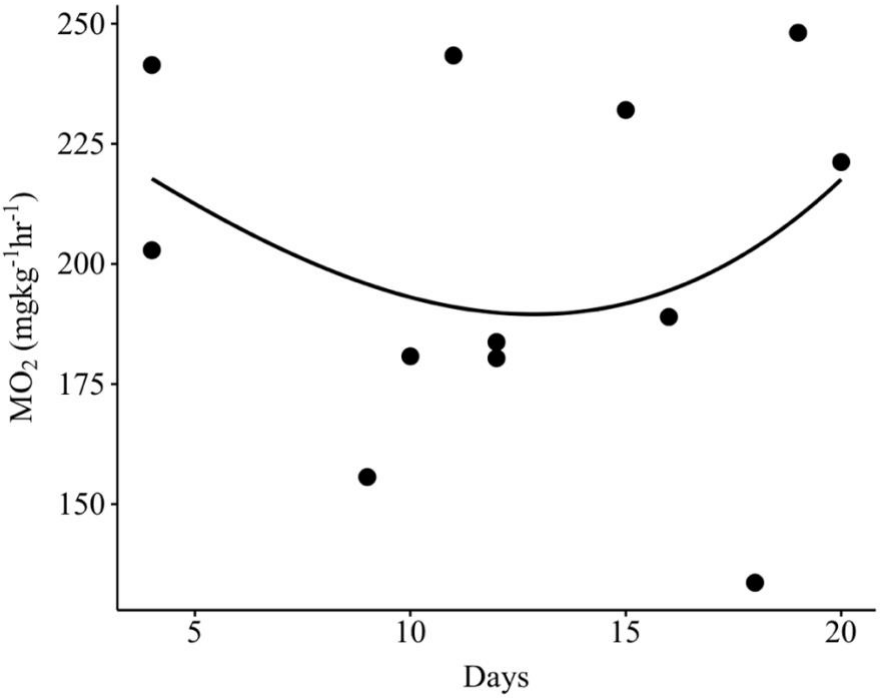

Figure 2.3 The relationship between the MO_2 of infected minnows and Days p.i. (n = 12, R^2 = -0.23).

2.4 Discussion

Most previous investigations have explored metabolic responses of hosts experiencing infections in tissues which directly control aerobic metabolic function through factors such as oxygen transport through the gills (Leef et al., 2007; Filipsson et al., 2017) or circulatory system (Wagner et al., 2005; Lapointe et al., 2014; Powell & Gamperl, 2016; Bruneaux et al., 2017) as well as the disruption of energetic absorption associated with infection of the digestive tract (Walkey & Meakins, 1970; Laxmareddy & Benarjee, 2013). Although decreases in metabolic rate during these infections can be attributed at least in part to metabolic disruption, many fish appear to maintain metabolic rates comparable to uninfected fish through a mitigation of these pathologies. Similar to previous studies, my results indicate a reduction of the inherent costs associated with infection with the metabolism of infected hosts eventually matching or moving below rates of uninfected fish at relatively low parasite burdens. This finding was somewhat surprising as this parasite is not known to directly influence metabolic function and brain tissue is known to be energetically expensive (Nilsson, 1996; Soengas & Aldegunde, 2002). It suggests that the observed host metabolic rates are a result of either parasite- or host-induced alterations of host energetics.

Although we did not measure other aspects of host energetics in this study, previous investigations indicate that hosts utilize multiple avenues of behavioural and physiological mitigation of infection-induced costs. There have been few previous studies of host metabolic rates during trematode infection, however, behavioural investigations suggest that fish are able to maintain relatively normal functions during the obligate growth period while experiencing a wide range of parasite numbers (Shirakashi & Goater, 2002; Voutilainen et al., 2008). These results indicate that the pathologies associated with infection are significantly reduced through host or parasite modification. As previously described, one study of host metabolism during infections with *Diplostomum sp.* found similar metabolic rates in uninfected and infected fish during the early stages of parasite development (Voutilainen et al., 2008). Metabolic rate was increased during the later stages of infection after cataract formation and was associated with an increase in foraging activity in an effort to offset impaired feeding abilities due to obstructed vision. To my knowledge this is the only previous study which included direct measures of aerobic metabolism, but it suggests that hosts play an important role in mitigating the increased energetic demands associated with the obligate growth period.

One of the most common methods employed by hosts to meet energetic demands associated with infection is the reallocation of energy from other functions such as activity and growth. Previous studies of fathead minnows during trematode infections have shown evidence of decreased activity (Shirakashi & Goater, 2002) and mass (James et al., 2008) as compensatory responses to infection. For example, *O. ptychocheilus*-infected fish significantly decreased activity during a short observation period. This only occurred at infection levels much higher than those reported in this experiment (150 ± 31 metacercariae) (Shirakashi & Goater, 2002). However, it is possible that infected fish with low to intermediate numbers of parasites may experience more subtle decreases in activity throughout the day. Changes in fish mass during *O. ptychocheilus* infection have not been investigated, but fathead minnows infected by another trematode, *Ornithodiplostomum sp.* – a parasite which encysts within the abdominal cavity and undergoes a similar developmental pattern to *O. ptychocheilus* (Matisz & Goater, 2010) – demonstrated significantly reduced mass in the middle of the obligate growth period compared to uninfected fish (James et al., 2008). Although parasite burdens ranged from 20-120 metacercariae, mean differences in mass between the low and high infection status groups were comparable among infected fish. If fathead minnows show similar compensatory responses to *O. ptychocheilus* infection, these results may suggest that hosts are able to meet the energetic demands of infection by reallocating energy from growth or by increasing reliance on energetic reserves. In this scenario, my results suggest that hosts may only be able to tolerate a very small number of parasites before it is necessary to utilize resources otherwise devoted to growth or storage.

Although the redistribution of energy is often an important strategy to meet energetic demands associated with infections, reducing the drain of other infection-related challenges can also play a significant role in host energy budgets. For example, one of the main challenges to host energetics during infection appears to be the maintenance of defensive systems. Specifically, modifications to stress responses (Madison et al., 2013) and immune function (Secombes & Chappell, 1996; Kutyrev et al., 2014) have been observed during infection. These host defenses have been directly linked to increases in metabolic rate (Skinner et al., 2010; Murray et al., 2017). Although this is the first investigation of host energetics during *O. ptychocheilus* infections, evidence of infection-induced alterations of fathead minnow physiological defenses have been found previously and may suggest parasite-related energetic

alterations. In one study, oxidative stress in liver tissue has been observed during the obligate growth period of *O. ptychocheilus* infection (Stumbo et al., 2012). Oxidative stress was only evident during day 5 of the early stage of parasite development (coinciding with extensive development of the microvillar network) suggesting that this response may be reduced or restricted to the immediate infection site thereafter. Although the specific mechanisms are not well understood, suppression of host physiological defensive systems such as immune function is often associated with infection by helminth parasites (Maizels & Yazdanbakhsh, 2003; Kutyrev et al., 2014). In my results, the steady decrease in metabolic rate associated with parasite burden may indicate that the successful suppression of host defensive systems increases with metacercariae numbers. Therefore, individual *O. ptychocheilus* may facilitate the protection of surrounding metacercariae from host defenses.

The suppression of host defensive functions is likely necessary for parasite survival, however, as discussed, significantly handicapping hosts during non-infective stages is maladaptive for trophically transmitted parasites. Therefore, parasite alterations of host defenses such as immunosuppression may be highly selective for specific functions. For example, helminth parasites have often been associated with a depression of the inflammatory immune response, which can cause increased tissue damage, while other immune activity such as host tissue repair and protection from parasite-released toxins is promoted (Gause et al., 2013). In terms of *O. ptychocheilus* infections, the inflammatory response has been observed following metacercarial encystment when parasite interactions with host tissue appear significantly reduced (Matisz et al., 2010a). This indicates that this immune response may be suppressed only during the obligate growth period. The return of normal immune function has significant implications for host energetics after the early stages of parasite development and supports previous findings of development-dependent effects of trematodes on host metabolism.

Overall, my results indicate that infection modifies host energetics and these changes are reflected in host resting metabolic function. Although it is difficult to determine the relative influence of parasites-induced changes and host compensatory responses in the observed metabolic outcomes, it is likely that both play a role in metabolic alterations. Future investigations of minnow metabolic rates over the course of *O. ptychocheilus* development and across a wide range of parasite burdens will provide a greater understanding of how host energetics are modified by various infection conditions. The persistence of the observed

metabolic alterations during trematode infections are not well understood. Unlike the majority of previous investigations of host metabolism during infection, *O. ptychocheilus* is a parasite that has been associated with reduced histopathology over time. After the completion of the obligate growth period, the metacercariae encysts, and appears to be in a dormant state. Although host recovery following the obligate growth period likely depends on parasite burden, this suggests that the parasite may not directly alter host metabolic function during later developmental stages.

The evaluation of the underlying physiological and behavioural factors which influence the observed metabolic outcomes are necessary to determine the mechanisms which modify metabolic rates during trematode infections. This can be accomplished through the inclusion of measures which illustrate infection-induced changes to host energetics such as growth, daily activity, immune responses, and metabolic capacity (e.g. oxygen transport and tissue absorption capabilities). The close association between parasite burden and depression of metabolic rate strongly suggest that physiological and behavioural changes influencing host energetics are responses to factors which increase with parasite burden such as histological damage or parasite-released products. Therefore, studies of the interactions between with host tissues and metacercariae may provide a greater understanding of how parasite presence modifies host metabolism during rest.

Chapter 3 - Infection-induced Visuomotor Processing Disruption and Predator Escape

3.1 Introduction

O. ptychocheilus infection and associated OMR disruption

As previously described, infection with some trophically transmitted parasites has been associated with alterations of host antipredator behaviours in such a way as to increase the transmission of the parasite to definitive hosts – known as adaptive manipulation (Worth et al., 2013). Although there are many controversies surrounding the precise definition of what constitutes a manipulative parasite strategy, it is generally agreed that increased transmission to target hosts is an essential component of this phenomenon. Indeed, Poulin's more inclusive definition of adaptive manipulation encompasses any parasite-induced alteration of hosts if it both increases transmission and the parasite's trait underlying host changes is heritable (Poulin, 2010). To consider a wide variety of mechanisms by which trophically transmitted parasites may facilitate their transmission to target hosts, I will frame this study under Poulin's broader definition of parasite manipulative strategies.

Although the infection site of *O. ptychocheilus* in the optic tectum and cerebellum provides an efficient avenue for parasite manipulation, antipredator responses have not previously been investigated in the trophically transmitted host (fathead minnow). However, previous investigations have indicated that *O. ptychocheilus* infection has been associated with the disruption of visually-mediated behaviours, presumably due to parasite preference for the superficial layers of the optic tectum. The optic tectum has been associated with the regulation of visually-mediated behaviours through mechanisms such as optomotor response (OMR) (Arnold, 1974; Springer et al., 1977). OMR is defined as the movement of an animal in response to visual stimuli as it moves relative to its background (Shirakashi & Goater, 2005). It is thought to be essential for schooling, the detection of movement, and orientation (Arnold, 1974). An earlier study found complete disruption of OMR-controlled behaviours in goldfish (*Carassius auratus*) following the removal of the optic tectum (Springer et al., 1977). More recent investigations in

zebrafish suggest that OMR disruption of the goldfish study was due to removal of deeper tectal regions, as zebrafish with these brain regions intact retained normal OMR (Roeser & Baier, 2003; Temizer et al., 2015). Interestingly, *O. ptychocheilus* infection is associated with a disruption of OMR performance comparable to the earlier investigation of goldfish with ablations of the optic tectum (Shirakashi & Goater, 2001). This suggests that the reduced OMR performance of infected fish is not necessarily a direct result of the histological damage of the superficial optic tectum layers associated with infection.

Similar to infection-induced pathologies of other host-parasite systems, the severity of OMR disruption in hosts is dependent upon *O. ptychocheilus* development and parasite burden. One study found that infection resulted in the overall decrease of OMR performance across ontogenetic stages, however, this difference was only significant during the obligate growth period (Shirakashi & Goater, 2005). This result is somewhat surprising as an intermediate host of a trophically transmitted parasite developmental stage is an obvious target of parasite manipulation. It suggests that OMR disruption was a pathology associated with the parasite-induced deterioration of host brain tissue during the initial stage of parasite development. In comparison, the relationship between OMR performance and parasite load was not linear, but rather followed an inverse U-shape relationship: OMR performance was reduced in fish with low (5-20 metacercariae) and very high (150 ± 31 metacercariae) parasite burdens while OMR performance of fish with more intermediate parasite numbers was comparable to that of uninfected fish (Shirakashi & Goater, 2002). The underlying mechanisms driving this relationship have not been determined. However, the duration of the obligate growth period has been shown to be inversely density dependent, with increased growth and size of metacercariae in lower level infections (Sandland & Goater, 2001). Observed OMR outcomes may be related to benefits associated with density-dependent inhibition of parasite growth at intermediate intensities. Beyond a threshold, the energetic demands of the parasites and subsequent histological damage may overwhelm the protection of density-limited parasite development. Despite the apparent recovery of hosts following the obligate growth period, mature *O. ptychocheilus* infections have been associated with inhibited visuomotor learning (Shirakashi & Goater, 2005) as well as reduced OMR in fish with lower parasite numbers suggesting that the influence of parasite burden on infection-induced changes persists overtime (Shirakashi & Goater, 2001).

Infection-induced interruption of visual processing: implications for visually-mediated fast-start responses

Vision is one of the main avenues of sensory information in aquatic systems (Hartman & Abrahams, 2000). As a result, many antipredator behaviours are visually-mediated and play a crucial role in survival (Godin, 1997; Temizer et al., 2015). Therefore, the disruption of visual processing associated with *O. ptychocheilus* infection has important implications for threat recognition, and consequently, appropriate displays of defensive behaviour (Blaxter & Fuiman, 1990; Domenici, 2002; Ferrari et al., 2010). An important component of the antipredator response is escape behaviour. Mediated by fast-acting Mauthner neurons as well as other reticulospinal neurons in the brainstem, the aptly named fast-start responses, or c-starts, are initiated during an imminent predator threat and are, therefore, directly linked to survival (Eaton et al., 1984; Korn & Faber, 2005). Their importance in prey survival has prompted investigations into the factors which influence escape performance such as distance travelled, speed and acceleration (Webb, 1976; Harper & Blake, 1990; Walker, et al., 2005), response latencies (Eaton et al., 1984; Binning et al., 2014), and response distance (Dill, 1974). Response latencies in particular have often been used as a measure of escape performance. Short response latencies have been associated with successful escape from live aerial (Lotem et al., 1991; Katzir & Camhi, 1993) and aquatic predators (Meager et al., 2006).

Despite the well-known influence of trophically transmitted parasites on antipredator responses, response latencies to visual stimuli have not been investigated in fish hosts with known infection-induced disruption of the visual system. However, increased response latencies during environmental visual disruption and experimental ablation of the optic tectum have been described. For example, slower responses of juvenile Atlantic cod (*Gadus morhua*) to threatening visual stimuli in turbid water, contributed to a 73% decrease in escape success compared to responses in clear water (Meager et al., 2006). In comparison, zebrafish with optic tectum ablations demonstrated longer response latencies and fewer fast-start responses, resulting in a significant decrease in escape probability (Temizer et al., 2015). Increased response latencies of fish in turbid environments as well as following optic tectum damage indicate that infection-induced disruption of vision has significant implications for host's ability to respond quickly and successfully avoid threatening visual stimuli. Also, as described, the infection site provides an

efficient route of parasite-induced modifications of host antipredator behaviours in an effort to increase transmission. Therefore, determining the vulnerability of fathead minnows housing developing and infective stages to target hosts and non-target predators is necessary to establish if alterations to minnow behaviour are in accordance with the adaptive manipulation hypothesis.

Fathead minnows are prey for many aerial and aquatic predators and must perceive both vertically and horizontally approaching stimuli. In order to simulate aerial and aquatic predators, we explored escape behaviours to a visual stimulus from two approach angles: directly above and forward approaching. Due to the importance of parasite developmental stage and number on the degree of OMR disruption experienced by hosts, I expected the intensity of infection-induced modification of host response latencies to depend on infection conditions.

3.2 Methods

Ethics statement and safety practices

All fish maintenance and experimental procedures were again conducted in accordance with Canadian animal research standards and Biosafety Containment Risk Level 2 protocols.

Fish exposure to O. ptychocheilus

One-year old minnows raised from eggs collected between June-August 2016 at Feedlot Pond were used in this experiment. Snails were again collected from Feedlot Pond and infection status was determined following previously described protocol. For this experiment, cercariae were obtained from two infected snails and combined in preparation for fish exposure. Intended parasite exposure doses were low: 15-30, intermediate: 55-70, high: 95-120, and none (clean system water) to provide a range of parasite burdens found in the wild. Exposure doses were higher than expected parasite loads, due to the assumption that many cercariae infection attempts would not be successful. Cercariae were exposed to minnows between 1-5 h after release to address concerns associated with cercariae viability during fish exposure for the respirometry experiment. Following the conclusion of the 1.5-2 h exposure period, fish were sorted according to infection dose and gently transferred to 38-L holding tanks. The location of fish from each treatment was concealed from observers and holding tanks were color-coded according to infection status. A total of 6 parasite exposures were staggered over the course of 5 weeks during September and October 2017 with all treatments included in each exposure period. This ensured

that experiments with fish during the early stage of infections occurred closer to the time frame of trials conducted during later parasite developmental stages. A group of fish containing later stages of infection from 2016 exposures were also included in the approaching visual stimulus experiments to compare responses with fish infection groups exposed in 2017.

Visual stimulus chambers

Vertically approaching stimulus experiments were conducted in a 66 x 66 x 41 cm corrugated plastic tank with a clear acrylic base. Inside the tank, a clear 63-cm diameter circular plastic insert ensured that the experimental fish did not use the corners of the arena as a shelter (Figure 3.2). To provide even lighting throughout the experimental chamber, LED light strips were wrapped around the sides of the tank. Water depth was approximately 9 cm during trials and maintained at 20°C to match holding tank temperature. The visual stimulus, a 10-cm diameter white disc with a 4.5 cm diameter black circle in the center, was connected to a remotely activated electromagnet via a 5 $^{4}/_{5}$-cm bolt at the center of the visual stimulus (Figure 3.1). The weight of the bolt, as well as the additional weight of four nuts evenly spaced around the edge of upper surface of the disc reduced horizontal motion of the stimulus during the fall. Following release, the movement of the disc towards the fish simulated a visual stimulus of a black circle increasing in size and is known to induce fast-start responses (Fuiman et al., 2005; Fuiman et al., 2006). The stimulus was connected to a wooden bar above the tank with braided fishing line to minimize the mechanical stimuli associated with the drop. When fully extended, the length of the fishing line held the visual stimulus just above the water level. I did not let the disc break the water surface, to reduce the possibility that the response of the fish was affected by mechanical cues, however, fish may have experienced air pressure waves associated with the drop. A mirror at a 45° angle was placed underneath the transparent bottom of the experimental tank, allowing the response of the fish to be recorded with an unobstructed view, using a high-speed camera (Casio Exilim EX-ZR100v) at 480 fps.

The horizontally approaching visual stimulus set up comprised of a 6.8-L glass aquarium placed on a tabletop with an overhanging metal frame. The side of the aquarium facing away from the observer was wrapped in opaque, black plastic while the side closest to the observer was covered in dark window tinting (5% visual light transmission). The tank end facing towards the stimulus was clear, and the fish was restricted to this side of the tank by a white corrugated

plastic divider arranged halfway along the long side. Attached to the metal frame above the tank was a 75-cm wooden dowel with the previously described visual stimulus (10-cm diameter white disc surrounding a 4.5-cm diameter black circle center). The disc was secured to an electromagnet 42 cm away from the tank face (Figure 3.3). Upon release, the disc swung forward towards the tank. An attached cord limited the stimulus arc to a point immediately prior to reaching the tank. This prevented mechanical stimuli from the disc striking the aquarium face. Lights attached to the metal frame as well as the tabletop below illuminated the set up. Again, a high-speed Casio Exilim EX-ZR100 camera recording at 480 fps, was positioned above the aquarium to simultaneous view both the experimental chamber and the stimulus.

Visual stimulus trials

In both vertically and horizontally moving visual stimulus experiments, fish were placed individually in experimental chambers and given 7 min to acclimate prior to stimulus release. Following this 7-min period, the high-speed video cameras were turned on and fish were observed until they moved into the zone of the experimental chamber where the observer would initiate stimulus drop. During the vertically approaching stimulus trials, the stimulus was only dropped if the fish was approximately one body length away or closer to the area beneath the disc. During horizontally approaching stimulus trials, the body of the fish had to be more than half-way over the dotted-line dividing the arena on the tank side facing the visual stimulus. For both visual experiments, the observer only initiated disc release if the fish was moving calmly and slowly in the experimental drop zone. This ensured that minnows were not already demonstrating fear responses such as dashing or freezing prior to the introduction of the stimulus. The average speed of stimulus approach during vertical trials was 0.12 $^{cm}/_{ms}$. During horizontal trials disc approach towards tank A was slightly faster than tank B with average approach speeds of 0.07 $^{cm}/_{ms}$ and 0.09 $^{cm}/_{ms}$, respectively.

After the drop, a fish was considered to have responded if it moved after stimulus release and prior to drop completion. The initial stimulus-motivated movement exhibited by the fish was recorded as a response. If the fish did not move at all during stimulus approach, it was described as showing no response to the visual stimulus. In the case of a non-response, the stimulus was reset for another attempt. Testing was concluded when fish either demonstrated a response to the disc or continued to show no response after 3 trials. This distinction was only relevant for the

horizontal stimulus trials as fish in the vertical stimulus trials consistently demonstrated escape c-starts to disc approach.

Video analysis

Videos of the visual stimulus trials were uploaded and enhanced in Shotcut version 18.01.02. Video length was trimmed to the time frame of the drop and edited to enhance the visibility of fish profile within the arena – increasing the accuracy of identifying the moment of fish response. Fish response latency and fish position within the tank after the initiation of disc release was recorded for both directions of stimulus approach. Again, only stimulus-motivated movement within the time period of the drop was considered a response. Response latency was found by calculating the difference between the moment of stimulus release and the beginning of fish response. Due to the observation of three distinct responses during horizontally approaching trials, response type – movement away (primarily full escape c-starts; Figure 3.4), stimulus inspection (c-start-like movement towards the disc; Figure 3.4), and non-response – was also recorded.

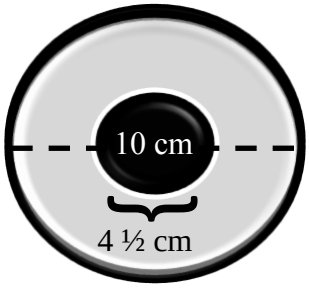

Figure 3.1 Diagram of stimulus used for visual experiments.

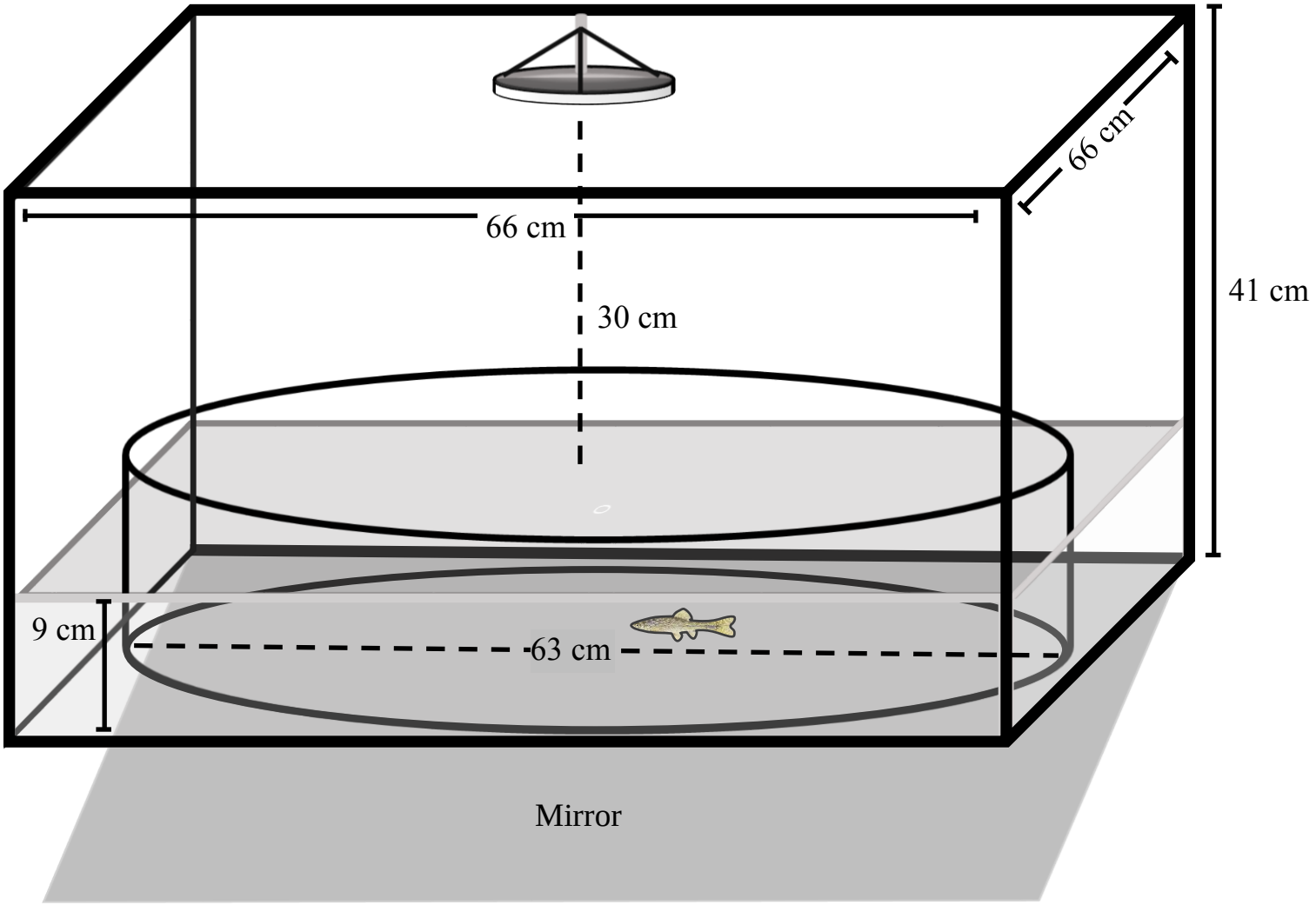

Figure 3.2 Vertically approaching stimulus experiment set up (diagram components not to scale, for descriptive purposes only).

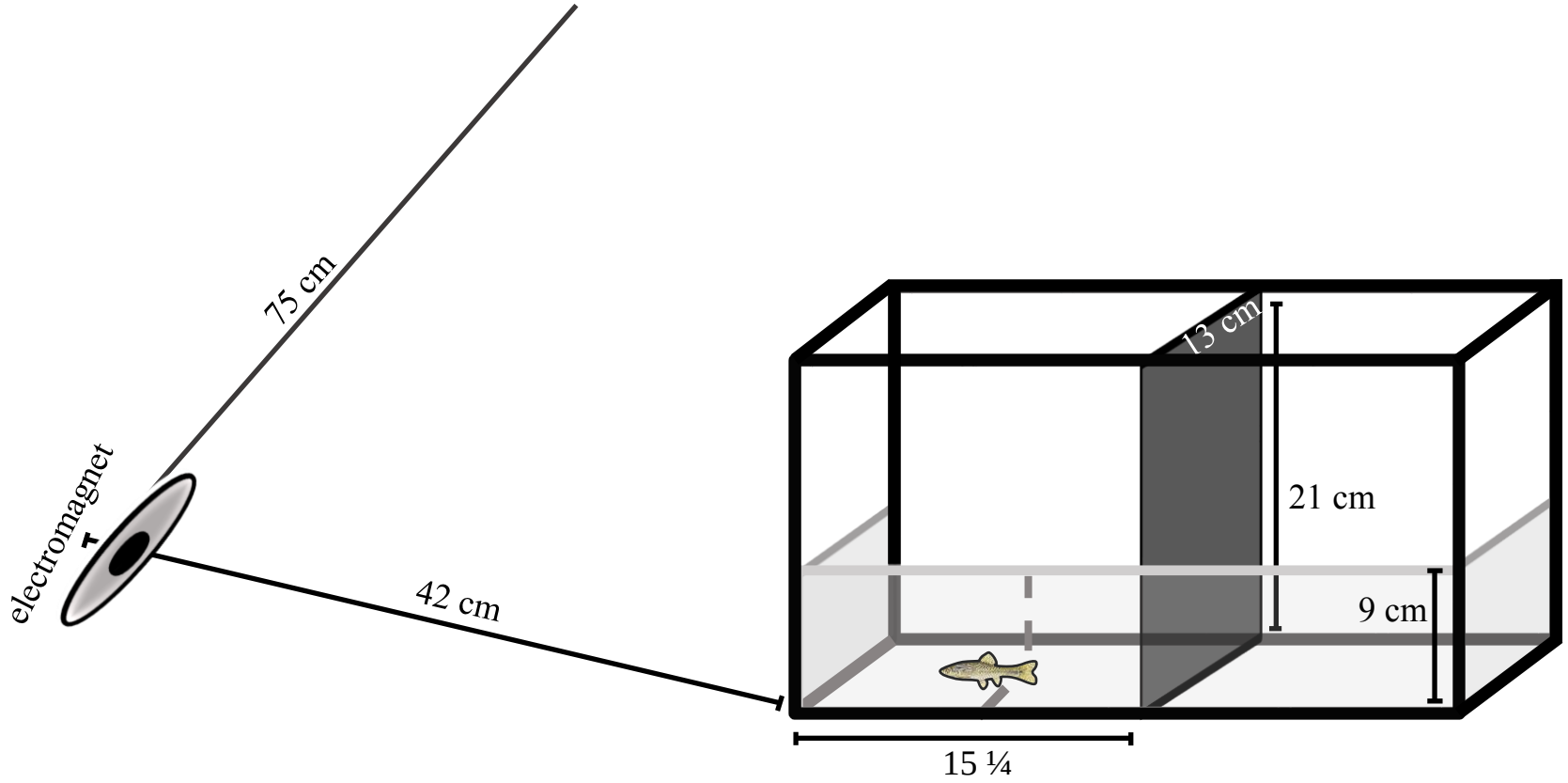

Figure 3.3 Horizontally approaching stimulus experiment set up (diagram components not to scale, for descriptive purposes only).

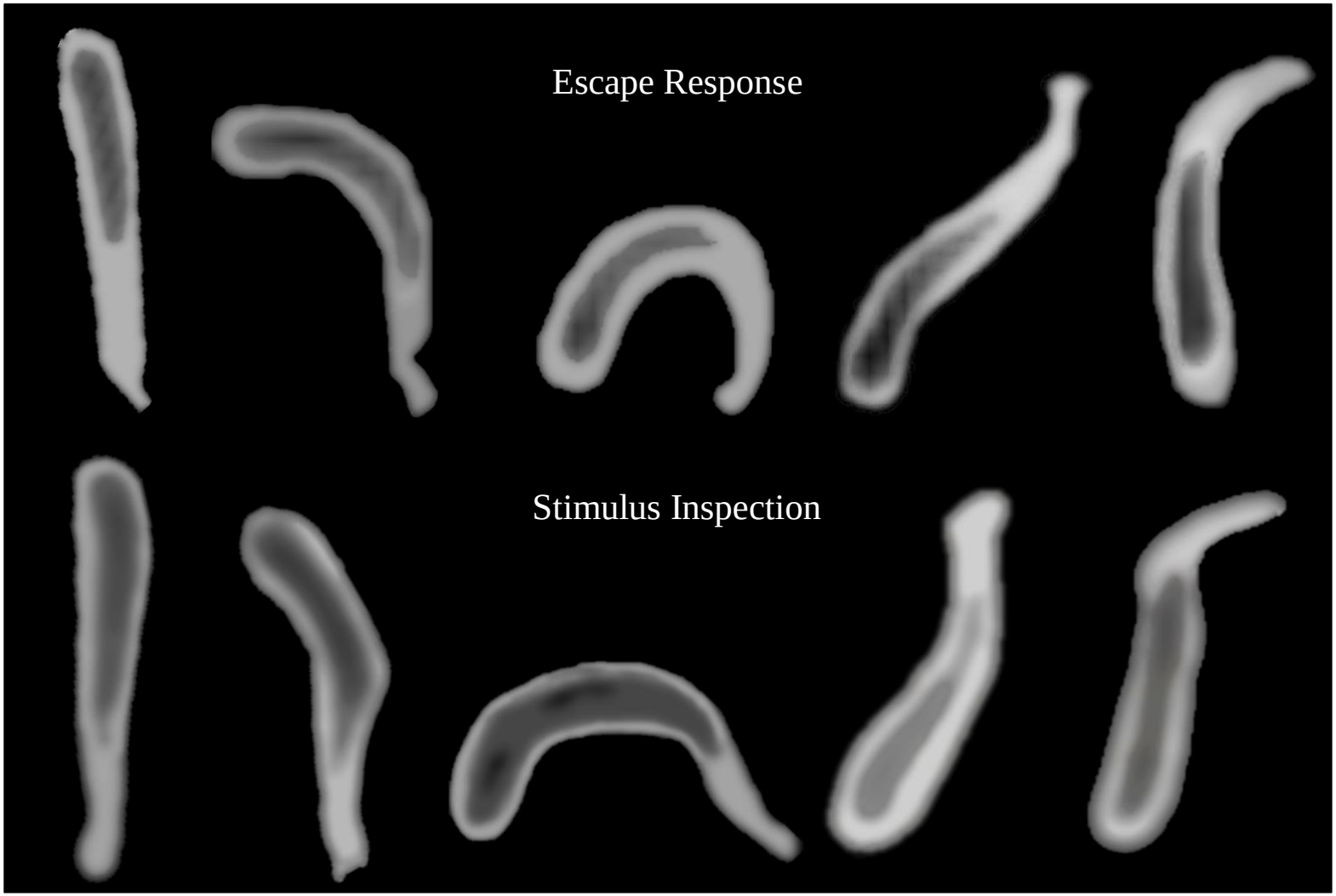

Figure 3.4 Fish images showing the stages of c-starts during escape and stimulus inspection behaviour.

Parasite counts

Following visual trials, fish were euthanized with a Tricaine overdose and previously described parasite count procedures were followed with one modification: fish dissection occurred immediately after euthanasia. Although previous parasite counts were considered accurate, procedures were modified because brain removal from freshly euthanized fish was achieved more successfully, and the movement of living parasites within the brain tissue increased parasite visibility. The DinoCapture 2.0 for Windows software was again used to take images of the brain, which were later were pieced together and edited in GIMP version 2.8.4. A sample of 10 fish (5 infected and 5 uninfected) were investigated for the presence of other parasitic infections by Dr. Elemir Simko of the Department of Veterinary Pathology at the University of Saskatchewan's Western College of Veterinary Medicine.

Statistical analysis

Statistical analyses were conducted in R 3.4.3 (www.rproject.org). Responses to both the vertically and horizontally approaching stimuli were analyzed with mixed-effect models (package lmerTest) with fish position within the experimental chamber and tank designated as random effects and time period post infection and infection status run as fixed. Response latencies for vertically approaching stimulus trials did not follow a normal distribution, however, mixed models are robust to deviations in normality and the large sample size of this experiment allowed for appropriate conclusions with a parametric model. The influence of infection on response latencies to a vertically approaching visual stimulus were analyzed with the model: latency = infection status * time period + (position/tank). Fish included in this experiment were separated into 4 infection groups (uninfected, low, intermediate, and high) for analysis. Time period post infection was delineated according to parasite development periods: obligate growth, development within a double-walled cyst, and infectivity to bird hosts. The previously described model was also used to analyze the influence of the late stage of parasite development on fish response latency. In this model, the factor "time period" encompassed early stage maturity (newly infective parasites) and late stage maturity (infections > 1-year-old). Due to the lower parasite burdens of fish following the 2016 exposures, high infection levels were excluded from the factor "infection status" to ensure that infection status groups were comparable. A Tukey's HSD test was used for *post hoc* analyses of significant fixed effects with a $p < 0.05$ considered

significant. The significance of random effects was determined via likelihood ratio tests, again, a $p < 0.05$ was considered significant. The regression function in R was used to determine the relationship between response latency and parasite load for infected fish.

Sixty-four of the fish run through the vertically approaching visual stimulus trials were also tested in response to the forward (horizontally) approaching visual stimulus. The influence of infection on fish response latencies to the horizontally approaching visual stimulus was analyzed with the model: latency = infection status * time period + (fish angle/tank) + (tank side/tank) + (run/tank). Due to the small sample size and higher numbers of fish with low infection rates, minnows were separated into 3 infection groups (uninfected, low, and intermediate-high) for analysis. Time period post exposure was defined according to the 3 parasite development periods described previously. A few fish in each infection status group experienced multiple trials due to initial non-responses. Therefore, the random factor "run" was included to determine if previous experience with the stimulus affected fish responses. The model for the forward approaching stimulus was used to analyze the influence of late stage parasite development on fish response latency by restricting the factor "time period" to early stage parasite maturity (newly infective parasites) and late stage parasite maturity (infections > 1-year-old), and by excluding fish with high parasite burdens from the factor "infection status".

The influence of infection and time period on response type was analyzed using the previously described model with "response type" replacing "latency" and the random factor "run" removed due to the inclusion of non-response fish: response type = infection status * time period + (fish angle/tank) + (tank side/tank). The influence of infection and time period on active response types (flight and stimulus inspection) was analyzed using the same model with the random factor "run" included to investigate if previous experience with the stimulus influenced the type of active response exhibited: response type = infection status * time period + (fish angle/tank) + (tank side/tank) + (run/tank). Again, a Tukey's HSD test was used for *post hoc* analyses of significant fixed effects and likelihood ratio tests were used for analyses of random effects with a $p < 0.05$ considered significant for both tests. The regression function in R was used to determine the relationship between response latency and parasite load for infected fish.

3.3 Results

Infection success

Experimental infections were more successful in infected fish used during the visual stimulus experiments. Parasite counts spanned a minimum of 1 to a maximum of 151 metacercariae, encompassing a wide range of parasite burdens observed in the wild. This suggests that the modifications to fish exposure procedures in preparation for visual experiments (i.e. the use of two infected snails as parasite sources as well as cercariae less than 5 h old) helped to achieve the desired scope of infection levels.

Further investigations of parasitic infection conducted by Dr. Simko discovered one other parasite present in *O. ptychocheilus*-infected fish. Experimentally infected females harbored large ovarian cysts containing a microsporidian parasite. Uninfected fish were found to be parasite-free indicating that this second infection was a result of contaminated parasite exposure water related to the use of wild-caught infected snails. Although the cysts may have influenced some aspects of host behaviours, their location suggests that the infection was unlikely to have directly modified responses to visual stimuli. Therefore, I consider the results to reflect *O. ptychocheilus*-induced alterations of host behaviour.

Vertically approaching visual stimulus

Infection status had a significant effect on fish response latencies to the visual stimulus released from above ($F_{3,104.8} = 3.64$, $p = 0.015$). *Post hoc* analyses revealed a pattern of intensity-dependent increases in response latencies (Figure 3.5). Fish with high parasite burdens (≥ 50 metacercariae) were slower to respond to the stimulus than any other infection status group, although only the comparison between minnows with high parasite burdens and uninfected fish was significant ($p = 0.002$). The relationship between minnows with low level infections and fish with high parasite burdens (1-20 metacercariae) neared significance ($p = 0.054$), while the trend of faster response latencies in minnows with intermediate parasite loads (21-49 metacercariae) compared to fish with high infection levels was not as strong ($p = 0.093$). Response latencies between minnows with low infection and intermediate infection levels and uninfected fish were comparable. A quadratic regression analysis further illustrated this intensity-dependent increase in response latencies of infected fish and showed an increase in response latency with parasite numbers ($F_{2,76} = 6.73$, $p = 0.002$; Figure 3.6). Again, this result appears to be primarily driven by

slower responses in fish with 50 parasites or more. This indicates that infection does not represent a significant disruption of appropriate fast-start responses to visual stimuli approaching from above until parasite burdens surpass a threshold number. Fish position within the tank did not influence the described patterns of minnow response latencies ($p > 0.99$), suggesting that stimulus visibility during release was similar across trials.

In contrast to previous studies of visually-mediated behaviours in *O. ptychocheilus*-infected minnows, the behaviour of infected fish did not appear to be influenced by parasite developmental stage. The pattern of response latencies across fish infection status groups was comparable regardless of parasite development ($F_{2, 105.5} = 1.17$, $p = 0.315$; Figure 3.7) or the maturity of the infective stage ($F_{1,16} = 0.16$, $p = 0.696$; Figure 3.8).

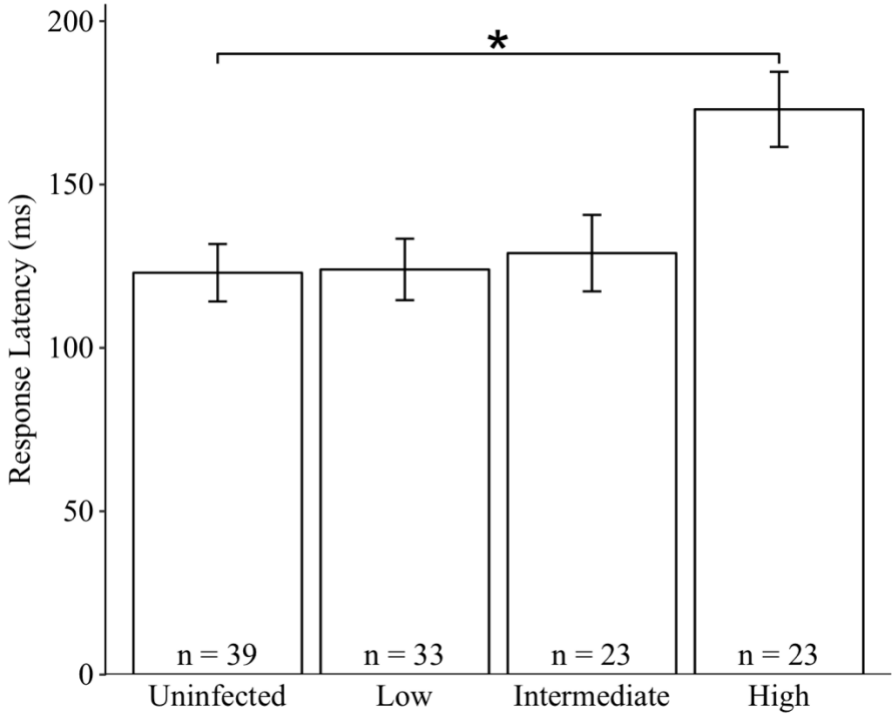

Figure 3.5 Mean ± SE response latencies elicited by the vertically approaching stimulus across infection status groups. Significant relationships are indicated by *.

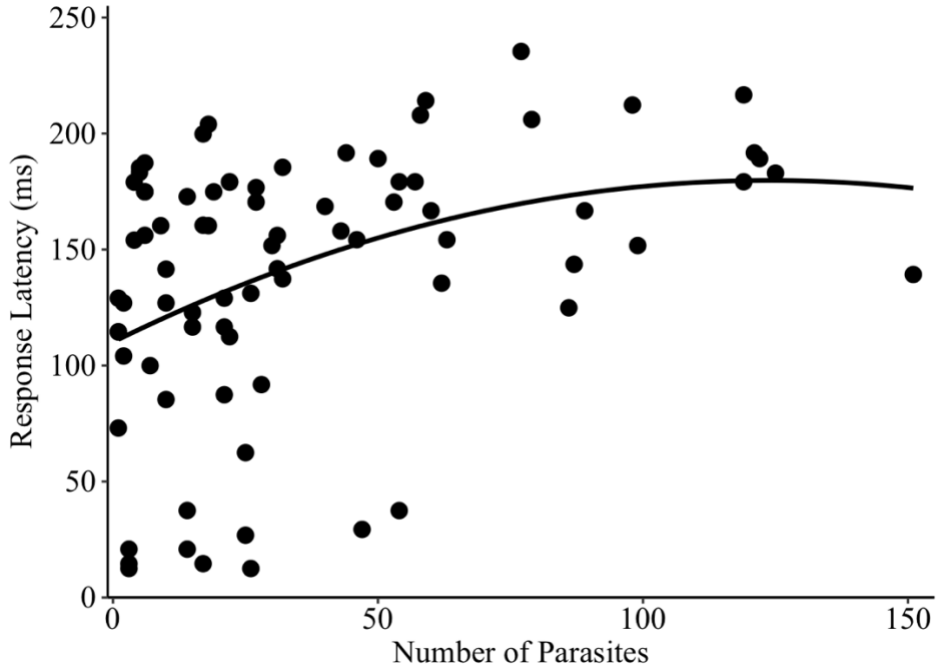

Figure 3.6 The relationship between the response latency of infected minnows to the vertically approaching stimulus and the number of parasites (n = 79, R^2 = 0.13).

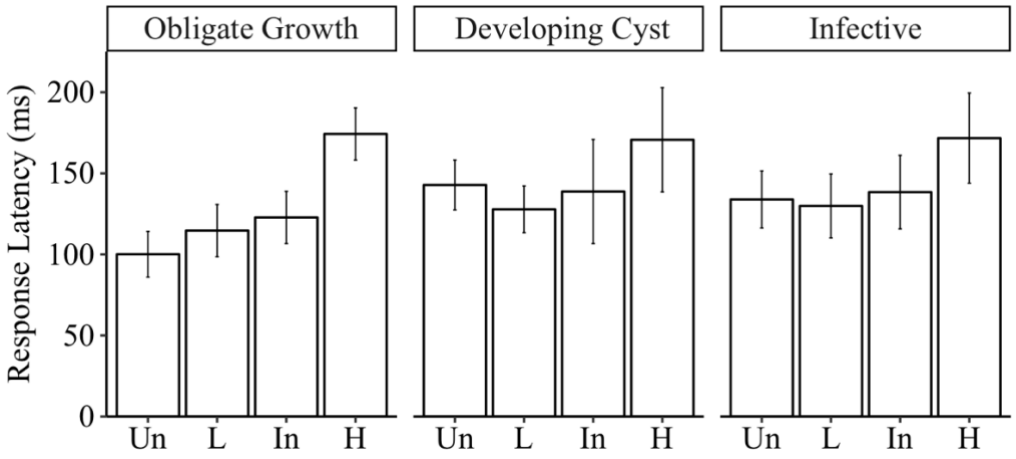

Figure 3.7 Mean ± SE response latencies of uninfected ("Un"), low ("L"), intermediate ("In"), and high ("H") fish infection groups to the vertically approaching stimulus during the time frame associated with each *O. ptychocheilus* developmental stage.

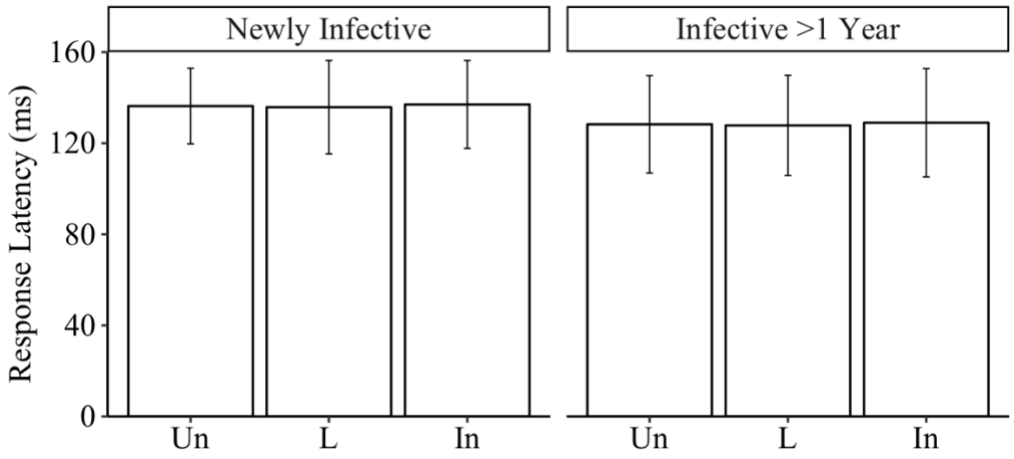

Figure 3.8 Mean ± SE response latencies of uninfected ("Un"), low ("L"), and intermediate ("In") fish infection groups to the vertically approaching stimulus according to the maturity of the late stage *O. ptychocheilus* infections.

Horizontally approaching visual stimulus

Similar to fish responses to visual stimuli released from above, response latencies of minnows to the forward approaching disc depended on infection status ($F_{2,44.4} = 7.18$, $p = 0.002$). The *post hoc* analysis again revealed that infected fish groups demonstrated slower responses compared to uninfected fish (Figure 3.9). However, unlike response latencies to the vertically approaching stimulus, this relationship was significant in fish with low parasite loads (1-20 metacercariae, $p = 0.03$) and fish with intermediate-high parasite burdens (> 20 metacercariae, $p = 0.041$). In contrast to response latencies to vertically approaching visual stimuli, the cubic regression analysis demonstrated no significant pattern between parasite numbers and fast-start response latencies of infected fish to forward approaching stimuli ($F_{3,31} = 0.45$, $p = 0.717$; Figure 3.10). Similar to response patterns to visual stimuli dropped from above, parasite developmental stage did not influence the relationship between infection status and response latency to the horizontally approaching stimulus ($F_{2, 44.8} = 1.06$, $p = 0.355$; Figure 3.11). The maturity of the infective stage did not affect response latencies: fish housing newly infective parasites responded comparably to fish with infections from the previous year ($F_{1,9} = 1.69$, $p = 0.249$; Figure 3.12). Fish position during the stimulus drop (body angle relative to the stimulus and nearness of fish to the aquarium sides) also did not influence responses ($p > 0.99$ for each factor).

As described, in contrast to the vertically approaching visual stimulus trials, fish demonstrated three distinct responses to stimulus approach on a horizontal plane: escape, no response, and stimulus inspection (described here as obvious movement towards the disc

following release). The results did not show a significant relationship between response type and infection status ($F_{2,53.4} = 1.94$, $p = 0.154$; Figure 3.13). However, only uninfected fish or minnows with low and intermediate intensity infections (1-32 parasites) were observed to demonstrate stimulus inspection behaviours following disc release. This suggests that parasite burden of infected fish has an effect on the expression of other antipredator behaviours and the lack of significance in the results may be a result of small sample size. Time post parasite or sham exposures did not appear to influence which of the three types of fish responses were exhibited ($F_{2,54.4} = 0.733$, $p = 0.485$). However, there was a difference in active response types between time periods ($F_{2,45.7} = 4.05$, $p = 0.024$). *Post hoc* analyses revealed that stimulus inspections occurring more often during the last time period than the first ($p = 0.053$) or the middle time frame post parasite or sham exposures ($p = 0.076$) although these trends were not significant (Figure 3.14). Fish position within the aquarium during the stimulus drop did not significantly affect fish response types ($p > 0.99$ and $p = 0.747$, body angle and fish proximity to aquarium sides, respectively).

A small number of fish from each infection group completed multiple trials due to initial non-response reactions to the stimulus (Figure 3.15). Experience with multiple trials did not significantly affect fish response latency ($p = 0.376$) or the type of active fish responses exhibited ($p > 0.99$), which indicates that fish did not modify responses to according to previous knowledge of the apparatus.

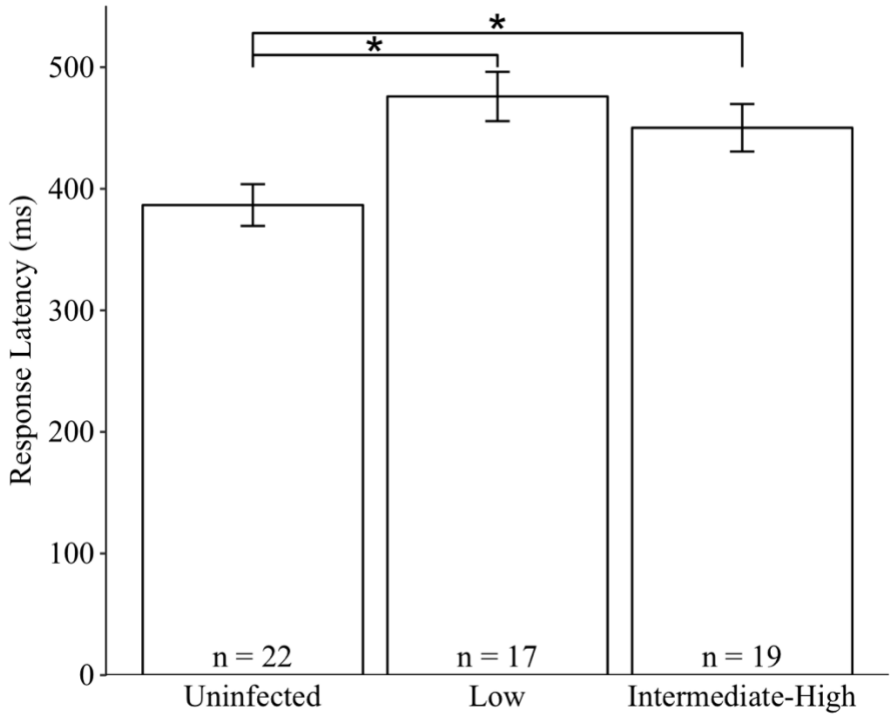

Figure 3.9 Mean ± SE response latencies of fish infection groups to the horizontally approaching stimulus. Significant relationships are indicated by *.

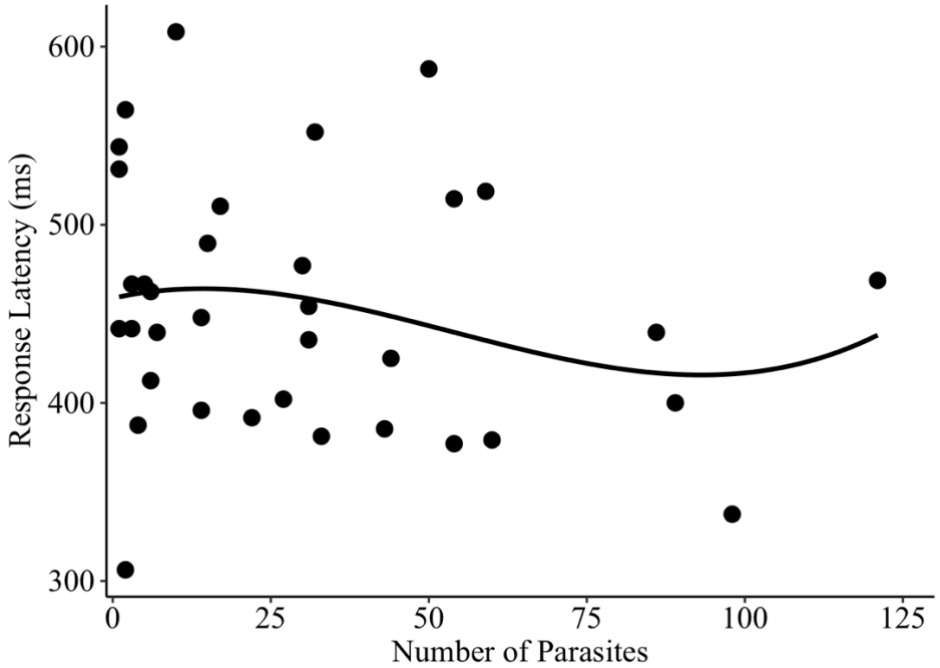

Figure 3.10 The relationship between the response latency of infected minnows to the horizontally approaching stimulus and the numbers of parasites (n = 35, R^2 = -0.05)

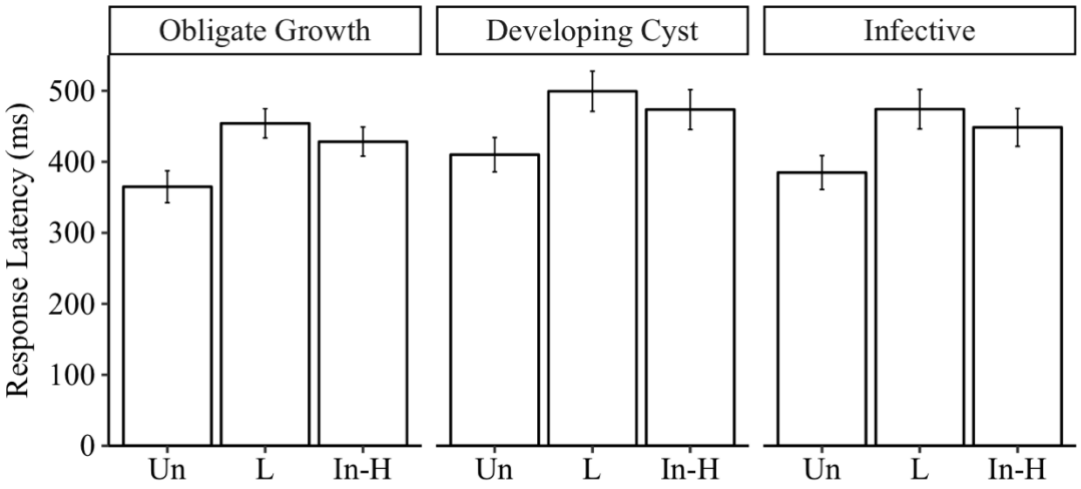

Figure 3.11 Mean ± SE response latencies of uninfected ("Un"), low ("L"), and intermediate-high ("In-H") fish infection groups to the horizontally approaching stimulus during the time frame associated with each *O. ptychocheilus* developmental stage.

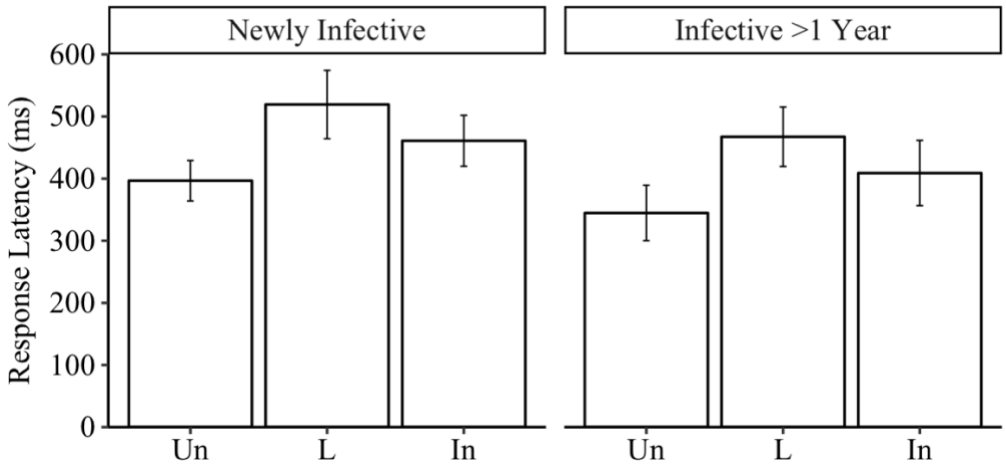

Figure 3.12 Mean ± SE response latencies of uninfected ("Un"), low ("L"), and intermediate ("In") fish infection groups to the horizontally approaching stimulus according to the maturity of the late stage *O. ptychocheilus* infections.

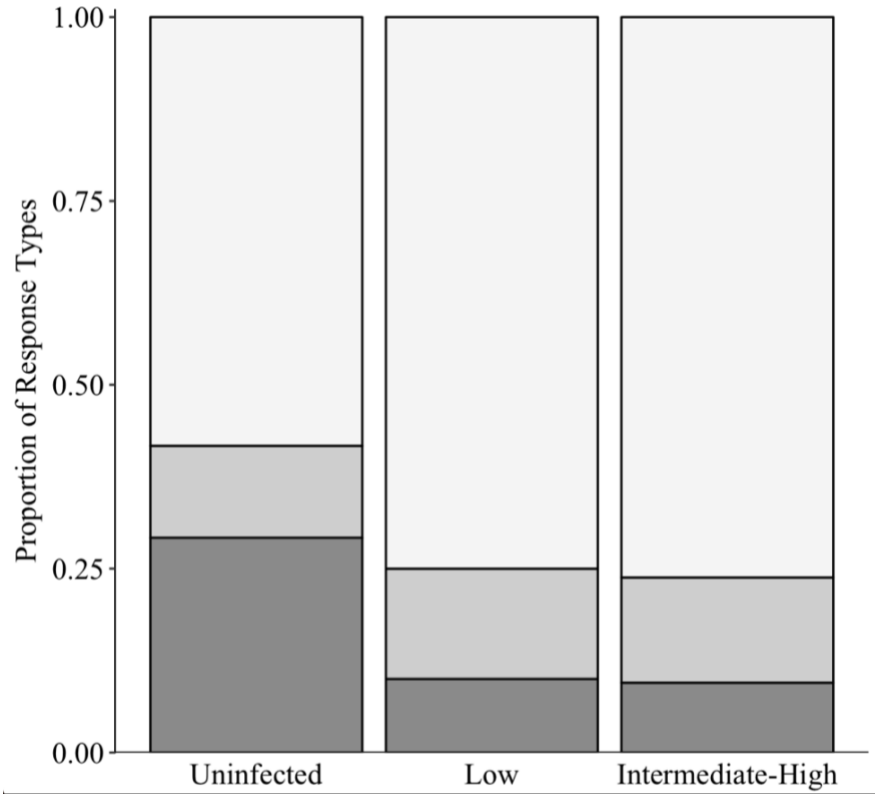

Figure 3.13 The proportion of minnows in each infection status group exhibiting different response types to the horizontally approaching stimulus. Stimulus inspection is represented in dark gray, non-response behaviours in light gray, and movement away in white.

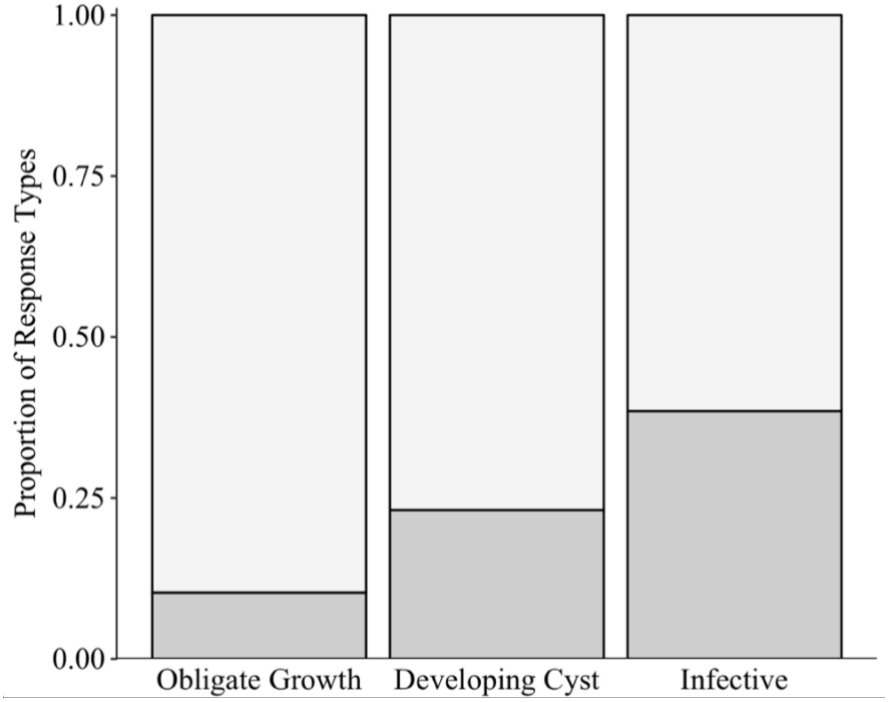

Figure 3.14 The proportion of actively responding minnows (movement away, and stimulus inspections) in each time period post parasite or sham exposure. Stimulus inspection and movement away are represented in light gray and white respectively.

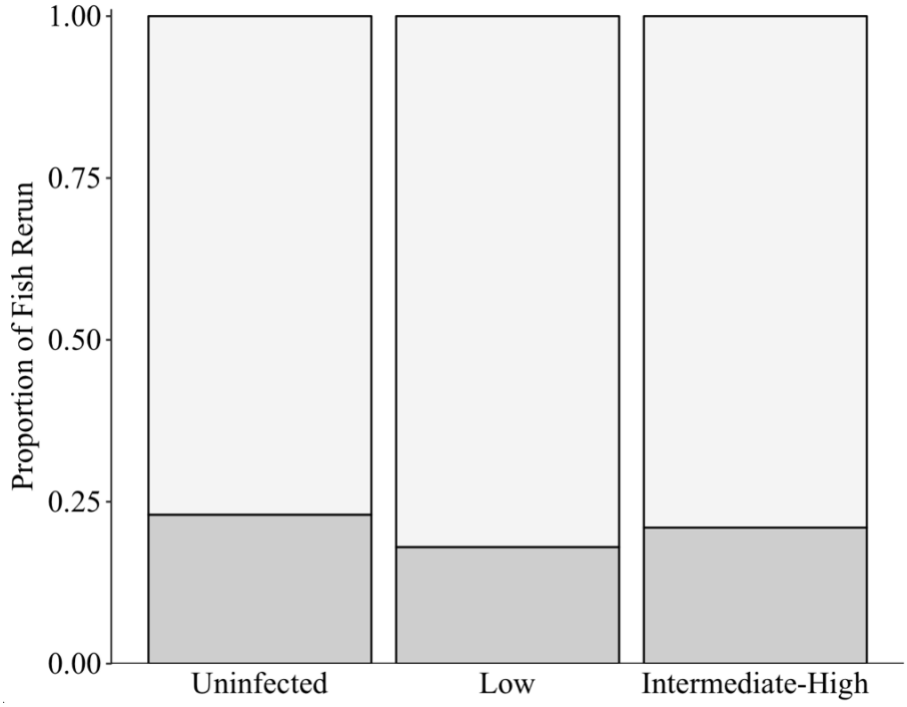

Figure 3.15 The proportion of actively responding minnows (movement away and stimulus inspections) in each infection status group which experienced multiple trials. Fish which demonstrated responses to the stimulus during the first trial are represented in white while fish experiencing multiple trials prior to a response are shown in light gray.

3.4 Discussion

Overall, infection appeared to increase response latencies to visual stimuli for both horizontal and vertical visual experiments. This follows patterns of previous reports suggesting an overall decrease in visual capabilities of infected fish (Shirakashi & Goater, 2002; 2005) and illustrates the importance of the superficial layers of the optic tectum for interpreting visual information (Avitan et al., 2016). However, the differences between the patterns of response latencies associated with parasite burden during vertical and horizontal visual trials was unexpected. The relationship between parasite burden and response latencies during the vertical stimulus experiment suggested that fish are relatively resistant to infection-induced visual processing disruption at lower parasite burdens. Any mitigation of the reduced visual capabilities associated with infection appeared overwhelmed as parasite numbers increased. This also was somewhat similar to observed patterns between high parasite burdens and changes in OMR performance found previously (Shirakashi & Goater, 2002; 2005). However, visual processing disruption was

associated with much higher parasite loads during OMR trials (150 $\pm$ 31 metacercariae) than experienced by the high parasite group in the vertical stimulus experiment ($\geq$ 50 parasites).

The relationship between response latencies to vertically approaching stimuli and parasite burden in this study suggests that this visually-mediated behaviour is more sensitive to the histopathology related to parasite development. This supports previous studies suggesting that the upper regions of the optic tectum play a more direct role during responses to approaching visual stimuli than during OMR (Roeser & Baier, 2003; Temizer et al., 2015). However, the relationship between parasite burden and response latencies to stimuli approaching on a horizontal plane more closely followed the pattern associated with previous investigations of OMR with the disruption of visually-mediated behaviours beginning at low parasite loads. The spread of parasite numbers of infected fish included in the horizontally approaching visual stimulus trials did not extend past 121 parasites, but, with a greater range of infection intensities, it is possible that I would have eventually seen a non-linear relationship similar to previous investigations of OMR (Shirakashi & Goater, 2001; 2002).

The observed differences in response latencies patterns between the visual stimulus experiments suggest that the results may be related to the quality of information across the visual field. A previous comparison between escape responses in turbid and clear water has shown that the amount and quality of visual information available to fish is important in determining responses latencies associated with fish assessment of risk (Meager et al., 2006). Similarly, the threshold of visual information required to elicit an escape response may change according to the region of the visual field. For instance, in an assessment of the visual field of two cyprinid prey species, although visual coverage extends above the head, the fronto-dorsal regions of the visual field were associated with the highest acuity (Pita et al., 2015). Therefore, the visual information threshold may be lower for rapidly approaching visual stimuli on a vertical plane. Thus, fish may consistently respond with escape behaviours to avoid costly mistakes. At higher infection levels when visual disruption is greatest, fish are presumably unable to perceive the stimulus as quickly and, consequently, demonstrate delayed responses. In comparison, the forward approaching visual stimulus was viewed from the proportion of the visual field with the highest acuity which allowed fish to increase the visual information gathered to determine risk. Uninfected fish were likely able to quickly accumulate visual information to assess risk and respond rapidly while infected fish may have required more visual information due to disrupted visual processing. The

similarities of response latencies between the two infected fish groups suggests that the effects of infection are mitigated across parasite burdens.

Although differences in visual information quality across the visual field could have influenced the observed patterns of response latencies between the horizontal and vertical stimuli, it is likely that the slow approach speed of the horizontal stimulus was an important factor. The speed of stimulus approach is known to influence fast-start response latencies because it is directly related to time until predator contact (i.e. risk of capture) (Tyrrell & Fernández-Juricic, 2015) and previous investigations of escape response latencies of fathead minnows have described slower turning movements and reduced response distance associated with slower aquatic predator approach (Webb, 1982). In my experiments, this may have resulted in a different determination of risk posed by the forward approaching stimulus among infection groups. Fish with presumably higher visual capabilities (i.e. uninfected fish) may have associated the slower stimulus with lower risk. Again, if fish from both infection groups required more visual information to initiate fast-start responses, visual processing disruption associated with infection could have contributed to slower responses compared to other infection groups. It also points to an explanation for the occurrence of stimulus inspection behaviours in uninfected fish and fish with low and intermediate-level infections (<32 metacercariae). Fish with higher infection levels demonstrated consistent escape c-start responses which may be related to a reduced ability to determine the level of risk associated with approach speed.

Despite the significant influence of infection on host responses latencies, one of the most surprising results was the discovery that response latencies of infected fish did not change over time during either visual experiment even in fish infected with metacercariae over 1-year-old. This suggests that fish may be largely able to reduce infection-induced pathologies during the obligate growth period until parasite burdens become too high. However, the overall increase in response latencies to both vertical and forward approaching visual stimuli indicate that infection does result in a significant disruption of visual processing during the obligate growth period which persists even after tissue repair. This is consistent with previous investigations of visually-mediated responses during infection with infected fish continuing to demonstrate reduced OMR performance even after regeneration of the superficial layers of the optic tectum (Sandland & Goater, 2001). The similarities between host behaviour during the parasite's infective and noninfective stages appear to indicate that the observed behavioural outcomes are a result of

pathology. However, the persistence of infection-induced disruption of visual processing during infective parasite stages supports prior suggestions of a subtle adaptive modification of host behaviour. The mechanisms of such behavioural alterations are unclear, however, during a previous study of OMR, it was suggested that site selection of the parasite could facilitate transmission by maintaining pathology within the site during infectivity (Shirakashi & Goater, 2005). More recent investigations have indicated that *O. ptychocheilus* infection of fathead minnows (Pan et al., 2016) as well as *Diplostomum phoxini* infection of the superficial layers of the optic tectum and cerebellum of Eurasian minnows (*Phoxinus phoxinus*) (Kekäläinen et al., 2014) may modify host personality traits such as boldness.

Slight modifications of host personality may increase host vulnerability without parasites incurring high fitness costs associated with conspicuous changes to host behaviour in a common prey species like the fathead minnow. Further, the differences found in the relationship between response latency and parasite burden when comparing the vertically and horizontally approaching stimuli indicate that *O. ptychocheilus* infection may result in a selective disruption of antipredator responses. Specifically, the comparability between response latencies of infected minnows to the forward approaching stimulus suggests that the influence of infection on vulnerability to aquatic predators is mitigated across parasite burdens. Thus, the fish appear able to avoid increases in vulnerability to non-target predators during high infection levels. In contrast, the results suggest that infected minnows are unable to reduce infection-induced disruptions of escape response to stimuli approaching from above as parasite numbers increase. If so, my results indicate that a threshold number of parasites are required to achieve desired behavioural alterations to facilitate transmission, a phenomenon which has also been described in killifish infected with *Euhaplorchis californiensis* (Lafferty & Morris, 1996). However, it is possible that more subtle alterations of host antipredator responses are occurring during low parasite burdens and that minnow vulnerability to aerial predation may be much higher than the current results suggest.

These findings may provide an important insight into infection-induced alterations of antipredator behaviours to multiple predator types, however, the possible role of visual stimulus approach velocities in determining behavioural outcomes, make it difficult to determine if the observed pattern between response latency and parasite burden are indicative of a selective disruption of host antipredator responses. Therefore, future investigations of fast-start responses

to approaching visual stimuli on vertical and horizontal planes with comparable stimulus velocities will provide a greater understanding of the role of infection-related alterations to host visual fields during escape responses. Further, a more direct determination of host susceptibility to aerial predation will provide a more conclusive answer to the central question of adaptive manipulation in the *O. ptychocheilus*-fathead minnow system. The inclusion of other fast-start response parameters such as reactive distance, flight distance, and fish acceleration in response to multiple stimulus velocities may help us better determine host evaluation of risk (Meager et al., 2006) as well as escape probabilities (Temizer et al., 2015).

Host visual fields and the location of the area associated with highest visual acuity may become particularly important in determining how parasites modify host behaviours during infective stages. For example, subtle modifications of behaviour may increase vulnerability to aerial predation if infection exaggerates visual capabilities across the visual field. Also, although the disruption of visual processing is likely an important avenue of defensive response alteration, infection of the cerebellum has significant implications for host susceptibility to predation as well. Ablation of the cerebellum has been associated with reduced motor and cognitive capabilities in fish (Rodríguez et al., 2005). Specifically, lesions of the corpus cerebelli have been found to disrupt both swimming abilities (Roberts et al., 1992) and classical fear conditioning (Yoshida et al., 2004) suggesting that damage to the cerebellum may have widespread consequences for host defensive responses. However, this is speculative as there is currently little understanding of both fathead minnow visual fields as well as how infection-induced disruption of the optic tectum and cerebellum modifies response capabilities. Also, the 90º angle of the vertical visual stimulus drop relative to the fish is likely an extreme angle of approach for an aerial predator (Lotem et al., 1991; Katzir & Camhi, 1993). It is clear that further investigations of more subtle alterations to host behaviour are needed to uncover possible *O. ptychocheilus* "manipulations". Future investigations into factors such as the repeatability of and correlations between host personality traits which may influence appropriate responses in an antipredator context as well as the changes in these parameters overtime may uncover significant infection-induced alterations of host antipredator responses.

Chapter 4 - Concluding Remarks

Following patterns of previous investigations of *O. ptychocheilus* pathologies, my results suggest that infection is associated with significant alterations to host physiology and behaviour. In particular, my observations provide further evidence for the significant role of parasite burden in the degree of infection-induced changes experienced by hosts (Sandland & Goater, 2001; Shirakashi & Goater, 2002; 2005). The parasite loads experienced by infected fish in the described experiments encompass a range of infection levels found in the wild and, therefore, have great implications for fish metabolic rates and antipredator responses of wild hosts. For example, the parasite burdens experienced by hosts during measures of SMR are comparable to parasite numbers experienced by various low infection rate populations, as well as many young of the year in populations with higher infection rates, and the higher levels of infection of fish during visual experiments reach the upper range of parasite burdens found in several populations around Saskatchewan and Alberta.

In contrast to previous investigations of visually-mediated behaviours, one of the most interesting results of my experiments is the continued infection-induced disruption of host visually-mediated escape behaviours during later parasite developmental stages, which suggests that the influence of *O. ptychocheilus* presence persists overtime. As discussed, this may be an example of a subtle adaptive manipulation and, in this scenario, my results suggest that higher parasite burdens are needed to significantly increase fish host vulnerability to piscivorous birds. Another possibility is that there is significant physiological compensation to mitigate infection-induced disruption of escape responses during the obligate growth period. This is indicated by the maintenance of relatively normal host behaviour during the developmental stages of *O. ptychocheilus* (Shirakashi & Goater, 2002; 2005), as well as the negative relationship between metabolic rate and parasite numbers I observed. However, as the specific underlying mechanisms of the metabolic outcomes in the results are unknown, it is not clear if this represents a compensatory response by hosts.

Comparisons between the results of the metabolic and behavioural experiments are tentative, as they were conducted during different years when minnows were different ages. In fact, it is likely that the minnows used during visual experiments experienced their first infections at an older age than fish in the wild, which may have influenced host responses. The scope of the investigation into infection-induced metabolic changes was also comparatively limited, with experimental fish representing a reduced range of infection conditions (i.e. parasite ontogeny and the infection rate experienced by hosts). Therefore, future investigations of *O. ptychocheilus* infection of fathead minnow hosts should determine the specific mechanisms underlying behavioural and metabolic alterations during different stages of a first infection within a single young host. Moreover, monitoring changes across multiple exposures as fish age may provide a more realistic set of fish infection conditions, since wild hosts often experience multiple infection events overtime, and therefore, house various ages of infections. Further, previous research suggests that fish may be able to better compensate for infection-related pathologies if smaller exposures occur over a longer period of time (Sandland et al., 2001). Therefore, the described results may represent host responses under more extreme infection conditions. The inclusion of these factors in future studies will lead to a better understanding of *O. ptychocheilus* modifications of host metabolism and antipredator behaviours. Also, as modifications to resting metabolic rate or SMR are indicative of hosts' ability to utilize energy (Biro & Stamps, 2010) and perform high energy activities (Slavík et al., 2017), investigations of the metabolic capacity related to aerobic scope will help us to understand how parasite-induced alterations to metabolism influence host behaviour. This not only has implications for a wide variety of host behaviours, including antipredator responses, it also is indicative of thermoregulatory capabilities (Lapointe et al., 2014; Bruneaux et al., 2017) and the ability to tolerate anoxia (Petersen & Gamperl, 2010).

Although the discussed limitations of my experiments make conclusions regarding and comparisons between metabolic and behavioural alterations challenging, my results provide an important insight into the *O. ptychocheilus*-induced phenotypic alterations of intermediate fathead minnow hosts. In particular, my investigations have significant implications for the respective roles of *O. ptychocheilus* and intermediate minnow hosts in the observed physiological and behavioural outcomes of infected fish. Beyond the *O. ptychocheilus*-fathead minnow system, my findings also contribute to the growing body of research describing the

possible presence of subtle manipulations of hosts behaviour by trophically transmitted parasites as well as explore the relatively unknown alterations of aerobic metabolism related to distinct periods of trematode development. These findings will provide a platform on which future investigations can build comprehensive explorations of similar trophically transmitted parasite systems.

Chapter 5 - Literature Cited

Anderson, R. M. (1986). The invasion, persistence and spread of infectious diseases within animal and plant communities. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *314*(1167), 533–570.

Arnold, G. P. (1974). Rheotropism in fishes. *Biological Reviews*, *49*(4), 515–576.

Avitan, L., Pujic, Z., Hughes, N. J., Scott, E. K., & Goodhill, G. J. (2016). Limitations of neural map topography for decoding spatial information. *Journal of Neuroscience*, *36*(19), 5385–5396.

Bairagi, N., & Adak, D. (2015). Complex dynamics of a predator-prey-parasite system: an interplay among infection rate, predator's reproductive gain and preference. *Ecological Complexity*, *22*, 1–12.

Ballabeni, P. (1994). Experimental differences in mortality patterns between European minnows, *Phoxinus phoxinus*, infected with sympatric or allopatric trematodes, *Diplostomum phoxini*. *Journal of Fish Biology*, *45*(2), 257–267.

Barber, I., & Crompton, D. W. (1997). The ecology of *Diplostomum phoxini* infections in two minnow (*Phoxinus phoxinus*) populations in Scotland. *Journal of Helminthology*, *71*(3), 189–96.

Barber, I., & Wright, H. A. (2005). Effects of parasites on fish behaviour: interactions with host physiology. *Fish Physiology*, *24*, 109–149.

Binning, S. A., Barnes, J. I., Davies, J. N., Backwell, P. R. Y., Keogh, J. S., & Roche, D. G. (2014). Ectoparasites modify escape behaviour, but not performance, in a coral reef fish. *Animal Behaviour*, *93*, 1–7.

Biro, P. A., & Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology & Evolution*, *25*(11), 653–659.

Blaxter, J. H. S., & Fuiman, L. A. (1990). The role of the sensory systems of herring larvae in evading predatory fishes. *Journal of the Marine Biological Association of the United Kingdom*, *70*(2), 413–427.

Bruneaux, M., Visse, M., Gross, R., Pukk, L., Saks, L., & Vasemagi, A. (2017). Parasite infection and decreased thermal tolerance: impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. *Functional Ecology*, *31*(1), 216–226.

Chabot, D., Steffensen, J. F., & Farrell, A. P. (2016). The determination of standard metabolic rate in fishes. *Journal of Fish Biology*, *88*(1), 81–121.

Chivers, D. P., & Smith, R. J. F. (1994). Fathead minnows, *Pimephales promelas*, acquire predator recognition when alarm substance is associated with the sight of unfamiliar fish. *Animal Behaviour*, *48*(3), 597–605.

Clark, T. D., Sandblom, E., & Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *Journal of Experimental*

Biology, *216*(15), 2771–2782.
Conn, D. B., Goater, C. P., & Bray, D. (2008). Developmental and functional ultrastructure of *Ornithodiplostomum ptychocheilus* diplostomula (*Trematoda: Strigeoidea*) during invasion of the brain of the fish intermediate host, *Pimephales promelas*. *Journal of Parasitology*, *94*(3), 635–642.
Crane, A. L., Fritts, A. K., Mathis, A., Lisek, J. C., & Barnhart, M. C. (2011). Do gill parasites influence the foraging and antipredator behaviour of rainbow darters, *Etheostoma caeruleum*? *Animal Behaviour*, *82*(4), 817–823.
Curtis, V. A. (2014). Infection-avoidance behaviour in humans and other animals. *Trends in Immunology*, *35*(10), 457–464.
Dill, L. M. (1974). The escape response of the zebra danio (*Brachydanio rerio*) I. The stimulus for escape. *Animal Behaviour*, *22*(3), 711–722.
Domenici, P. (2002). The visually mediated escape response in fish: predicting prey responsiveness and the locomotor behaviour of predators and prey. *Marine and Freshwater Behaviour and Physiology*, *35*(1–2), 87–110.
Eaton, R. C., Nissanov, J., & Wieland, C. M. (1984). Differential activation of Mauthner and non-Mauthner startle circuits in the zebrafish: implications for functional substitution. *Journal of Comparative Physiology A*, *155*(6), 813–820.
Ferrari, M. C. O., Lysak, K. R., & Chivers, D. P. (2010). Turbidity as an ecological constraint on learned predator recognition and generalization in a prey fish. *Animal Behaviour*, *79*(2), 515–519.
Filipsson, K., Brijs, J., Näslund, J., Wengström, N., Adamsson, M., Závorka, L., … Höjesjö, J. (2017). Encystment of parasitic freshwater pearl mussel (*Margaritifera margaritifera*) larvae coincides with increased metabolic rate and haematocrit in juvenile brown trout (*Salmo trutta*). *Parasitology Research*, *116*(4), 1353–1360.
Fisk, D. M., Powell, M. D., & Nowak, B. F. (2002). The effect of amoebic gill disease and hypoxia on survival and metabolic rate of Atlantic Salmon (*Salmo salar*). *BULLETIN-EUROPEAN ASSOCIATION OF FISH PATHOLOGISTS*, *22*(3), 190–194.
Fredensborg, B. L., & Longoria, A. N. (2012). Increased surfacing behavior in longnose killifish infected by brain-encysting trematode. *Journal of Parasitology*, *98*(5), 899–903.
Fuiman, L. A., Cowan Jr, J. H., Smith, M. E., & O'neal, J. P. (2005). Behavior and recruitment success in fish larvae: variation with growth rate and the batch effect. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(6), 1337–1349.
Fuiman, L. A., Rose, K. A., Cowan Jr, J. H., & Smith, E. P. (2006). Surival skills required for predator evasion by fish larvae and their relation to laboratory measures of performance. *Animal Behaviour*, *71*(6), 1389–1399.
Gause, W. C., Wynn, T. A., & Allen, J. E. (2013). Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths. *Nature Reviews Immunology*, *13*(8), 607–614.
Goater, C. P., Bray, D., & Conn, D. B. (2005). Cellular aspects of early development of *Ornithodiplostomum ptychocheilus* metacercariae in the brain of fathead minnows, *Pimephales promelas*. *Journal of Parasitology*, *91*(4), 814–821.
Godin, J. G. (1997). Evading Predators. In *Behaviour Ecology of Teleost Fishes* (pp. 191–236).
Gopko, M., Mikheev, V. N., & Taskinen, J. (2015). Changes in host behaviour caused by immature larvae of the eye fluke: evidence supporting the predation suppression hypothesis. *Behavioral Ecology and Sociobiology*, *69*(10), 1723–1730.

Griffin, M. J., Khoo, L. H., Steadman, J. M., Ware, C., Quiniou, S. M., Mischke, C. C., … Wise, D. J. (2014). Chronic pathology and longevity of *Drepanocephalus spathans* infections in juvenile channel catfish. *Journal of Aquatic Animal Health*, *26*(4), 210–218.

Harper, D. G., & Blake, R. W. (1990). Fast-start performance of rainbow trout *Salmo gairdneri* and Northern pike *Esox lucius*. *Journal of Experimental Biology*, *150*(1), 321–342.

Hartman, E. J., & Abrahams, M. V. (2000). Sensory compensation and the detection of predators: the interaction between chemical and visual information. *Proceedings of the Royal Society of London B: Biological Sciences*, *267*(1443), 571–575.

Hodos, W. (2009). Evolution of the cerebellum. In *The Encyclopedia of Neuroscience* (pp. 1240–1243).

James, C. T., Noyes, K. J., Stumbo, A. D., Wisenden, B. D., & Goater, C. P. (2008). Cost of exposure to trematode cercariae and learned recognition and avoidance of parasitism risk by fathead minnows *Pimephales promelas*. *Journal of Fish Biology*, *73*(9), 2238–2248.

Jones, M. A., Powell, M. D., Becker, J. A., & Carter, C. G. (2007). Effect of an acute necrotic bacterial gill infection and feed deprivation on the metabolic rate of Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms*, *78*(1), 29–36.

Katzir, G., & Camhi, J. M. (1993). Escape Response of Black Mollies (*Poecilia sphenops*) to Predatory Dives of a Pied Kingfisher (*Ceryle rudis*). *Copeia*, *1993*(2), 549–553.

Kekäläinen, J., Lai, Y. T., Vainikka, A., Sirkka, I., & Kortet, R. (2014). Do brain parasites alter host personality? Experimental study in minnows. *Behavioral Ecology and Sociobiology*, *68*(2), 197–204.

Kelly, D. W., Thomas, H., Thieltges, D. W., Poulin, R., & Tompkins, D. M. (2010). Trematode infection causes malformations and population effects in a declining New Zealand fish. *Journal of Animal Ecology*, *79*(2), 445–452.

Klein, S. L. (2003). Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. *Physiology and Behavior*, *79*(3), 441–449.

Korn, H., & Faber, D. S. (2005). The mauthner cell half a century later: review a neurobiological model for decision-making? *Neuron*, *47*(1), 13–28.

Kutyrev, I. A., Franke, F., Büscher, J., Kurtz, J., & Scharsack, J. P. (2014). In vitro effects of prostaglandin E2 on leucocytes from sticklebacks (*Gasterosteus aculeatus*) infected and not infected with the cestode *Schistocephalus solidus*. *Fish and Shellfish Immunology*, *41*(2), 473–481.

Lafferty, K. D., & Morris, K. A. (1996). Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology*, *77*(5), 1390–1397.

Laitinen, M., Siddall, R., & Valtonen, E. T. (1996). Bioelectronic monitoring of parasite-induced stress in brown trout and roach. *Journal of Fish Biology*, *48*(2), 228–241.

Lapointe, D., Vogelbein, W. K., Fabrizio, M. C., Gauthier, D. T., & Brill, R. W. (2014). Temperature, hypoxia, and mycobacteriosis: effects on adult striped bass *Morone saxatilis* metabolic performance. *Diseases of Aquatic Organisms*, *108*(2), 113–127.

Lawlor, E. (2016). *Interactions of Disease and Temperature on the Aerobic Scope of Freshwater Fish and Implications for Changing Climates*. Murdoch University.

Laxmareddy, B., & Benarjee, G. (2013). Intestinal histopathology of trematode infected fish, *Channa striatus*. *Biolife*, *1*(1), 29–31.

Leef, M. J., Harris, J. O., & Powell, M. D. (2007). Metabolic effects of amoebic gill disease (AGD) and chloramine-T exposure in seawater-acclimated Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms*, *78*(1), 37–44.

Lima Jr, D. P., Giacomini, H. C., Takemoto, R. M., Agostinho, A. A., & Bini, L. M. (2012). Patterns of interactions of a large fish – parasite network in a tropical floodplain. *Journal of Animal Ecology*, *81*(4), 905–913.

Lotem, A., Schechtman, E., & Katzir, G. (1991). Capture of submerged prey by little egrets, *Egretta garzetta garzetta*: strike depth, strike angle and the problem of light refraction. *Animal Behaviour*, *42*(3), 341–346.

Madison, B. N., Woo, P. T. K., & Bernier, N. J. (2013). Duress without stress: Cryptobia infection results in HPI axis dysfunction in rainbow trout. *Journal of Endocrinology*, *218*(3), 287–297.

Maizels, R. M., & Yazdanbakhsh, M. (2003). Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology*, *3*(9), 733–744.

Matisz, C. E., & Goater, C. P. (2010). Migration, site selection, and development of *Ornithodiplostomum sp.* metacercariae (*Digenea: Strigeoidea*) in fathead minnows (*Pimephales promelas*). *International Journal for Parasitology*, *40*(13), 1489–1496.

Matisz, C. E., Goater, C. P., & Bray, D. (2010a). Density and maturation of rodlet cells in brain tissue of fathead minnows (*Pimephales promelas*) exposed to trematode cercariae. *International Journal for Parasitology*, *40*(3), 307–312.

Matisz, C. E., Goater, C. P., & Bray, D. (2010b). Migration and site selection of *Ornithodiplostomum ptychocheilus* (*Trematoda: Digenea*) metacercariae in the brain of fathead minnows (*Pimephales promelas*). *Parasitology*, *137*(4), 719–731.

McElroy, E. J., & de Buron, I. (2014). Host performance as a target of manipulation by parasites: a meta-analysis. *Journal of Parasitology*, *100*(4), 399–410.

Meager, J. J., Domenici, P., Shingles, A., & Utne-palm, A. C. (2006). Escape responses in juvenile Atlantic cod *Gadus morhua L.*: the effects of turbidity and predator speed. *Journal of Experimental Biology*, *209*(20), 4174–4184.

Meakins, R. H., & Walkey, M. (1975). The effects of parasitism by the plerocercoid of *Schistocephalus solidus* Muller 1776 (*Pseudophyllidea*) on the respiration of the three-spined stickleback *Gasterosteus aculeatus L*. *Journal of Fish Biology*, *7*(6), 817–824.

Mikheev, V. N., Pasternak, A. F., Taskinen, J., & Valtonen, E. T. (2010). Parasite-induced aggression and impaired contest ability in a fish host. *Parasites & Vectors*, *3*(1), 17.

Nilsson, G. (1996). Brain and body oxygen requirements of *Gnathonemus petersii*, a fish with an exceptionally large brain. *Journal of Experimental Biology*, *199*(3), 603–607.

Nilsson, J. Å., Granbom, M., & Råberg, L. (2007). Does the strength of an immune response reflect its energetic cost? *Journal of Avian Biology*, *38*(4), 488–494.

Pan, T., Gladen, K., Duncan, E. C., Cotner, S., Cotner, J. B., McEwen, D. C., & Wisenden, B. D. (2016). Bold, sedentary fathead minnows have more parasites. *Zebrafish*, *13*(4), 248–255.

Parker, G. A., Ball, M. A., Chubb, J. C., Hammerschmidt, K., & Milinski, M. (2009). When should a trophically transmitted parasite manipulate its host? *Evolution*, *63*(2), 448–458.

Perrot-Minnot, M. J., Sanchez-Thirion, K., & Cézilly, F. (2014). Multidimensionality in host manipulation mimicked by serotonin injection. *Proceedings of the Royal Society of London B: Biological Sciences*, *281*(1796), 20141915.

Petersen, L. H., & Gamperl, A. K. (2010). Effect of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (*Gadus morhua*). *Journal of Experimental Biology*, *213*(5), 808–819.

Pita, D., Moore, B. A., Tyrrell, L. P., & Fernández-Juricic, E. (2015). Vision in two cyprinid fish: implications for collective behavior. *PeerJ*, *3*, e113.

Poulin, R. (2010). Parasite manipulation of host behavior: an update and frequently asked questions. In *Advances in the Study of Behavior* (1st ed., Vol. 41, pp. 151–186). Elsevier Inc.

Poulin, R., & Maure, F. (2015). Host manipulation by parasites: a look back before moving forward. *Trends in Parasitology*, *31*(11), 563–570.

Powell, M. D., & Gamperl, A. K. (2016). Effects of *Loma morhua* (*Microsporidia*) infection on the cardiorespiratory performance of Atlantic cod *Gadus morhua* (L). *Journal of Fish Diseases*, *39*(2), 189–204.

Powell, M. D., Speare, D. J., Daley, J., & Lovy, J. (2005). Differences in metabolic response to *Loma salmonae* infection in juvenile rainbow trout *Oncorhynchus mykiss* and brook trout *Salvelinus fontinalis*. *Diseases of Aquatic Organisms*, *67*(3), 233–237.

Radabaugh, D. C. (1980). Encystment site selection in the brain-inhabiting metacercariae of *Ornithodiplostomum ptychocheilus* (*Trematoda: Strigeoidea*). *Journal of Parasitology*, *66*(1), 183–184.

Raffel, T. R., Martin, L. B., & Rohr, J. R. (2008). Parasites as predators: unifying natural enemy ecology. *Trends in Ecology and Evolution*, *23*(11), 610–618.

Robar, N., Murray, D. L., & Burness, G. (2011). Effects of parasites on host energy expenditure: the resting metabolic rate stalemate. *Canadian Journal of Zoology*, *89*(11), 1146–1155.

Roberts, B. L., van Rossem, A., & de Jager, S. (1992). The influence of cerebellar lesions on the swimming performance of the trout. *Journal of Experimental Biology*, *167*(1), 171–178.

Rodríguez, F., Durán, E., Gómez, A., Ocaña, F. M., Álvarez, E., Jiménez-Moya, F., … Salas, C. (2005). Cognitive and emotional functions of the teleost fish cerebellum. *Brain Research Bulletin*, *66*(4–6), 365–370.

Roeser, T., & Baier, H. (2003). Visuomotor behaviors in larval zebrafish after GFP-Guided laser ablation of the optic tectum. *Journal of Neuroscience*, *23*(9), 3726–3734.

Sandland, G. J., & Goater, C. P. (2000). Development and intensity dependence of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows (*Pimephales promelas*). *Journal of Parasitology*, *86*(5), 1056–1060.

Sandland, G. J., & Goater, C. P. (2001). Parasite-induced variation in host morphology: brain-encysting trematodes in fathead minnows. *Journal of Parasitology*, *87*(2), 267–272.

Sandland, G. J., Goater, C. P., & Danylchuk, A. J. (2008). Population dynamics of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows (*Pimephales promelas*) from four Northern-Alberta Lakes. *Journal of Parasitology*, *87*(4), 744–748.

Santos, E. G. N., & Santos, C. P. (2013). Parasite-induced and parasite development-dependent alteration of the swimming behavior of fish hosts. *Acta Tropica*, *127*(1), 56–62.

Schleppe, J. L., & Goater, C. P. (2004). Comparative Life Histories of Two Diplostomid Trematodes, *Ornithodiplostomum ptychocheilus* and *Posthodiplostomum minimum*. *Journal of Parasitology*, *90*(6), 1387–1390.

Secombes, C. J., & Chappell, L. H. (1996). Fish immune responses to experimental and natural infection with helminth parasites. *Annual Review of Fish Diseases*, *6*, 167–177.

Seppälä, O., Karvonen, A., & Valtonen, E. T. (2011). Eye fluke-induced cataracts in natural fish populations: is there potential for host manipulation? *Parasitology*, *138*(2), 209–214.

Seppänen, E., Kuukka, H., Huuskonen, H., & Piironen, J. (2008). Relationship between standard metabolic rate and parasite-induced cataract of juveniles in three Atlantic salmon stocks. *Journal of Fish Biology*, *72*(7), 1659–1674.

Seppänen, E., Kuukka, H., Voutilainen, A., Huuskonen, H., & Peuhkuri, N. (2009). Metabolic

depression and spleen and liver enlargement in juvenile Arctic charr *Salvelinus alpinus* exposed to chronic parasite infection. *Journal of Fish Biology*, *74*(3), 553–561.
Shaw, J. C., Hechinger, R. F., Lafferty, K. D., & Kuris, A. M. (2010). Ecology of the brain trematode *Euhaplorchis californiensis* and its host, the California killifish (*Fundulus parvipinnis*). *Journal of Parasitology*, *96*(3), 482–490.
Shaw, J. C., Korzan, W. J., Carpenter, R. E., Kuris, A. M., Lafferty, K. D., Summers, C. H., & Øverli, Ø. (2009). Parasite manipulation of brain monoamines in California killifish (*Fundulus parvipinnis*) by the trematode *Euhaplorchis californiensis. Proceedings of the Royal Society of London B: Biological Sciences*, *276*(1659), 1137–1146.
Shaw, J. C., & Øverli, Ø. (2012). Brain-encysting trematodes and altered monoamine activity in naturally infected killifish *Fundulus parvipinnis*. *Journal of Fish Biology*, *81*(7), 2213–2222.
Shirakashi, S., & Goater, C. P. (2001). Brain-encysting parasites affect visually-mediate behaviours of fathead minnows. *Ecoscience*, *8*(3), 289–293.
Shirakashi, S., & Goater, C. P. (2002). Intensity-dependent alteration of minnow (*Pimephales promelas*) behavior by a brain-encysting trematode. *Journal of Parasitology*, *88*(6), 1071–1074.
Shirakashi, S., & Goater, C. P. (2005). Chronology of parasite-induced alteration of fish behaviour: effects of parasite maturation and host experience. *Parasitology*, *130*(2), 177–183.
Slavík, O., Horký, P., Douda, K., Velíšek, J., Kolářová, J., & Lepič, P. (2017). Parasite-induced increases in the energy costs of movement of host freshwater fish. *Physiology and Behavior*, *171*, 127–134.
Soengas, J. L., & Aldegunde, M. (2002). Energy metabolism of fish brain. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *131*(2), 271–296.
Springer, A. D., Easter Jr, S. S., & Agranoff, B. W. (1977). The role of the optic tectum in various visually mediated behaviors of goldfish. *Brain Research*, *128*(3), 393–404.
Stumbo, A. D., Goater, C. P., & Hontela, A. (2012). Parasite-induced oxidative stress in liver tissue of fathead minnows exposed to trematode cercariae. *Parasitology*, *139*(12), 1666–1671.
Svendsen, M. B. S., Bushnell, P. G., & Steffensen, J. F. (2016). Design and setup of intermittent-flow respirometry system for aquatic organisms. *Journal of Fish Biology*, *88*(1), 26–50.
Temizer, I., Donovan, J. C., Baier, H., & Semmelhack, J. L. (2015). A visual pathway for looming-evoked escape in larval zebrafish. *Current Biology*, *25*(14), 1823–1834.
Thomas, G. R., Taylor, J., & de Leaniz, C. G. (2014). Does the parasitic freshwater pearl mussel *M. margaritifera* harm its host? *Hydrobiologia*, *735*(1), 191–201.
Trubiroha, A., Kroupova, H., Wuertz, S., Frank, S. N., Sures, B., & Kloas, W. (2010). Naturally-induced endocrine disruption by the parasite *Ligula intestinalis* (*Cestoda*) in roach (*Rutilus rutilus*). *General and Comparative Endocrinology*, *166*(2), 234–240.
Tyrrell, L. P., & Fernández-Juricic, E. (2015). 12 Sensory systems and escape behavior. In *Escaping from predators: An integrative view of escape decisions* (p. 322).
Vissio, P. G., Cánepa, M. M., & Maggese, M. C. (2008). Brain-derived neurotrophic factor (BDNF)-like immunoreactivity localization in the retina and brain of *Cichlasoma dimerus* (*Teleostei, Perciformes*). *Tissue and Cell*, *40*(4), 261–270.
Voutilainen, A., Figueiredo, K., & Huuskonen, H. (2008). Effects of the eye fluke *Diplostomum*

spathaceum on the energetics and feeding of Artic charr *Salvelinus alpinus*. *Journal of Fish Biology*, *73*(9), 2228–2237.
Wagner, G. N., Hinch, S. G., Kuchel, L. J., Lotto, A., Jones, S. R. M., Patterson, D. A., … Farrell, A. P. (2005). Metabolic rates and swimming performance of adult Fraser River sockeye salmon (*Oncorhynchus nerka*) after a controlled infection with *Parvicapsula minibicornis*. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(9), 2124–2133.
Walker, J. A., Ghalambor, C. K., Griset, O. L., McKenney, D., & Reznick, D. N. (2005). Do faster starts increase the probability of evading predators? *Functional Ecology*, *19*(5), 808–815.
Walkey, M., & Meakins, R. H. (1970). An attempt to balance the energy budget of a host-parasite system. *Journal of Fish Biology*, *2*(4), 361–372.
Webb, P. W. (1976). The effect of size on the fast-start performance of rainbow trout *Salmo gairdneri*, and a consideration of piscivorous predator-prey interactions. *Journal of Experimental Biology*, *65*(1), 157–177.
Webb, P. W. (1982). Avoidance responses of fathead minnow to strikes by four teleost predators. *Journal of Comparative Physiology*, *147*(3), 371–378.
Worth, A. R., Lymbery, A. J., & Thompson, R. C. A. (2013). Adaptive host manipulation by *Toxoplasma gondii*: fact or fiction? *Trends in Parasitology*, *29*(4), 150–155.
Yoshida, M., Okamura, I., & Uematsu, K. (2004). Involvement of the cerebellum in classical fear conditioning in goldfish. *Behavioural Brain Research*, *153*(1), 143–148.