

Direct and indirect effects of a trematode parasite
on the endemic freshwater fish *Galaxias anomalus*

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Te Whare Wānanga o Otāgo

Dedication

For my Father

*From a young age you inspired the scientist within me,
I am forever grateful for your unconditional support and belief in me*

Abstract

Fish have critical periods during early life where mortality is extremely high. There are a multitude of factors which can influence mortality, however, parasitism is often overlooked as a causal factor of mortality during this period. Understanding the extent to which biotic and abiotic factors, such as parasites, can influence fish survival, is paramount for effective management of threatened species. The purpose of this study was to investigate the effects of a trematode parasite, *Telogaster opisthorchis* (a parasite known to cause malformations in young fish), on the critical larval stage of its second intermediate fish host *Galaxias anomalus*, a small, endemic, freshwater fish vulnerable to extinction. The influence of *T. opisthorchis* on the condition, size, and survival of *G. anomalus* fish was tested by experimentally infecting larval fish of different ages with different doses under standard conditions. Additionally, the likelihood that parasites cause malformations and their influence on the swimming ability of their hosts were also investigated. Swimming tests were carried out using a unidirectional propeller-driven flume where individual fish swimming ability was monitored over a fixed period. Results demonstrate that *T. opisthorchis* can affect *G. anomalus* survival directly via reduced condition, and indirectly via malformations and lowered swimming ability. However there was no effect of parasitism on condition or size, nor was there any effect of infection level on the presence of skeletal malformations, the reasons for which are discussed in the text. The effect of parasitism in early life stages of fish influences survival, and in wild populations, may be exacerbated by additional stressors, with consequences at both the population and community level.

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Chapter 1:

General introduction



R. Paterson

Galaxias anomalus with many parasite cysts seen through the skin of the fish as small white dots (Photo used with permission from Rachel Paterson)

1.1 Parasitism as a driver of population dynamics

Many biological and environmental processes can influence the health and survival of a population (Fogarty 1993, Letcher et al. 1996, Ebert 2005a, Townsend et al. 2011). Although there are numerous factors which can influence survival, the literature consistently draws our attention to well-known biotic effects such as competition or predation (Krebs et al. 1995, Hanna et al. 1997, Begon et al. 2005, Ebert 2005a, Forchhammer et al. 2008, Johnson & Zuniga-Vega 2009). Lack of competition or predation can alter population dynamics by increasing survival, growth or fecundity, whereas an increase in competition or predation, or the introduction of a new competitor/predator, could have the opposite effect (Berven 1990, Delahay et al. 1995, Hebblewhite et al. 2002, Dalkvist et al. 2011). Recently, ecologists have become increasingly aware of additional biotic influences on species survival. For example, parasites can impact host survival at the individual level, and may further influence dynamics at the population and community levels (Lafferty et al. 2008, Tompkins et al. 2011).

In recent years, there has been an increasing amount of research on the role of parasitism in population dynamics (Combes 1991, Minchella & Scott 1991, Grenfell & Gulland 1995, Ebert 2005a). Populations respond differently under the influence of parasites, with the latter either reducing host density, causing host population fluctuations, pushing a host population to extinction, or at times, having no apparent effect (Minchella & Scott 1991, Kohler & Wiley 1992, Cattadori et al. 2005, de Castro & Bolker 2005b, Ebert 2005a, Lopez 2005, Redpath et al. 2006, Rosa et al. 2011, Townsend et al. 2011). Furthermore, Anderson and May (1978), and Murdoch (1994) developed models demonstrating that parasites can actually help to stabilise populations. The variation in population response to parasitism is due to the complex nature of population dynamics. A myriad of environmental and biological factors, including parasitism, each with a varying influence on the population, makes it difficult to tease apart and determine the relative impact of each component (Scott & Dobson 1989, Minchella & Scott 1991, Cattadori et al. 2005, Ebert 2005a, Reeves et al. 2010, Townsend et al. 2011). Likewise, it can be challenging to isolate the impact of one

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particular type of parasite on a host as individuals and populations are often simultaneously influenced by multiple parasite species.

Studies on fish provide good model systems to elucidate the relative roles of different population drivers, including parasitism. There have been many studies looking at population dynamics of both marine and freshwater fish in the wild. Many focus on food availability and predation, which are known to influence populations (Treasurer et al. 1992, Sirois & Dodson 2000a, Pettorelli et al. 2011). Even though the influence of parasitism on populations is becoming recognised, most studies focus on adult fish, and comparatively few researchers have specifically looked at survival of early life stages and the repercussions it has on the structure of the population as a whole (Anderson & May 1978, May & Anderson 1978, Grosberg & Levitan 1992, Ebert 2005a, Grutter et al. 2010). The majority of studies on larval fish have focussed on survival and recruitment (Anderson 1988, Grimes & Finucane 1991, Letcher et al. 1996, Cribb et al. 2000), with starvation and predation identified as the main factors determining recruitment of young fish (Sirois & Dodson 2000b). However there are numerous alternative explanations for variability in recruitment including temperature, food availability, water currents, and parasitism (Grimes & Finucane 1991, Houde 1997, Rutherford et al. 1997, Sirois & Dodson 2000b, Grutter et al. 2010).

Survival curves of fish show a high mortality rate in the early stages of development, typical of a type III survival curve (Dahlberg 1979, Houde 1987, Leis 1991, Ebert 2005a). Type III survival curves are characteristic of species where parents have many offspring, yet there is little to no parental care for those offspring (Dahlberg 1979). It has been estimated that less than 1% of the juvenile cohort make it through to maturity (Bourque et al. 2006). The time of highest mortality is called the critical period, and typically spans through the egg, yolk-sac larvae, and post-larval stages. The reasons behind the high number of fatalities are often attributed to eggs or newly hatched larvae not developing correctly, or not being able to cope with stressful environmental conditions (Houde 1987, Anderson 1988, Letcher et al. 1996, Houde 1997, Kattel & Closs 2007, Sun et al. 2012). As larval fish are very small, they are much more sensitive to abiotic factors such as chemicals, turbulence, and temperature, as well as biotic factors,

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including foraging ability, evading predators, or parasites (Treasurer et al. 1992, Sirois & Dodson 2000b). Therefore, surviving the larval stage is crucial, and is a key determinant of population stability. Previous studies have shown that larger juveniles are more likely to escape predation and navigate strong currents, therefore, fast growth which limits the amount of time young fish are most vulnerable to predators (the ‘stage duration’ hypothesis) is greatly advantageous (Anderson 1988, Drucker 1996, Letcher et al. 1996, Hare & Cowen 1997).

Mortality and recruitment are highly variable in juvenile fish, and as such, they are rarely used in laboratory and field studies (Grosberg & Levitan 1992). As a consequence, there is much less known about the impact of parasitism on age-0 fish (Longshaw et al. 2010). For instance, we know that many parasitic infections often have little impact on adult hosts, however, the impact of parasitic infection in young fish is less well known, and may previously have been overlooked as a main factor driving population dynamics (Strathmann et al. 2002, King & Cone 2009, Poulin et al. 2012). Even Anderson (1988), who produced a thorough review of factors affecting the survival of juvenile fish, neglected to mention any impact of parasitism. Since then, however, the number of relevant studies has increased. It has been acknowledged that parasites have the potential to drastically reduce the condition and survival of juveniles within a population and they can be a determining factor in the size of the subsequent adult population (Berven 1990, Williams & Jones 1994, Rigby & Dufour 1996, Bourque et al. 2006, Longshaw et al. 2010, Sun et al. 2012).

As well as having a direct impact on survival by reducing the condition of the fish, parasites may influence mortality indirectly, for example changing the behaviour or conspicuousness of the host could increase the likelihood of predation (Barber et al. 2000). Previous studies of fish have investigated the influence of parasitism on predator response (Poulin 1993a), shoaling behaviour (Poulin & Fitzgerald 1989b), and swimming ability (Butler & Milleman 1971), all of which can be influenced by parasites. Often these changes in behaviour are a by-product of infection and do not always lead to increased transmission of the parasite by predation or other means (Poulin 1995). However, over time, selection has favoured many trophically-transmitted parasites that modify their host in such

a way that will increase the likelihood of predation of the current host by the next one in the parasite's life cycle (Poulin 2007). To understand this concept, one first needs to consider the life history of parasites and the transmission challenges it involves.

1.2 Host parasite relationships and parasite life cycles

Parasitism is a symbiotic relationship between a host and parasite (Cheng 1991). In this relationship, the parasite benefits from having a refuge and exploiting resources from its host (Lemly & Esch 1984). The host is therefore at a disadvantage and is often weakened by the presence of the parasite (Cheng 1991, Begon et al. 2005). Not surprisingly, it is in any potential hosts' best interest to avoid being parasitised, and hosts have therefore evolved strategies to reduce the likelihood of infection (Sheldon & Verhulst 1996, Poulin 2007). Consequently, the evolution of traits which increase the likelihood of a parasite encountering its next host is equally important. Parasite lifecycles vary widely in their level of complexity. Some parasites have a direct life cycle comprising a free living stage and only one host species in which they reproduce. Others have what is called an indirect or complex life cycle which require one or more intermediate hosts as well as a definitive host where the parasites reproduce sexually (Poulin 2001).

Trematodes (flukes, phylum Platyhelminthes) provide good examples of parasites with complex life cycles. Generally a trematode life cycle consists of an invertebrate first intermediate host, usually a mollusc, either a vertebrate or invertebrate second intermediate host, and a vertebrate definitive host. Although complex life cycles have evolved many times independently, which suggests they are advantageous, there can be major costs associated with having such a complex lifestyle (Poulin 2001, Rauch et al. 2005). For instance, the odds of any one parasite surviving the transmission through several hosts to its final destination within its definitive host are exceptionally low (Poulin & Fitzgerald 1988, Iwasa & Wada 2006).

1.3 Strategies employed by a parasite to increase the likelihood of transmission

Parasites can fine-tune their lifecycle using strategies which increase the chance of transmission at each stage of the lifecycle (Haas et al. 1995, Poulin 2007). For example, they can time egg release with environmental cues such as temperature, rain, or photoperiod, when external conditions favour the survival of the parasite, or when the next target host is more abundant (Tinsley 1990, Poulin 2007). As well as employing these non-invasive strategies, evolution has also favoured many parasites which use manipulative strategies to alter the host's phenotype, sometimes in fairly drastic ways. By altering the behaviour, morphology, and physiology of their hosts, they can increase the exposure or conspicuousness of their intermediate host to the definitive host, thereby making them more susceptible to predation and increasing the likelihood of transmission (Bakker et al. 1997). This process is known as parasite increased trophic transmission.

There are many examples of parasites altering host phenotype. A textbook example is that of the digenean parasite *Leucochloridium* spp., which alter the size, shape, colour, and movement of the tentacles of its snail intermediate host, attracting the definitive host to consume the snail and its parasites. Another example is that of practically any acanthocephalan parasite, which all appear capable of modifying the behaviour and colour of their intermediate crustacean host by altering activity levels, photoreaction, and habitat choice, which again increase the exposure of the crustacean, and therefore transmission of the parasite to its definitive host (Moore 1984). Although the supposed increase in transmission success is not documented, many of these cases of host modification by parasites appear adaptive.

1.4 Parasite-induced malformations

Some parasites using amphibians as intermediate hosts cause severe physical malformations when they infect during the host's larval stage (Johnson et al. 2002, Bowerman & Johnson 2003, Johnson & Sutherland 2003, Schotthoefer et al. 2003a, Rohr et al. 2008). Initially, it was presumed that malformations

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occurring in amphibians were due to pollution; however, in the past few years it was established experimentally that widespread malformations in amphibians in North America were due mainly to trematode parasitism (genus *Ribeiroia*) and not to pollution (Johnson et al. 2001). Although they are very small, it is likely that trematode cysts can interfere with development if they encyst at the right time and in the right location within the host body (Stopper et al. 2002, Schotthoefer et al. 2003a, Holland 2009, Jayawardena et al. 2010). In particular, if the *Ribeiroia* parasite encysts near the limb bud of a developing tadpole, the result may be a frog with several limbs from a single limb bud (Figure 1.1) or even the absence of a limb altogether (Johnson et al. 2001). Malformations in frogs are likely to decrease the condition of individuals, ultimately leading to a greater chance of predation by the parasite's avian host (Johnson et al. 1999, Rohr et al. 2008). Because malformations caused by the parasites may lead to increased predation and transmission of the parasite to its definitive host (in this case, a frog-eating bird), it has been assumed that they may be adaptive (Goodman & Johnson 2011).



Figure 1.1: Malformed Oregon spotted frog (*Rana pretiosa*), with two extra, poorly developed hind limbs. The malformations are attributed to the trematode *Ribeiroia* sp. This figure (from Johnson & Sutherland 2003), reproduced with permission from Elsevier.

As well as malformations in amphibians, there are numerous reports of malformed fish in the literature. Malformed fish are rarely observed in the wild, presumably because they don't survive to maturity, however they are frequently observed in aquaculture hatcheries. Malformations typically occur when fish are in the larval phase before their bones are fully formed and mineralised (Lall & Lewis-McCrea 2007). Numerous factors can impede normal development of the larvae and result in skeletal malformations (Cahu et al. 2003). Anomalies in hatchery raised fish have previously been attributed to rearing temperature (Bertolini et al. 1991, Polo et al. 1991), nutrient deficiencies (Barahonafernandes 1982, Koven et al. 1990, Dedi et al. 1995, Takeuchi et al. 1995), water currents (Divanach et al. 1997), pollution (Muscatello et al. 2006), and genetics (Lettice et

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al. 2003). However, much less is known about the causes of malformations in wild fish.

Recently, Kelly et al. (2010b) discovered a natural population of the New Zealand fish *Galaxias anomalus* (Stokell 1959), with fin and spinal malformations they later attributed to the trematode parasite *Telogaster opisthorchis*. Malformations included multiple curvatures of the spine, and multiple, asymmetric, or missing fins (Figure 1.2) (Kelly et al. 2010b). It is likely that the mechanisms by which trematode parasites cause malformations in fish are similar to that of trematodes such as *Ribeiroia* and their amphibian hosts in North America. Adult *G. anomalus* are often found infected with many *T. opisthorchis* parasites but rarely with malformations. However, when these fish are experimentally infected at a young age, malformations occurred in over 80% of fish (Kelly et al. 2010b). Furthermore, Kelly et al. (2010b) also found that the frequency of malformed individuals decreases rapidly over time in natural populations, suggesting that malformed fish incur higher mortality, a phenomenon with potential consequences for the dynamics of infected populations. Little is known about the implications of malformations at both the individual and population level as parasite-induced malformations have only recently been discovered. Therefore, my thesis will build on the pioneering work of David Kelly and his colleagues, investigating the impact of *T. opisthorchis* parasites and malformations in juvenile *G. anomalus*, including survival when exposed to parasites at different ages or varying levels of exposure, and comparing swimming ability of both infected and uninfected fish.

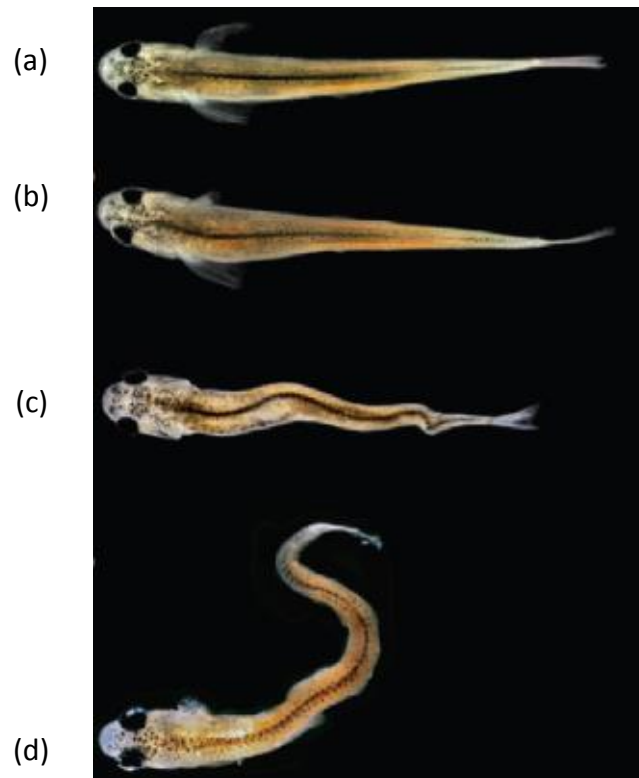


Figure 1.2: Normal and malformed larval *G. anomalus* (2–3 weeks post-hatch) from the wild. Normal fish (a), lateral spinal anomalies (b, c, d). Lateral malformations of one (b), five (c) and four (d) spinal deviations. (Photograph used with permission from David Kelly)

1.5 The parasite *Telogaster opisthorchis*

Telogaster opisthorchis is a digenean trematode (family Cryptogonimidae) with a complex life cycle involving three hosts: a snail, a small fish, and an eel (Figure 1.3). All three of these hosts must be utilised in the right order, for the mature worm to sexually reproduce, making the chances of any one parasite surviving and reproducing a rare event.

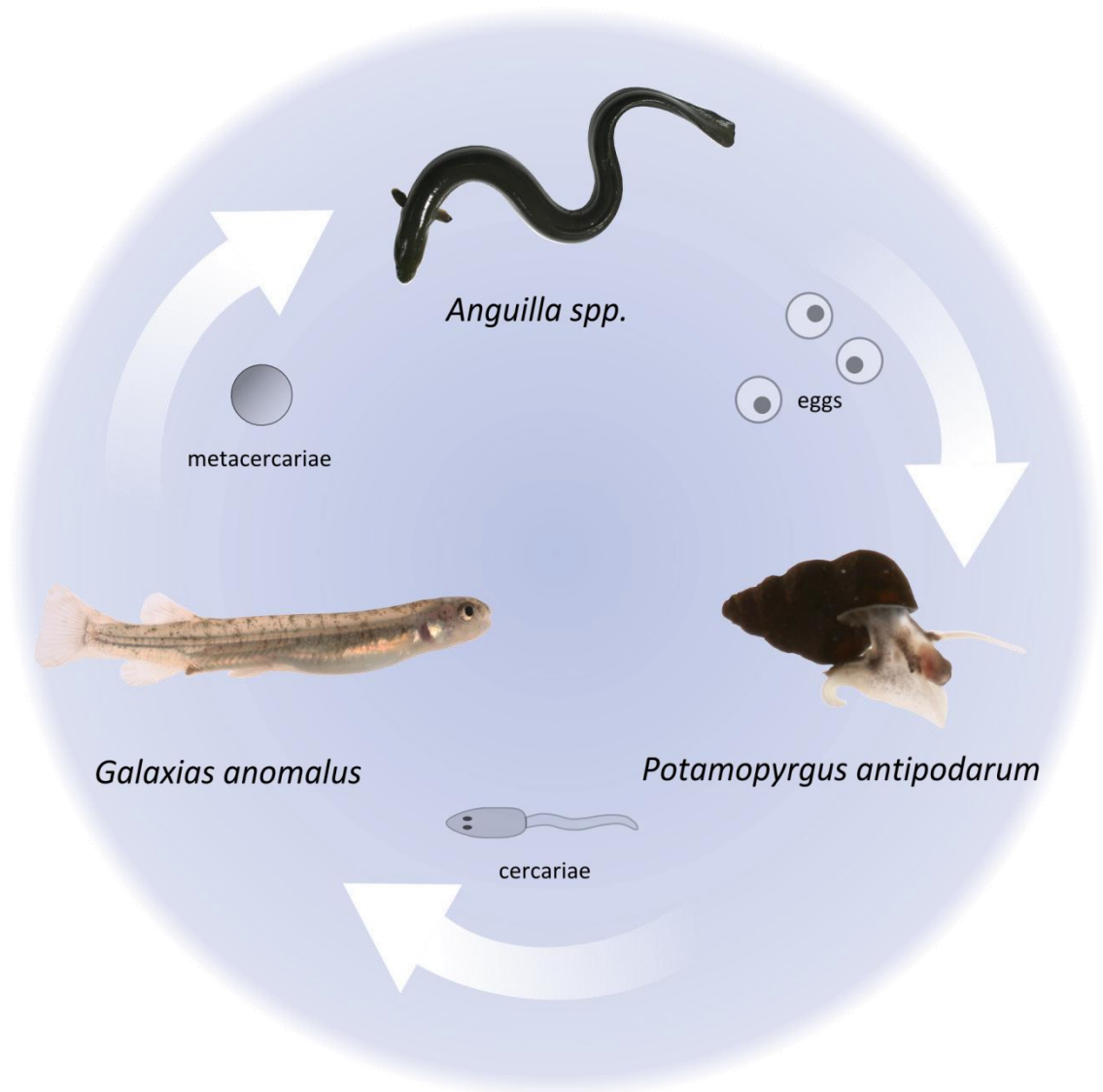


Figure 1.3: Typical complex life cycle of a trematode. The trematode *T. opisthorchis* hatches in the water column before infecting the snail (*P. antipodarum*), its first intermediate host. Cercariae leave the snail host and infect the second intermediate host, a fish (*G. anomalus*) through the skin. The parasite encysts in the tissue and waits for the fish to be ingested by the parasite's definitive host, an eel (*Anguilla spp.*), where it lays its eggs which will then be released back into the water column. (Eel image used with permission from Alvin Setiawan)

Mature parasites reside in the posterior part of the intestine in a New Zealand longfin or short-finned eel (*Anguilla dieffenbachii* or *A. australis*) where they reproduce sexually (MacFarlane 1945). Eggs are released in the intestine and

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pass out into the water column with faecal matter. The eggs hatch into small ciliated larvae known as miracidia which then seek out and infect the first intermediate host, the snail *Potamopyrgus antipodarum*. Miracidia develop further into rediae within the snail before producing tailed cercariae through asexual multiplication. Cercariae emerge from the snail into the water column. These cercariae are strongly attracted to light and vigorously swim toward the surface of the water before relaxing and allowing themselves to descend down again - possibly increasing their chances of coming across a suitable second intermediate host, of which there are thought to be at least 15 species of small fish, mostly galaxiids and electroids (Macfarlane 1952, Hine et al. 2000). Cercariae penetrate the fish host through the skin, generally on the fins but also through the snout or eyes (MacFarlane 1945). They attach themselves using their oral sucker and use enzymes and cuticular spines to tear through the skin before dropping their tails and pushing their way through into the muscle tissue (MacFarlane 1945). After finding a suitable place to reside, generally in the muscle tissue, but sometimes in the head or body cavity, they form a cyst wall around themselves as they transform into metacercariae, which can quite often be seen through the skin of the fish (Figure 1.4). Transmission back to the definitive eel host occurs when an eel feeds on a fish harbouring these metacercariae.



Figure 1.4: *Galaxias anomalus* with many *T. opisthorchis* parasites visible as small white dots through the skin on the fish.

1.5.1 Hosts of *Telogaster opisthorchis*

1.5.1.1 Snail first intermediate host: *Potamopyrgus antipodarum*

Potamopyrgus antipodarum (Hydrobiidae: Gray (1843)), the New Zealand mudsnail, is the only known first intermediate host for *T. opisthorchis*. It is a small snail, generally 4-6 mm in length (Winterbourn 1970), found in a range of freshwater habitats, and is widely distributed throughout New Zealand (Winterbourn 1969). *Potamopyrgus antipodarum* is host to at least 14 trematode species, the prevalence of any particular parasite being generally less than 5%, although their occurrence and prevalence vary greatly geographically (Winterbourn 1973, McArthur & Featherston 1976, Jokela & Lively 1995).

1.5.1.2 Fish second intermediate host: *Galaxias anomalus*

Telogaster opisthorchis has been found in at least 15 species of freshwater fish, mostly of the genera *Gobiomorphus* (Eleotridae) and *Galaxias* (Galaxiidae), but also infect exotic fish such as trout (*Salmo trutta*) (Hine et al. 2000). However, *G. anomalus* is the only fish for which spinal malformations have been recorded, and is therefore the fish host used in the following study. *Galaxias anomalus* is endemic to New Zealand, and is non-diadromous, meaning it does not have a marine phase as part of its life cycle. However, like many fish, they have a pelagic phase during early life stages before becoming benthic (Baker et al. 2003). *Galaxias anomalus* are only found within the Taieri and Clutha catchments in Otago, New Zealand (Baker et al. 2003). These catchments are also subject to wide seasonal temperature changes that may exert additional stress on developing *G. anomalus* juveniles.

Adult *G. anomalus* grow to a maximum size of approximately 140 mm (McDowall 2006), however they are seldom found greater than 90 mm long. It has been estimated from size that males generally become sexually mature at one year of age and females at two years of age (David Kelly, pers comm.). Spawning takes place from August to early October (Allibone & Townsend 1997). *Galaxias anomalus* tend to have high fecundity, early age at maturity, and a short life span in comparison to other Taieri galaxiids, supporting an *r*-selected life history strategy (Allibone & Townsend 1997). *Galaxias anomalus* prey mostly on

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chironomids and *Deleatidium* mayflies (Allibone & Townsend 1998), and they themselves are vulnerable to predation by trout, eels and other galaxiids (Crowl et al. 1992, McIntosh et al. 1994). In many locations, *G. anomalus* have been eradicated due to the presence of trout (Townsend & Crowl 1991, Townsend & Simon 2006), causing remaining populations to become isolated. The highly restricted distribution of this fish means they are more susceptible to population fluctuations and the possibility of extinction by chance events such as flooding (Townsend & Crowl 1991, Moore et al. 1999, Allibone 2000, Department of Conservation 2009, Allibone et al. 2010). Presently *G. anomalus* is categorised as vulnerable (Department of Conservation 2009, Allibone et al. 2010).

1.5.1.3 Definitive hosts *Anguilla dieffenbachii* and *Anguilla australis*

Telogaster opisthorchis has two definitive eel hosts, both from the family Anguillidae: the New Zealand longfin eel, *Anguilla dieffenbachii* (Gray 1842), and short-finned eel, *A. australis* (Richardson 1841). Collectively these two species are widely distributed throughout freshwater systems in New Zealand and can be found in high densities (McDowall 1990). Short and long-fin eels are opportunistic feeders, but their main diet consists of small freshwater fish including galaxiids. As a result of both their diet and long lifespan, they are exposed to a variety of parasites including Protozoa, Myxosporea, Trematoda, Cestoda, Nematoda, Acanthocephala, Copepoda and Mollusca over their long life span (Hine et al. 2000).

1.6 Aims and objectives

To further our knowledge on the effects of *T. opisthorchis* on *G. anomalus*, I have expanded on the work already undertaken by Kelly et al. (2010b). The main objective of this thesis was to examine the ways in which parasites may directly and indirectly influence the ecology of fish hosts using the parasite *T. opisthorchis* and its fish host *G. anomalus*. More specifically, I wanted to discover whether infection by *T. opisthorchis* influences health and survival in young fish, and how the parasite and parasite-induced malformations affect the ecology of the fish. To address these objectives I tackled the following questions:

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- 1 Does the length of exposure or the number of infected snails, influence parasite load and number of malformations?
- 2 Is the likelihood of host survival influenced by infections and/or malformations in juvenile fish?
- 3 Does the age at which young fish are exposed to parasites influence their acquired parasite load and the likelihood of developing a malformation following infection?
- 4 Do infections and malformations affect the swimming ability of juvenile fish?

These results help increase awareness of the importance of early life stages to ecologists and conservationists, and potentially aid in new approaches to the conservation of galaxiids and other animals. This project can also inform water management policies and Best Management Practices (BMPs), as water abstraction in streams and other human activities can lead to areas of low flow, higher temperatures and decreased water quality (Allibone 2000, Baker et al. 2003), conditions very favourable to the proliferation of trematodes in snails and their subsequent transmission to fish.

Chapter 2:

The effect of trematode *Telogaster opisthorchis* on the survival and early development of *Galaxias anomalus*



Telogaster opisthorchis parasites encyst in tissues of a larval *Galaxias anomalus* fish causing severe spinal malformations.

2.1 Introduction

As the list of declining and endangered species continues to grow, it becomes more important to work out the reasons behind their (often sudden) declines. Although there are usually multiple factors involved in the decline of a species which may work independently or synergistically, it is essential to figure out the key components and relative impacts of each in order to best manage and prevent its extinction. New Zealand freshwater ecosystems have been particularly susceptible to species declines, with most of them generally attributed to the impacts of either introduced species or changes in catchment land use (Townsend 1996, Matthaei et al. 2006). The focus of the present study is *Galaxias anomalus*, commonly known as the roundhead galaxiid, a small freshwater fish endemic to the Taieri and Clutha catchments of the South Island, New Zealand. The current status of *G. anomalus* is ‘vulnerable’ which means it is considered at risk of near-future extinction in the wild (Department of Conservation 2009, Allibone et al. 2010).

Among the many factors that may lead to the decline of a population or species, very few studies have focussed on the role of parasitism. Parasites can have a large impact on demography and community dynamics (Anderson & May 1978, Dobson & May 1987, Minchella & Scott 1991, Sheldon & Verhulst 1996, Szuroczki & Richardson 2009). However the impact of parasites is not equally distributed among all individuals in a host population, and patterns of parasite distribution have therefore been well studied in an array of different species. Often parasites are aggregated among hosts rather than being randomly distributed, leading to several scenarios about how this may then impact the condition and survival of individuals and populations (Gregory & Woolhouse 1993, Poulin 1993b). Aggregated distributions consist of most hosts harbouring few parasites while some hosts harbour many, as animals already infected with parasites are often more likely to acquire more, creating a typical negative binomial distribution (Poulin 1993b).

The comparison of highly infected individuals with lightly infected or uninfected individuals is often used to evaluate the effects of parasitism which can

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be estimated in a number of ways. A proxy of host health is often estimated using condition factors. For fish, condition factors are most simply calculated using just length and weight (Ferron & Leggett 1994, Tadiri et al. 2013). In basic terms, it is assumed that the greater the weight of a fish for a given length, the better its physiological condition, when compared with fish of the same species (Bagenal & Tesch 1978, Bolger & Connolly 1989). As parasites generally weaken their hosts and deplete their energy stores, as well as lowering their foraging efficiency, condition indices can be used to assess the impact of parasites on fish (Lemly & Esch 1984).

The effects of parasites on their hosts can also be measured by quantifying survival rates of hosts. Parasite-associated host mortality can result in individuals with the highest parasite counts disappearing from the population, allowing only hosts with intermediate levels of infection to live longer (Latham & Poulin 2002). Even so, parasite loads of individual hosts generally increase with size or age as hosts come into contact with more parasites over time (Poulin 2000). This is especially true for trematodes in their second intermediate hosts where parasites reside within their hosts for long periods of time, awaiting ingestion by a definitive host. Naturally, size also increases with age so it is often difficult to determine whether age (duration of exposure) or host size is a better predictor of parasite load. These relationships make it difficult to measure the impacts of parasites on both condition factors and mortality of fish in natural populations.

The biogeography of *G. anomalus* is narrow and patchy. Populations are only found in the Clutha and Taieri catchments in New Zealand, and their distribution has become fragmented due to waterfalls, the presence of trout, and the damming of streams (Townsend & Crowl 1991, Allibone 2000, Jellyman & McIntosh 2008). The estimated number of sub-populations has decreased from 27 in 2004 to just 17 in 2009 (Department of Conservation 2009). Many habitat sites are exposed to the effects of farming, such as run-off and water abstraction. Chemical run-off (Allibone 2000, Kiesecker 2002, Johnson et al. 2007, Johnson & Hartson 2009, Koprivnikar 2010, Hock 2012, Hock & Poulin 2012) and altered water and temperature levels caused by water abstraction (Poulin & Fitzgerald 1989a, Allibone 2000, Paull & Johnson 2011), can improve conditions necessary

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for trematode parasites to emerge from their snail hosts and indirectly lead to high parasite loads in *G. anomalus* (Kelly et al. 2010a). Exposure to a greater number of infected snail hosts result in higher parasite loads in both frogs and fish (Johnson & Hartson 2009, Kelly et al. 2010b), possibly lowering survival and decreasing the number of fish reaching sexual maturity (McDonald et al. 2006); the conditions in which many *G. anomalus* populations exist are certainly compatible with such effects.

A common parasite of *G. anomalus* is *T. opisthorchis* (Cryptogonimidae), as *T. opisthorchis* infected snails commonly coexist with *G. anomalus* in the wild. *Telogaster opisthorchis* has a typical three-host life cycle consisting of a first intermediate snail host, second intermediate fish host and an eel definitive host (see Chapter 1 for more detail). *Telogaster opisthorchis* must utilise all three hosts in order to mature and sexually reproduce. As *T. opisthorchis* infect their second intermediate fish host by penetrating the skin, the parasite, by chance alone, should have a higher encounter rate with larger fish. Larger fish have a greater surface area exposed for potential parasites to come in contact with, and a greater volume of tissue for parasites to reside in. These can all contribute to larger, older fish having higher parasite loads (des Clers 1991, Grutter & Poulin 1998, Poulin 2000).

However, it may not be the number of parasites in a host, but the timing of their arrival, that ultimately determines the severity of their impacts on the host. Young fish are likely to be more susceptible to infection or the associated impacts of infection. For instance, the trematode parasite *Ribeiroia* not only encysts in the tissues of frogs, but is the cause of frog limb malformations, which occur when a parasite encysts near a limb bud during early stages of development (Johnson et al. 2002, Jayawardena et al. 2010). Recently, Kelly et al. (2010b) documented the first cases of parasite induced malformations in roundhead galaxiids. Upon examination of parasite placement along the skeletons of malformed fish, it was concluded that the same disruption processes occurring in frogs are likely to cause the malformations in roundhead galaxiids. Malformed frogs may suffer from bone malformations such as extra or missing toes or limbs (Johnson et al. 2001, Stopper et al. 2002) and Kelly et al. (2010b) observed spinal and fin malformations in fish.

Experiments have demonstrated that when frogs are exposed to parasites across a range of ages, the number of malformations induced by infection drops as the age of exposed tadpoles increases (Schotthoefer et al. 2003a, Jayawardena et al. 2010). When frogs are in the early stages of life, their bones have not fully calcified, leaving a ‘window’ of opportunity early during development for parasites to permanently disturb bone formation (Johnson et al. 2011). A similar ‘window’ of opportunity is likely to occur in fish in the late larval and early juvenile stage (Kelly et al. 2010b). The next step in researching malformations in galaxiids is to narrow down the stage in development where parasites permanently affect bone development. Nevertheless, age may still dictate the impacts that infection has on the host, even in the absence of a malformation.

2.1.1 Aims and hypotheses

I aimed to examine the role of *T. opisthorchis* in altering the life history of *G. anomalus*. As well as obtaining information about parasite distributions and the effect of exposure duration vs host size on the parasite loads of *G. anomalus*, I looked into the repercussions those parasites have on the life of the fish. For example, how parasites affect host condition, likelihood of survival, likelihood of developing parasite-induced malformations, and in which stage of development parasites can cause malformations. So far very little is known about the malformations observed in *G. anomalus* fish in the wild, and the time frame during which fish can become permanently affected by a malformation. Here I tested experimentally how exposure to various numbers of trematode-infected snails affect young *G. anomalus* fish of different ages. More specifically, I tested the prediction that fish exposed to a greater number of infected snails will incur higher parasite loads, and consequently, a greater number of malformations. As parasites are expected to reduce condition of the fish, infection status was also related to fish condition coefficients and survival. To understand more about the critical window of opportunity, young fish were exposed at different ages in an attempt to identify when during development the ‘critical window’ lies. By using young fish from the same cohort, known to be previously uninfected, I had an opportunity to tease apart whether size or duration of exposure had the most influence on parasite loads and survival.

2.2 Materials and methods

2.2.1 Animal collection and care

Infected *P. antipodarum* snails, the first intermediate host of *T. opisthorchis*, were used as a source of trematode infective stages. On the 17th November 2009, snails were gathered from an unnamed spring-fed stream of the Upper Taieri River in Central Otago, New Zealand (45°06'45"S, 170°15'18"E (GPS coordinates)), 130 km north of Dunedin (Figure 2.1). This is a site where *T. opisthorchis* infection is 100% in *G. anomalus* fish. Snails were collected by dragging dip nets around vegetation within the stream and kept in plastic containers with fresh stream water before being taken back to the laboratory. Once taken back to the laboratory, snails were kept in aerated aquaria before determining their infection status.

On the 17th and 24th November 2009, larval *G. anomalus* fish approximately six weeks old were collected using a push net in Swin Burn stream, Central Otago (45°08'50"S, 170°17'35"E), 8 km away from the collection site of the snails (Figure 2.1). The Swin Burn is a site described by Kelly et al. (2010b) as having low parasite prevalence so larval fish were less likely to be infected. Once brought back to the laboratory, fish were housed in a large glass holding aquaria (1.10 x 0.45 x 0.45 m) filled with approximately 140 L of aged, aerated water. A random subsample of fish larvae was checked to verify the absence of parasites; all were uninfected. Both the fish and snails were kept in a laboratory room at an ambient temperature of between 15-20°C for the duration of the experiments. Parasites generally emerge from snail hosts when temperatures are elevated; as room temperature was slightly higher than the temperatures snails would be experiencing in their natural habitat, snails were therefore expected to be constantly shedding infective stages (cercariae). Fish larvae were fed on fine commercial pellet food twice daily and snails were fed algae pellets. The holding tank and pump filters were cleaned regularly, and food and fish waste was removed daily.

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All collection of animals, caring, experimenting and euthanizing procedures were carried out humanely following the University of Otago code of animal ethics and under the ethics approval number # 63/09.

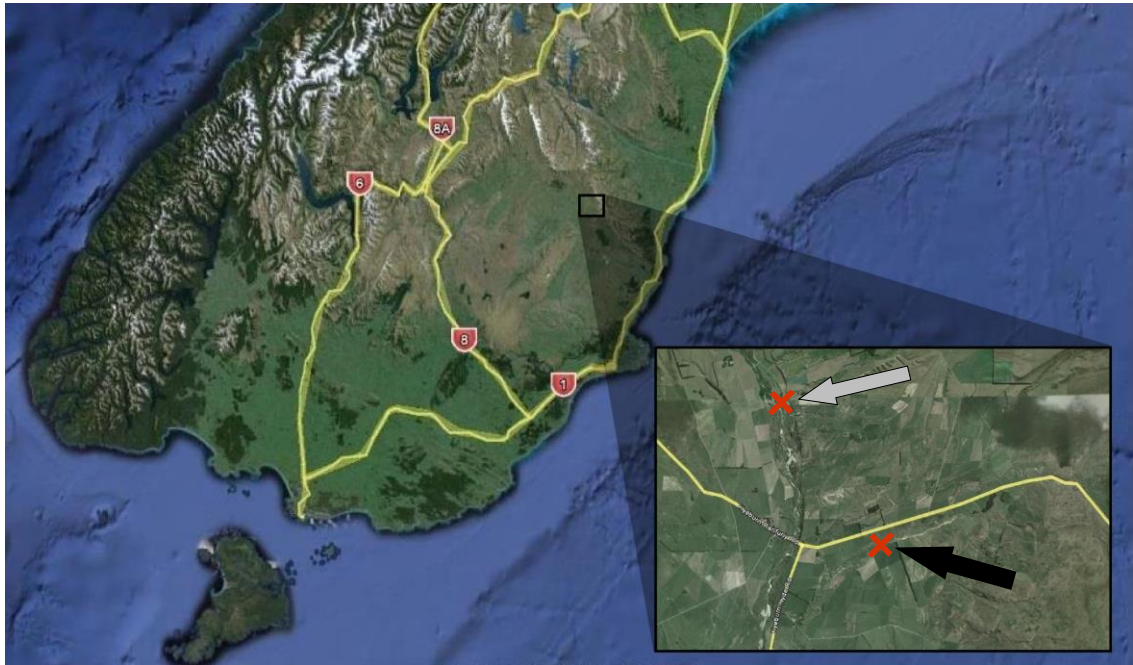


Figure 2.1: Map of the southern South Island, New Zealand. Arrows indicate the location of collection sites. Fish were collected from the Swin Burn stream (black arrow) and snails collected from a small un-named spring fed stream (grey arrow).

2.2.2 Experimental treatments

Trematode infected snails were identified according to the presence of cercarial emergence. To determine whether snails were infected with *T. opisthorchis*, they were placed in 12 well culture plates (12 per well). Wells were half filled with water then plates were incubated under increased temperatures (5°C above ambient) and intense light, to promote the release of parasites. Snails from infected wells with *T. opisthorchis* cercariae were redistributed into a new plate (one snail per well) and allowed to shed again to identify exactly which snail(s) were infected. All plates were screened for parasites twice daily under a dissecting microscope. Snails were considered uninfected if no cercariae were shed after five screenings. Infected and uninfected

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snails were separated into separate aquaria until ready to be used in experiments. Approximately 8% of snails were infected with *T. opisthorchis* parasites, a relatively high prevalence compared to <2% seen in most Otago populations.

One hundred and fifty fish, estimated to be 12-weeks of age were taken from the holding tank and randomly allocated into 15 smaller four litre experimental tanks, 10 fish per tank, and left to acclimatise for 48 hours. Each small tank had four litres of aged aerated tap water and a bubbler to keep the water oxygenated. All tanks had a fitted plastic and mesh lid, allowing easy observation of the fish while allowing air flow and preventing snails from escaping. There were three experimental groups, each with five replicates. After the acclimatisation period, four snails were added to each tank. Four uninfected snails were added to control tanks, two infected plus two uninfected snails were added to low dose tanks, and four infected snails were added to high dose tanks (Figure 2.2).

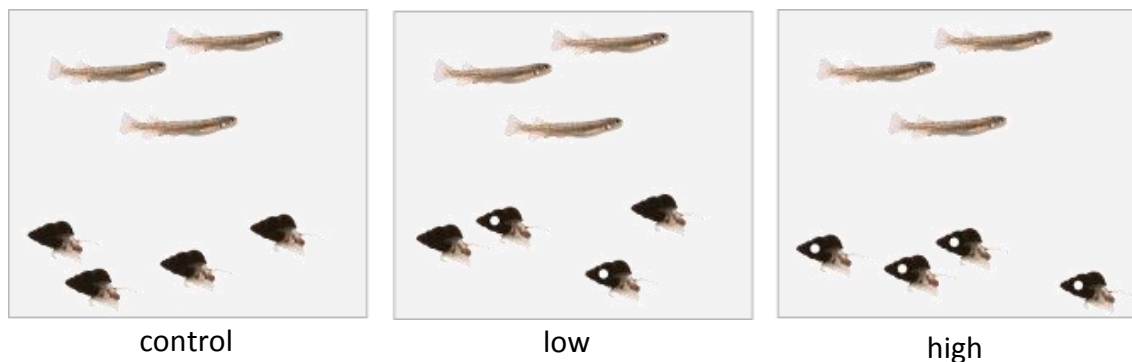


Figure 2.2: Tank snail dosage levels. Number of infected and uninfected snails in each tank. White spots indicate infected snails (Note: each tank contained not three but ten fish each).

Dosage levels were chosen based on a prior study by Kelly et al. (2010b) and my own pilot study. I wanted to create some variation between dosage levels while minimising fatalities of hosts before the end of the experiment, a difficult task when dealing with such young fish. During the pilot study, all fish (estimated to be 10-weeks old) exposed to parasites died within a week, suggesting that fish were too young to experiment on, or that dosage levels were too high. Dosage levels were lowered from those used in the pilot study but still estimated to be

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within the limits these fish may experience in the wild. Snails were kept in tanks with the fish for 20 days, at the end of which the snails were taken out. Fish which died prior to the termination of the experiment were removed as quickly as possible and fixed in 10% buffered formalin for at least one week before being transferred to 70% ethanol. Fish in all tanks continued to be fed fine commercial pellet food twice daily during the experiment. Fish waste and surplus fish food were removed twice daily and topped up with fresh water to keep tanks as clean as possible. When cleaning experimental tanks, extra care was taken to prevent water and wet equipment from being transferred to adjacent tanks so as to not move cercariae from one tank to the next. Photoperiod was set to imitate the natural austral summer light/dark cycle over the course of the experiments between December and February.

The same experiment was repeated with another 150 fish a month after the commencement of the first experiment (when fish were approximately 16-weeks of age), to observe the effect of host age on the likelihood of malformations and survival. All fish still alive at the termination of the experiments were kept for use in a further short laboratory experiment where they were not exposed to any more parasites (explained in Chapter 3). Following that, fish were euthanized with an overdose of MS-222 before being fixed in 10% buffered formalin for at least one week and then transferred to 70% ethanol.

2.2.3 Clearing and staining protocol

Fish were cleared and stained using bone and cartilage staining procedures modified from Hanken and Wassersug (1981). The protocol is specifically used for analysing skeletal development and is especially useful here as animals can be kept whole throughout the entire process. Stains were used to allow unmineralised (cartilaginous) structures, and mineralised (bony) structures to be easily distinguished. During the process, cartilage was stained blue by being submersed in Alcian blue for 24 hours, and bones and teeth then stained red using alizarin red for another 24 hours. The two stains allowed identification of the stage of mineralisation in the fish and made spinal malformations much easier to evaluate. Tissue was then cleared using a graded series of potassium hydroxide and

glycerine over 72 hours. Clearing with potassium hydroxide allowed a clearer view of the skeleton and metacercarial cysts through the tissue.

2.2.4 Measures and statistical analyses

The total length and weight of each fish was measured. For each fish a condition coefficient (K) was calculated using the formula $K = W/L^b$ where W is the weight in mg, L is the length in mm, and b is the slope of the best fit line of the logarithmic regression of weight on length. Sex could not be determined due to the young age of the fish used in this study.

Each fish was examined under a dissecting microscope to identify and count malformations, paying close attention to the spine and fins, where malformations had previously been found. The gut and eyes of fish were dissected and inspected for metacercarial cysts, the rest of the body did not need to be dissected as cysts within the tissue were easily seen through the tissue. The number and location (eyes/brain, gut, or muscle tissue) of metacercarial cysts were recorded throughout the body (see Figure 2.3).

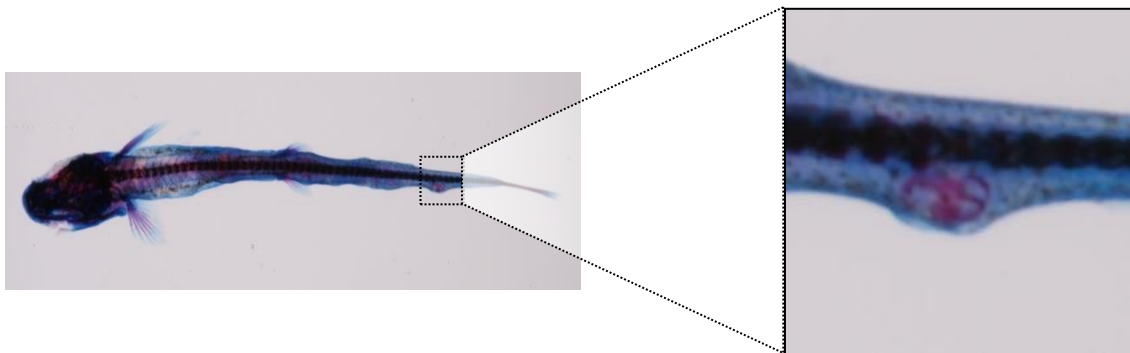


Figure 2.3: A fish infected with *T. opisthorchis*. A close up of a cyst can be seen on the right, with parts of the worm stained red within the cyst.

A common index used to measure parasite aggregation is the index of dispersion or variance-to-mean ratio $D = \frac{\sigma^2}{\mu}$ where σ^2 is variance and μ is the mean number of parasites per host in a given sample (Poulin 2007). A random

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distribution of parasites within hosts would see the mean and variance being equal. When the variance is greater than the mean, the distribution shifts away from random (Poisson) distribution, and toward aggregation (negative binomial distribution) (Linden & Mantyniemi 2011).

Data was analysed with simple parametric tests (regressions, ANOVAs, chi squared) and are mentioned along with their results in the following section. Statistical analyses were carried out using the statistical software package SPSS version 16 (SPSS Inc., Chicago, Illinois, U.S.A). Unfortunately, in the second trial with 16-week old fish, only one low dosage and one high dosage tank had infected fish at the end of the trial, therefore all results and statistical analyses refer only to the first trial (12-week old fish) except for one analysis comparing dosage and age of infection on parasite load. Note that where necessary, variables were $\log(x+1)$ -transformed if zero values were present in order to meet the assumptions of normality.

2.3 Results

Of 12-week old fish exposed to infected snails, a total of 86 out of 100 (86%) fish became infected. Most fish had few parasites though several had a greater number of parasites, with parasite loads ranging from 0 up to 64 cysts per fish (Figure 2.4). The mean number of trematodes per host was $12.5 (\pm 1.3)$ and over two thirds of fish had fewer than 15 cysts. The variance-to-mean ratio of parasites per host was greater than one (14.2), suggesting that parasites are aggregated among a few hosts rather than being randomly distributed. This aggregation is evident in Figure 2.4, where the distribution is skewed toward zero, resembling that of a negative binomial distribution.

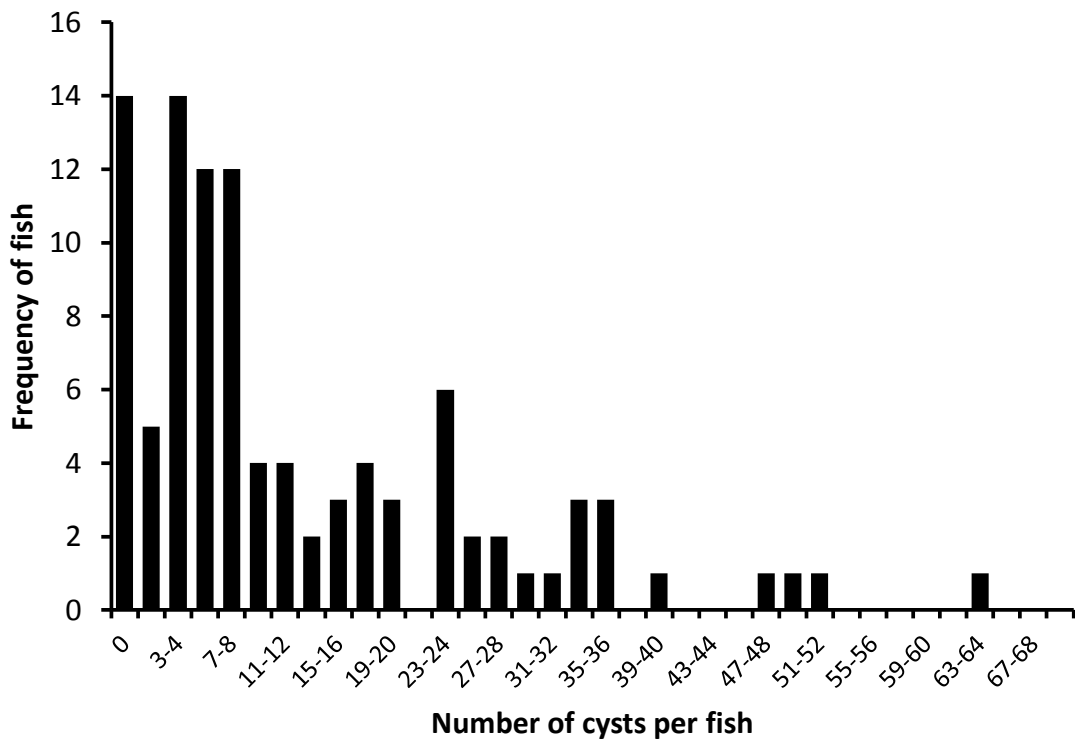


Figure 2.4: Frequency distribution of the number of *T. opisthorchis* cysts per fish in 12-week old roundhead galaxias (*G. anomalus*) exposed to infected snails. N = 100.

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There was a relationship between fish length and parasite load in 12-week old fish (Figure 2.5). Although a linear relationship was expected, a quadratic relationship provided a better fit to the data although the power is still poor (quadratic regression R^2 : 0.1773, $p < 0.001$). The longest fish sampled here harboured fewer parasites relative to their size.

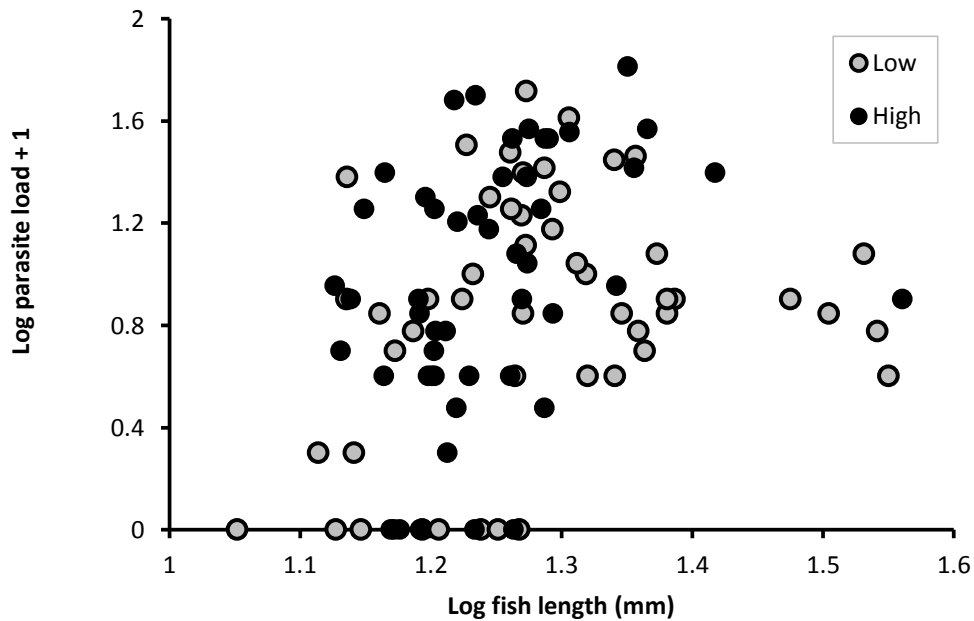


Figure 2.5: Relationship between fish length and parasite load acquired during experimental trials with 12-week old roundhead galaxiids (*G. anomalus*) exposed to infected snails. Figure displays high and low exposure to infected snails separately, however statistical analysis combines both. N fish = 50 (low) and 50 (high).

In 12-week old fish, twice as many fish from the high treatment group died during the trial in comparison to the low treatment group (38 vs 19 fish). Of fish that died prior to the end of the experiment, there was a significant positive linear relationship between days of exposure and parasite load for fish exposed to a high number of snails (linear regression: $R^2 = 0.514$, $p < 0.001$) (Figure 2.6). However, there was no relationship between days of exposure and parasite load in fish exposed to a low number of infected snails.

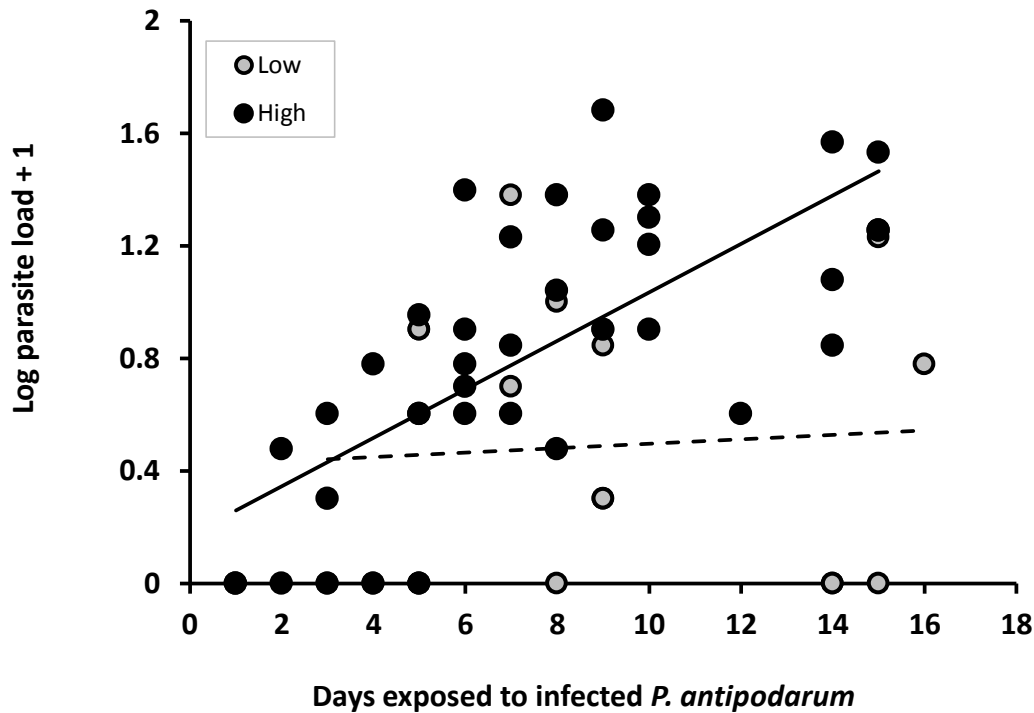


Figure 2.6: Parasite loads over the course of the experiment: Relationship between the number of days a fish was exposed to parasites, and the subsequent parasite load of fish, among fish that died during the experiment. Fish were exposed to either a high (n = 38, solid trend line) or low (n = 19, broken trend line) number of *T. opisthorchis* infected *P. antipodarum* snails.

Survivorship decreased over the trial period in all groups, including control fish not exposed to parasites (Figure 2.7). There was no difference in survival between the control and low groups during the trial. However, there were significant differences in survival between the control and high groups from day nine onwards. At the conclusion of the experiment, control groups had the highest survival with 66% (± 11.7), while fish in the high dose groups had much lower survival at 28% (± 9.2) (X^2 test: $X^2 = 13.36$, $p = <0.001$).

Parasite loads of 12-week old fish depended on dose, with fish exposed to a higher number of infected snails accumulating a significantly higher parasite load (Two way ANOVA: $F_{1,96} = 7.861$, $p = <0.01$) (Figure 2.8). However, fish that died prior to the end of the experiment did not have significantly more parasites than those that continued to survive, (Two way ANOVA: $F_{1,96} = 1.264$, $p = 4.63$).

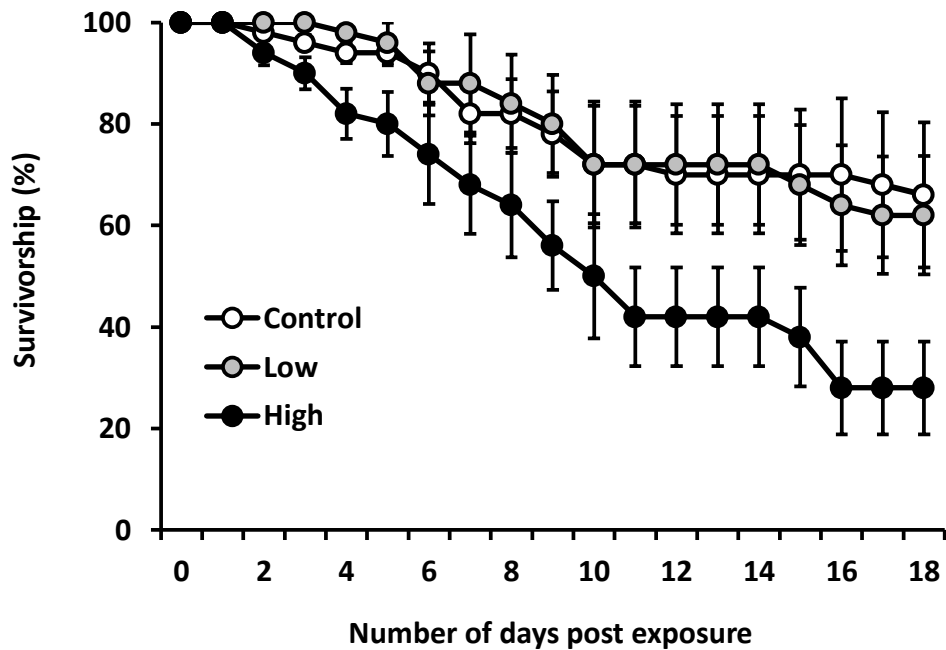


Figure 2.7: Survivorship curves for the groups of *G. anomalus* exposed to different number of *T. opisthorchis* infected snails. Percentage surviving (mean \pm 1 SE) in each treatment each day from the start of exposure.

There was no relationship between parasite load and condition coefficient for fish exposed to either a high or low number of infected snails (Figure 2.9). However, fish that died prior to the end of the experiment had condition coefficients significantly higher than those which survived until the end of the experiment (Two way ANOVA: $F_{1,98} = 0.514$, $p < 0.001$) (Figure 2.10). Dose had no effect on condition coefficient, nor was there any interaction effect.

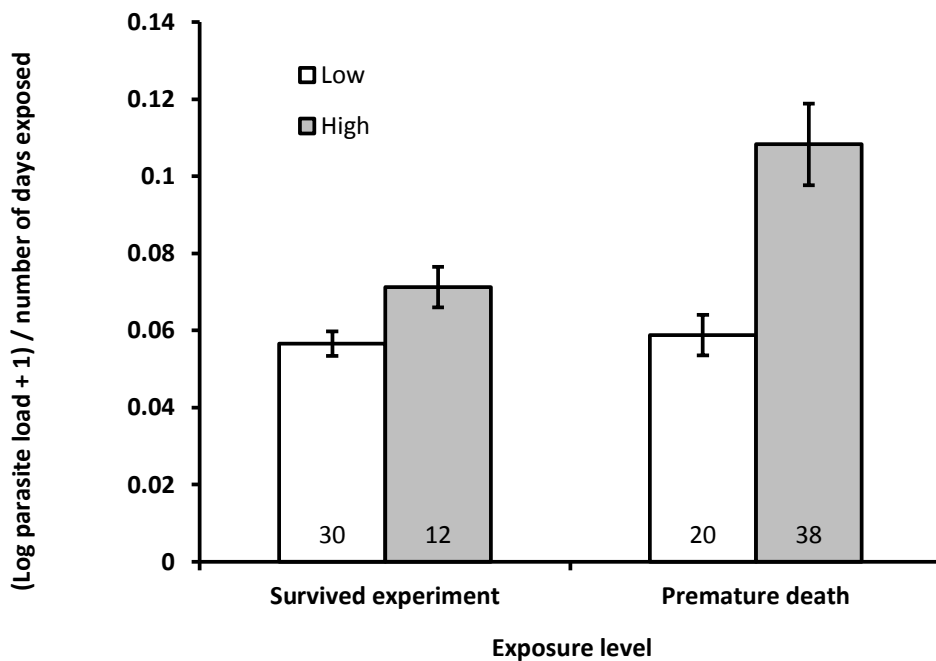


Figure 2.8: Parasite loads of dead or surviving fish: Parasite loads of fish which died prior to the termination of the experiment, in comparison to fish which survived the entirety of the experiment before being euthanized. Varying lengths of exposure were taken into account by dividing each fish's parasite load by the number of days that they were exposed. Numbers in bars indicate sample size.

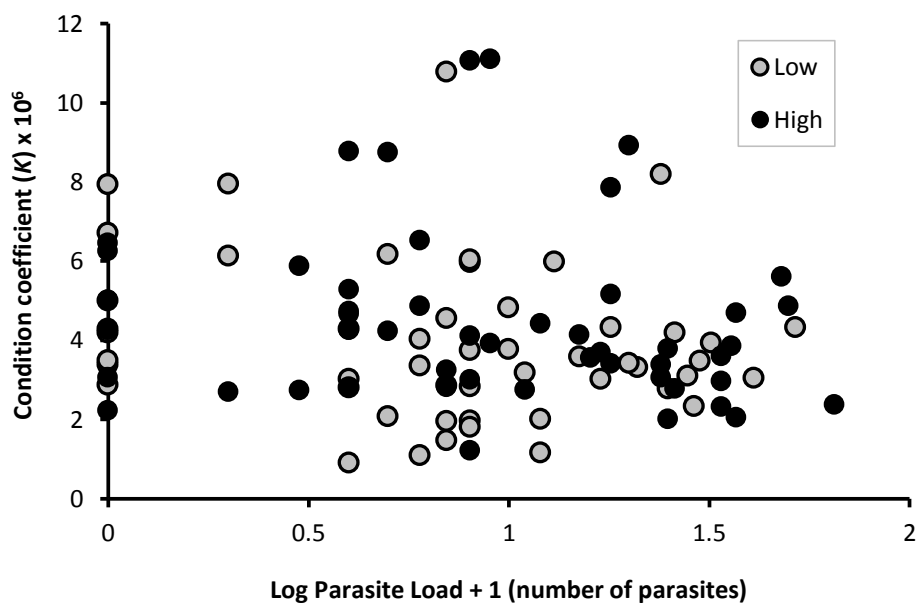


Figure 2.9: Comparing parasite load and condition: Relationship between fish condition coefficient and the number of parasites acquired during experimental trials.

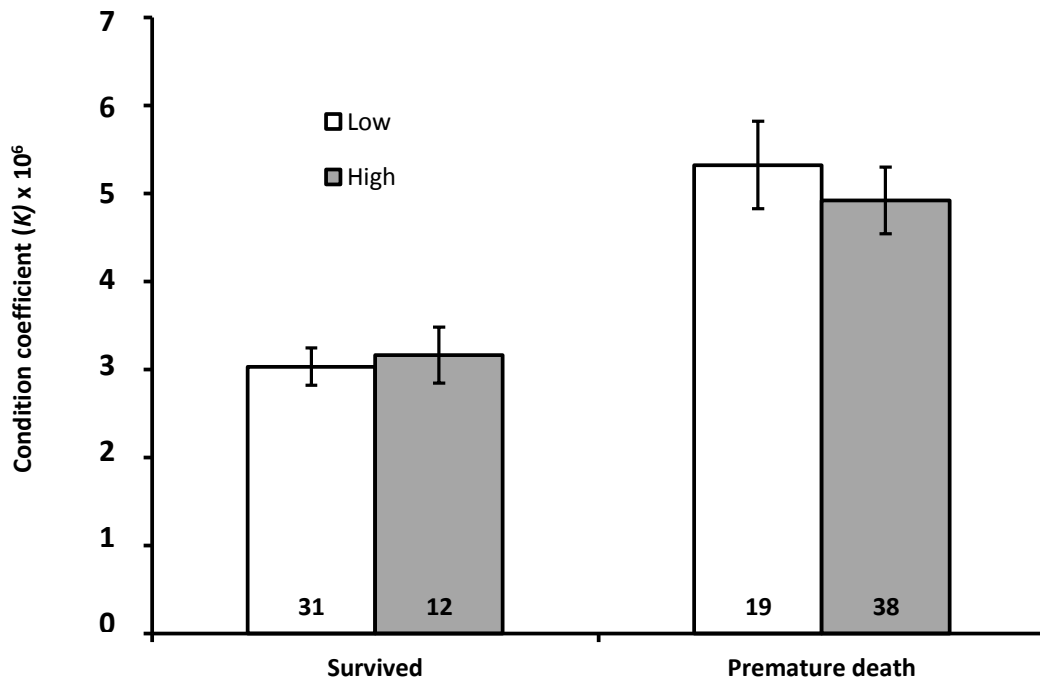


Figure 2.10: Condition of dead and surviving fish: Condition coefficient of fish which survived the entirety of the experiment and were euthanised in comparison to fish which died prior to the termination of the experiment. Numbers in bars indicate sample size.

No fish from the control group developed a spinal malformation. However, for those fish which were exposed and became infected, the proportion of fish with spinal malformations was higher in fish exposed to a higher number of infected snails than those exposed to fewer infected snails (X^2 test: $X^2 = 6.69$, $p = 0.010$). In the low treatment group only one out of 42 infected fish developed spinal malformations compared with the high treatment group where a total of 18 out of 44 infected fish developed a malformation (Figure 2.11). Only 10 of the 19 fish with a malformation survived the entirety of the experiment.

I was unable to determine whether there was a significant difference between the mean number of spinal deviations of fish exposed to either a low or high number of infected snails as only one fish in the low treatment group developed a malformation (Figure 2.12).

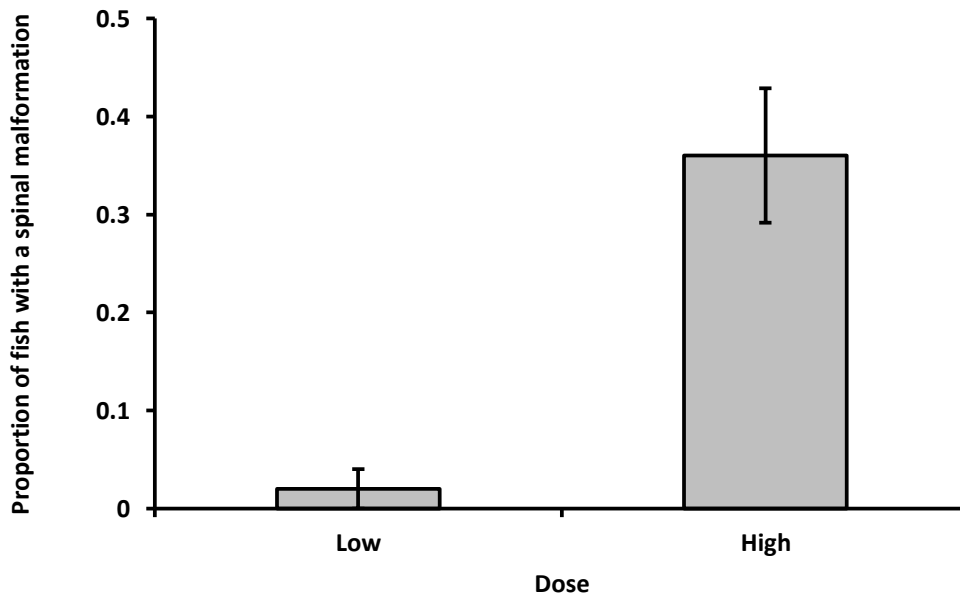


Figure 2.11: Proportion of fish with a spinal malformation: Mean (± 1 SE) proportion of infected *G. anomalous* which developed a malformation among individuals exposed to low and high numbers of infected snails. N (fish) = 42 (low) and 44 (high).

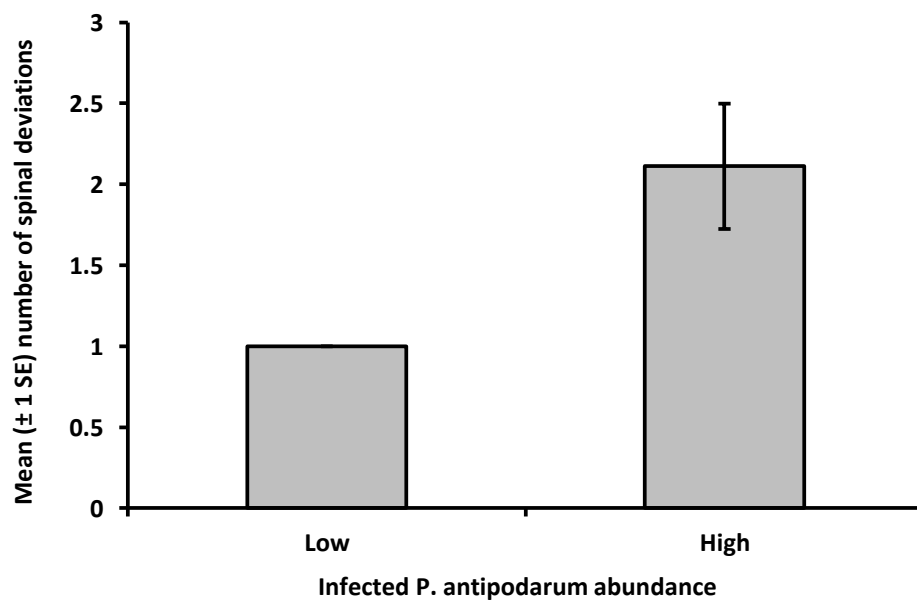


Figure 2.12: Mean (± 1 SE) number of spinal deviations per fish among malformed fish exposed to a high or low number of infected snails during infection trials. N (fish) = 1 (low) and 18 (high).

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There was no difference in survivorship between infected normal and infected non-malformed fish (Figure 2.13).

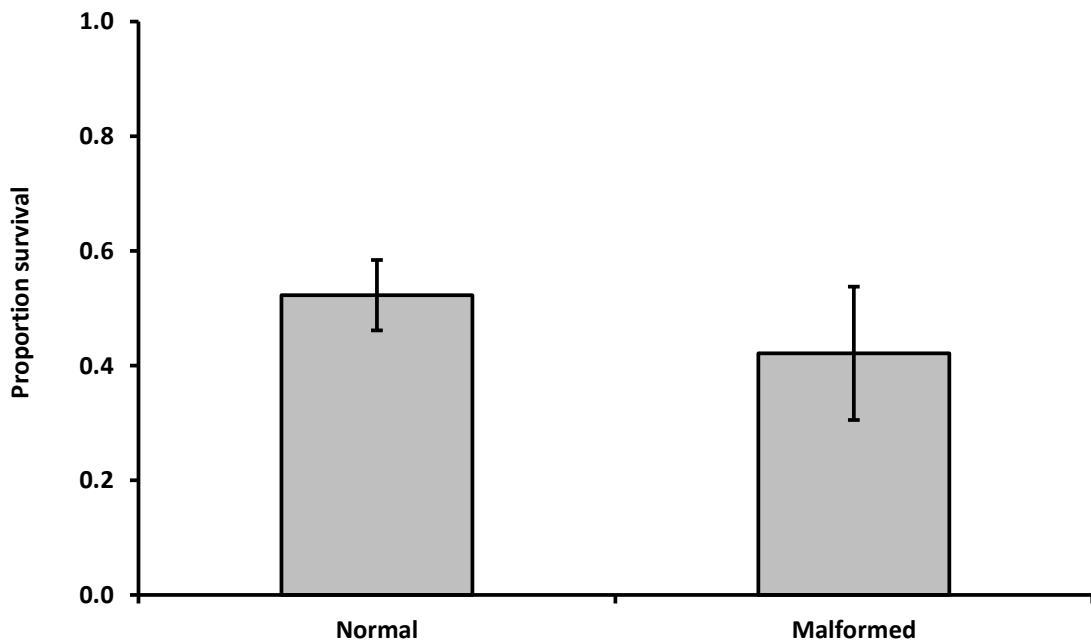


Figure 2.13: Proportion of surviving infected non-malformed, and infected malformed fish. N (normal) = 67, n (malformed) = 19

Lastly, I compared fish from both experiments to assess the effect of fish age (12-week or 16-week), and dosage, on infection levels. Parasite loads were influenced by dosage (number) of infected snails added to the tank and age (Figure 2.14). Exposure to a greater number of infected snails (4) compared with a lesser number of infected snails (2), accumulated a greater number of parasites two-way ANOVA: $F_{(1,116)} = 8.809$, $p = 0.004$). Furthermore, the younger, 12-week old fish, accumulated more parasites per day on average than 16-week old fish (two-way ANOVA: $F_{(1,116)} = 9.110$, $p = 0.003$).

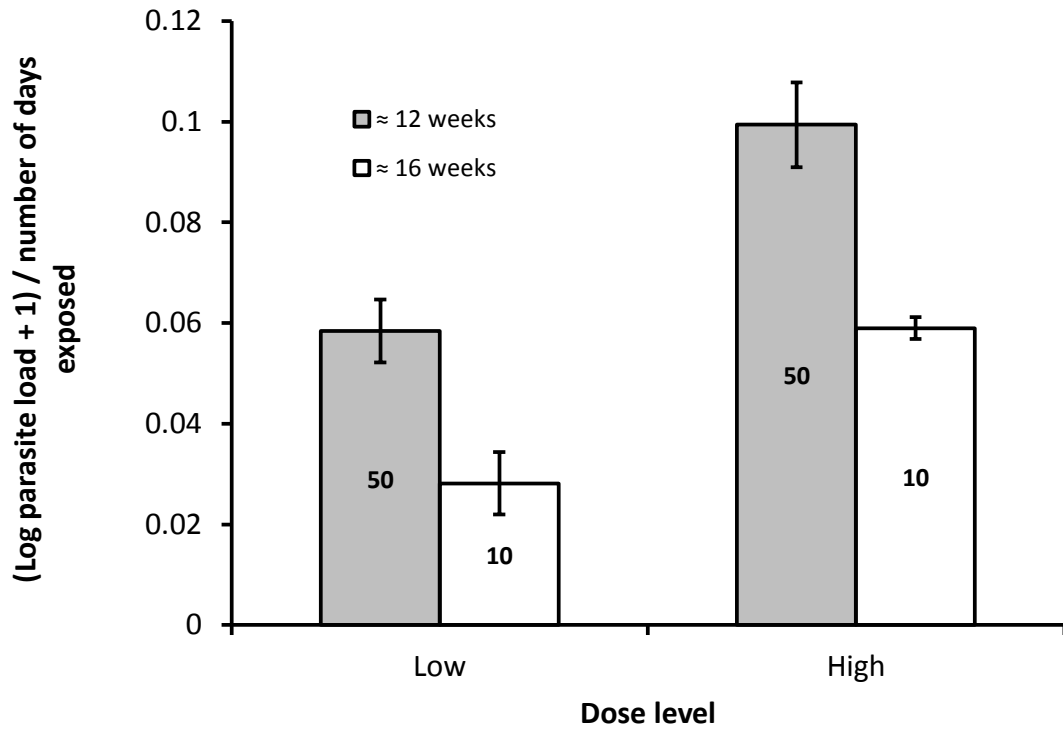


Figure 2.14: Comparing the effect of dose and age on parasite load: Parasite loads of fish exposed to a high or low dose (number) of infected snails at 12 and 16-weeks of age. Numbers in bars indicate fish sample size.

2.4 Discussion

Parasite infections can directly impact fish, making them more susceptible to further infection, causing malformations, and lowering host survival. These effects combine to contribute to the high mortality rate we see in young fish and may even impact population survival. This study focused on the impacts of a trematode parasite on the condition, development and survival of *Galaxias anomalus*, a threatened New Zealand endemic species. Its results indicate that this parasite has the potential to significantly impact wild populations.

In my experimental infections, the distribution of parasites among hosts was similar to that which we would see in nature, and congruent with past studies of trematode distribution (see Anderson & Gordon 1982, Poulin 2013). Karvonen et al. (2004) found that fish exposed to trematodes in the wild had aggregated distributions, but in the laboratory they had random parasite distributions, contrasting the laboratory experiments in the present study. Aggregated distributions are usually attributed to variation in host immunity or heterogeneity in exposure due to the environmental patchiness they may experience in the wild. In this experiment, fish have been exposed in a homogeneous environment, yet the data show the distribution of parasites among hosts was still aggregated. Therefore, variation in immune (or some other form of) response is the likely cause of aggregation here. It is possible that a host already infected by a parasite has a higher chance of being re-infected. Not only could some fish be less resistant to parasites in terms of immune defence, but the chance acquisition of a first parasite may alter normal behaviour of the host in some way which increases the likelihood of reinfection (Poulin et al. 1991, Lafferty & Morris 1996, Bakker et al. 1997, Fredensborg & Longoria 2012). Still, it is likely that aggregation of *T. opisthorchis* in *G. anomalus* is due to a combination of processes, with the net result being that even under standardised conditions, not all fish will incur the same effects of parasitism.

Commonly, there is a linear relationship between fish size (or age) and trematode parasite load (des Clers 1991, Poulin et al. 1991, Poulin 2000, Wilson et al. 2002). However, a polynomial curve fitted the data from the present

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experiment better than a linear curve. The lack of a linear trend may simply be explained by the narrow size range used, as all fish were of the same age class, whereas linear relationships are usually explored across a range of age classes. King and Cone (2009) who also worked on larval fish, did not speculate on the lack of relationship between fish length and parasite load in their study. Thus, the narrow range in larval fish sizes in their study may also have prevented a clear relationship. Alternatively, a reason for the polynomial trend may be because fish which survived this experiment were used for a swimming trial (explained in the following chapter) nine weeks later. Therefore, those fish lived longer and became larger in size, yet they were not exposed to parasites during that time meaning largest fish wouldn't necessarily have more parasites, potentially creating the polynomial curve.

Fish survivorship decreased in all three groups during the trial, including those where fish were housed with uninfected control snails. Survival of the high exposure group became significantly lower during the course of the experiment, congruent with past studies on fish and frogs (see Lemly & Esch 1984, Delahay et al. 1995, Johnson & Dick 2001, Schotthoefer et al. 2003b, Bourque et al. 2006). Not surprisingly, fish exposed to a greater number of infected snails, or those exposed for a longer period of time, had higher parasite loads. The greater the number of infected snails in each experimental tank, and the longer the length of time exposed to shedding snails, meant more cercariae could be released into the water. Hence, those fish exposed to more cercariae over time inevitably had heavier infections.

If exposure to a greater number of infected snails, or exposure for a greater length of time, means fish have a greater parasite load, and higher parasite loads increase the likelihood of fish mortality, then we would predict that a higher exposure dose would lead to an increase in mortality (Kelly et al. 2010b), just as the trematode *Ribeiroia* has a similar effect on frogs (Johnson et al. 1999, Johnson et al. 2001, Schotthoefer et al. 2003b).

Fish that died prematurely did not have a higher parasite load than survivors, so we may assume that parasite-induced host mortality (PIHM) was not

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occurring here. In many other host-parasite associations, heavily infected hosts are more likely to die prematurely as a result of infection (Lemly & Esch 1984, Rousset et al. 1996, Bakker et al. 1997, Knudsen et al. 2002, Jacobson et al. 2008, Ferguson et al. 2012). It must be remembered that in field conditions there are additional factors at play. Fish would also be exposed to predation, other parasites, competition, variable food availability, and temperature fluctuations. Thus, survival rates are likely to be much lower in the wild than those observed here (Seppala et al. 2004). For *G. anomalus*, a major threat to their survival is land use. Fine sediment run-off from farms support high *P. antipodarum* snail numbers (Matthaei et al. 2006), while chemical run-off and an increase in temperatures due to water abstraction, enhance replication and release of trematode parasites from their snail host (Allibone 2000, Paull & Johnson 2011, Hock & Poulin 2012). Consequently, *T. opisthorchis*, in combination with the effects of farming may exacerbate juvenile mortality for young galaxiids.

Even though condition factors are widely used as general indices of wellbeing, they have long been criticised for their simplistic approach, and therefore should be used with caution (Cone 1989, Jones et al. 1999, Camara et al. 2011). Here, a condition coefficient has been used to estimate fish health. Since parasites lower the energy reserves of their hosts, we may expect hosts with higher parasite loads to suffer from lower condition coefficients. Poulin (1993a) found the parasite load (specifically the trematode *T. opisthorchis*) of an adult population of upland bullies (*Gobiomorphus breviceps*), to be negatively related to host condition. However, Tadiri et al. (2013) found that ectoparasites actually favoured and aggregated among guppies with higher condition indices. Here, I found no relationship between parasite load and condition coefficient of fish during the infection trials. If condition factors can also predict the likelihood of survival, we would have expected fish which died prior to the end of the experiment to have lower condition coefficients than those that survived. In contrast, fish which died prematurely had condition coefficients significantly higher than those which survived to the end. Lemly and Esch (1984) found parasites to decrease condition and total body lipid, and considered condition indices to be a good assessment of the effects of parasitism provided

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conformational changes in body shape with age are taken into account. Since natural changes in body shape occur rapidly during early growth, the comparison of larvae differing by even a few weeks of age may explain the difference in condition factors seen here. Alternatively, it is possible that the food provided to the fish during the experiment negated any impact of infection of their condition.

A number of fish which became infected also developed malformations of the spine, just like those Kelly et al. (2010b) had observed in the same species. However, Kelly et al. (2010b) found both spinal and fin anomalies, whereas I only observed malformations of the spine. Fish in the high exposure group tended to have a higher parasite load, and therefore by chance, we would expect those fish to have more metacercariae encysted near the spine, each potentially causing a malformation. As it was, the high exposure group did have a significantly higher proportion of malformed fish. We would also expect the high exposure group to have a greater number of malformations per fish than the low exposure group. Unfortunately, there was insufficient data to statistically test this prediction, although it is likely that this would be the case. Kelly et al. (2010b) found the number of spinal deviations in fish exposed to a higher abundance of infected snails to be significantly higher than those exposed to a low number of infected snails.

When looking at the survival of malformed individuals, Goodman and Johnson (2011) found a difference in survival between malformed and infected normal frogs, yet there was no statistical difference in survivorship between malformed and infected normal fish in the current experiment. The difference being that experiments carried out in the present study were conducted in a laboratory, whereas Goodman and Johnson (2011) used field studies. In the wild, skeletal malformations are likely to affect swimming ability, thereby decreasing foraging ability or making fish more susceptible to predation and further infection. This suggests that malformed individuals would be at a disadvantage in the wild and laboratory data may underestimate the difference in survival between these two groups. Kelly et al. (2010b) study also noticed a decrease in the number of malformed individuals in a wild population over the season sampled. Unfortunately, only three of the malformed fish in the present study survived long

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enough to be used for subsequent experiments on swimming ability (see Chapter 3). Still, it is interesting to note that toward the end of the experiment, several malformed fish were seen to be swimming erratically in circles on a number of occasions (pers. obs.), similar to fish with whirling disease. If such swimming behaviour occurs in malformed fish in the wild, they may be more easily noticed by predators and thus more likely to be removed from the population (Butler & Milleman 1971). This raises the question of whether malformations induced by parasites are adaptive to the parasites by improving their transmission success to their next host (predatory eels in the case of *T. opisthorchis*). However, as all malformed individuals died by the time they were four months old, any improved transmission via predation would be offset by a reduced lifespan. Johnson et al. (1999) found that less than 50% of malformed Pacific tree frogs make it to sexual maturity, suggesting it is unlikely that malformations benefit the parasites as their window of opportunity for transmission by predation (to birds in this case) would be very limited.

When looking at the ecology of larval fish compared with mature fish, there are disparities in the importance of growth, reproduction, and avoidance of predators. For example, the most important determinant of success for a larval fish is to eat and grow as fast as possible (Anderson 1988, Letcher et al. 1996, Hare & Cowen 1997). The larger they are, and the faster they get through the larval stage, the more energy they are able to spend on fighting infection, evading predators, and reproduction (Hare & Cowen 1997). As a result, smaller, younger fish, lacking the extra energy for immune responses, and being relatively small compared to their parasites, suffer more severe effects of parasitic infection than adults do in terms of both infection level and mortality (Ryce et al. 2004, McDonald et al. 2006). Resistance to infection can change over time (Minchella & Scott 1991). The ability of young fish to resist parasitic infections is partially due to a genetically inherited immune response of which there will always be natural variation among individuals (Paterson et al. 1998). However, parasite resistance in an individual can also change over time; younger fish spend comparatively more energy and a greater immune response to resisting parasites than adults (Nordling et al. 1998). The reason for the ontogenic change in investment into immune

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responses emerges from a simple cost-benefit analysis. Adult fish have matured and as well as having the resources to cope with costly parasites, they may have already produced some offspring, therefore they have much less to lose in comparison to a juvenile in terms of potential reproductive output. For an adult, the energy spent on resisting parasites might often be greater than the cost of accepting the parasite (Poulin 1993a). The opposite is true for juvenile fish, as parasitism poses a greater threat on available energy, reducing chances of survival to reproduction or the quality of future offspring (Rosenqvist & Houde 1997). I found a significant difference in the number of parasites harboured by fish exposed at different ages. As both age groups used here were very young with only four weeks difference in the age of exposure, we may have expected the input into resisting parasites to be the same as they are of the same age class, therefore neither group had yet had a chance to reproduce, which would make the potential loss in terms of reproduction the same. However, the younger 12-week old fish accumulated a greater number of parasites per day on average, highlighting that small changes in age and development during the larval period can greatly influence susceptibility of infection. Still, this result must be interpreted with caution due to the uneven sample sizes of 12-week and 16-week old fish, with much lower sample sizes in the 16-week old age fish.

In many species, the coming of age where young fish become better able to cope with parasite infection may coincide with settlement from a pelagic to benthic existence (Altizer et al. 2011). Grutter (2010) noted a large increase in the number of infected damselfish between the pelagic (larval) and benthic (juvenile) stages. Young fish may utilise a pelagic environment where water flow is greater to avoid being close to the substrate where infected snails dwell, possibly breaking parasite life cycles (Strathmann et al. 2002), or just avoiding them until they are more able to cope with infection. Galaxiids also undergo a change in environment with age, from the pelagic zone of estuaries or lakes, to life in shallow streams. Perhaps if fish had been sampled at ages either side of the shift from a pelagic to stream-dwelling existence, there may be evidence to support that parasitism of fish in the juvenile phase is costly enough to be a driver in the evolution of the pelagic phase and diadromy (Poulin 1993a, Combes 2001, Strathmann et al. 2002,

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McDowall 2009, Grutter et al. 2010, Poulin et al. 2012). A preliminary study was planned to assess parasite loads of pelagic vs older, stream-dwelling dwelling fish by restricting fish to a specific level in the water column. Unfortunately, the study had to be abandoned due to a shortage of infected snails. However, a recent analysis by Poulin et al. (2012), suggests that life away from the substrate, characteristic of young galaxiids, serves as an escape from infection.

Studies using lab experiments in order to explain processes occurring in the wild have been criticised as to their ecological relevance, a possible limitation of this study. As we are now well aware, hosts in the wild are partial to a myriad of biotic and biotic factors influencing their ecology, many of which may work synergistically to exacerbate or pacify effects. I studied the effects of parasitism in isolation, while controlling for other factors so the results observed here may predict, but not truly reflect, the effects of parasitism in real situations.

2.4.5 Conclusion

Our knowledge of the effect of *T. opisthorchis* parasites on larval *G. anomalus* is still in its infancy. The current study has allowed us to support the initial findings of Kelly et al. (2010b) and also to extend them further, and more closely analyse how condition factors, malformations, age, and survivorship are associated with infection at the individual level. This new knowledge cannot necessarily be extrapolated to effects at the population level (Ebert 2005b) and long term effects at that level are still unknown. Although it is unlikely that parasites alone could cause the extinction of a host (de Castro & Bolker 2005a, de Castro & Bolker 2005b), the severity of parasitic infection is certainly capable of rising rapidly following environmental changes. Considering that *G. anomalus* are only found in the Taieri and Clutha catchments, that populations are fragmented, and that streams are influenced by the effects of agriculture, the future of *G. anomalus* as a species could be a concern.

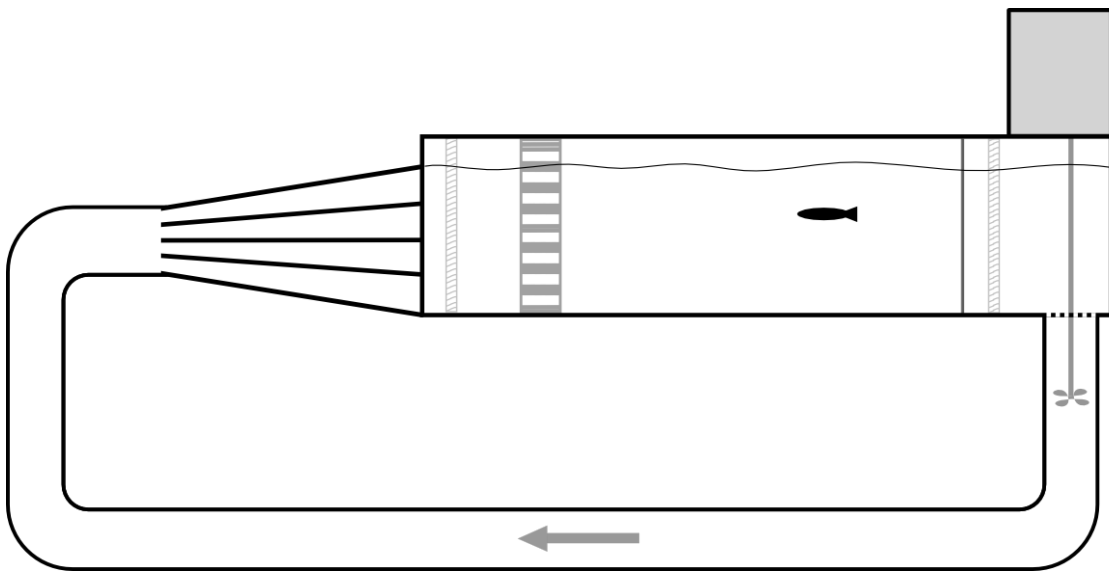
Chapter 3

The effect of the trematode

Telogaster opisthorchis on the

swimming performance of *Galaxias*

anomalus



Propeller driven swimming flume used to test fish swimming ability

3.1 Introduction

Swimming is fundamental to many aquatic organisms, increasing their chances of survival by allowing them to move freely about their environment. Fish rely on the ability to swim not only to maintain their position in the water column, obtain food, and to reach spawning grounds, but also faster ‘burst’ swimming to avoid dangers such as predation or strong currents (Drucker 1996, Plaut 2001, Doehring et al. 2012). The larval and juvenile stages of a fish coincide with the period of time between spawning and settlement, both of which are associated with high mortality. As swimming is essential for migration and recruitment, poorer swimmers are likely to make up the majority of mortalities during this time (Sale 1991, Doehring et al. 2012). Fish size, condition, swim bladder inflation, muscle strength, and behaviour all affect a fish’s swimming performance (Szekely et al. 2009, Ellerby & Herskin 2013). These factors can indirectly affect fish survival as poorer swimmers are more likely to be predated upon, are less able to avoid unfavourable conditions, and are at higher risk of being swept downstream (Taylor & McPhail 1986, Grorud-Colvert & Sponaugle 2006, Ferguson et al. 2012, Taeubert & Geist 2013).

Parasitism is also known to affect swimming ability. A range of parasites, both internal and external, have been found to alter the swimming performance of a fish, and can do so in a number of ways (Marin et al. 2009, Santos et al. 2011, Binning et al. 2013). Locomotion may be influenced behaviourally or physically. For example, behaviourally, the parasite may alter neurological processes, causing a change in behaviour that sees fish swim closer to the surface of the water, spend less time seeking the cover of vegetation, or increase noticeable, erratic-type swimming, all of which increase conspicuousness of the host to a predator (Poulin et al. 1992, Barber et al. 2000, Coyner et al. 2001, Johnson et al. 2006). Physically, parasites can essentially get in the way of the normal functioning of the host, reducing the fish’s physical fitness and decreasing foraging ability and predator avoidance (Butler & Milleman 1971, Minchella & Scott 1991, Mages & Dill 2010, Binning et al. 2013). For instance, trematode worms can clog up the heart or main arteries, reducing blood flow to the muscles (Coleman 1993), or larval bivalves attaching to gills can reduce respiratory efficiency (Taeubert &

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Geist 2013). These behavioural and physiological alterations can result in higher standard metabolic rates, poorer anaerobic capacities and lower swimming speeds, all of which can indirectly elevate parasite transmission rates, and some of which are adaptive traits for certain parasites transmitted via predation (Fox 1965, Butler & Milleman 1971, Barber et al. 2000, Wagner et al. 2003, Wagner et al. 2005, Bourque et al. 2006, Szekely et al. 2009, Poulin 2010, Santos & Santos 2013, Umberger et al. 2013). As parasitism in fish is ubiquitous, swimming performance is paramount to survival, and parasites have the ability to alter swimming performance, parasites are likely to play a key role in population dynamics merely through their impairment of fish swimming ability (Coleman 1993, Umberger et al. 2013).

To take advantage of their three-dimensional environment, fish have evolved specialised skeletal and muscular systems to be able to move through water efficiently (Jayne & Lauder 1994). There are two main muscle groups used for swimming; red muscle and white muscle. Red muscle makes up only 1-14% of total muscle tissue but is responsible for most of the fish's swimming as it is used for continuous, slow, routine swimming over long periods of time. In contrast, white muscle, which makes up a much larger percentage of the total muscle mass, is used much less often as it is used for faster 'burst' swimming. Burst swimming can only be sustained for short periods of time as there are less blood vessels within the white muscle tissue (Taylor & McPhail 1986, Jayne & Lauder 1994).

Many trematode species migrate and encyst within host muscle tissues, potentially reducing fish swimming ability by damaging muscle fibres or mechanically impairing muscle function (Baldwin et al. 1967, Pascual et al. 2006). The size of a trematode cyst is typically very small, reaching up to 0.5-1.0 mm in diameter for *Telogaster opisthorchis* cysts (Poulin et al. 2012). In young, heavily infected hosts, these cysts can take up a much greater proportion of a fish's body mass than for a larger fish, so parasitism may have greater consequences in smaller fish. For example, Umberger et al. (2013) found that swimming speeds of small nematode-infected flounder were impaired compared to small uninfected flounder, yet parasitism had negligible effect on swimming speeds in larger fish.

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The trematode parasite *T. opisthorchis*, has a complex three-host life-cycle involving an eel, where the parasite sexually reproduces and eggs are released in eel faeces, a snail, where the parasite asexually multiplies, and a small fish, such as *G. anomalus*, where the parasite encysts and awaits ingestion by an eel. As this chapter focusses specifically on the host *G. anomalus*, I will further explain the processes involved in parasite infection and encystment at this particular stage of the lifecycle.

When *T. opisthorchis* cercariae come into contact with a small fish host such as *G. anomalus*, they attach themselves using their oral sucker (See Figure 3.1). They then use enzymes and penetration spines to burrow their way through the skin, usually through the fins but sometimes through the snout or eyes (MacFarlane 1945). As they burrow through the skin into the tissue they drop their tails and migrate through the muscle tissues in search of a suitable site to encyst. The parasite moves by thrusting its anterior end and cuticular spines forward, the spines grip the tissue and pull the cercariae further in (MacFarlane 1945). This tearing action damages muscle fibres (Pascual et al. 2006) and causes haemorrhaging (Baldwin et al. 1967), which may lessen the effectiveness of the muscle as a whole. According to MacFarlane (1945) *T. opisthorchis* takes three minutes to completely burrow through the skin and another half an hour migrating through the muscle tissue to seek out a suitable site to encyst.

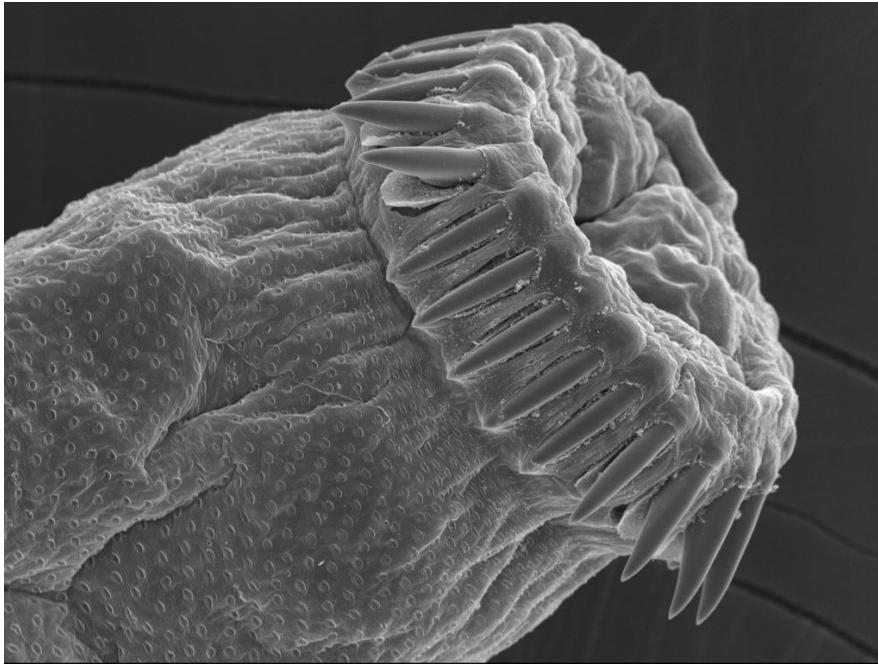


Figure 3.1: Scanning electron micrograph of the oral sucker, and its ring of spines, on an excysted metacercaria of *T. opisthorchis* (Photo: Haseeb Randhawa & Matthew Downes)

After embedding in the tissue, cercariae secrete a cyst wall around themselves as protection from host defences (Lemly & Esch 1984, Ogawa et al. 2004, Feist & Longshaw 2008). Host response begins approximately a day later when macrophages appear at the site of the parasite causing inflammation and developing a fibrous capsule around the parasite cyst which, in some fish species, can continue for up to three weeks before being melanised (Hunter & Hamilton 1941). If there are many parasites, the energy spent on encapsulating cysts, repairing muscle and skin tissues, which may then be prone to secondary infection, as well as surrendering to the parasites' continual energy demand for growth and development, may be a large cost to the host (Lemly & Esch 1984, Barber et al. 2000). High energy demands can lower fish condition, and leave less energy available for tasks such as swimming (Tinsley 1990). Hence, those with lower condition are more likely to have impaired swimming ability.

Parasites may not only interfere with muscles involved in swimming, but can also affect the skeletal structures necessary for efficient swimming. If a parasite encysts near the spine or fin bud while the host is still growing and calcifying its skeleton, the cyst may permanently alter the normal growth of bone in that area (Kelly et al. 2010b), hindering swimming ability further. Poor swimming ability of malformed individuals may be associated with the decrease in the proportion of malformed juveniles Kelly et al. (2010b) observed in a wild population of *G. anomalus* over the summer season.

3.1.1 Aims and hypotheses

Parasite-associated mortality has been well studied in a range of host-parasite relationships. As parasites generally take advantage of their host's energy stores, leaving them weakened, heavily parasitised hosts tend to have lower condition factors (Lemly & Esch 1984). Swimming ability relies heavily on the muscle mass and fitness of a fish, so those with lower condition factors are likely to have decreased swimming performance. Furthermore, as swimming is fundamental to fish survival, and poor swimming ability can contribute to the demise of a fish, swimming ability may be used as a proxy to assess fish health. Little is known about the effects of parasites on *G. anomalus*, and there is not yet any information on swimming ability in relation to parasites. Therefore, the purpose of the present study is to find out whether the parasite *T. opisthorchis* affects the swimming ability of the roundhead galaxiid, *G. anomalus*, and what consequences that may have for the survival of both the host and parasite.

Swimming tests will be conducted using *G. anomalus* hosts exposed to infected and uninfected snails to determine whether parasitism with *T. opisthorchis* has any effect on swimming ability. To assess swimming ability, a clear plexiglas recirculating flume will be used to maintain a constant water flow in which fish can be observed. It is hypothesised that fish which perform better in swimming tests will have fewer parasites than those which perform poorly. It is also hypothesised that those which perform better will also have higher condition factors and be larger in size.

3.2 Methods

3.2.1 Experimental subjects

Fish which survived the parasite exposure experiments from Chapter 2, were then used as subjects for the swimming experiment. Because fish from the parasite exposure experiment had been subjected to a varying number of infected snails including a control group exposed to uninfected snails, it was expected that there would be a range of parasite loads among fish (See Chapter 2 for methodology regarding exposure of fish to *T. opisthorchis* infected snails). Fish were exposed over the same period to either four uninfected snails (control), two infected and two uninfected snails (low exposure), or four infected snails (high exposure) over 20 days. At the commencement of the swimming experiment, 29 control fish and 11 fish exposed to snails were alive and able to be used in the trial, including three fish with obvious spinal malformations.

All previous experimental infections and the current swimming test were endorsed by the University of Otago Ethics committee (approval number # 63/09).

3.2.2 Flume tank

A propeller driven flume, initially created and used by Hurd et al. (1994) to observe the movement of seawater around macrophytes, was used to test the swimming ability of each fish. The plexiglas recirculating flume was a small 46 L capacity flume which integrated an expander section and a collimator (Figure 3.2). The collimator was made up of a 5 cm thick panel of plastic drinking straws, splitting the flow and allowing a more uniform movement through the test section. Mesh screens at each end of the test section were used as baffles and also prevented fish from being sucked into the propeller.

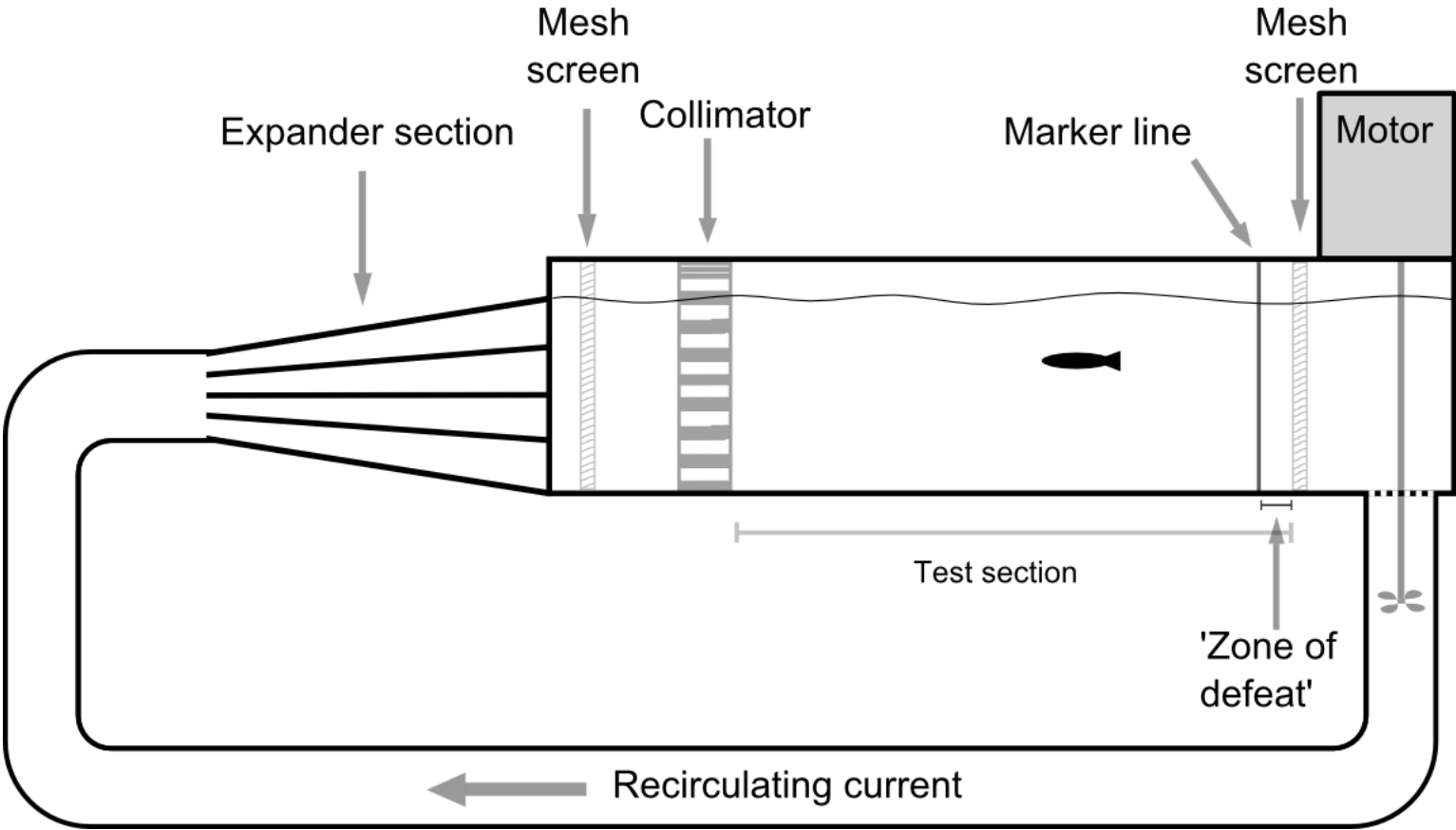


Figure 3.2: Profile view of the propeller driven recirculating flume. Diagram adapted from Hurd et al. (1994)

Chapter 3: Swimming experiments

Flumes can be used to test swimming ability in several different ways. For instance, critical swimming speeds can be measured by slowly increasing flow at regular intervals until the fish becomes fatigued, burst swimming speed tests fish in faster flowing water to simulate emergency response type swimming, or intermediate level of swimming (Taylor & McPhail 1986, Videler & Wardle 1991, Jayne & Lauder 1994, Hammer 1995, Nikora et al. 2003). Even though all three swimming modes are necessary for fish, and all can be used as an indicator of fitness, an intermediate level of swimming was chosen to test fish in this instance. This is also relevant to galaxiid fish, which must maintain their position in stream currents in order to feed. The majority of juvenile *G. anomalus* prefer flows $<0.1 \text{ m s}^{-1}$, whereas the majority of adults favour flows between 0.1 m s^{-1} and 0.3 m s^{-1} (Baker et al. 2003). Fish are able to hold their position in water currents due to their opto-motor response (Plaut 2001), so fish were expected to be swimming until fatigued.

3.2.3 Swimming tests

On the 2nd of March 2010, swimming tests on all remaining fish from the previous exposure experiment were carried out. The flume was filled with aged aerated tap water, and the flow set to a constant 0.15 m s^{-1} , slightly higher than flows favoured by juveniles in the wild. A marker line was drawn on the flume three cm from the back mesh and dubbed the ‘zone of defeat’ (Figure 3.2). One fish was observed at a time by two observers. Fish were placed in the running flume and given one minute to acclimatise. After the acclimatisation period, the fish was observed for three minutes. I recorded the length of time spent in the zone of defeat, after failing to maintain its position upstream, during the three-minute time period. Immediately after the test, fish were euthanized with an overdose of MS-222 then fixed in 10% buffered formalin for at least one week before being transferred to 70% ethanol.

3.2.4 Clearing and staining protocol

Clearing and staining of each fish was carried out using the same protocol as described in Chapter 2. Dissecting of fish and counting of cysts was also carried out in the same way.

3.2.5 Measures and statistical analyses

Condition factors are simple indices, frequently used to assess the health of a fish (Tadiri et al. 2013). Fish condition factors can be estimated using just length and weight, however there are many variations of the original equation (Jones et al. 1999). In essence, it is assumed that the greater the weight of a fish for a given length, the better its condition. The equation used to estimate condition is the same as the one used in Chapter 2.

Unfortunately during the fish tests, most fish spent either all or none of their time swimming in the zone of defeat, making it difficult to relate their number of parasites with their swimming ability. The distribution of the time spent in the zone of defeat was clearly bimodal, and could not be transformed in any way to remedy this prior to analysis. Therefore, data for the few fish which spent time in both zones were omitted from statistical analyses leaving two distinct groups; those which spent the whole time in the zone of defeat, assumed to be the weaker swimmers, were grouped in the 'low fitness' category, while those which never entered the zone of defeat, the stronger swimmers, were grouped in the 'high fitness' category. From there I was able to compare the two groups, strong swimmers and weak swimmers, and then compare their parasite loads and other factors which may influence swimming ability. One-way ANOVAs were used to compare the two groups in each case.

Statistical analyses were carried out using the statistical software package SPSS version 16 (SPSS Inc., Chicago, Illinois, U.S.A). Note that where necessary, variables were $\log(x+1)$ -transformed if zero values were present in order to meet the assumptions of normality.

3.3 Results

Of the 40 fish which were tested in the swimming flume, 11 were infected and 29 were uninfected. Sixteen fish were grouped in the high fitness category and 10 in the low fitness category. The proportion of time spent in the zone of defeat for fish in the medium fitness category ranged from 0.01-0.98. Infected fish had the highest proportion of individuals in the low fitness category, while uninfected fish had the highest proportion of individuals in the high fitness category (Table 3.1).

Table 3.1: Distribution of fitness levels between infected and uninfected fish

	High swimming fitness	Medium swimming fitness	Low swimming fitness	Total
Infected fish	2	4	5	11
Uninfected fish	14	10	5	29
Total	16	14	10	40

The majority (>80%) of *T. opisthorchis* cysts were located within the hosts' muscle tissue. Just less than 20% of cysts were found in the gut of the fish, and no parasites were found encysted in the head (Table 3.2).

Table 3.2: Distribution of metacercarial cysts found throughout the host body.

	High swimming fitness	Medium swimming fitness	Low swimming fitness	Total
Muscle	13	23	21	57
Body cavity	4	3	6	13
Head	0	0	0	0

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Of the 16 fish in the high fitness category, two were infected, however among the group of 10 fish in the low fitness category, five were infected. Although there was much variation, fish in the low fitness group had a significantly higher parasite load than those in the high fitness group (One Way ANOVA: $F_{1,25} = 4.494$, $p = 0.045$) (Figure 3.3 a). There was no difference in either fish length or condition factor between the high and low fitness groups (Figure 3.3 b, c), nor was there a significant correlation between fish length and parasite load of fish used in this experiment.

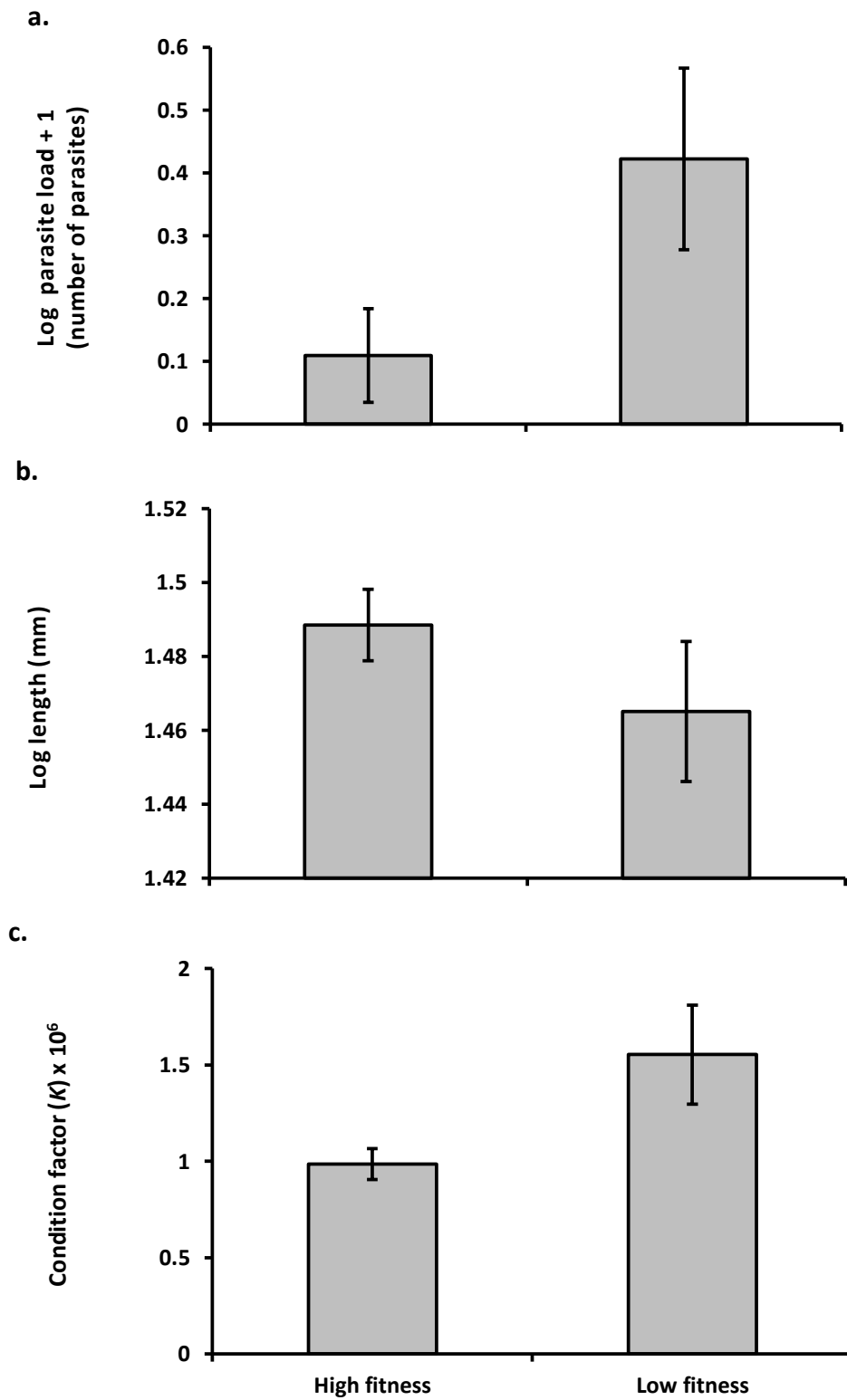


Figure 3.3: Comparing (a) parasite load, (b) length, and (c) condition with swimming ability of fish grouped into high (n = 16) and low (n = 10) fitness categories

3.4 Discussion

Parasites affect the swimming ability of fish in various ways. The type of parasite, host and site of infection within the host body can affect fish differently (Butler & Milleman 1971, Santos & Santos 2013). Here, the trematode parasite *T. opisthorchis*, a muscle encysting trematode, was used to assess the effects of parasitism on fish swimming ability in *G. anomalus*.

Telogaster opisthorchis is well recognised as a muscle encysting trematode, yet cysts were located in both the muscle tissue and the body cavity. Swimming ability varied amongst infected and uninfected individuals, however, fish with low swimming ability had significantly more parasites than those with a high swimming ability. This means infected individuals are more likely to experience mortality either by fish being purged from the flood-prone Taieri and Clutha catchments, by decreasing predator avoidance, or reducing foraging ability leading to starvation. This is consistent with recent research concerning parasites and swimming performance (see Wagner et al. 2005, Palstra et al. 2007, Mages & Dill 2010, Santos et al. 2011, Binning et al. 2013). For example, Palstra et al. (2007) noticed an effect on swimming and swim bladder efficiency in eels, where highly infected eels performed poorly in swimming tests, and Mages and Dill (2010) found sea lice to reduce swimming endurance in juvenile salmon. Fish with higher parasite loads are likely to have more damaged muscle tissues as a result of the migration of parasites through tissues, and therefore less efficient muscle, making swimming a lot more taxing than for fish with lower parasite loads. Yet, the extent to which damaged muscle tissue interferes with the efficiency of muscle fibres is not well studied. Jones and Moffitt (2004), and Butler and Milleman (1971) suggested that the timing of exercise tests after trematode infection may be more important than the parasite load per se, as tests conducted later would give hosts a chance to repair damaged tissues. This suggests the migration of the trematode prior to encysting could be more influential on swimming ability than just the presence or absence of parasites. Butler and Milleman (1971) tested newly infected fish as well as fish which had been infected 15 days prior to swimming tests. They found the effect of trematodes on swimming ability to be more pronounced in fish infected within the previous 96 hours, whereas fish with older

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encysted parasites, which had been experimentally infected at least 15 days earlier, showed little difference to controls. The length of time between exposure and swimming tests in the present study may account for the low difference between high and low fitness groups and the considerable variation in the medium fitness level group. In my own experiment, fish had between 16 days and two months from exposure to parasites until the swimming experiment, meaning damaged muscle fibres resulting from trematode migration would probably have healed, although scar tissue may still remain. Furthermore, the present data may underestimate the actual effects fish may be experiencing in the wild as it is assumed fish would be constantly exposed to parasites and therefore constantly be spending energy on encapsulating cysts and repairing skin and muscle tissues. In the wild this may cause hosts to spend more time foraging to meet the energy costs associated with harbouring parasites, increasing exposure time to predators and potentially exacerbating the effects of parasitism (Ferguson et al. 2012).

Even though parasites were not expected to be migrating through tissue when swimming tests were carried out, and already encysted parasites may only minimally alter swimming ability (Butler & Milleman 1971, Abrous et al. 2001), the size of the metacercariae in relation to the small size of the fish may still be important (McDonald et al. 2006, Mages & Dill 2010, Umberger et al. 2013). While most studies concerning parasites and swimming ability of fish have used adults, the current study used juvenile fish, so the relative mass of trematode cysts in comparison to total body mass in juveniles may be much greater than in adults. For example, five parasites in a larval host are likely to have more of an impact on swimming ability than five parasites infecting an adult host. As I did not test any adult fish and there is no record of swimming tests being performed on infected *G. anomalus* in the past, the swimming ability of infected adult and smaller juvenile fish cannot be compared. Nevertheless, data on fish length, which would be expected to be representative of fish mass, is available for the current study. Unfortunately, using fish from the same age class gave a very low range of fish lengths and I found no relationship between fish length and parasite load.

It would be expected that larger fish, or fish with higher condition factors, would be associated with better swimming ability as fish size and condition have

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both been an indicator of swimming ability in the past (Grorud-Colvert & Sponaugle 2006). Basaran et al. (2007) observed the critical swimming speed (U_{crit}) of sea bream to be higher in larger individuals. And Ferguson et al. (2012) believed fish size was strongly linked to swimming stamina. However there was no difference in length between the high and low fitness groups which, again, may be due to the narrow range in fish lengths and a small sample size.

Another factor which may influence swimming ability is the location of parasite cysts within the host body (Umberger et al. 2013). Parasite location was categorised into three groups where *T. opisthorchis* cysts have previously been found; in the muscle tissue, body cavity and head. *Telogaster opisthorchis* is recognised as a muscle encysting trematode, and the majority of cysts were found in muscle tissue as expected. However, approximately 20% of cysts were located in the body cavity of the fish. Parasites encysting in the body cavity may affect swimming ability in a different way, or not at all. As far as muscle encysting trematodes are concerned, the specific type of muscle tissue each cyst was found in was not defined. This may have been an important oversight as a parasite encysting near the base of the pectoral fin may hinder swimming ability more than it would in other muscle tissues (Umberger et al. 2013). Unfortunately, it is impossible to manipulate cyst position in order to better compare how cyst location may affect swimming ability.

Regrettably, analysis of the swimming ability of malformed individuals could not be carried out as there were only three malformed fish still alive after the previous experiment. Malformations are likely to have a substantial effect on swimming ability, and therefore survival, as seen in previous studies where lordosis (curvature of the spine) and other skeletal malformations decreased parasite avoidance and survival (Chatain 1994, Goodman & Johnson 2011). There are very few documented accounts of malformations in native New Zealand fish, most commonly referring to malformed jaws. One documented example is provided by Allibone (2000) who noted *G. depressiceps*, closely related to *G. anomalus*, as having malformations of the head, jaw and sometimes fins. He did not consider parasites as a potential cause of these malformations but suggested environmental stressors as a likely reason for the observed

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malformations. It is known that *T. opisthorchis* parasites are abundant in the area where Allibone (2000) obtained his fish, and are able to infect other galaxiid species including *G. depressiceps*. Yet it is unknown whether *T. opisthorchis* is responsible for the malformations seen by G.P. Wallis and J.M. Waters (pers. comm.) and Allibone (2000) in several species of galaxiid in recent years. Even though it has been demonstrated that parasites can cause malformations in a controlled laboratory environment in non-polluted water, the addition of pesticides may increase parasite loads in fish by creating an environment which may enhance the release of cercariae from snail hosts and therefore increase the likelihood of a malformation (Johnson & Chase 2004, Johnson et al. 2007, Rohr et al. 2008, Kelly et al. 2010a, Hock & Poulin 2012). Hock and Poulin (2012) monitored the release of cercariae from *Potamopyrgus antipodarum* snails in various concentrations of glyphosate, the active ingredient in the widely used pesticide Roundup®. They found an increase in cercarial emergence at low concentrations of the chemical compared to clean water. Furthermore, Kelly et al. (2010a) found *G. anomalus* fish exposed to *T. opisthorchis* infected *P. antipodarum* snails, in a low concentration of the same chemical, to have higher parasite loads than those in clean water. This relatively new information, coupled with the fact that snail densities are higher in areas of intense land use, where these types of chemicals leech into waterways, suggests a potential increase in parasites within the whole system, and may influence population dynamics of *G. anomalus* in the long term. Moreover, Goertzen et al. (2011) found that polluted water was detrimental to fish swimming ability in a study of normal (non-malformed) fish. So fish swimming performance may be affected by parasite load and water quality, regardless if malformations are present.

The biggest limitation of this study may have been testing swimming ability itself. The bimodal nature of the data suggests improvements could have been made to the study design. The flow rate used in the flume to test swimming ability might have been too high, causing many fish to tire, or simply give in, before the acclimatisation period was over. This could be resolved by testing fish at a lower velocity for a longer period of time in an attempt to gather data with a normal distribution. The limitations not only refer to study design, but also the

design of the flume itself. As with any laboratory experiment, mimicking the natural environment is extremely difficult (Abrous et al. 2001, Plaut 2001) and swimming flumes have been widely criticised as a reliable method of testing swimming ability (Ellerby & Herskin 2013). Perfect laminar flow will always be challenging to achieve (Beamish 1978), and even though baffles were implemented in attempt to keep water flow as laminar as possible, fish were able to find areas of slower water velocity, e.g. near the sides of the tank (pers. obs.). Furthermore, deciding on a reliable way to test swimming ability is challenging, and no method is perfect. Alternatively, fish could have been tested at several stages post-exposure to parasites in order to determine whether early infection and migration have an effect on swimming ability.

3.4.1 Conclusion

Telogaster opisthorchis parasites can indirectly affect the swimming ability of *G. anomalus*. However the evidence in the present study is limited. Poorer swimming ability did correspond with higher parasite loads, yet we are no closer to knowing whether size or condition of juvenile fish have an impact on their swimming ability and potentially their survival. Locations of *T. opisthorchis* cysts within the host body were varied and greater sample sizes would be needed in order to compare the effects of body cavity and musculature cysts or pectoral and flank muscle cysts for example, as manipulative placement of parasites is not an option. While we now know that *T. opisthorchis* can affect swimming ability, we are still unaware of the impact of other parasites or combined effects of multiple parasites on *G. anomalus*. From the parasites' point of view, decreasing swimming ability would be beneficial as it would result in an increase in transmission rate, however, this is unlikely to be an adaptive trait. Even though the negative effects of parasitism such as decreased swimming performance may have an impact at the individual level, they may not necessarily flow on to the population level which could be devastating to the survival of a threatened species like *G. anomalus*. Long term field studies may help to further assess the impact of parasitism on the future of *G. anomalus*.

Chapter 4:

General discussion



Collecting snails in Tomahawk lagoon, Dunedin

4.1 Discussion

In fish, great fecundity is balanced by high mortality during early development (Dahlberg 1979). The critical period during which mortality is highest typically spans early development from the egg to post-larval stages, after which, the likelihood of reaching maturity dramatically increases (Sun et al. 2012). During this critical period, fish are much more vulnerable to biological factors, physical factors, and the interactions between these two sets of factors (Fogarty et al. 1991). These combine to influence survival of early life stages. And change in the factors that hinder survival during the critical stage can go on to modify population dynamics.

This thesis aimed to uncover the role of parasitism in the development and survival of larval fish. More specifically I intended to further our knowledge on the host-parasite relationship between the trematode *T. opisthorchis* and the larval stage of its' second intermediate fish host, *G. anomalus*. The primary aims of the study were, firstly, to validate initial experiments carried out by Kelly et al. (2010b). This involved experimentally testing whether exposure to a greater number of infected snails would lead to higher parasite loads in fish, a greater number of malformations, and a reduction in host survival. Secondly, I wanted to explore further, and find out whether host age, or the length of time hosts are exposed to parasites, influenced parasite load or the likelihood of developing a malformation. Additionally, I wanted to find out whether infection and malformations affect the swimming ability of juvenile fish.

The findings of the present study support those of Kelly et al. (2010b) work on the trematode *T. opisthorchis* and its intermediate fish host *G. anomalus*. My results show that under experimental conditions, *T. opisthorchis* is indeed a threat to juvenile *G. anomalus*, as survival of *G. anomalus* individuals is compromised when parasite loads are high, or when infection results in a malformation, yet there is no difference in survival at low exposure. As expected, parasite loads of fish were directly influenced by the number of infected snails each host is exposed to, and the length of time the host was exposed. Finally, the swimming ability of

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G. anomalus is influenced by *T. opisthorchis*, where fish with poor swimming ability were found to have higher parasite loads.

These results suggest trematode infection reduces survivorship of juvenile *G. anomalus*, exacerbating type III survival curves, both directly and indirectly. Directly via infection and its associated pathology, and indirectly via reduced swimming fitness which may lead to mortality either by increased probability of being overcome by strong currents, decreased ability to avoid predators, or decreased foraging ability leading to starvation. The impact of parasites at this fragile life stage highlights the importance of larval survival to the success of host populations. Even though predation and starvation, not parasitism, are still likely to be the biggest causes of larval mortality, parasitism can potentially exacerbate these mechanisms (Hudson & Greenman 1998, Bourque et al. 2006, Townsend et al. 2011, Sun et al. 2012). As a fish's critical period is characterised by both high susceptibility to infection and a phase of high predation, the infection of a larval host instead of an adult host may seem like the fastest gateway for a trematode to reach its definitive host. However, cercariae infecting intermediate hosts must first metamorphose into mature metacercariae to become infective to, and successfully live within the definitive host. Metamorphosis of trematode parasites within a second intermediate host usually requires a period of weeks (Roberts & Janovy 2000), so immature parasites being consumed by a definitive host would not survive. It is also unclear whether eels consume very small galaxiids, like the ones among which malformations are common. Regardless, *G. anomalus* will still be especially vulnerable to parasite-induced population disturbances as they are a non-diadromous fish with a fragmented distribution and limited gene flow between populations, factors putting the species further at risk (King & Wallis 1998, Department of Conservation 2009, Allibone et al. 2010).

Human actions can also contribute to a cascade of events, ultimately threatening the survival of *G. anomalus* by altering the dynamics of pre-existing ecological processes. For instance, changes in land use, or farming practices such as water abstraction, or actions resulting in chemical or sediment run-off, can increase stream temperatures or increase snail densities. These can increase cercarial output from snail hosts, leading to increased parasite loads and

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prevalence of malformations in fish which can then alter survival rates and possibly population dynamics (Kelly et al. 2010a). Therefore, these findings are important to inform farmers, land developers, policy makers, and the general public of the significance of human activities on ecosystem dynamics. Increased awareness helps to facilitate better management programs and adjust BMPs for farmers and other land users in an attempt to minimise human influence on freshwater ecosystems (Rhodes et al. 2007).

4.1.1 Further research

The current research revealed new information on host-parasite relationships between trematodes and larval fish hosts, however it has also identified areas where further research is required.

As we are in the initial stages of research in this area, I studied the effects of *T. opisthorchis* parasites on *G. anomalus* in isolation, experimentally controlling for all other factors, yet follow-up experiments are required in order to assess how larval hosts respond to multiple factors simultaneously. Galaxiids in New Zealand are infected by numerous different species of parasites, and not solely by *T. opisthorchis* (Hine et al. 2000). Infection by multiple species of parasite at once, or in combination with varying abiotic factors such as temperature may further influence the likelihood of infection, malformation, or mortality (Sun et al. 2012). By considering synergism between multiple factors we can establish a more realistic interpretation of host-parasite relationships in nature.

Combining experimental studies in the laboratory with field surveys in wild populations allows the backup of laboratory results with real-world observations. Even though Kelly et al. (2010b) carried out field surveys looking into the effect of *T. opisthorchis* on a *G. anomalus* population over time, the surveys only followed one population over a single summer. Data collected over a number of years would allow a better picture of population dynamics in the long term. Likewise, sampling multiple populations, or sampling in areas of differing land use, varying snail infection prevalence, various climates, presence/abundance of native and exotic predators, or the presence of channels or other man-made

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alterations of stream flow, would contribute to a better understanding of the role of parasitism in population dynamics.

This study assumed poorer swimming ability of fish would naturally correspond with a higher probability of predation, as previous experiments on fish and frogs have demonstrated (Taylor & McPhail 1985, Blake et al. 2006, Mages & Dill 2010, Goodman & Johnson 2011). However, this is yet to be tested specifically on *G. anomalus*. To verify this assumption, a swimming experiment like the one described here should be carried out followed by predation experiments using a predator housed with both infected and uninfected *G. anomalus* fish. Alternatively, *G. anomalus* fish of varying parasite loads or with or without malformations could be used. Conducting both swimming and predation experiments would allow comparison of known swimming ability with likelihood of predation.

Lastly, the experiments conducted here could be replicated on other intermediate host species of *T. opisthorchis* to compare how infection impacts larval fish species differently. It would be especially interesting to see whether *T. opisthorchis* parasites can cause malformations in other fish species. Comparisons could also be made between diadromous and non-diadromous fish, since juveniles of the former species leave freshwater habitats (where exposure to the parasite occurs) for much of their juvenile stages.

4.1.2 Conclusion

The experimental trials carried out in this study emphasise the importance for ecologists of considering the role of parasitism on survival, population dynamics, and other ecosystem dynamics. Trematode parasites can both directly and indirectly increase mortality rates in larval fish, contributing to the mass mortality observed during critical periods and possibly impacting overall host population levels. As multiple factors interact simultaneously to shape a population, parasitism will affect populations differently depending on the presence of other environmental influences, making the future of fragmented fish populations such as *G. anomalus* challenging to predict (Anderson & May 1978, Redpath et al. 2006, Longshaw et al. 2010, Townsend et al. 2011, Sun et al. 2012).

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The classification of *G. anomalus* as ‘vulnerable’, along with the knowledge we have gained on *G. anomalus* here, suggest that numbers of *G. anomalus* will continue to be stable, but that management of the species should be carefully monitored and BMPs must continue to be encouraged in order to prevent decline. Following BMPs will not only benefit *G. anomalus*, but a myriad of other fish and invertebrate species whilst helping to maintain biodiversity and ecosystem functions.

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